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Hereditary Nonpolyposis Colorectal Cancer: Diagnostic Strategies and Their Implications

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The Centers for Disease Control and Prevention (CDC) requested and provided funding for this report. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to **epc@ahrq.gov.**

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Structured Abstract

Objectives: Hereditary Nonpolyposis Colorectal Cancer (HNPCC) has been defined clinically and genetically. The disorder has traditionally been recognized in kindreds with a clustering of related cancers in association with mutations in DNA mismatch repair genes. HNPCC is associated with a substantially increased risk for several forms of malignancy but particularly colorectal and endometrial cancer.

There were three main objectives of this report: (1) to assess the sensitivity, specificity, and reliability of laboratory and genetic tests commonly used in evaluating patients for HNPCC (analytic validity); (2) to summarize the accuracy of commonly used clinical and laboratory characteristics for predicting the presence of HNPCC in patients with colorectal cancer (clinical validity) and use these estimates to describe the efficiency of various strategies for identifying patients with a mismatch repair mutation; (3) to describe the benefits and harms related to screening and testing patients with colorectal cancer and their family members for HNPCC.

Data Sources: Published literature identified through an electronic search (through April 2006), review of relevant bibliographies, and suggestions from technical experts.

Review Methods: We evaluated studies critically and summarized the data qualitatively or by meta-analysis when studies used similar methodology and endpoints. We used decision trees to describe the efficiency of various strategies for identifying patients with HNPCC from a hypothetical population of patients with colorectal cancer.

Results: We included a total of 104 studies of which 40 addressed issues related to clinical validity, 3 to analytic validity, and 61 to benefits and harms.

We identified only three studies on analytic validity and thus there exists a major gap in the published literature with regard to the accuracy and reliability of specific tests used in the evaluation of HNPCC.

Among unselected patients with colorectal cancer who fulfilled the Amsterdam I criteria, 44% (95% CI: 35, 52%) carried pathogenic mismatch repair mutations (mainly in the MLH1 and MSH2 genes). The proportion was somewhat higher (51% [95% CI: 35, 66%]) among studies that performed sequencing on all available samples. The prevalence of MMR mutation carriers may be higher when genetic testing includes evaluation for large genomic deletions/rearrangements and when testing is also performed on MSH6 and PMS2. Approximately 71% (95% CI 63, 78%) of colorectal cancers from patients who fulfilled the Amsterdam I criteria demonstrated microsatellite instability while 40% (95% CI: 28, 53%) demonstrated loss of protein expression by immunohistochemistry.

Of nine clinical strategies considered for detecting the presence of mismatch repair mutations in patients with colorectal cancer, the combination of three clinical predictors (age less than 50 years old at diagnosis; or a history of colorectal or endometrial cancer in a first degree family member; or the presence of multiple, synchronous or metachronous colorectal or endometrial cancers in the proband) combined with either immunohistochemistry (IHC) or MSI testing of tumor tissue identified a similar number of patients with mismatch repair mutations as other more complex strategies.

There was little published information regarding potential harms associated with screening individuals with HNPCC-related cancers using clinical criteria (e.g., the Amsterdam criteria), MSI or IHC testing. Limited data suggested that testing probands for MMR mutations was not associated with severe psychological impact following formal counseling. Pre-test genetic counseling had good efficacy in improving knowledge about HNPCC and resulted in a high likelihood of proceeding with genetic testing, satisfaction in the decision to undergo genetic testing, and decreasing depression and distress levels among family members of HNPCC probands with cancer and among asymptomatic individuals from HNPCC families.

Identification of HNPCC mutations was associated with an increase in the likelihood that family members of probands with CRC would undergo cancer-screening procedures. HNPCC family members who underwent cancer-screening procedures had a lower risk of developing HNPCC-related cancers and lower mortality rates than those who did not take actions. However, all of the relevant studies suggesting these benefits had important limitations. Survival was increased among asymptomatic HNPCC family members who received colonoscopy screening, regardless of their mutation status. There was limited direct evidence related to harms of the cancer-screening procedures in family members of probands with HNPCC. However, complication rates associated with these procedures in other settings are probably similar.

Conclusions: This report characterizes the accuracy of clinical and laboratory predictors of MMR mutations that can be used to identify patients with an increased risk of having MMR mutations. However, the sensitivity, specificity, and reliability of the tests used to evaluate individuals for suspected HNPCC is not known confidently. Data regarding the net benefits and harms associated with predictive genetic testing in patients with HNPCC-related cancers and their families members is incomplete but suggest that such testing improves compliance with screening procedures. At-risk family members who undergo screening colonoscopy have a reduced risk of developing HNPCC-related cancers and lower mortality. However, all studies supporting these benefits had important limitations.

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Appendixes and Evidence Tables for this report are provided electronically at
http://www.ahrq.gov/downloads/pub/evidence/pdf/hnpcc/hnpcc.pdf

Executive Summary

Introduction

Individuals with a familial predisposition to cancer pose an increasing challenge for healthcare systems hoping to provide state-of-the-art care. Optimal strategies for recognizing them, performing (and interpreting) genetic testing, and preventing cancers in at-risk family members have not been well established when considering overall benefits, harms, and costs. The challenges involved will likely become increasingly complicated with advances in understanding of the molecular genetics underlying cancer risk. In this report, we attempt to clarify many of these issues for one form of hereditary cancer, Hereditary Nonpolyposis Colorectal Cancer (HNPCC). HNPCC is associated with a substantially increased risk for several forms of malignancy but particularly colorectal and endometrial cancer.

HNPCC has been defined clinically and genetically. As a genetic disease, it is inherited as an autosomal dominant disorder (with variable penetrance) caused by mutations in DNA mismatch repair (MMR) genes. As a clinical disorder, it can be defined as a clustering of related cancers across generations in a kindred. The disorder has also been referred to as the "Lynch syndrome" in recognition of Henry Lynch, who in 1966 described familial aggregation of colorectal cancer with gastric and endometrial cancer in two large kindreds (although it was first reported by the eminent pathologist Aldred Warthin in 1913).

These definitions are not entirely distinct since the disorder is typically recognized in patients or kindreds with clinical expression of the disease who have an associated genotype. However, the ability to perform predictive genetic testing in individuals with cancer and asymptomatic family members makes it imperative to fully understand its implications. There remain many uncertainties regarding how HNPCC should be identified in patients presenting with associated malignancies, and the full spectrum of implications related to screening and management options for the patient and their at-risk family members. Does, for example, the identification of MMR genotypes in family members of patients with HNPCC-related cancers improve their outcomes compared with management approaches that do not involve predictive genetic testing? What is the likelihood that a family member with a MMR mutation is destined to develop HNPCC-related cancers, and does screening for these cancers improve outcomes?

There were three main objectives of this report: (1) to assess the sensitivity, specificity and reliability of laboratory and genetic tests commonly used in evaluating patients for HNPCC (analytic validity); (2) to summarize the accuracy of commonly used clinical and laboratory characteristics for predicting the presence of HNPCC in patients with colorectal cancer (clinical validity) and to describe the efficiency of various strategies for identifying patients with a mismatch repair mutation; and (3) to describe the benefits and harms related to screening and testing patients with colorectal cancer (CRC) and their family members for HNPCC.

This report is based upon a systematic review of the literature. The Key Questions that it addresses were proposed through the Agency for Healthcare Research and Quality (AHRQ) on behalf of the Centers for Disease Control and Prevention (CDC) Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Project. EGAPP is a three-year model project developed by CDC's Office of Genomics and Disease Prevention to address the increasingly urgent need for timely and objective information that will allow health care providers,

consumers, policy makers and payers to distinguish tests that are safe and useful, and to guide their appropriate use in practice.

The Key Questions Addressed Include the Following:

Key Question 1: Does risk assessment and HNPCC mutation testing in patients with newly diagnosed CRC lead to improved outcomes for the patient or family members, or is it useful in medical, personal, or public health decision making? (Over-arching question).

Key Question 2a: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has a mismatch repair mutation?
2b: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has MSI?
2c: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has abnormal protein expression by immunohistochemistry?
2: How accurate are various predictors, assuming a genetic definition of the Lynch Syndrome?

Key Question 3: What are the harms associated with screening high-risk individuals for HNPCC?

Key Question 4: What is known about the analytic (sensitivity, specificity, reproducibility, reliability) and clinical validity of tests that identify HNPCC mutations?

Key Question 5: What are the harms associated with screening for high-risk individuals?

Key Question 6a: What are the management options for CRC patients who are HNPCC positive?
6b: Does the identification of HNPCC mutations lead to improved patient outcomes in terms of early detection, mortality/morbidity or management decisions (e.g., counseling, surveillance, treatment, other decision making) by patients and providers?

Key Question 7: What are the harms associated with subsequent management options after identification of HNPCC mutations in CRC patients?

Key Question 8a: What is the efficacy of pre-test genetic counseling for informing family members of potential risks and benefits of testing?
8b: What is the accuracy of HNPCC testing in family members in predicting the risk of CRC?
8c: Do other factors, such as race/ethnicity, age, gender, or co-morbidities affect the accuracy of the testing?

Key Question 9: What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations?

Key Question 10a: What are the management options for family members of CRC patients who have a positive HNPCC test?

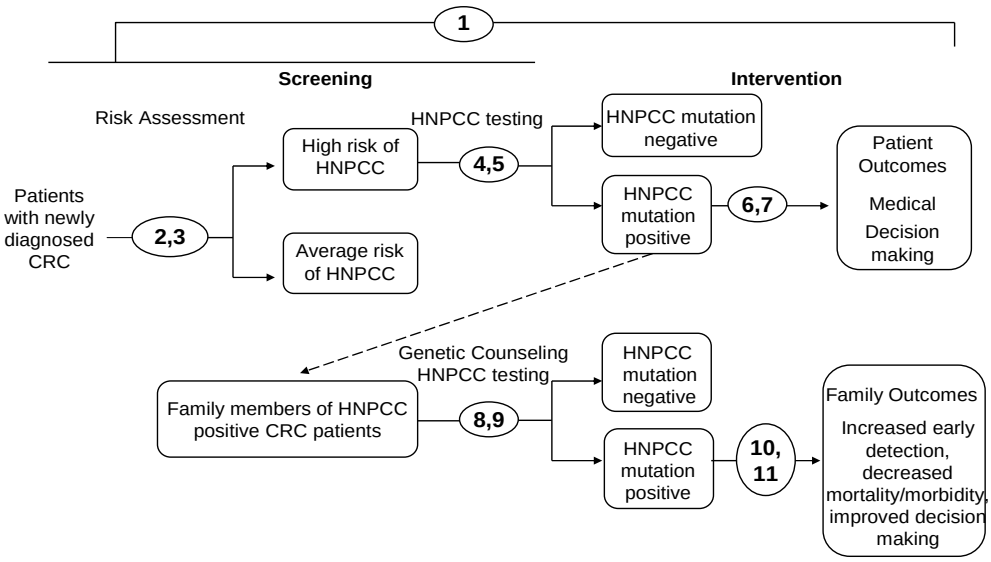
10b1: Does the identification of HNPCC mutations lead to improved outcomes in terms of decision making by patients, family members and providers, or public health policy?

10b2: Does the identification of HNPCC mutations lead to improved outcomes in terms of early detection and mortality/morbidity of patients, family members?

Key Question 11: What are the harms associated with subsequent actions or interventions for family members?

These questions were based upon an analytic framework that begins with a patient with colorectal cancer and examines the full spectrum of implications of identifying HNPCC in the patient and their at-risk family members, and the potential benefits and harms of this process from the perspectives of the patient, provider, family member and the public health.

Screening for HNPCC in Newly Diagnosed CRC Patients and Their Family Members



Methods

We performed an electronic search of the literature followed by review of abstracts and then full-text review of potentially relevant studies. We also retrieved additional studies based on bibliographies of retrieved articles and suggestions from a technical expert panel. We evaluated all studies critically based upon pre-specified criteria.

We reviewed the full-text of 523 publications. One hundred fifteen papers fulfilled eligibility criteria but 11 were duplicate reports and were therefore excluded (or used to provide supplementary information). Thus, 104 unique studies were included for this review (40 were pertinent to Key Question 2 – clinical validity; 3 were pertinent to Key Question 4 – analytic

validity; and 61 to the remaining Key Questions – benefits and harms), while 408 did not meet the inclusion criteria and were rejected.

We reviewed each study for its quality. We assigned an overall quality score (A, B or C) to provide a short hand appraisal of the overall validity of the study but also performed analysis of specific components of study quality that may have influenced the conclusions.

We combined data in studies that used similar methodology and definitions of endpoints in similarly selected CRC populations using meta-analysis to provide a point estimate and 95% confidence interval, mainly for questions pertaining to clinical validity.

We constructed decision trees models to calculate the reported outcomes of various strategies for identifying HNPCC among patients with colorectal cancer. The models were based upon parameters estimated from data presented in this report.

Results

Analytic Validity

Key Question 4: What is known about the analytic (sensitivity, specificity, reproducibility, reliability) and clinical validity of tests that identify HNPCC mutations?

The major laboratory tests used in the evaluation of patients suspected of having HNPCC include testing of tumor tissue using immunohistochemistry (IHC), microsatellite instability (MSI) testing, or germline (generally from peripheral blood mononuclear cells) testing for mismatch repair defects. Analytic validity may also apply to the accuracy of the family history.

Family members generally undergo only germline genetic testing (unless they have also developed a relevant cancer), ideally based upon the genotype of the proband. Detection of a pathogenic mutation (i.e., one known to be associated with HNPCC) in a proband permits testing of at-risk family members for the genotype. Family members with the same genotype have HNPCC while HNPCC can be excluded in those who do not. The situation is more complex when the probands do not have a detectable DNA alteration associated with HNPCC or when an alteration with unclear clinical significance is detected.

There was little published information on the analytic validity of laboratory testing in patients or family members suspected of having HNPCC. Thus, the analytic validity of the tests used to evaluate HNPCC is substantially uncertain. However, there was heterogeneity in the type of testing offered by commercial laboratories, and one study demonstrated variability in the accuracy of protein staining by IHC across facilities.

Additional information that could shed light upon analytic validity is available but would require evaluation of non-published data sources. For example, information has been collected through an external proficiency-testing program for MSI conducted by the College of American Pathologists and through internal testing performed by individual laboratories. Committees of experts could also review the strengths and limitations of specific testing techniques based upon clinical experience with these methods in HNPCC and in other genetically based disorders.

Clinical Validity

Key Questions 2a, 2b and 2c seek to evaluate the prevalence of MMR mutations, suggestive MSI and IHC, respectively, among patients fulfilling the Amsterdam I criteria or the Amsterdam

II criteria. We considered several variables in making comparisons across studies such as how populations were selected, the methods used for genetic testing, whether and how mutations were described as being pathogenic, and whether testing for MMR genes other than MLH1 and MSH2 was performed.

Key Question 2a: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has a mismatch repair mutation?

Most eligible studies evaluated only MLH1 and MSH2 genes; only three studies assessed other MMR genes. Among CRC fulfilling Amsterdam I criteria, the random effects summary prevalence of MLH1 and MSH2 gene mutations was 44% (95% CI: 35, 52; n=19 studies, 464 patients), with evidence for substantial between-study heterogeneity ($p<0.01$; $I^2=52\%$). The six studies that performed sequencing among all Amsterdam I patients had a summary prevalence of 51% (95% CI: 35, 66%).

For patients fulfilling the Amsterdam II criteria, the corresponding prevalence values were 39% (95% CI: 30, 49%; 10 studies 279 Amsterdam II patients) and 40% (95% CI: 30, 52%) based upon two studies that performed sequencing on all 87 Amsterdam II patients.

The three studies examining additional genes (MSH6, PMS1 and PMS2) in Amsterdam I patients did not identify any additional MMR mutations. Two additional MSH6 mutations (in addition to five MLH1 and MSH2 mutations) were found among 20 Amsterdam II patients in a single study.

There were limited studies that performed more comprehensive genetic testing for pathogenic MMR genotypes associated with HNPCC or evaluated all the MMR genes that have been associated with HNPCC. For example, only one study performed sequencing in all samples and tested for large genomic mutations/rearrangements for three MMR genes (MLH1, MSH2, MSH6). The study included only 22 Amsterdam I patients; the prevalence of MMR mutation carriers was 64%.

Limited data suggested that approximately one-fourth to one-third of genotypes associated with HNPCC are related to deletions or rearrangements that would be missed through sequencing alone. As a result, the prevalence of mutations in Amsterdam I or II patients assessed from studies that performed sequencing alone are likely to be underestimates. Accounting for this effect, one may derive a prevalence of MMR mutations of approximately 63% to 67% in Amsterdam I patients. For Amsterdam II patients the corresponding prevalence values would be 50% to 53%.

Furthermore, limited data suggest that approximately 10% of MMR genotypes involve MMR genes other than MLH1 and MSH2. Thus, assuming an additional 10% increase in mutations by assessing more genes would result in an overall prevalence of up to 70% to 75% for Amsterdam I patients and 55 to 59% for Amsterdam II patients.

Because of the limited data, these calculations should be considered as being highly imprecise. Furthermore, other considerations, such as how patients are selected and how genotypes are classified as being pathogenic, may affect these estimates.

Key Question 2b: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has MSI?

Among patients fulfilling the Amsterdam I criteria, 71% (95% CI: 63, 78%; n=11 studies, 159 patients) of tumors were MSI-H. Among patients who fulfilled the Amsterdam II criteria, the corresponding summary prevalence was 68% (95% CI: 58, 76%; n=4 studies, 102 patients).

Key Question 2c: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has abnormal protein expression by immunohistochemistry?

Among patients fulfilling the Amsterdam I criteria the overall prevalence of tumors with loss of protein expression by IHC was 40% (95% CI: 28, 53%; n= 6 studies, 63 patients) with no evidence for between-study heterogeneity (p=0.75, I^2=0%).

Only one eligible study provided relevant data for 20 patients fulfilling the Amsterdam II criteria. Eight out of 20 tumors had suggestive IHC for the MLH1, MSH2 or MSH6 genes (40% [95% CI: 9, 64%]).

MSI and IHC testing often depended upon practical and logistical considerations (e.g., patient availability and consent and availability of tumor tissue). Thus, not all patients had all tests and it was unclear whether additional bias may have been introduced in selecting patients for testing.

Key Question 2: How accurate are various predictors assuming a genetic definition of the Lynch Syndrome?

Five studies provided information on eight clinical predictors and on suggestive MSI testing for predicting the presence of MMR mutations among unselected incident CRC. All were limited by verification bias. The table below summarizes the sensitivity and specificity of the predictors most commonly reported (see Executive Summary Table 1).

Executive Summary Table 1. Diagnostic ability of various predictors to detect MMR mutations among unselected incident CRC

Predictor	Unselected CRC probands		
	N	Sensitivity [%] (95% CI)	Specificity [%] (95% CI)
Amsterdam I criteria	2	45 (29, 63)	99 (74, 100)
Amsterdam II criteria	2	28 (15, 47)	99 (97, 100)
Modified Amsterdam criteria	0	ND	ND
Bethesda guidelines	1	73 (39, 94)	82 (80, 84)
Revised Bethesda guidelines	1	91 (59, 100)	77 (75, 79)
Age <50 years	3	31 (18, 47)	95 (94, 96)
1st degree family history of CRC or EC	4	76 (50, 91)	87 (86, 89)
Multiple CRC or EC tumors in the same patient	3	38 (25, 54)	97 (91, 99)
Age <50 years, family history of CRC or EC, or multiple tumors in same patient	3	88 (60, 97)	77 (74, 81)
Suggestive MSI[a]	2	100 (88, 100)	90 (88, 92)
Suggestive IHC	0	ND	ND

CRC: colorectal cancer; EC: endometrial cancer; N: Number of studies; ND: no data.
[a] Estimates are the same for combined MSI-H and MSI-L versus MSS and for MSI-H versus MSS

Decision Tree Modeling of Genetic Testing Strategies. We evaluated nine strategies for identifying patients with CRC with MMR mutations based upon combinations of clinical features, laboratory testing of tumor tissue (i.e., IHC and MSI testing). These can be conceptually organized into four general strategies:

- Group 1: Perform genetic test on everyone
- Group 2: Screen with a set of clinical criteria

- Group 3: Screen tumor tissue with a laboratory test
- Group 4: Screen using two serial tests: a set of clinical criteria first, and then a laboratory test of tumor tissue

Of these, Group 4 strategies selected relatively fewer patients for genetic testing (one out of twenty-five or fewer) and missed at most in approximately 27% of patients with HNPCC. Mismatch repair mutation testing would be performed in less than 6% of CRC patients using these strategies. In contrast, for strategies in groups 1 to 3, more than 13% and up to 100% of newly diagnosed CRC patients would be genetically tested, and approximately 5% to 16% of patients with HNPCC would be missed. Similar results were obtained for both the low (0.90%) and the higher (2.75%) estimate for the prevalence of mutation carriers among incident unselected CRC (see Executive Summary Table 2).

Executive Summary Table 2. Expected number of MMR, MSI or IHC tests and expected MMR testing results with the nine strategies, assuming a population of 100,000 incident cases of CRC

Strategy	Received tests			Number of MMR tests that were			Unidentified MMR mutation carriers
	MMR	MSI	IHC	Positive	True positive	Inconclusive	
Low prevalence estimate for MMR mutation carriers (0.90%)							
MMR-All	100,000	0	0	1,351	855	252	45
BethR-All	23,612	0	0	892	778	61	122
3Clinical-All	23,585	0	0	866	752	61	148
MSI-All	13,738	100,000	0	877	812	37	88
IHC-All	13,702	0	100,000	843	778	37	122
BethR-MSI	3,741	23,612	0	754	739	12	161
3Clinical-MSI	3,716	23,585	0	730	715	11	185
BethR-IHC	1,828	0	23,612	659	654	6	246
3Clinical-IHC	3,684	0	23,585	700	685	11	215
Higher prevalence estimate for MMR mutation carriers (2.75%)							
MMR-All	100,000	0	0	3,098	2,612	257	138
BethR-All	24,870	0	0	2,489	2,377	69	373
3Clinical-All	24,787	0	0	2,410	2,299	69	451
MSI-All	15,255	100,000	0	2,545	2,481	46	269
IHC-All	15,145	0	100,000	2,440	2,377	45	373
BethR-MSI	5,285	24,870	0	2,273	2,258	20	492
3Clinical-MSI	5,206	24,787	0	2,198	2,184	20	566
BethR-IHC	3,220	0	24,870	2,002	1,997	14	753
3Clinical-IHC	5,110	0	24,787	2,106	2,092	20	658

In the hypothetical population, for the low prevalence estimate 900/100,000 patients are assumed to carry MMR mutations; for the high prevalence estimate 2750 people are assumed to carry MMR mutations. Strategies are presented with respect to the group (1 to 4) to which they belong. MMR-All: test all for mismatch repair mutations; BethR-All: test all using the revised Bethesda criteria; 3 Clinical-all: Perform MMR testing only among those fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis, family history of CRC or endometrial cancer, or multiple tumors, synchronous or metachronous, in the same patient); MSI-All: Perform MSI testing on all patients, followed by MMR testing only among those with suggestive MSI test; IHC-all: Perform immunohistochemistry (IHC) testing on all patients followed by MMR testing only among those with suggestive IHC test; BethR-MSI: Perform MSI testing on patients fulfilling the revised Bethesda guidelines, perform MMR only among those with suggestive MSI test;3 Clinical-MSI: Perform MSI testing on patients fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis, family history of CRC or endometrial cancer or multiple tumors, synchronous or metachronous, in the same patient); perform MMR only among those with suggestive MSI test; BethR-IHC: Perform IHC testing on patients fulfilling the revised Bethesda guidelines; perform MMR only among those with suggestive IHC test; 3 Clinical-IHC: Perform IHC testing on patients fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis, family history of CRC or endometrial cancer or multiple tumors, synchronous or metachronous, in the same patient; perform MMR only among those with suggestive IHC test.

The overall (strategy-level) sensitivity and specificity are shown in Executive Summary Table 3. The overall specificity was high in all strategies. Group 4 strategies had the lowest overall sensitivity (ranging between 73% and 82%). Overall sensitivity and specificity estimates were similar for both the low (0.90%) and the higher (2.75%) prevalence estimates.

Executive Summary Table 3. Overall sensitivity and specificity for each of the nine strategies

Strategy	Sensitivity of strategy (%)	Specificity of strategy (%)
MMR-All	95.0	99.3
BethR-All	86.5	99.8
3Clinical-All	83.6	99.8
MSI-All	90.3	99.9
IHC-All	86.5	99.9
BethR-MSI	82.1	100.0
3Clinical-MSI	79.4	100.0
BethR-IHC	72.6	100.0
3Clinical-IHC	76.1	100.0

Strategies are presented with respect to the group (1 to 4) to which they belong. In the above estimates the sensitivity and the specificity of the whole strategy was calculated. Inconclusive MMR tests were assumed to be false negative (for the calculation of overall sensitivity for each strategy) or false positive (for the calculation of overall specificity for each strategy).

Thus, of the nine clinical strategies, the presence of at least one of three clinical predictors: (i) age less than 50 years old at diagnosis, or ii) a history of colorectal or endometrial cancer in a first degree family member, or iii) the presence of multiple, synchronous or metachronous colorectal or endometrial cancers in the proband, combined with testing of tumor tissue for either IHC or MSI, identified a similar number of patients with colorectal cancer who had mismatch repair mutations associated with HNPCC (and failed to identify a similar number) compared with other, more complex approaches. There were relatively more studies demonstrating test characteristics of MSI testing compared with IHC and thus greater precision in the estimates of sensitivity and specificity of MSI testing.

Benefits and Harms

Key Question 1: Does risk assessment and HNPCC mutation testing in patients with newly diagnosed CRC lead to improved outcomes for the patient or family members, or is it useful in medical, personal, or public health decision making? (Over-arching question)

No study directly addressed Key Question 1.

Key Question 3: What are the harms associated with screening high-risk individuals for HNPCC?

Studies were considered eligible for Key Question 3 if they reported harms of a risk assessment process (e.g., Amsterdam, Bethesda and/or MSI, IHC) used to identify CRC patients at increased risk for HNPCC.

No study described harms of the risk assessment process in CRC patients at increased risk for HNPCC.

Key Question 5: What are the harms associated with screening for high-risk individuals?

Studies were considered eligible for Key Question 5 if they reported the harms associated with testing CRC patients for MMR mutations. Common harms that are thought to be associated with genetic testing are labeling, discrimination in health coverage, and emotional distress.

Three quantitative, comparative studies of quality A and B, and one qualitative study of B quality reported harms associated with MMR mutation testing in CRC patients. One 1-year prospective study (Grade A) compared the psychological impact of MMR mutation testing between mutation carriers and non-carriers. Subjects in this study were CRC probands or relatives from HNPCC families with a prior diagnosis of any cancer (excluding non-melanoma skin cancer). Anxiety, depression, and quality of life measures did not change over time, and there were no differences in these measures between mutation carriers and non-carriers. Distress levels were significantly decreased 2 weeks and 6 months after revealing the genetic testing results but were not significantly different from the baseline at 1-year follow-up. There was no difference in the distress levels between mutation carriers and non-carriers.

Another 1-month prospective study (Grade B) found that three of the 27 probands (11%) had minor depression at 1 month after revealing the genetic testing results, but the prevalence of minor depression was not significantly different compared to the prevalence at baseline or between mutation carriers and non-carriers. Of the six probands who received a positive result, two (33%) felt severe guilt regarding their children.

One prospective study (Grade B) reported changes in the psychological outcomes of CRC patients from self-completed questionnaires pre- and 4-6 weeks post-genetic counseling. There was no genetic testing performed in this study. There was a trend toward greater anticipated ability to cope with a positive gene test after counseling, as reflected by a decrease in anxiety and cancer-specific distress.

The qualitative study of 111 newly diagnosed CRC patients reported a high acceptance and understanding about information on HNPCC. Nineteen percent of participants rated their current level of worry caused by the genetics information at or above the midpoint of 4 on a 1 (not at all) to 7 (all the time) scale.

Key Question 6a: What are the management options for CRC patients who are HNPCC positive?

6b: Does the identification of HNPCC mutations lead to improved patient outcomes in terms of early detection, mortality/morbidity or management decisions (e.g., counseling, surveillance, treatment, other decision making) by patients and providers?

There are three aspects to Key Question 6: 1) Are management options for patients with CRC with a MMR mutation different from those without a MMR mutation? 2) Does the knowledge of MMR mutation status change management decisions by patients and providers? 3) Does changing management options for MMR positive patients with CRC improve outcomes (e.g., prognosis and survival) compared to standard approaches for patients with CRC?

We encountered a variety of surgical and medical management options in patients with CRC who were MMR positive but there were no comparative studies.

Indirect evidence from one study suggested that identification of HNPCC mutations was associated with better prognosis of CRC. However, there were no data on whether management options for CRC differed based on MMR mutation status.

Indirect evidence from one study showed no difference in survival of patients with endometrial cancer, comparing those who were mutation positive to those who were mutation negative.

In five studies with indirect evidence, there was no evidence in favor or against differences in survival, when comparing CRC patients who fulfilled different clinical criteria for HNPCC or screened positive for HNPCC by suggestive laboratory testing with those who did not.

Key Question 7: What are the harms associated with subsequent management options after identification of HNPCC mutations in CRC patients?

No study described harms associated with subsequent management options after identification of HNPCC mutations in patients with CRC or other forms of HNPCC-related cancers.

One study (involving two centers) described the types of colorectal surgery performed on CRC patients who were part of an Amsterdam criteria-positive family, and compared rates of metachronous cancers that followed each type of index operation. The overall rate of second surgeries for metachronous cancer were 23% in patients who underwent right colectomy, 17% in patients who underwent left/sigmoid colectomy or proctosigmoidectomy, 0% in patients who underwent total/subtotal colectomy, and 44% in patients who underwent segmental colectomy. The two centers had significantly different second resection rates for metachronous cancer.

One study described the survival rate of 45 patients with gastric cancer from HNPCC families with MMR mutations. Many of these patients had already had treatments for other HNPCC-related cancers, including CRC. The 5-year survival was higher in patients in whom radical surgery was performed (48%) than in patients in whom radical palliative surgery or explorative laparotomy alone was performed (15%).

Key Question 8b: What is the accuracy of HNPCC testing in family members in predicting the risk of CRC?

8c: Do other factors, such as race/ethnicity, age, gender, or co-morbidities affect the accuracy of the testing?

Only two studies of B quality reported the risk of CRC in family members of probands with positive MMR mutations (the proposed framework). The lifetime risk of CRC was 68.7% for men and 52.2% for women with MMR mutations in one study, and it was 74% and 30% respectively in the other study. Men had higher lifetime risk of CRC than women in both studies.

In a study that reported the risk of CRC in family members of probands with HNPCC based on clinical criteria, the cumulative risk of CRC by age 75 years old was 57% and 41% in families that fulfilled the Amsterdam I and II criteria, respectively. The cumulative risk of CRC by age 75 years old was 42% and 23% for men and women, respectively, from families that fulfilled Bethesda criteria. In another study, family members of CRC probands who were younger than 50 years old at cancer diagnosis had a higher risk of CRC, compared to family members of CRC probands who were 50 years old and older. The risk of CRC was increased three-fold, comparing

first-degree relatives of CRC probands who developed a second primary in the HNPCC spectrum with the single primary group in a third report.

In a study that reported the risk of CRC in kindreds with HNPCC based on genetic criteria, the cumulative risk of CRC by age 70 yr was 82% in MLH1/MSH2 mutation carriers. The cumulative incidence of CRC was 100% in men and 54% in women. There was overall a higher risk of CRC in men, but the sex difference was not consistent among HNPCC kindreds with different MMR mutations.

Key Question 9: What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations?

Key Question 8a: What is the efficacy of pre-test genetic counseling for informing family members of potential risks and benefits of testing?

Studies were considered eligible for Key Question 8a if they summarized the efficacy of pre-test genetic counseling immediately after counseling and before performing genetic testing. Studies addressing Key Question 9 were considered together with those addressing Key Question 8a because there was substantial overlap in the studies addressing these questions. The studies addressed the long-term efficacy of pre-test genetic counseling or harms associated with screening high-risk individuals (such as by using clinical criteria, MSI, or IHC), genetic testing, and informing/counseling family members or with subsequent testing for HNPCC mutations.

Four studies addressed the efficacy of pre-test genetic counseling immediately after counseling and before performing MMR genetic testing. Of these, three were of B quality and one was of C quality.

Eight comparative and four qualitative studies addressed the harms associated with genetic testing for HNPCC mutation or with informing/counseling family members or with subsequent testing for HNPCC mutations. Of these, one study was of A quality, six of B quality, and five of C quality.

Overall, pre-test genetic counseling had good efficacy in improving knowledge about HNPCC, and resulted in high likelihood of proceeding with genetic testing, satisfaction in the decision to undergo genetic testing, and decreasing depression and distress levels among family members of HNPCC probands or among asymptomatic individuals from HNPCC families. Family members of HNPCC who received positive MMR mutation test results had higher psychological distress levels and anxiety compared to those who received negative test results, but this difference generally disappeared with time. However, all of these psychological measures were within the normal ranges for the general populations. There were no differences in quality of life, comparing mutation carriers to non-carriers or the general population.

Key Question 10a: What are the management options for family members of CRC patients who have a positive HNPCC test?

b1: Does the identification of HNPCC mutations lead to improved outcomes in terms of decision making by patients, family members and providers, or public health policy?

b2: Does the identification of HNPCC mutations lead to improved outcomes in terms of early detection and mortality/morbidity of patients, family members?

We included studies evaluating all forms of cancer related to HNPCC since family members of CRC probands are potentially at risk for all such cancers.

Six studies examined the impact of mutation testing on the decision to undergo specific management recommendations among family members of CRC patients or asymptomatic individuals from HNPCC families. Of these, four were of B quality and two were of C quality. No study directly examined the impact of HNPCC mutation testing on public health policy or decision making by insurance providers.

Two studies (in three publications) of B and C quality indirectly addressed outcomes of early detection and mortality/morbidity in relation to the identification of MMR mutations in family members of CRC probands or asymptomatic individuals from HNPCC families. These studies were limited by potential selection bias, and/or unclear effects from treatments or subsequent interventions.

Identification of HNPCC mutations was associated with improved outcomes in terms of decision making to undergo screening for cancers in family members of HNPCC. HNPCC family members who took subsequent actions or interventions had a lower risk of developing HNPCC-related cancers and lower mortality rates, compared to those who did not take actions. Most data pertained to screening for CRC with colonoscopy while there was less information about screening for other forms of HNPCC-related cancer.

Key Question 11: What are the harms associated with subsequent actions or interventions for family members?

We included studies that reported any outcome relating to subsequent management options or interventions in HNPCC family members.

Nine studies reported outcomes related to subsequent actions or interventions among family members. Of these, six were of B quality and three were of C quality. Four of these nine studies reported harms or adverse events associated with subsequent actions or interventions for family members. In addition, one study of C quality examined the psychological impacts associated with colonoscopies. Some of these studies did not have a control group of subjects who declined to undergo surveillance and most results did not adjust for potential confounders such as age, personal history of cancer, and educational levels.

Less than 0.5 percent of family members experienced harms associated with screening or surveillance examination or surgical procedures in the studies we evaluated. However, complication rates associated with these interventions in the non-HNPCC setting are probably applicable. There was some negative psychological impact associated with colonoscopies.

Implications for Future Research

Our report identified several areas for future research; we considered the following to be particularly important priorities:

- There is very little information regarding the analytic validity of tests used in the diagnosis of HNPCC. Studies specifically addressing sensitivity, specificity and reliability of all of the laboratory and genetic testing methods in HNPCC are needed. Such studies should focus on contemporary testing methods and compare them against well-defined reference standards in tissue samples representative of the spectrum of genotypes associated with HNPCC. Unpublished information regarding analytic validity is also available; it may be feasible to

obtain information from commercial or private laboratories performing such testing. It may also be possible to obtain data from the College of American Pathologists regarding their MSI proficiency program once experience has accumulated. Experience with genetic testing methods in other conditions is likely to be relevant to HNPCC; a review of such information could be conducted by groups of experts on genetic testing techniques.

- Additional studies are needed to clarify the validity of specific clinical and laboratory predictors of HNPCC in patients with CRC who are representative of the general population of patients with CRC.

- Future studies should consider all forms of genetically based CRC cancer predisposition to fully understand the effectiveness of various diagnostic strategies. Such studies should consider all known genetic causes of cancer predisposition and the accuracy of clinical and laboratory testing in identifying these disorders in individuals who are representative of the general population.

- Additional studies are needed to establish the availability of genetic testing centers that can provide adequate counseling and whether there are barriers to access them. Such studies may involve electronic, mail, or telephone surveys.

- More studies are needed to understand what forms of surveillance should be offered to MMR mutation carriers for HNPCC-related cancers other than CRC. Well-designed controlled trials comparing various surveillance (or other management) strategies could be helpful.

- More studies are needed to clarify the risk of cancer in family members of probands with an HNPCC-related cancer who are found to carry MMR mutations. Such studies would ideally be prospective, fully account for interventions (such as cancer screening procedures) in those at-risk, and have a well-defined control population of individuals at average risk for cancer.

- Standards for reporting studies of genetically based diseases (including those addressing all aspects of the ACCE model) should be developed. A consensus development process with publication of a guideline(s) could be helpful.

Evidence Report

Chapter 1. Introduction

Background

Individuals with a familial predisposition to cancer pose an increasing challenge for healthcare systems hoping to provide state-of-the-art care. Optimal strategies for recognizing them, performing (and interpreting) genetic testing, and preventing cancers in at-risk family members have not been well established when considering overall benefits, harms, and costs. The challenges involved will likely become increasingly complicated with advances in understanding of the molecular genetics underlying cancer risk. In this report, we attempt to clarify many of these issues for one form of hereditary cancer (hereditary nonpolyposis colorectal cancer).

This evidence review is based upon a systematic review of the literature. The Key Questions that it addresses were proposed by the Agency for Healthcare Research and Quality (AHRQ) on behalf of the Centers for Disease Control and Prevention (CDC) Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Project. EGAPP is a three-year model project developed by CDC's Office of Genomics and Disease Prevention to address the increasingly urgent need for timely and objective information that will allow health care providers, consumers, policy makers, and payers to distinguish tests that are safe and useful, and to guide their appropriate use in practice.

Hereditary Nonpolyposis Colorectal Cancer (HNPCC) can be defined clinically or genetically as will be described below. As a genetic disease it is inherited as an autosomal dominant disorder (with variable penetrance) and is caused by mutations in DNA mismatch repair genes. HNPCC is associated with a substantially increased risk for several forms of malignancy but particularly colorectal and endometrial cancer[1-3] (see Table 1).

Table 1. Lifetime cancer risk in HNPCC

Cancer	HNPCC %	General Population %
Colorectal	80-82	5-6
Endometrial	50-60	2-3
Gastric	13	1
Ovarian	12	1-2
Small bowel	1-4	0.01
Bladder	4	1-3
Brain	4	0.6
Kidney, renal pelvis	3	1
Biliary tract	2	0.5

From Chung, DC. Ann Intern Med 2003; 138:560; HNPCC: Refers to individuals with Lynch Syndrome.

The disorder has also been referred to as the "Lynch syndrome" in recognition of Henry Lynch, who in 1966 first described familial aggregation of colorectal cancer with gastric and

endometrial cancer in two large kindreds (although it was first reported by the eminent pathologist Aldred Warthin in 1913).[4] Lynch I syndrome (also referred to as HNPCC I) refers to kindreds in which colorectal cancer predominates, while Lynch II syndrome (HNPCC II) refers to kindreds who also have extracolonic tumors. The Muir-Torre syndrome (sebaceous gland tumors with or without keratoacanthomas associated with visceral malignancy) and Turcot syndrome (HNPCC-related tumors associated with glioblastoma multiforme) describe additional subsets of HNPCC with tumor types that appear to cluster in affected families. These distinctions have become less clear as more families have been studied, and as a result, these clinical classifications are being supplanted by genetic classification.[5-7] The most common mismatch repair mutations associated with HNPCC are MLH1 and MSH2 (which together are believed to account for about 80 percent of cases) while MSH6 and PMS2 are less common.[7]

The precise cancer burden due to HNPCC has not been well defined. However, a germline mismatch repair mutation associated with HNPCC has been described in 1-5% of patients diagnosed with colorectal cancer in various reports.[8-12] Thus, HNPCC accounted for approximately 1400 to 7300 cases of colorectal cancer in 2005 in the United States based upon the overall estimate of 145,290 new cases of colorectal cancer.[13] Similarly, approximately 0.5-2% of patients with endometrial cancer has a history compatible with HNPCC.[14,15] More than 1 in 3100 people between the ages of 15 and 74 are estimated to carry a defective DNA mismatch repair gene associated with HNPCC and thus are at risk for developing an HNPCC-related cancer.[16]

The recognition of a heightened cancer risk in carriers of mismatch repair mutations provides hope for offering screening, intensive surveillance or other measures (such as colectomy or hysterectomy) aimed at reducing the risk that cancer will develop. Furthermore, identification of a specific gene defect allows for testing of family members potentially sparing them the worry, bother, and expense associated with lifelong cancer surveillance if they do not carry the mutation. Notably, HNPCC has been excluded in 97 living members of the original family described by Henry Lynch based upon genetic testing.[17]

However, many uncertainties remain on the sequence of events that should occur in selecting patients with cancer to undergo testing for HNPCC and the spectrum of implications that screening, surveillance, and other management strategies have on affected patients and their families. The following summarizes many of these issues, while outlining those that are the subject of this report.

The framework for this report begins with patients with colorectal cancer, and explores the issues of screening and testing patients with colorectal cancer, identifying, counseling and testing at-risk family members, and the benefits and harms of subsequent management options for the probands and family members. We evaluate these issues from the perspectives of the patient, caregiver, family member, and policy-maker. The analytic framework is described in further detail in Chapters 2 and 3.

Who Should be Screened?

Screening for HNPCC has been most widely advocated in patients with colorectal cancer while there is much less information about screening in patients with other forms of HNPCC-related cancers. This report focuses only on colorectal cancer. However, recognition of HNPCC may also be possible in patients presenting with other HNPCC-related cancers. In one study, for example, one-half of women with HNPCC (defined by the Amsterdam criteria) presented with

endometrial cancer.[18] It may also be feasible to identify an HNPCC kindred from individuals without a personal family history of an HNPCC-related cancer by obtaining a detailed family history.

Several studies have described estimates of the proportion of patients with colorectal cancer who have a mismatch repair mutation, fulfill clinical criteria for a familial cancer predisposition, or have clinical (e.g., tumor location or histology) or laboratory (e.g., abnormal staining for mismatch repair proteins [IHC] or microsatellite instability [MSI]) characteristics of their tumor tissue that suggest the diagnosis. In this report, we evaluate such studies in detail to produce estimates of these parameters and attempt to explain variability across studies to an extent possible. These parameters are important for defining cost-effective pathways for evaluating patients with colorectal cancer for HNPCC and caring for family members.[19] However, this report does not include a formal cost-effectiveness analysis.

How Should HNPCC be Defined?

The definition of HNPCC continues to evolve. Some authorities consider HNPCC to represent a superset of individuals with a mismatch repair mutation of whom a subset is considered to have the familial cancer clustering described by Henry Lynch. Such an approach recognizes the uncertainty that remains regarding the penetrance of cancer in carriers of mismatch repair mutations; the risk of cancer in a family with Lynch Syndrome may be substantially different compared with the risk associated with a mismatch repair mutation alone without such a history.[20]

A different view is that Lynch Syndrome represents a description of familial cancer clustering, a subset of which is caused by mismatch repair mutations. Patients with familial clustering of HNPCC-related cancers but without a mutation may have a different form of hereditary cancer (e.g., cancers caused by mutations in the MYH gene)[21] while others may have HNPCC caused by a mismatch repair mutation that was not sought, or from a false negative result of testing. These distinctions are important because they identify subgroups of patients with variable cancer risk, and they may influence how family members are screened and subsequent surveillance strategies. For example, at least one report suggested that families who fulfill the Amsterdam criteria for Lynch syndrome (described below), but do not have an identifiable mismatch repair mutation, might be at lower cancer risk compared with those in whom a mutation is identified.[22]

An advantage of this view is that it establishes a reference standard for Lynch syndrome that is relatively more objective than one based upon a clinical definition. For example, it may be possible to establish the diagnosis of HNPCC in a proband with colorectal cancer who does not fulfill classical criteria for Lynch syndrome. In addition, a genetically based reference standard provides a framework for calculating sensitivity, specificity and predictive values of various predictors of mismatch repair mutation that might be useful in selecting patients for genetic studies (see Table 2).

Table 2. 2x2 table for test characteristics when considering the presence of a mismatch repair mutation as the reference standard

Patients with Colorectal Cancer		Pathogenic Mismatch Repair Mutation	
Test		Detected	Not detected
Microsatellite instability Abnormal immunohistochemistry Amsterdam Criteria I Amsterdam Criteria II Bethesda Guidelines Revised Bethesda Guidelines Other	Positive	A	B
	Negative	C	D

As will be described further in the methods section, in this report we take both views on HNPCC depending upon which Key Question is being addressed. For questions related to the test characteristics of predictor tests (such as clinical criteria or laboratory tests of tumor tissue described below), HNPCC can be defined as the presence of a pathogenic mismatch repair mutation in a patient with an HNPCC-related mutation (see Table 2). In this model, test characteristics depend upon how comprehensively mutation testing (e.g., the number of mismatch repair mutations tested and methods of testing, and the accuracy of the specific laboratory methods used), and predictor tests on tumor tissue (e.g., the quality of IHC analysis and the specific methods used to determine MSI status) were carried out and whether a mutation was known to be pathogenic. We describe the type of testing performed in all studies included in this report and provide a general description of how they are used in Chapters 2 and 3.

Not all mismatch repair mutations that can be identified using modern genetic testing are known to be pathogenic, leaving some uncertainty as to whether a mismatch repair mutation found in a patient with an HNPCC-related cancer is responsible for the increased cancer risk. The strongest evidence that a mutation is pathogenic is when its presence correlates strongly with the clinical expression of the disease. A mutation may also be considered pathogenic when its predicted protein sequence is expected to lead to a dysfunctional protein. In this report, the methods by which the authors attempted to define pathogenic mutations (if at all) were recorded for all eligible studies.

However, in some cases, the observed genotype has an unclear relationship to the clinical expression of the disease. Thus, even under the best of circumstances, genetic testing may produce an ambiguous result, making it unclear how the patient and their family should be counseled.[2]

Test characteristics are also vulnerable to several other features of study design, particularly selection bias, spectrum effects and verification bias, potentially helping to explain the variable results that have been described in the literature. In this report, we attempt to define these issues clearly to permit valid comparisons among studies. For example, a study applying clinical criteria to an unselected, consecutive population of patients with colorectal cancer may produce substantially different estimates of the accuracy of the Amsterdam criteria compared with a study enrolling patients who were referred to a tertiary care medical center because of multiple occurrences of cancer in the family. The latter group would be expected to have a higher prevalence of HNPCC and correspondingly better predictive values for the Amsterdam criteria.

In considering a clinical diagnosis of Lynch syndrome, sensitivity can also be defined as the proportion of patients with Lynch syndrome (defined by the Amsterdam criteria) with a specific

predictor (see Table 3). Specificity would be the proportion of unselected patients with colorectal cancer in whom these predictor variables are present.

Table 3. 2x2 table for test characteristics when considering Amsterdam criteria I as the reference standard

Patients with Colorectal Cancer		Lynch syndrome (Amsterdam criteria I)	
Test		Present	Absent
Microsatellite instability Abnormal immunohistochemistry	Positive	A	B
	Negative	C	D

However, this view permits only a limited understanding of the sensitivity or specificity of clinical criteria used to screen patients for HNPCC since many such criteria are included in the Amsterdam criteria. Nevertheless, the proportions determined using a clinical or genetic reference standard for HNPCC are complementary since they all describe relationships among predictor test, mutations, and the clinical syndrome of familial cancer clustering.

Identifying HNPCC in Patients With Colorectal Cancer

Most patients with colorectal cancer do not have a mismatch repair mutation making it impractical to consider universal genetic testing, especially when considering the cost (about $3,000 for comprehensive mutation testing).[23] As a result, several strategies have been proposed to identify patients who should undergo additional testing. As a general rule, these have been based either upon clinical criteria, predictive laboratory testing of tumor tissue, or a combination of both. Statistical models incorporating these approaches have also been proposed.[11] These approaches can be characterized quantitatively by determining their sensitivity, specificity and predictive values as described above.

In this report, we attempt to understand test characteristics of various approaches to identifying HNPCC in patients with colorectal cancer. We used these parameters to develop models (based upon decision-analysis) that explore different strategies for recognizing patients who carry the mutations.

Clinical Criteria That Suggest the Diagnosis

The recognition that certain types of cancers cluster in families with HNPCC and that cancer develops at relatively early ages compared with the general population provided the rationale for development of criteria that could be used to aid in the diagnosis. Two sets of criteria (the Amsterdam criteria and Bethesda guidelines) developed by a consensus of experts, have been most widely accepted and best studied, although many similar criteria have been proposed.[24,25] Both have been revised since their initial development.[26,27] These criteria have applied by healthcare providers and genetic counselors during interviews with the patient and/or their family and/or with assistance of written documents such as a survey.

The Amsterdam criteria (see Table 4) were designed to establish the diagnosis of HNPCC based upon familial clustering of HNPCC-related tumors. As described above, some authorities consider the Amsterdam criteria as the formal description of Lynch syndrome.

Table 4. Original and revised Amsterdam criteria

Original (Amsterdam I)	Revised (Amsterdam II)
• At least 3 relatives with colorectal cancer, one of whom must be a first degree relative of the other two • Involvement of 2 or more generations • At least 1 case diagnosed before age 50 • Familial adenomatous polyposis has been excluded	• At least 3 relatives with HNPCC-associated cancer • One should be 1st degree relative of other two • At least 2 successive generations affected • At least 1 diagnosed before age 50 • Familial adenomatous polyposis excluded • Tumors should be verified by pathologic examination

By contrast, the Bethesda guidelines (see Table 5) were designed to help predict which patients with colorectal cancer are likely to have a mismatch-repair mutation and should thus undergo further testing. However, both the Amsterdam criteria and Bethesda guidelines have been studied for predicting the presence of mismatch repair mutations. The Amsterdam criteria are much stricter than the Bethesda guidelines and thus have lower sensitivity but higher specificity. The Bethesda guidelines are also more applicable in small families.

Table 5. Original and revised Bethesda guidelines

Original	Revised
• Individuals with cancer in families that meet the Amsterdam criteria • Patients with two HNPCC-related cancers, including synchronous and metachronous colorectal cancer or associated extracolonic cancers (endometrial, ovarian, gastric, hepatobiliary, small bowel, or transitional cell carcinoma of the renal pelvis or ureter). • Patients with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma with one of the cancers diagnosed before age 45 years, and the adenoma diagnosed before age 40 years. • Patients with right-sided colorectal cancer having an undifferentiated pattern (solid/cribriform) on histopathologic diagnosis before age 45 years. • Patients with signet-ring cell type colorectal cancer diagnosed before age 45. • Patients with adenomas diagnosed before age 40.	• Colorectal cancer (CRC) diagnosed in a patient <50 • Presence of synchronous, metachronous colorectal or other HNPCC-associated tumors regardless of age • CRC with the MSI-H-like histology diagnosed in a patient less than 60 • CRC diagnosed in a patient with one or more 1st degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 • CRC in a patient with two or more 1st or 2nd degree relatives with HNPCC-related tumors, regardless of age

Although the Bethesda guidelines and Amsterdam criteria continue to be used widely, several studies evaluating them (both the original and revised) have underscored the limitations of their accuracy in predicting the presence of mismatch repair mutations.[11,12,20,28,29] A 2006 review of the literature reported that the sensitivity of the original Amsterdam criteria ranged from 54 to 91%.[7] Such a wide range of estimates leaves substantial uncertainty as to the role of

the Amsterdam criteria as a screening test for mismatch repair mutations. As described, above, there are many potential explanations for the variability across studies. In this report, we attempt to clarify important differences across studies that may help explain the variability.

In addition to the limitations regarding their predictive accuracy, there are practical problems with policies based on the implementation of these clinical criteria. Patients' report of the family history may not be accurate, particularly for cancers other than colorectal that are potentially related to HNPCC.[30] Issues of uncertain paternity may also be relevant in some families while some families may be too small (or have insufficient contact among family members) to obtain a clinically meaningful family history. In addition, the criteria are not always remembered or practical to obtain; as a result many caregivers (including oncologists) fail to obtain a detailed family history, and among those who do, many do not act appropriately upon it.[30,31]

Testing All Cancers Regardless of the Family History

Because of the limitations of relying on clinical criteria to guide testing, some authorities have proposed that tumors from patients with colorectal cancer be evaluated for markers of HNPCC regardless of the family history.[8,32] One of the largest studies evaluating this approach[8] included 1066 patients with colorectal cancer whose tumors were tested for MSI. Patients with suggestive MSI results were tested for germ-line mutations in the mismatch repair genes (MSH2, MLH1, MSH6, and PMS2) by IHC, genomic sequencing, and deletion studies. A mutation causing HNPCC was detected in 23 patients (2.2 percent) of whom ten were older than 50 and five did not meet the Amsterdam criteria or Bethesda guidelines.

These data suggest that the Amsterdam or Bethesda criteria alone may miss as many as 22 percent of patients with HNPCC. However, only five additional individuals from the cohort of 1066 subjects (one-half of 1 percent) would have been identified by routine molecular analysis of all colon cancers fulfilling the Bethesda criteria, making such an approach impractically expensive for routine clinical use. Furthermore, the detailed laboratory analysis the authors performed on tumor tissue is not widely available. Despite these considerations, we include a strategy of testing all patients with colorectal cancer for mismatch repair mutations for comparison against alternative strategies.

Combinations of Family History and Laboratory Testing

Most expert guidelines on HNPCC suggest a combination of sequential laboratory testing in patients who fulfill the Amsterdam criteria or Bethesda guidelines to minimize costs and maximize test accuracy.[1,33] Approaches based on such a strategy have been considered to be cost-effective.[19,34] However, the exact methods and order of testing are unsettled. Proposed strategies include initial testing of tumors for MSI with or without IHC for loss or expression of mismatch repair proteins, with germline gene sequencing reserved for patients with suggestive results. Certain histologic features of HNPCC-related tumors may also raise clinical suspicion, but none is sufficiently specific to establish the diagnosis.[35,36] One report identified a specific oral manifestation (Fordyce granules) as highly predictive of mismatch repair mutations, but this observation has not yet been confirmed.[37] Several other strategies for selecting patients for genetic testing have been described, but none has been widely adopted. Thus, this report focuses mainly on the predictive accuracy of testing tumor tissue for MSI and IHC.

Microsatellite instability occurs as a result of "slippage" of DNA polymerase during DNA replication of microsatellite DNA sequences (short dinucleotide or mononucleotide repeats).[1] These are normally repaired by DNA repair mechanisms. In the presence of deficient mismatch repair functions (such as in HNPCC) these errors are not corrected, leading to a state that is referred to as microsatellite instability. The United States National Cancer Institute (NCI) defines the MSI-high (MSI-H) when two of five microsatellite markers from a standard panel display instability and the MSI-low phenotype when only one marker is unstable. Tumors without instability are labeled as microsatellite stable (MSS).

The NCI panel has been widely adopted in recent years, although some centers use additional or different markers. MSI-H tumors are generally more predictive of mismatch repair mutations than MSI-low tumors. However, approximately 10 to 20 percent of spontaneous colorectal cancer test positive for MSI-H, not all laboratories test for the full panel of microsatellite markers suggested by the National Cancer Institute, MSI-testing is not widely available, and archived tissue may not be readily available to perform such testing.

In some studies, MSI-H and MSI-L tumors are combined and compared to MSS, while in others each category has been considered separately. In this report, we record the specific methods used for determining MSI status and how tumors were categorized to permit valid comparisons among studies.

IHC techniques can identify the expression of mismatch repair proteins.[38,39] Testing for other mismatch repair proteins has not been performed routinely, although it may be important in some families. This approach is less costly than MSI testing and is technically much easier. Mutations associated with HNPCC generally lead to the absence of a detectable gene product, although some may lead to a dysfunctional protein that can still be detected (and hence cause a false negative result).

Some studies have suggested that almost all tumors in which MSH2 or MLH2 is absent by IHC demonstrate MSI-H, while approximately 8 percent of MSI-H tumors will demonstrate retained immunostaining.[39] However, the extent to which IHC and MSI correlate with one another is not known precisely. Nevertheless, some authorities have proposed that IHC may be a suitable alternative to MSI testing while others consider the two to be complementary. In this report we attempt to clarify these issues by providing test characteristics of each approach used alone or in combination.

Analytic Validity

As noted above, there are several laboratory methods used for predictive testing for mismatch repair mutations and for genetic testing itself. The accuracy of these methods can be influenced by several factors such as the definition of the reference standard, how tissue was collected and processed, and the specific method by which it was analyzed. Laboratory errors (e.g., mislabeling of a specimen, contamination, incorrect interpretation of results) all weigh into overall accuracy. These considerations have been collectively referred to as analytic validity.

A related issue is the reliability of testing (both within a laboratory and between laboratories). In some clinical areas, reliability has been assessed by a method known as "proficiency testing" in which samples of known positive and negative biological materials are submitted blindly to a laboratory.[40] Results can be used to determine sensitivity, specificity, and reliability. Proficiency testing for MSI became available in the United States in 2006.

In this report we attempt to define analytic validity and reliability of the predictive laboratory tests (i.e., IHC and MSI) and genetic testing methods. However, there are several limitations to attempting to assess these parameters using a literature-based approach:

- The literature search was based upon HNPCC, not the specific laboratory techniques, thus limiting the pool of potentially relevant studies.

- Many of the techniques described in the literature are experimental or old and thus do not reflect contemporary methods.

- There is likely to be publication bias since information regarding reliability and accuracy of the laboratory methods have been evaluated by individual laboratories or by manufacturers of the testing methods.

- Because it can be difficult to establish a clear reference-standard, many studies attempting to define analytic validity included the predictor tests in the reference standard, thereby making the results of test characteristics uninterpretable.

We attempted to clarify these points both by adhering to strict criteria for literature selection and in analyzing individual studies.

Benefits and Harms of Screening, Testing, and Subsequent Management Strategies

Genetic testing for a cancer predisposition has profound implications for the affected patients and their families. The genetic test results have the potential to prevent cancer in the affected patient and their families, and may influence how patients with cancer are managed, but they can also lead to harm from discrimination, the risks associated with surveillance strategies or other interventions (such as colectomy or hysterectomy), and the psychological impact of recognizing a cancer predisposition. These are issues that are common to all forms of genetically based diseases and represent areas of intense study. In this report we critically evaluate the literature exploring these issues in patients and their families with HNPCC from the perspective of the affected patients, their family and from the point of view of providers and policy-makers.

Enthusiasm for genetic testing is based upon the belief that knowledge of the genetic basis for a disease will allow for improved treatment and prevention. How these objectives can be best achieved in the care of patients and families with HNPCC has not been well established. Few high-quality studies have evaluated the effectiveness of screening strategies based upon the results of genetic testing for HNPCC. It is generally agreed that patients undergoing genetic testing should fully understand the implications of a positive and a negative result and the level of certainty of a positive or negative result as a predictor of disease.[41] The knowledge-base used to counsel patients on these parameters is still evolving.

Correlation of genotypes with phenotypes in HNPCC is incompletely understood, as are the corresponding implications for screening for HNPCC-related tumors. At least two studies[42,43] and a cost-effectiveness analysis [19] suggested a reduction in colorectal cancer and mortality from colorectal cancer screening based upon results of genetic testing. However, the primary studies

were not randomized and were vulnerable to selection bias. There is even less information regarding the effectives of screening for other forms of HNPCC-related cancers (particularly endometrial cancer).[44,45]

The uncertain benefits must be balanced against the potential for harms, which include the risks associated with screening procedures, the potential for false-positive results leading to further, possibly unnecessary testing, and the psychological, social, and economic implications from stigmatization. An observational study that included 16 HNPCC and HNPCC-like families illustrated the difficulties that may be encountered when attempting to implement a program of genetic screening and counseling.[46] Problems encountered included lack of compliance, ambiguous results of genetic tests, incomplete documentation of pathologic materials or medical history, poor cooperation among family members and/or their physicians, patient fear and anxiety and perception of insurance discrimination, and lack of knowledge among referring physicians. Thus, the realities of implementing a program for testing patients with colorectal cancer for HNPCC must be understood along with the full spectrum of implications of various strategies for establishing the diagnosis and testing family members

Chapter 2. Methods

Key Questions Addressed in This Report

The following Key Questions are addressed in this evidence report:

Key Question 1: Does risk assessment and HNPCC mutation testing in patients with newly diagnosed CRC lead to improved outcomes for the patient or family members, or is it useful in medical, personal, or public health decision making? (Over-arching question).

Key Question 2a: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has a mismatch repair mutation?
2b: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has MSI?
2c: Assuming a clinical definition of the Lynch Syndrome, what proportion of patients has abnormal protein expression by immunohistochemistry?
2: How accurate are various predictors assuming a genetic definition of the Lynch Syndrome?

Key Question 3: What are the harms associated with screening high-risk individuals for HNPCC?

Key Question 4: What is known about the analytic (sensitivity, specificity, reproducibility, reliability) and clinical validity of tests that identify HNPCC mutations?

Key Question 5: What are the harms associated with screening for high-risk individuals

Key Question 6a: What are the management options for CRC patients who are HNPCC positive?
6b: Does the identification of HNPCC mutations lead to improved patient outcomes in terms of early detection, mortality/morbidity or management decisions (e.g., counseling, surveillance, treatment, other decision making) by patients and providers?

Key Question 7: What are the harms associated with subsequent management options after identification of HNPCC mutations in CRC patients?

Key Question 8a: What is the efficacy of pre-test genetic counseling for informing family members of potential risks and benefits of testing?
8b: What is the accuracy of HNPCC testing in family members in predicting the risk of CRC?
8c: Do other factors, such as race/ethnicity, age, gender, or co-morbidities affect the accuracy of the testing?

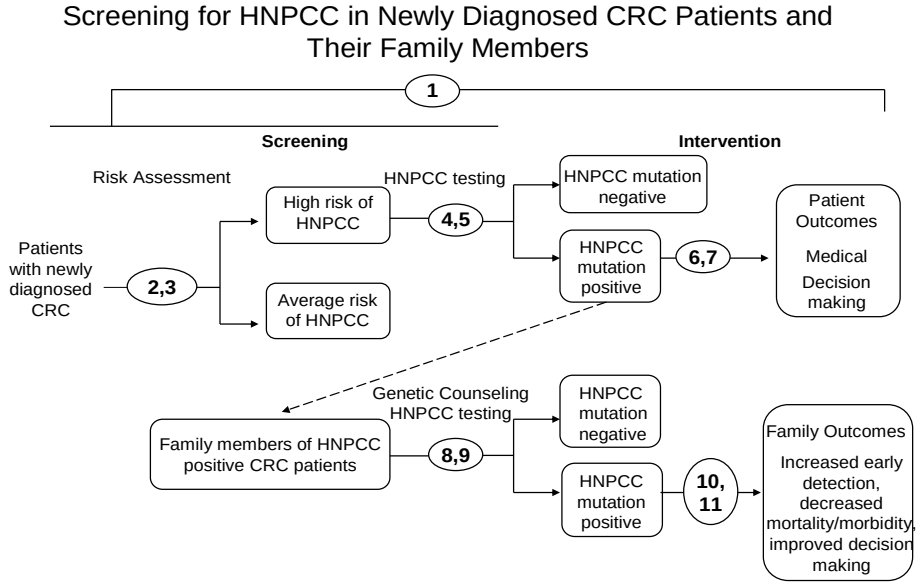
Key Question 9: What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations?

Key Question 10a: What are the management options for family members of CRC patients who have a positive HNPCC test?
10b1: Does the identification of HNPCC mutations lead to improved outcomes in terms of decision-making by patients, family members and providers, or public health policy?
10b2: Does the identification of HNPCC mutations lead to improved outcomes in terms of early detection and mortality/morbidity of patients, family members?

Key Question 11: What are the harms associated with subsequent actions or interventions for family members?

These questions were formulated by EGAPP based upon an analytic model that begins with a patient with CRC and proceeds to genetic testing of family members. They broadly reflected the conceptual framework proposed by the ACCE Project from the Office of Genomics and Disease Prevention at the Centers for Disease Control and Prevention (CDC). The aim of the project is to develop a model system for assembling, analyzing, disseminating and updating existing data on the safety and effectiveness of DNA-based genetic tests and testing algorithms (See http://www.cdc.gov/genomics/gtesting/ACCE.htm for details). ACCE takes its name from its four components of evaluation—analytic validity, clinical validity, clinical utility and associated ethical, legal and social implications. It is intended to provide a model process for evaluating data on emerging genetic tests. The process includes collecting, evaluating, interpreting, and reporting data about DNA (and related) testing for disorders with a genetic component in a format that allows policy makers to have access to up-to-date and reliable information for decision making. The CDC Office of Genomics and Disease Prevention has previously published a mini review on HNPCC based upon the ACCE framework, which underscored the need for a more comprehensive review (see http://www.cdc.gov/genomics/gtesting/ACCE/fbr.htm).

Figure 1. Analytic framework that served as the basis of the key questions proposed by the CDC Office of Genomics and Disease Prevention 2005



The Key Questions were refined in several teleconferences with members of EGAPP and the Technical Expert Panel (TEP), and following review of draft reports.

The Key Questions and subset questions were divided conceptually into three general domains: clinical validity; analytic validity; and benefits and harms of screening, genetic testing and various management strategies from the patient, family member, provider, and public health perspectives. (Table 6) These domains were based upon overlapping concepts implied by the Key Questions and reflect the core components of the ACCE model. Thus, Key Questions were grouped according to the domain, and studies that addressed one of the three domains were often relevant to more than one Key Question, but generally not to more than one domain.

Table 6. Summary of key questions

Domain	Key questions addressed
Analytic validity	4
Clinical validity	2, 2a, 2b, 2c
Benefits and harms (including clinical utility and associated ethical, legal and social implications in the ACCE model)	1, 3, 4, 5, 6a, 6b, 7, 8a, 8b, 8c, 9, 10a, 10b1, 10b2, 11

We undertook the following steps in conducting this review:

- Performed an electronic search of the literature followed by review of relevant abstracts and then full-text review of potentially relevant studies.
- Retrieved additional studies from bibliographies of retrieved citations and suggestions of the TEP.
- Included or excluded studies based upon prespecified criteria.
- Identified duplicate reports of the same patients by comparing authors and study centers. Data were included only once, except when duplicate studies reported complementary information (e.g., data at one month and then one year).
- Developed data extraction forms for each domain (i.e., three data extraction forms) and tested them until we achieved consensus on the meaning of the data elements in each extraction form.
- Evaluated each study critically according to quality criteria described below.
- Verified all data with at least two extractors.
- Summarized data in tables that addressed specific Key Questions (or groups of Key Questions) that corresponded to the three domains described above. Because only a few studies addressed analytic validity, we described them in the text of the report rather than in tables.

- Reviewed drafts of the summary tables with members of the TEP.

- Pooled data where studies used similar methodology and definition of endpoints using meta-analysis to provide a point estimate and 95% confidence interval, mainly for questions pertaining to clinical validity. We performed multiple sensitivity analyses to explore possible explanations when studies demonstrated statistically significant heterogeneity.

- Constructed models depicting various strategies for identifying HNPCC among patients with colorectal cancer using decision trees. The models were based upon parameters estimated from data presented in this report.

- Prepared a draft report, which underwent peer review and addressed each comment in a revised final report.

Literature Search Strategy

We conducted a literature search of MEDLINE® using PubMed on January 10, 2006. We used MEDLINE® subject headings and text words to capture relevant English language publications of human studies. Additional sources of potentially relevant studies included technical experts and hand searching of bibliographic references of reviews. An automatic updated search results from PubMed was received on April 1, 2006 after which additional studies were included only if the investigators or TEP considered them to provide substantive new information that might influence the conclusions (see Appendix A*).

We reviewed all abstracts for their relevance to the Key Questions and retrieved the full-text article of potentially relevant citations. We reviewed bibliographies of studies included in the report (as well as previous review articles or meta-analyses, which were not included) to identify additional citations, all of which were retrieved for review. We identified duplicate reports of the same patients by comparing authors and study centers. Duplicate reports were excluded unless they provided complementary information (such as outcomes at different time points) in which case they were considered together.

Inclusion/Exclusion Criteria and Data Extraction

We applied prespecified inclusion and exclusion criteria in considering each study. The criteria corresponded to the three domains described above.

Studies Related to Analytic Validity

We required the following for studies of analytic validity:

- The study evaluated biological material from patients with colorectal cancer considered to be at risk for HNPCC. This criterion was selected to choose studies evaluating laboratory techniques that were directly applicable to the spectrum of genotypes associated with

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

HNPCC. We did not consider it feasible to conduct a systematic review on the individual laboratories tests used for other conditions.

- Reported any of the following (these criteria were selected to choose studies evaluating the most common tests performed in HNPCC):
    1. Proportion of tumors that were MSI-H with National Cancer Institute (NCI) panel of markers (i.e., BAT-25, BAT-26 D2S123, DS346 and D17S250) versus other markers
    2. Sensitivity or specificity of MSI-H using NCI markers compared with a reference standard that the study claimed was better
    3. Sensitivity or specificity of IHC compared with an immunohistochemical standard that study claimed was better
    4. Sensitivity or specificity of a genetic technique compared with a reference standard (or combination of standards)
    5. Reliability of MSI, IHC or genetic methods across laboratories or within a laboratory.

- Data were extractable into 2x2 tables.

We excluded studies that included the index test in the reference standard. For example, some studies attempted to calculate sensitivity of a specific NCI MSI marker by comparing it with a reference standard that included the marker plus additional markers. Such an approach produces an invalid estimate of sensitivity since the calculation includes the index test (i.e., the specific NCI marker) in the numerator and the denominator.

Studies Related to Clinical Validity

We required all three of the following criteria to be met for studies of clinical validity with extractable data:

- Enrolled patients with colorectal cancer

- Compared an index test to genetic testing (*at least* one of the following: suggestive family history, MSI, or IHC)

- Sought mutations using DNA sequencing (or other similar genetic approaches) for a minimum of MLH1 and MSH2.

HNPCC has been defined clinically and genetically as described in Chapter 1. We used both approaches depending upon which Key Questions were being addressed. In all cases, the definitions used are described explicitly in the tables and figures.

Key Questions 2a-2c focus on the prevalence of MMR mutations, suggestive MSI or ICH among patients with a clinical definition of HNPCC. We defined Lynch Syndrome I and Lynch Syndrome II using the Amsterdam I and II criteria, respectively. Studies that reported this proportion or provided sufficient data for it to be calculated were eligible.

We required that studies evaluated all available patients who fulfilled the Amsterdam criteria or a representative (random) sample thereof. We excluded studies that did not evaluate all

patients because they selected a non-random patient sample (e.g., patients who fulfilled the Amsterdam criteria who were also younger than a certain age).

Key Question 2 pertains to the predictive accuracy of clinical or laboratory features for determining the presence of mismatch repair mutations. We assessed the performance of different clinical and laboratory predictors (preliminary tests) to identify carriers of MMR gene mutations. We considered all studies that provided relevant data. When the primary study reported proportions rather than actual counts, we reconstructed the 2x2 tables using the information conveyed by the proportions and their 95% confidence intervals.

We analyzed the following predictors:

Laboratory predictors:

1. MSI high versus MSI stable.
2. MSI high and low versus MSI stable.
3. Suggestive IHC versus non suggestive IHC.

Clinical predictors specified *a priori*:

1. Amsterdam I criteria fulfilled versus not fulfilled.
2. Amsterdam II criteria fulfilled versus not fulfilled.
3. Modified Amsterdam criteria fulfilled versus not fulfilled.
4. Bethesda guidelines fulfilled versus not fulfilled.
5. Revised Bethesda guidelines fulfilled versus not fulfilled.
6. Young age of onset (<50 years) versus later age of onset .
7. Presence of CRC or HNPCC related cancer in first degree family versus sporadic CRC cases.
8. Presence of CRC or HNPCC related cancer in family (any definition) versus sporadic CRC cases (irrespectively of how they were selected).

Clinical predictors specified *a posteriori*:

9. Presence of multiple tumors in a CRC proband versus probands without multiple tumors.
10. Presence of young age of onset (<50 years) or suggestive family history of cancer or multiple tumors in a CRC proband (i.e., predictors #6 or #7 or #8) versus absence of all three characteristics.

We focused only on patients with CRC who received at least some form of genetic testing to minimize the effects of verification bias. Verification bias occurs when patients with a negative test result are not evaluated with the reference test.[47] We generally did not accept screening with MSI or IHC as a substitute for genetic testing. A single exception pertained to studies assessing clinical predictors among newly diagnosed, unselected, non-referral CRC, because this was of particular clinical importance and there were few studies available for analysis. For such studies we accepted the authors' assumption that patients who were not tested for mutations based on MSI and IHC test results were indeed mutation negative.

Studies Related to Benefits and Harms

For studies related to benefits and harms, we accepted studies of virtually any design and using any definition of HNPCC that reported any outcomes or other findings pertinent to patients or families with HNPCC, or provided insights into these issues from a public health perspective. We did not confine our inclusion criteria to studies of patients with colorectal cancer even though this report focuses on patients with colorectal cancer. Benefits and harms of genetic testing, counseling and other management approaches are pertinent to the full spectrum of tumors associated with HNPCC. For example, a woman with colorectal cancer who is found to have HNPCC is at risk for other forms of HNPCC-related cancer (such as endometrial cancer). Thus, the benefits and harms related to genetic testing may not only be relevant to clinical issues related to colorectal cancer management (and prevention of metachronous cancers) but also management (and prevention) of other HNPCC-related cancer in the patient or her family members. We expanded the scope of all pertinent Key Questions to provide as comprehensive a view on these issues as possible.

Although we attempted to be as comprehensive as possible in including studies related to benefits and harms, we occasionally encountered studies that did not report any outcomes or other findings that appeared to be relevant to the Key Questions and thus excluded them. As noted above, the reasons that specific studies were excluded are summarized in the Appendix D[*]. List of Excluded Studies.

Evaluation of Study Quality

We evaluated the quality of each study based upon multiple quality features. Evaluation of study quality is a complex process since there is no established method that can comprehensively describe all features that are pertinent to the validity of a study. The included studies varied in the rigor with which they were designed, conducted, analyzed, and reported. Deficiencies in any of these areas can lead to biased reporting and interpretation of results.

Furthermore, the quality of a study and its applicability are not always related; a study that is considered to be of relatively low quality may be more applicable to a specific question (i.e., answers it directly) than a study of higher quality. In addition, assessment of quality is based upon information reported and not necessarily how the study was conducted since there may be omissions or editorial constraints in the published manuscript.

We hoped to critically assess the quality of each study and to present studies based upon their applicability to each Key Question. Thus, we attempted to feature studies that answered Key Questions most directly and reported a composite quality score (A, B or C, described below) to allow comparison among studies. We based the composite quality score upon an overall assessment of the degree to which individual elements describing study quality were fulfilled and the implications of those that were not. There was an element of judgment for the final scoring of each study but we attempted to be as consistent as possible.

The composite quality scores are helpful for giving a shorthand, qualitative appraisal of the overall study quality, but they do not necessarily reflect particular deficiencies or strengths that might be important for fully understanding the results of the study or interpreting the body of knowledge. Thus, strengths and weaknesses of individual studies might be important when

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

considering their validity and relevance for specific Key Questions. We attempted to highlight study features that we considered to be most relevant while making comments as to specific deficiencies to allow readers to have a view on this that was transparent and succinct. The components of the individual quality scores for each study are available in Appendix C*. In addition, important features of each study are summarized in the tables included in the body of the text following each Key Question to allow for easy comparison among them.

We used the composite quality score formally for sensitivity analysis, mainly for studies of clinical validity to determine whether study quality correlated with test characteristics. For example, we determined whether sensitivity and specificity were better for high compared with low quality studies.

The quality criteria selected for this report (summarized below) were based upon discussions with the TEP and quality elements that have been proposed to be relevant for the specific types of studies that we included. All investigators discussed details of the meaning of each quality element to help assure that they were applied uniformly. Investigators discussed the quality of individual studies whenever there were questions related to the overall score that the study should receive until consensus was achieved.

The Tufts-NEMC EPC has used similar systems for several other evidence reports. However, it must be acknowledged that the reliability and validity of quality scoring systems used for systematic reviews, including the one used for this report, have not been extensively evaluated.[48]

Table 7. Interpretation of overall quality grading of individual studies

Grade	Explanation for quality scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

We used the following approach for grading studies related to clinical validity. These criteria were adapted, in part from the Centers for Disease Control and Prevention and the QUADAS tool for assessing diagnostic test accuracy.[49,50]

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Table 8. Quality criteria used to characterize studies of clinical validity

Item	Criteria	Yes	No	Un-clear
	General quality criteria			
1	Were unselected patients with CRC included (i.e., were representative of patients seen in clinical practice not selected based upon a suggestive family history or other criteria that may cause selection bias)?			
2	Inclusion criteria clear?			
3	Did the whole sample or a random selection of the sample (i.e., total population of patients with CRC) receive verification using gene sequencing?			
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing (i.e., was there blinding)?			
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e., was there blinding)?			
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g., badly stained tissues etc)?			
7	Were withdrawals from the study explained?			
8	Did the authors report *AND* analyze results for deleterious MMR mutants?			
	Additional relevant quality items			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g., either a full description or relevant references)?			
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
12	Was there a clear description of which mismatch repair mutations were being tested for?			
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing.... i.e., avoid verification bias)?			
14	Were quality control methods described for the molecular and genetic tests?			
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e., this helps minimize the effect of random errors.)			

We used the following quality criteria for studies related to analytic validity. These criteria were adapted from those proposed by the Centers for Disease Control and Prevention.[49]

Table 9. Quality criteria used to characterize studies of analytic validity

	Study quality	Yes	No	Unc
1	Was the description of how MSI or IHC and other genetic techniques described in sufficient detail that others could replicate it (e.g., either a full description or relevant references)?			
2	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
3	Was MSI, IHC, other genetic testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
4	Was there a clear description of which mismatch repair mutations were being tested for?			
5	Were quality control methods described for the molecular and genetic tests?			
6	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
7	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e., this helps minimize the effect of random errors).			
8	Was microdissection (technique for removing only tumor tissue from gross specimen) performed?			
9	Did the study specify whether the biological tissues were from patients known to have HNPCC clinically?			
10	Did the study include a control group in which biological material was obtained from patients known not to have HNPCC clinically			

We used the following quality criteria for studies related to benefits and harms. These criteria were adapted from the Cochrane Collaboration Handbook for Systematic Reviews of Health Promotion and Public Health Interventions for Evaluation of Studies Related to Public health.[51]

Table 10. Quality criteria for studies on benefits and harms

Domain/question	*Place an "X" in one*					*Overall rating*		
Selection bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						

Table 10. Quality criteria for studies on benefits and harms (continued)

Domain/question	*Place an "X" in one*					*Overall rating*		
Withdrawals and dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e., intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e., intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Synthesis, Analysis, and Reporting

Questions Related to Analytic Validity

As will be discussed in Chapter 3, we encountered only a few studies of analytic validity, and thus these are described in the text rather than in tables. There were insufficient data for a pooled analysis.

Questions Related to Clinical Validity

Studies relevant to each Key Question were grouped together, organized according to their applicability to the specific Key Question and their overall quality score. In some cases, Key Questions that had substantive overlap in the type of findings reported were grouped together to provide a comprehensive yet succinct overview of the literature.
Questions related to clinical validity: For questions related to clinical validity, sensitivity and specificity were based upon the definitions summarized in Chapter 1. We presented studies by categorizing them for important features (such as use of similar definitions and selection criteria) so that similarities and differences would be as transparent as possible.

Prevalence of MMR Mutations, MSI or Suggestive IHC Among Patients Fulfilling Amsterdam I and II Criteria. For each study we estimated the proportion (and exact binomial 95% confidence interval) of Amsterdam I and II patients with MMR mutations, MSI or suggestive IHC. We estimated the corresponding summary prevalence values with random effects meta-analyses.[52,53] For the quantitative syntheses, proportions from each study were *logit* transformed to stabilize variances, and then back-transformed to their natural scale. Analyses using the more drastic Tuckey-Freeman *arcsin* transform yielded largely similar estimates, and thus are not reported.

Diagnostic Ability of Predictors (Preliminary Tests) To Identify MMR Mutation Carriers. As mentioned above, the preliminary tests that identify MMR mutation carriers are either sets of clinical criteria, or laboratory tests (namely MSI and IHC). Different considerations are applicable to the analysis of clinical and laboratory predictors across a selection of studies.

Considerations on study populations for clinical predictors (preliminary tests). In HNPCC the clinical preliminary tests are highly susceptible to spectrum effects across populations that have been selected with eligibility criteria of a clinical nature. Although the detailed analysis of this statement is cumbersome, it can be intuitively evaluated through an illustrative example. Assume that the preliminary test of interest is the clinical criterion of "age less than 50 years at diagnosis of CRC". It is expected that the sensitivity and specificity of this criterion will be different among unselected people with CRC compared to CRC patients who were less than 55 years at diagnosis. The second population has already been "pre-selected" based on a clinical criterion (less than 55 years at CRC diagnosis) that is quite similar to the evaluated "preliminary test" (less than 50 years at CRC diagnosis). This pre-selection alters the composition of the population with respect to the clinical predictor in a systematic and non-random way, both among MMR mutation carriers and among non-carriers. The net effect is a change in the

apparent sensitivity and specificity across populations with increasing prevalence of MMR mutation carriers (spectrum effects).

Because of the aforementioned consideration we decided *a priori* not to synthesize the sensitivity and specificity of clinical predictors across populations that had been defined using different clinical eligibility criteria. Identifying such homogeneous populations is challenging, especially given the incomplete descriptions of the studied populations in many of the assessed studies.

Thus we decided to focus on populations fulfilling sets of criteria that are most frequently used for HNPCC, are well known, and presumably assessed identically by different research teams. In order of increasing prevalence of MMR mutation carriers these homogeneous populations were: unselected, incident CRC, patients selected by the Bethesda guidelines and revised Bethesda guidelines, modified Amsterdam criteria, and Amsterdam II and I criteria. We also created separate estimates for the sensitivity and specificity for patients who were pre-selected based on suggestive MSI and/or IHC results.

Considerations on study populations for laboratory predictors (preliminary tests). The above consideration may not be as important for laboratory predictors (e.g., IHC) because there is no strong reason to assume that pre-selection with a clinical eligibility criterion (e.g., "age less than 55 at CRC diagnosis", as above) in a study would be correlated with IHC results both in MMR carriers and in those who are not MMR mutation carriers. However, it is possible that some laboratory predictor tests (e.g., MSI analysis) may be vulnerable to spectrum effects.[54] Thus, for laboratory predictors, we did not require the strict similarity of populations across studies as above, and considered all studies together.

Separate analyses of sensitivity and specificity. For each study we estimated the sensitivity and specificity (and exact binomial 95% confidence intervals thereof) for the clinical and laboratory predictors (preliminary tests) of interest. As described before, we derived summary sensitivity and specificity estimates with random effects syntheses using logit-transformations of proportions for the meta-analyses. As a general rule, this approach tends to underestimate the diagnostic performance, because it ignores the correlation between the sensitivity and the corresponding specificity from the same study. However, it provides a pooled estimate (and 95% confidence interval) of sensitivity and specificity that can be useful for providing overall appraisal of test performance.

Summary receiver operating characteristic curve analyses. For laboratory predictors only, we summarize test performance graphically using summary receiver operating characteristic curve (SROC) analyses. SROC curve analysis can be used to graphically describe the tradeoff between sensitivity and specificity across studies. The sensitivity and specificity of each study are represented in the graph, thereby depicting the operating characteristics of a group of studies.

The tradeoff between sensitivity and specificity generally reflects the threshold for calling a test positive or negative. Such a tradeoff can be understood easily when considering a single study that assessed various cutoff values of a diagnostic test. By contrast, the threshold implied in an SROC curve analysis is not always apparent. Different estimates of sensitivity and specificity across studies may be due to several considerations such as variations in how the tests were implemented (e.g., differences in the number of microsatellite markers used) or in interpretation of results. With regard to the latter, the same specimen (e.g., a tumor stained for IHC) may be interpreted differently when the interpreter believes the test is being used for

screening versus confirmation of a high-risk patient. Thus, by grouping studies according to specific study features, SROC analysis can be used to help explain differences in test characteristics across studies that seemingly used the same diagnostic test. However, it does not necessarily define an explicit threshold effect.

The area under a receiver operating curve has been used to describe the overall diagnostic ability of a test; the greater the area, the better the test. However, calculating the area under a SROC curve requires extrapolation outside the range of the sensitivity and specificity values of the analyzed studies and thus may not produce a valid appraisal of the test's accuracy. As a result, we did not attempt to calculate the area under the SROC curve.

Subgroup and Sensitivity Analyses. We defined a priori factors that might explain heterogeneity of pooled estimates. These included:

Overall quality. We compared results from high and low quality studies (as defined by the A, B or C classification described above).

Study size. For the questions relating to the proportion of patients with the Lynch Syndrome with mutations or abnormal MSI or IHC results, we used a cutoff of 20 patients (≥20 versus <20) to distinguish larger and smaller studies. Although this was a largely arbitrary cutoff, a single misclassification in smaller studies would result in more than a 5% misclassification rate, which we considered clinically important. Similarly, we used a cutoff of at least 40 people in the 2 by 2 table for studies that were used to assess sensitivity and specificity.

Characteristics of how genetic testing was performed. Studies were categorized in groups according to the comprehensiveness of the genetic testing strategy they used. As least comprehensive, we classified studies that used only gene screening methods to detect MMR mutations, and did not perform sequencing on any sample; performed sequencing on samples with suggestive gene screening analyses; or performed sequencing on all available samples. As most comprehensive we considered studies that performed sequencing and analyses for large genomic deletions/rearrangements in all samples. The application of more advanced techniques such as conversion analysis or mono allelic mutation analysis (MAMA) was only sporadic or in the context of demonstrating their feasibility in only a few samples, and thus did not comprise a separate category. Examples of genetic screening strategies are single-stranded conformation polymorphism (SSCP), conformation sensitive gel electrophoresis (CSGE), denaturing gradient gel electrophoresis (DGGE), and denaturing high-pressure liquid chromatography (DHPLC). Examples of methods to detect large genomic deletions are: southern blotting and multiplex ligation-dependent probe amplification (MLPA). These methods are described in more detail on Chapter 3 in the section on Analytic Validity.

Whether and how the study defined pathogenic mutations. We determined if and how studies defined mutations as being pathogenic (i.e., known to be associated with HNPCC versus a variant of unknown significance). It is possible that the same mutations might have been defined as pathogenic in one study and non pathogenic in another.[55] Furthermore, not all definitions are equally valid and the methods used to define pathogenicity may not have been conducted with equal rigor. For example evidence from functional studies (i.e., in which the function of the mutated gene was assessed) may provide strong support that the mutation is pathogenic, whereas absence of the mutation in a small sample of healthy controls is not as strong.

Characteristics of MSI testing. We assessed whether studies that used the panel of markers recommended by the NCI and whether they performed microdissection. Microdissection helps assure that the sample analyzed was from malignant tissue and did not contain DNA from surrounding, healthy colonic tissue. Microdissection is not pertinent to germline MMR mutation testing or IHC. Germline mutations are typically assessed in blood samples (non-malignant tissue). For IHC, a pathologist studies a tissue section to evaluate MMR protein expression in regions of malignancy (microdissection it typically not needed).

The sample selection process. We attempted to identify studies that had a biased sample selection process. Studies with transparent sample selection process clearly stated that they selected their samples among all available patients using a set of eligibility criteria. Studies with non-transparent sample selection process did not report that they applied the same criteria to all available patients. For example, they may have used a convenience sample of cases from various sources.

Despite our efforts, we caution that studies that applied seemingly transparent eligibility criteria may still have a highly biased selection process. For example, studies from a referral center may have recruited patients with a relatively higher prevalence of familial cancer compared with studies that recruited consecutive patients with colorectal cancer in the community.

Whether the study used consecutive, unselected patients with CRC. We identified studies that used consecutive CRC (including retrospective studies that assessed all unselected patients that were diagnosed during a specific time period) that were otherwise nonselective or representative of the general population. Populations evaluated in studies from specialized centers or studies that imposed clinical or other criteria to select their populations were not considered to be representative of the general population.

Decision Tree Model Methods. We performed analyses using decision trees to model the expected outcomes with different testing strategies from the payers'/third party perspective. The outcomes were the number of incident CRC with positive diagnosis for HNPCC, and the number of tests (MMR, MSI, or IHC) needed to detect them. We also assessed how many patients found to be mutation carriers with each strategy would be truly positive.

The decision trees pertain to a cross-section in time. We used a hypothetical population of 100,000 incident cases of CRC. This number is in the order of incident cases expected annually in the US (approximately 150,000, given an annual incidence of 50/100,000 and a population of 300 million) and is a number convenient for calculations.

We assessed nine different strategies, which were most commonly represented in the studies summarized in this review. The strategies used clinical criteria, MSI, IHC or a combination of clinical criteria with MSI or IHC to select patients for MMR mutation testing. The probabilities that were used in the decision trees were derived from the eligible studies. See Chapter 3 for a complete description of the modeled strategies and the probabilities that were used.

Questions Related to Benefits and Harms

For studies related to benefits and harms, we grouped studies according to their relevance to the Key Questions, presenting comparative trials and higher quality studies first followed by

qualitative and other types of descriptive studies. Statistical comparisons made within each study are presented; we did not attempt to recalculate these comparisons; however, specific comments about study methods are included in footnotes.

The majority of studies related to benefits and harms were qualitative. Among the comparative trials, there were insufficient studies of similar design to allow for meta-analysis.

We present major findings that were considered to be clinically important or addressed (directly or indirectly) the Key Questions and subset questions. We described together studies that used similar endpoints (e.g., quality of life instruments or depression scales) and interventions (e.g., surveillance colonoscopy) whenever possible.

Evidence and Summary Tables

Evidence tables offer a detailed description of the studies that addressed each of the Key Questions. Each study appears once regardless of how many interventions or outcomes were reported. We did not attempt to construct evidence tables for all the extracted studies but all data extracted for each study are included in data extraction forms that are available in Appendix C[*].

Summary tables succinctly report summary measures of the main study features and outcomes evaluated. They are designed to facilitate comparisons and synthesis across studies. Individual studies may appear more than once if the data they contain are relevant to more than one Key Question. The summary tables are featured in sections corresponding to each Key Question in Chapter 3.

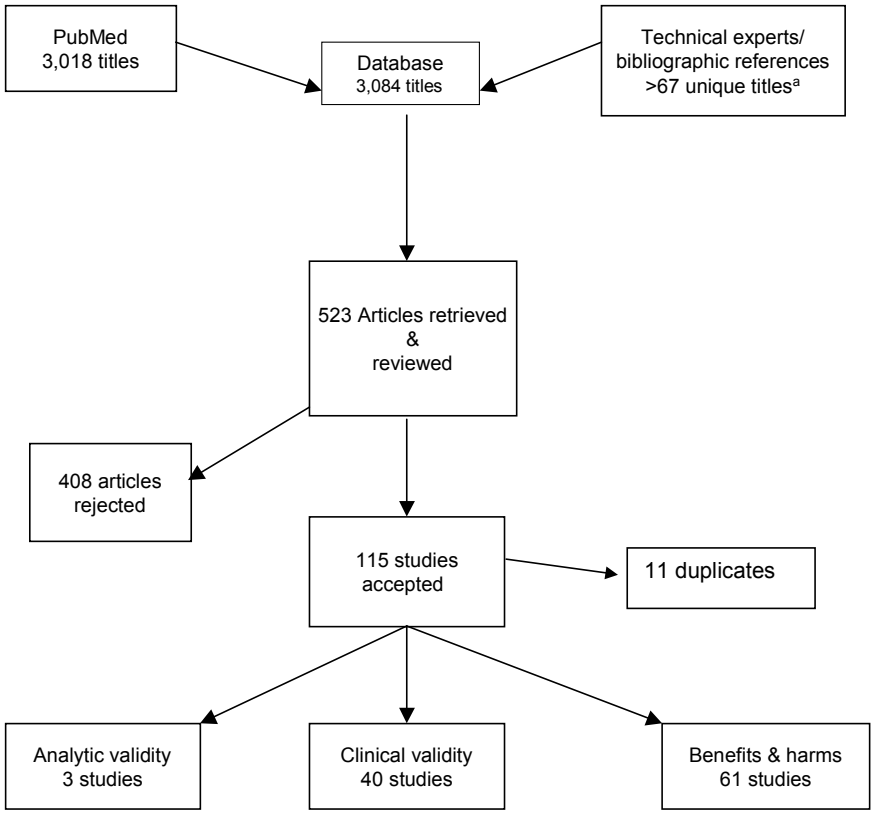
[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Chapter 3. Results

Overview

The MEDLINE® literature search produced 3,018 abstracts, which were screened for their relevance. The full text article was retrieved for all studies that appeared to be relevant to the Key Questions. With the addition of titles from technical experts and reviews of bibliographies, 523 publications were retrieved for review. One hundred fifteen papers fulfilled eligibility criteria but 11 were duplicate reports and were therefore excluded (or used to provide supplementary information as described in Chapter 2). Thus, 104 unique studies were included for this review (40 for clinical validity, 3 for analytic validity and 61 for benefits and harms) while 410 did not meet the inclusion criteria and were rejected (see Figure 2; see also Appendix D*).

Figure 2. Literature search results



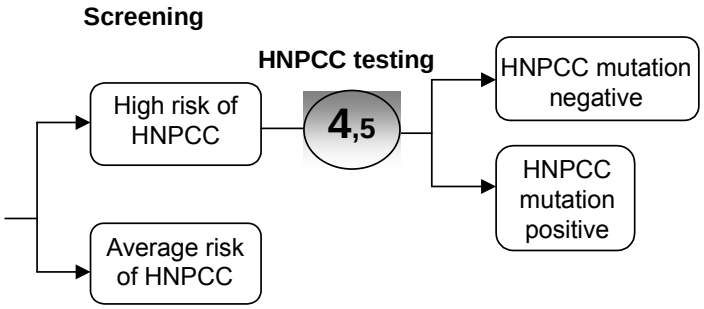
[a] Additional titles overlapping with PubMed not counted.

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

In the following sections, we present results for Key Questions as described in Chapter 2. The Key Questions pertaining to each domain (i.e., clinical validity, analytic validity, and benefits and harms) are presented within each section.

Analytic Validity

Key Question 4: What is Known About the Analytic (Sensitivity, Specificity, Reproducibility, Reliability) and Clinical Validity of Tests That Identify HNPCC Mutations?



Definition

The analytic validity of a laboratory test refers to its accuracy and reliability for identifying a finding of interest (such as a genotype). There are four general elements of analytic validity (see http://www.cdc.gov/genomics/gtesting/ACCE.htm for further details):

1) Sensitivity (i.e., the ability to detect a finding when it is present)
2) Specificity (i.e., the ability to exclude a finding when it is absent)
3) Laboratory quality control (i.e., the procedures used in a testing laboratory to ensure that results fall within specified limits)
4) Assay robustness (i.e., how resistant the assay is to changes in pre-analytic and analytic variables).

Laboratory Tests Used in HNPCC

The major laboratory tests used in the evaluation of patients suspected of having HNPCC include testing of tumor tissue using IHC, MSI testing, or germline (generally from peripheral blood mononuclear cells) testing for mismatch repair defects. Family members generally undergo only germline genetic testing (unless they have also developed a relevant cancer), ideally based upon the genotype of the proband. Detection of a pathogenic mutation (i.e., one known to be associated with HNPCC) in a proband permits testing of at-risk family members for the genotype. Family members with the same genotype have HNPCC while HNPCC can be excluded in those who do not. The situation is more complex when the proband does not have a detectable DNA alteration associated with HNPCC or when an alteration with unclear clinical

significance is detected. In such cases, it may not be possible to exclude HNPCC in family members; as a result, they may be empirically offered enhanced cancer surveillance.

The laboratory tests used in HNPCC have been described in several reviews, including a comprehensive summary published in 2006 to which we refer readers seeking a detailed review.[7] The specific tests and order of testing used in the studies included in this review are summarized in Table 11.

Table 11. Genetic testing strategies used by studies included in quantitative analyses

Study, year	Brief description of genetic strategy	MMR genes other than MLH1, MSH2	Sequencing	Gene screening	Deletion analysis
Syngal, 2000 Wahlberg, 2002 Rossi, 2002 Wolf, 2005 Lee, 2005 Moslein, 1996 Debniak, 2000 Peel, 1999	PCR → Sequencing	No	All samples	X	X
Farrington, 1998	PCR → IVSP → Sequencing PCR → Sequencing	No	All samples	√	X
Barnetson, 2006	PCR → Sequencing (MSH6, some MLH1 and MSH2 exons) PCR → DHPLC → Sequencing	MSH6	All samples	√	X
Wang, 1999	PCR → IVSP → Sequencing PCR → HD → Sequencing in vivo MLH1 expression analysis PCR → Sequencing (of all other samples)	MSH6 PMS1 PMS2	Practically all samples[a]	√	√
Durno, 2005	[used PTA and Sequencing]	No	Some samples?[b]	√	X
Aaltonen, 1998	MSI → PCR → DGGE (some samples) → Sequencing; MSI → PCR → Sequencing (all other samples) PCR for founder mutations in MLH1 (all samples)	No	Some samples	√	√
Salovaara, 2000	MSI → PCR → Sequencing PCR for founder mutations in MLH1 gene	No	Some samples	X	√
Liu, 2004 Colombino, 2005 Yuan, 2004 De Abajo, 2005	PCR → DHPLC → Sequencing	No	Some samples	√	X
Southey, 2005	PCR → DHPLC → Sequencing MLPA (in only 10 tumors)[c]	MSH6 PMS2	Some samples	X	√
Curia, 1999 de Leon, 1999 Dieumegard, 2000 Yuan, 1998	PCR → SSCP → Sequencing	No	Some samples	√	X
Katballe, 2002 Christensen, 2002	PCR → SSCP & HD →Sequencing	No	Some samples	√	X
Luce, 1995	PCR → IVTT → Sequencing	No	Some samples	√	X

Table 11. Genetic testing strategies used by studies included in quantitative analyses (continued)

Study, year	Brief description of genetic strategy	MMR genes other than MLH1, MSH2	Sequencing	Gene screening	Deletion analysis
Zhu, 2005	MLPA → Sequencing	No	Some samples	X	√
Park, 1999	PCR → SSCP Southern blotting	PMS1 PMS2[d]	None	√	√
Callistri, 2000	PCR → SSCP	No	None	√	X
Lamberti, 1999	PCR → SSCP RT-PCR, PTA	No	None	√	X
Samowitz 2001	MSI → PCR → Sequencing MSI → PCR for founder mutations in MLH1	No	Some samples	X	√
Raedle, 2001 Pistorius, 2000	MSI → PCR → Sequencing	No	Some samples	X	X
Terdiman, 2001	MSI → PCR → DGGE → Sequencing	No	Some samples	√	X
Nakahara, 1997	MSI → PCR→ SSCP → Sequencing	No	Some samples	√	X
Casey, 2005	MSI, IHC → PCR → Sequencing MSI, IHC → Conversion analysis MSI, IHC → Deletion analysis	No	All available[e]	X	√
Pinol, 2005	MSI, IHC → PCR → Sequencing MSI, IHC → MLPA	No	Some samples	X	√
Stormorken, 2001	Not described	MSH6	Unclear	Unclear	Unclear

DHPLC: denaturating high performance liquid chromatography; DGGE: Denaturating gradient gel electrophoresis; HD: heteroduplex formation; IHC: immunohistochemistry; IVSP: *in vitro* synthesized protein test; IVTT: *in vitro* transcription translation; MLPA: multiplex ligation-dependent probe amplification; MSI: Microsatellite instability testing; PCR: polymerase chain reaction; SSCP: Single-stranded conformation polymorphism.
Shown are the genetic strategies used in the various studies that were assessed in the quantitative analyses of this report. All studies assessed MLH1 and MSH2 at minimum.

[a] In Wang 1999 essentially all patients were sequenced, but not completely; however, complete sequencing was performed for those testing negative with other methods.

[b] Durno 1999 does not describe the used strategy in detail.

[c] The 10 tumors were MSI positive, IHC negative and MMR negative with the usual strategy.

[d] In Park 1999 PMS1 was assessed in 27 samples and PMS2 on 24 samples out of 123 samples for MLH1 and MSH2 (all were negative, unclear how the 27 and 24 were selected).

[e] All available patients were tested with full sequencing. However, the sample was assembled mainly on the basis of suggestive MSI testing (85 out the 89 patients had suggestive MSI testing).

Immunohistochemistry. Pathogenic mutations in mismatch repair proteins usually lead to the absence of a detectable gene product providing the rationale for IHC techniques used to detect underexpression. Tumors from patients suspected of having HNPCC (based upon clinical or pathologic findings) can be stained for mismatch repair proteins. The surrounding normal colonic tissue can be used as a positive control.

As noted in the table below, testing is available commercially for MLH1, MSH2, MSH6 and PMS2, although the extent to which various laboratories stain for all of these proteins is unclear. Furthermore, such staining is relatively easy to perform and available in kits. As a result, local pathologists, who may select to stain for some or all of these proteins, can perform it.

Knowledge of how the mismatch repair proteins interact during DNA repair can help interpret the results of such testing and be useful for guiding germline genetic testing. For example, MSH2 forms a heterodimer with MHS6; MLH1 complexes with PMS2 and binds to the MSH2-MSH6 heterodimer. When MSH2 is not expressed in a tumor, MSH6 is also not

expressed. Because MSH2 and MLH1 are the most common mismatch repair proteins implicated in HNPCC, a patient who has a tumor that does not stain for MSH2 (and whose normal surrounding colonic mucosal demonstrates preserved staining) is most likely to have a mutation in MSH2 (but could also possibly have a mutation in MHS6). Similarly, MSH6 may be the likely gene involved in a patient with a tumor that expresses MLH1 and not MSH6 (again with normal staining patterns in surrounding colonic mucosal). The situation is more complex with lack of expression of MLH1; promoter hypermethylation of MLH1 is common with sporadic colorectal cancer and may lead to its underexpression. Some laboratories offer methylation analysis to help determine whether lack of expression is due to promoter hypermethylation. Such an approach has been suggested in various reviews of HNPCC.

Immunohistochemistry has an advantage over other techniques (particularly MSI testing) since it is much easier to perform and is less expensive. However, the technique is vulnerable to the quality of tissue preparation, staining, and interpretation. This concern is not merely, hypothetical; our literature search revealed that sensitivity to detect loss of MSH2 expression ranged from 84 to 100 percent in a study of 18 participating centers performing such testing.[56] Variability in specificity was even greater (see below).

Microsatellite Instability. Microsatellite instability (MSI) refers to a variety of patterns of microsatellite repeats observed when DNA is amplified from a tumor with defective mismatch repair compared with DNA amplified from surrounding normal colonic tissue. Repetitive mono- or dinucleotide DNA regions are particularly vulnerable to defective mismatch repair. For example, the mononucleotide sequence "AAAAAAAAAAAAAAAAAAAAAAAAA" is located on chromosome 2P, near the MSH2 gene locus. This sequence is referred to as the "Big A Tract-26" (BAT26). As a result, a tumor suspected to result from mismatch repair defects can be tested for the presence of these repeats.

MSI testing involves amplification of a standardized panel of DNA markers; five markers were agreed upon by a consensus panel convened by the National Institutes of Health in 1997 (BAT25, BAT26, D2S123, D5S345, and D17S250) as described in Chapter 1. Three categories of MSI have been recognized based upon these panels: MSI-high (instability of two or more markers), MSI-low (instability of one marker), and MS-stable (no instability). More recently, some laboratories have begun using ten or more markers. In such cases MSI is defined as "stable" when fewer than 10% of markers are unstable, "low" when 10 to 30% of markers are unstable, and "high" when greater than 30% of markers are unstable (some laboratories use 40%). We recorded the markers used in each study included in this review (see section on clinical validity and accompanying tables).

There are several pitfalls of MSI testing. First, it is labor intensive, relatively costly (compared with IHC), and requires expert pathologic services. In addition, tissue to be amplified should ideally be microdissected to avoid amplifying DNA from normal colonic mucosa. We systematically recorded whether microdissection was performed for all studies related to clinical validity. As a practical consideration, tissue may not always be available since the diagnosis of HNPCC may not be suspected when the cancer was first diagnosed.

Genetic Testing. Multiple methods have been used for genetic testing in HNPCC. The methods used should ideally be able to detect the many potential genotypes associated with HNPCC (e.g., nonsense, missense, and frameshift mutations, genomic deletions, duplications, and rearrangements). We recorded the specific methods and order of testing used in all studies included in this report and, in the case of clinical validity, attempted to discern whether the

specific testing methods were associated with the accuracy of HNPCC testing (see sections and corresponding tables on clinical validity).

A detailed review of these methods is beyond the scope of this report. However, the following summarizes major categories of testing that are used currently, and that were reported in the studies included in this report.

High Output Screening Techniques. High output screening techniques include single stranded conformation polymorphisms (SSCP), conformation sensitive gel electrophoresis (CSGE), denaturing gradient gel electrophoresis (DGGE) and denaturing high-pressure liquid chromatography (DHPLC). These methods all take advantage of the observation that alteration of DNA (due to a polymorphisms or mutation) confers chemical properties that allow it to be differentiated from normal DNA. These approaches can be performed relatively rapidly and allow more detailed studies (such as DNA sequencing) to be targeted to specific regions of DNA.

DNA Sequencing. DNA sequencing can be used following a high output screening technique or as a primary approach (particularly when IHC patterns allow for targeting of a specific mismatch repair gene). It is considered the method of choice for detecting most mismatch repair gene mutation. However, it does not reliably allow for detection of deletions or rearrangements, which are important in HNPCC. DNA sequencing has become automated in recent years, greatly reducing the required time, costs, and expertise.

Conversion analysis. Conversion analysis involves converting diploid cells to haploid cells so that only a single allele is analyzed at a time. The rationale is based upon the observation that a wild-type allele can mask the presence of a mutant allele when performing DNA sequencing (thereby obscuring the presence of a mutation). Conversion testing can increase the yield of genetic testing in HNPCC but is technically complicated, expensive, and, as a result, not widely available. We recorded whether conversion analysis was performed in all studies included in this report.

Methods To Detect Large Structural DNA Abnormalities. Large structural DNA abnormalities (such as large genomic deletions, rearrangements) are potentially important in HNPCC but are not detected by the high output screening techniques or DNA sequencing. There are several methods for detecting these defects. We recorded the specific methods used for all studies included in this report.

Southern blotting involves digestion of genomic DNA (which breaks it into pieces), separation of fragments using electrophoresis, transfer of the fragments to a membrane, and hybridization using probes to recognize deletions, duplications or rearrangements. Southern blotting has not yet been automated to the extent of DNA sequencing and is time-consuming.

Multiplex ligation-dependent probe amplification (MLPA) is a newer technique. It involves measurement of the relative copy number of DNA sequences. MLPA has evolved to become a standard approach for analyzing mismatch repair genes for deletions.

Family History. The family history (and related risk assessment tools such as the Amsterdam criteria) can also be considered as a type of laboratory testing, which can be applied to both the probands and their family members. The accuracy of the family history in predicting the presence of germline mismatch repair mutations is described in the sections on clinical validity.

By contrast, the analytic validity of the family history can be considered the accuracy with which individuals are able to report their family history.

We did not identify any studies that assessed the analytic validity of the family history in patients or families with HNPCC. However, we confined our literature search specifically to HNPCC, while there are several studies that have assessed the validity of the family history for a variety of tumor types, including those associated with HNPCC such as colorectal or endometrial cancer.[57-60] A systematic review of these data found that (in individuals without a personal history of cancer) the positive and negative likelihood ratios of a family history for colon cancer in a 1st degree relative were 23.0 (95% CI 6.4-81.0) and 0.25 (95% CI 0.1-0.63), respectively.[59] These values were 14.0 (95% CI 2.2-83.4) and 0.68 (95% CI 0.31-1.52), respectively for endometrial cancer. In another report, the degree of relationship to the probands, type of cancer, age at diagnosis of the probands, and source of ascertainment of probands were all statistically significant predictors of the accuracy of reporting.[60] The extent to which these data can be generalized to patients and families with HNPCC is unclear.

Limitations of a Literature-Based Approach to Analytic Validity

We encountered only three studies that were eligible based upon our inclusion criteria, and these are summarized below. This was disappointing but expected, since a literature-based approach to understanding analytic validity has severe limitations as described in Chapter 1:

A) Many of the laboratory techniques (e.g., DNA sequencing) are used not only in testing for HNPCC but also in testing for a variety of genetic disorders. As a result, a comprehensive search of issues related to analytic validity should consider the entire literature evaluating such testing, an undertaking that is neither practical nor feasible. A search in MEDLINE® on DNA sequencing, for example, will yield more than 416,000 citations.

   As noted in the methods section, we confined our search to studies that used biological materials from patients or family members suspected of having HNPCC to make the results as applicable to the population of interest as possible. The accuracy of these methods used for other disorders may not be generalizable to HNPCC. The extent to which such sources of indirect evidence may be applicable to HNPCC was beyond the scope of this review.

B) The methods used in laboratory testing evolve rapidly. The specific methods used are commercialized by companies or individuals who developed them. As a result, the published literature may not (and often does not) reflect the actual testing performed in clinical laboratories. It is customary for commercial laboratories to assess at least some components of analytic validity for these contemporary tests, but laboratories are under no obligation to publish the results.

   A partial list of laboratories offering testing services for HNPCC is available through "Genetests" an organization funded through the National Institutes of Health (see http://www.genetests.org/servlet/access?id=8888891&key=AmmF6UdFjuxHB&fcn=y&fw=vDlR&filename=/). A summary of the tests listed on this Website is presented

in Table 12 below. We attempted to contact one of the laboratories (chosen at random) to determine whether they would share information related to analytic validity. Our attempt had limited success. Such a review of "gray" literature may be helpful for understanding the analytic validity of these tests but would require appropriate resources, including incentives, for participating laboratories.

Some commercial laboratories participate in proficiency testing, but the results are generally not published. Proficiency testing involves distributing samples of tissue that are known to be positive or negative for findings of interest. Laboratories participating in such programs can obtain a benchmark for how they are performing in detecting (or excluding) such findings (e.g., genotypes of interest). Proficiency testing also helps understand issues related to reliability.

A major limitation of proficiency testing is that it does not consider the pre-analytic components of testing; for example, the accuracy of a test may be greatly affected by the methods used to acquire tissue. In addition, proficiency testing does not allow for a detailed understanding of the spectrum of abnormalities (e.g., various genotypes) that may be clinically relevant.

Proficiency testing for MSI was introduced in 2006 and is being conducted by the College of American Pathologists. Further information is available at their Website (see http://www.cap.org/apps/cap.portal) but the initial results of their program have not been made public.

C) Studies evaluating analytic validity often suffer from serious methodologic limitations that potentially invalidate the results. Particularly problematic was the inappropriate use of reference standards or lack of clear definitions of references standards altogether. For example, a common pitfall was the inclusion of the test under evaluation as part of the reference standard.

We chose inclusion and exclusion criteria carefully to select for high quality studies while excluding studies whose results are misleading or clinically irrelevant. There were remarkably few high-quality studies directly addressing analytic validity using biological materials from probands or family members proven or suspected to have HNPCC.

Eligible Studies. We identified only three studies that fulfilled eligibility criteria for analytic validity.

- One study compared conversion analysis (in which alleleles are separated into hybrids prior to mutation screening) with DNA sequencing alone to detect heterogeneous germline mutations in MLH1, MSH2 and MSH6 in patients with colorectal cancer.[61] The authors estimated that conversion analysis provided an increased yield of 56% (35/63 cases) compared with DNA sequencing alone. The study was rated a methodologic quality B because the investigators were not blinded to the method used.

- Another study included 20 patients with CRC with known mutations in MLH1 or MSH2.[56] A set of two unstained slides from each case were sent to participating medical centers with capability of performing immunoperoxidase assays for MLH1 and MSH2.

  Of 18 participating centers 2 were excluded: one because slides were damaged in transit and the other because of insufficient staining. Sensitivity for detecting loss of MSH2 expression ranged from 84 to 100%; 10 centers identified all six. Five out of six false positive results were in the same case suggesting that staining or interpretation were not random. Fourteen out of 16 laboratories showed 100% specificity (one laboratory had 93% specificity due to staining failure on one slide and one lab demonstrated 45% specificity due to weak or absent staining in most cases.)

  Re-review of returned MSH2 slides showed lack of internal positive control staining in at least 2 of the 6 MSH2-negative cases from 8 of 16 centers. The other 8 centers had 100% sensitivity and 93-100% specificity on re-review. The slides that lacked internal positive control staining were largely accounted for by two cases.

  The study was rated a methodologic quality B because of its small sample size, and because it did not describe quality control methods or whether microdissection had been performed when preparing the specimens.

- A third study evaluated the sensitivity and specificity of DHPLC analysis compared with DNA sequencing in 46 patients with colorectal cancer from families with HNPCC.[62] DHPLC analysis identified 19 changes previously identified by DNA sequencing and 16 new alterations not previously described. DHPLC was considered to be highly sensitive, detecting a mutation in all patients with no false negative results. The study was rated methodologic quality C because of several deficiencies in reporting.

Summary. In summary, the analytic validity of the tests used to evaluate HNPCC is substantially uncertain. However, there is heterogeneity in the type of testing offered by commercial laboratories and the available data suggest that there may be variability across testing facilities. Additional information that could shed light upon analytic validity is available but would require evaluation of non-published data sources. Committees of experts could also review the strengths and limitations of specific testing techniques based upon clinical experience, with particular reference given to experience with these methods in other genetically-based disorders.

Table 12 presents information from North American laboratories offering genetic testing for HNPCC. Most of the data was retrieved through the website, GeneTests.org. When available, links to the clinical laboratory websites offering genetic testing service were accessed to gather additional detail on type of tests offered. The information presented is not comprehensive as reporting to GeneTests.org is voluntary.

Table 12. North American laboratories offering clinical testing for Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)*

Clinical laboratories Location	Analysis of entire coding region: Sequence analysis	Sequence analysis of select exons	Analysis of entire coding region: Mutation scanning	Targeted mutation analysis	Linkage analysis	Microsatellite instability testing (MSI)	Deletion/ duplica-tion analysis	Mutation scanning of select exons	Immuno-histochemistry	Sequence analysis of RNA
ARUP Laboratories, Inc. Salt Lake City, UT	---	---	---	---	---	PCR: BAT25, BAT26, D2S123, D5S346, D17S250 in MSI low (instability<30%) samples, additional markers available: BAT40, MYCL1, TGS-beta-R2, D10S197, D18S58	---	---	---	---
Baylor College of Medicine Medical Genetics Laboratories Houston, TX	PCR-based assay: MLH1, MSH2, MSH6 [a]MLH1 promoter methylation assay (in combination with MSI analysis): MLH1, MSH2, MSH6	---	---	---	---	Multiplex PCR-based assay (in combination with MSI analysis): BAT25, BAT26, NR21, NR22, D2S123, D17S250, D5S346, D18S35, DIS2883	PCR-based assay: MLH1, MSH2	---		---
Boston University School of Medicine Center for Human Genetics Boston, MA	MLH1, MSH2, MSH6	MLH1, MSH2, MSH6	---	---	---	---	MLPA	---	---	---

Table 12. North American laboratories offering clinical testing for Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)* (continued)

Clinical laboratories Location	Analysis of entire coding region: Sequence analysis	Sequence analysis of select exons	Analysis of entire coding region: Mutation scanning	Targeted mutation analysis	Linkage analysis	Microsatellite instability testing (MSI)	Deletion/ duplica-tion analysis	Mutation scanning of select exons	Immuno-histochemistry	Sequence analysis of RNA
Children's Hospital of Eastern Ontario Molecular Genetics Diagnostic Laboratory Ottawa, Ontario, Canada	MLH1, MSH2	---	---	---	---	---	MLH1, MSH2	---	---	---
City of Hope Clinical Molecular Diagnostic Laboratory Duarte, CA	√	---	Fluorescent sequencer: MLH1, MSH2, MSH6	√	---	Amplification ≥5 markers by denaturing polyacrylamide gel electrophoresis: MSI ≥2 markers, low MSI = 1 marker	---	---	MLH1, MSH2, MSH6	---
Creighton University Medical Center Creighton Medical Laboratories Omaha, NE	---	---	---	---	---	√	---	---	---	---
Fox Chase Cancer Center Clinical Molecular Genetics Laboratory Philadelphia, PA	---	---	---	---	---	√	---	√	---	---

Table 12. North American laboratories offering clinical testing for Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)* (continued)

Clinical laboratories Location	Analysis of entire coding region: Sequence analysis	Sequence analysis of select exons	Analysis of entire coding region: Mutation scanning	Targeted mutation analysis	Linkage analysis	Microsatellite instability testing (MSI)	Deletion/ duplica-tion analysis	Mutation scanning of select exons	Immuno-histochemistry	Sequence analysis of RNA
Huntington Medical Research Institutes Molecular Oncology & Cancer Genetics Laboratory Pasadena, CA	MSH2, MLH1, MSH6	MSH2, MLH1	---	---	MSH2, MLH1	PCR: MSH2, MLH1	√	---	---	√
London Health Sciences Centre Molecular Diagnostic Laboratory London, Ontario, Canada	MSH2, MLH1	---	---	---	---	√	√	---	MLH1 and MSH2 in combination with MSI analysis	---
Mayo Clinic Molecular Genetics Laboratory Rochester, MN	MLH1, MSH2, MSH6	---	---	---	---	---	MLPA: MLH1, MSH2	---	PCR: MLH1, MSH2, MSH6, PMS2	---
Memorial University of Newfoundland Molecular Genetics Laboratory St. John's, Newfoundland, Canada	---	---	---	MSH2, Del 50 CODONS	---	---	---	---	---	---

Table 12. North American laborato ries offering clinical testing for Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)* (continued)

Clinical laboratories Location	Analysis of entire coding region: Sequence analysis	Sequence analysis of select exons	Analysis of entire coding region: Mutation scanning	Targeted mutation analysis	Linkage analysis	Microsatellite instability testing (MSI)	Deletion/ duplica-tion analysis	Mutation scanning of select exons	Immuno-histochemistry	Sequence analysis of RNA
Myriad Genetics Laboratory Salt Lake City, UT	MLH1, MSH2, MSH6 (Southern Blot available for MLH1 and MSH2	---	---	√	---	---	√	---	---	---
North York General Hospital Molecular Genetics Laboratory North York , Ontario, Canada	---	---	---	---	---	√	---	---	---	---
Ohio State University Molecular Pathology Laboratory Columbus, OH	---	---	---	---	---	BAT 25, BAT 26	---	---	---	---
Quest Diagnostics, Inc. Molecular Genetics Laboratory San Juan Capistrano, CA	MLH1, MSH2, MSH6	---	---	---	---	Multiplex polymerase chain reaction (PCR): BAT 25, BAT 26, D2S123, D5S346, D17S250	√	---	---	---
Saint Louis University Health Science Center DNA Diagnostic Laboratory Saint Louis, MO	---	---	---	---	---	√	---	---	---	---

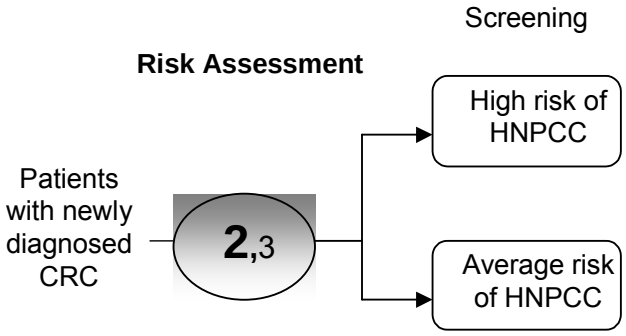
Table 12. North American laborato ries offering clinical testing for Hereditary Nonpolyposis Colon Cancer (Lynch Syndrome)* (continued)

Clinical laboratories Location	Analysis of entire coding region: Sequence analysis	Sequence analysis of select exons	Analysis of entire coding region: Mutation scanning	Targeted mutation analysis	Linkage analysis	Microsatellite instability testing (MSI)	Deletion/ duplica-tion analysis	Mutation scanning of select exons	Immuno-histochemistry	Sequence analysis of RNA
UCLA Medical Center Diagnostic Molecular Pathology Laboratory Los Angeles, CA	---	---	---	---	---	√	---	---	---	---
University of Alberta Molecular Diagnostic Laboratory Edmonton, Alberta, Canada	---	---	MSH2, MLH1	---	---	---	√	---	---	---
University of Pennsylvania School of Medicine The Genetic Diagnostic Laboratory Philadelphia, PA	MSH2, MLH1 (If negative, Southern blot optional)	---	√	---	√	---	√	---	---	---

*May include Muir-Torre Syndrome, Turcot Syndrome.
√ = Genetests.org indicates offered by laboratory, but no additional information given.
Abbreviations: PCR, Polymerase Chain Reaction/Fragment Analysis.
[a] due to space limitations promoter methylation assay was reported in this column.

Clinical Validity

Key Question 2 and Pertinent Subquestions



HNPCC has been defined clinically and genetically as described in Chapter 1. The following sections present analyses using either the clinical or the genetic definition of the condition, depending on which Key Question was being addressed.

Key Questions 2a to 2c seek to evaluate the prevalence of MMR mutations and suggestive MSI and IHC among patients with the Lynch Syndrome. Thus, they assume a clinical definition of the condition.

Key Question 2 pertains to the ability of clinical and laboratory predictors to identify the presence of MMR mutations. Thus, it assumes a genetic definition of the condition.

We first present analyses based on a clinical definition of HNPCC. In these analyses we estimated the frequency of mismatch repair gene mutations and tumors with MSI and IHC among CRC patients fulfilling the Amsterdam I and II criteria.

We present subsequent analyses based on the genetic definition of the Lynch syndrome. These help define test characteristics of various strategies (such as the combination of a clinical history with laboratory testing of tumor tissue) for predicting the presence of mismatch repair mutations.

Finally, we used a decision tree model to calculate the expected number of patients with MMR mutations among unselected patients presenting with CRC using various predictive strategies. The parameters used in the calculations are based on our best estimates derived from this systematic review.

Key Question 2a: Assuming a Clinical Definition of the Lynch Syndrome, What Proportion of Patients has a Mismatch Repair Mutation?

Among CRC fulfilling Amsterdam I criteria, the random effects summary prevalence of MLH1 and MSH2 gene mutations was 44% (95% CI: 35, 52; n=19 studies, 464 CRC patients), with evidence for substantial between-study heterogeneity ($p<0.01$; $I^2=52\%$). The six studies that performed genetic testing among all Amsterdam I patients had a summary prevalence of 51% (95% CI: 35, 66%; 84 CRC patients).

For patients fulfilling the Amsterdam II criteria, the corresponding prevalence values were 39% (95% CI: 30, 49%; 10 studies, 279 CRC patients) and 40% (95% CI: 30, 52%) based upon 2 studies that performed sequencing on all 87 Amsterdam II patients.

Only three studies examined other MMR genes (MSH6, PMS1 and PMS2) in Amsterdam I patients, without identifying any additional mutations. Two additional MSH6 mutations were found among 20 Amsterdam II patients in a single study.

Frequency of MMR Gene Mutations Among Patients Fulfilling Amsterdam Criteria I.

Description of studies. Nineteen studies (described in 21 papers [63-83]) provided data for the prevalence of MMR mutations among CRC probands who fulfilled the Amsterdam I criteria. The median number of patients with relevant data in each study was only 13 (interquartile range: 10, 22), and the actual number ranged between 3 and 154 patients. However, study sizes (all included patients) ranged between 19 and 509. Only five studies included at least 20 probands fulfilling the Amsterdam I criteria.[67,72,76,79-81] Nine studies were rated grade B for their overall methodologic quality[63,65-67,70,71,74,77,79-81] while the rest were rated grade C (Appendix F-1*).

Six studies performed bidirectional sequencing on all patients who fulfilled the Amsterdam I criteria.[69,75,77,79,81,83] (Colombino 2005[83] performed full sequencing only among familial CRC cases, which included Amsterdam I patients). Ten studies performed full sequencing only on patients who were selected by gene screening methods such as DHPLC or SSCP.[63,66-68,70,71,73,74,76,82] The remaining three studies did not describe the presence of any mutation with bidirectional sequencing in any patient[64,72] or did not provide any details on the genetic testing strategy they used.[78] None of these 19 studies used conversion analysis to detect mutations.

Assessment of mutations in MMR genes other than MLH1 and MSH2. All reports assessed mutations in the MLH1 and MSH2 genes. Additional MMR genes were assessed in three studies.[76,78,81] Park 1999[76] assessed the PMS1 and PMS2 genes in a minority of their Amsterdam I patients (24 and 27 patients respectively, less than 20% of their sample), without identifying any additional mutations(Appendix F-1*). Wang 1999[81] also tested for MSH6, PMS1 and PMS2, but found no additional mutations among the subgroup of patients fulfilling the Amsterdam I criteria. Stormorken 2001[78] also tested for MSH6 mutations, but found none among Amsterdam I patients.

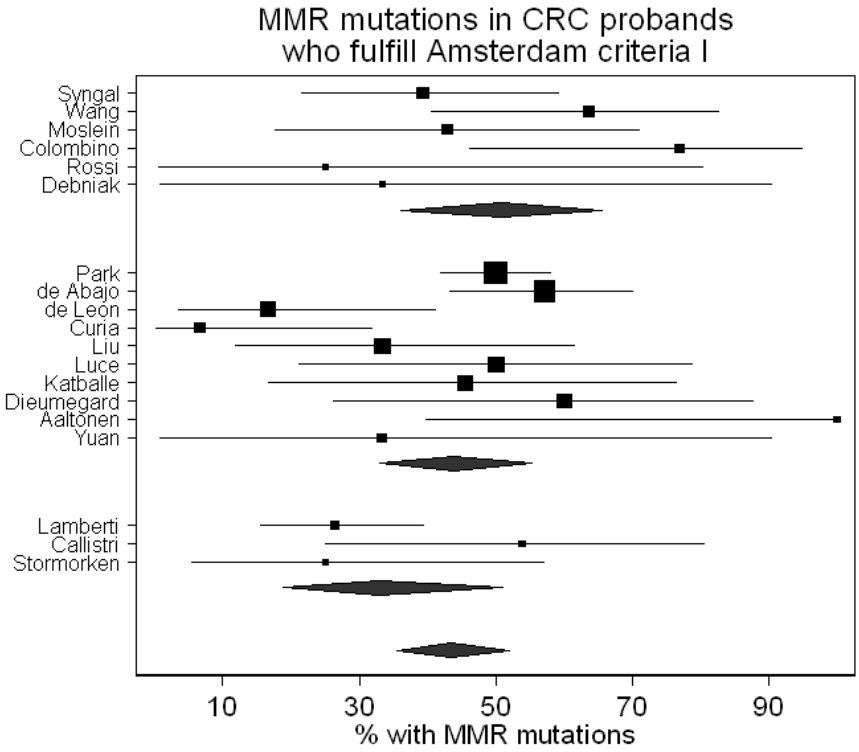
Analyses. Overall, the random effects summary estimate from the 19 studies was 44% (95% CI: 35, 52%), with evidence for substantial between-study heterogeneity ($p<0.01$; $I^2=52\%$) (Table 13, Figure 3). The six studies that performed full sequencing in all available samples identified mutations in 51% (95% CI: 35, 66%) of 84 patients fulfilling the Amsterdam I criteria (Table 13). The corresponding prevalence estimate was 44% when sequencing was performed after suggestive SSCP, DHPLC or other gene screening methods, and only 33% among studies that used screening methods without further confirmation by sequencing (Table 13). The prevalence of MMR mutations did not differ beyond chance with respect to overall study quality score, whether the study was small (i.e., analyzed fewer than 20 patients), or whether the authors described how they classified mutations as being pathogenic.

Finally, three studies (n=28 eligible patients) that sampled unselected, nonreferral patients with CRC found a higher prevalence of MMR mutations (66%) among patients who fulfilled the

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Amsterdam I criteria, compared to studies that sampled among referral or otherwise selected cases (prevalence 41%; Table 13). The precision of these estimates is uncertain because of the very small number of eligible patients.

Figure 3. Prevalence of mismatch repair gene mutations among colorectal cancer patients fulfilling the Amsterdam I criteria.



Three panels are shown, representing three subgroups of studies. Top: Studies that performed sequencing on all Amsterdam I patients. Middle: Studies that performed sequencing only after a suggestive gene screening method. Bottom: studies that did not perform sequencing on any patient. Within each subgroup, studies are ordered by decreasing number of patients. The bottom diamond represents the overall random effects estimate. MMR: mismatch repair.

Table 13. Summary estimates of the prevalence of MMR mutations among CRC fulfilling Amsterdam I criteria

Summary	Number of studies (CRC patients fulfilling AM1)	% with mutations (95% CI)	Heterogeneity P-value, (I^2 [%])	Between-subgroup heterogeneity, P-value
Overall	19 (464)	44 (35, 52)	<0.01 (52)	NA
Overall quality scale				
B [a]	9 (162)	49 (37, 61)	0.09 (41)	0.12
C	10 (302)	39 (28, 51)	0.01 (58)	
Sequencing				
All samples	6 (84)	51 (36, 66)	0.17 (35)	0.02
Some [b]	10 (298)	44 (33, 55)	0.04 (50)	
None	3 (82)	33 (19, 51)	0.16 (46)	
Deletion analysis and sequencing in all samples				
Yes	1 (22)	64 (41, 83)	NA	0.09
No	18 (442)	42 (34, 51)	0.03 (51)	
Assessment of additional MMR genes (other than MLH1 and MSH2)				
Yes	3 (188)	49 (34, 65)	0.12 (54)	0.12
No	16 (276)	42 (32, 52)	0.01 (51)	
Any definition for pathogenic mutations				
Yes	11 (233)	47 (34, 60)	<0.01 (63)	0.90
No	8 (231)	40 (30, 51)	0.13 (33)	
Total number of patients fulfilling Amsterdam I criteria				
≥20	5 (317)	47 (35, 59)	<0.01 (73)	0.28
<20	14 (147)	41 (30, 53)	0.06 (40)	
Sampling among unselected, non-referral CRC				
Yes	3 (28)	66 (35, 88)	0.17 (44)	0.09
No	16 (436)	41 (31, 50)	0.01 (52)	

AM1: Amsterdam I criteria; CI: confidence interval; CRC: colorectal cancer patients.
None of the studies was rated A in the overall quality scale.
Gene sequencing was performed only to those selected by gene screening methods.

Frequency of MMR Gene Mutations Among Patients Fulfilling Amsterdam II Criteria.

Description of studies. Ten studies assessed the prevalence of MMR mutations among 271 CRC probands who fulfilled the Amsterdam II criteria (Appendix F-2[*]). They were described in 12 papers.[65,67,71,72,77-80,84-87]

The total number of patients in the various study populations ranged from 45 to 535 patients with CRC. However, the median number of patients fulfilling Amsterdam II criteria was 19.5 (interquartile range: 5, 35). Only five studies assessed more than 20 probands from families fulfilling the Amsterdam II criteria.[67,72,79,80,86,87] Eight out of ten studies received grade B in the overall quality rating while two[72,78] received grade C.

Four studies performed bidirectional sequencing in all patients fulfilling Amsterdam II criteria.[77,79,84,86] Four studies performed full sequencing only to patients who were selected by gene screening methods.[65,67,71,85,87] The remaining two studies did not describe the presence of any mutation with bidirectional sequencing in any patient[72] or did not provide any details on the genetic testing strategy they used.[78] None of these 10 studies used conversion analysis.

Assessment of mutations in MMR genes other than MLH1 and MSH2. All studies tested for MLH1 and MSH2 mutations. Only Stormorken 2001[78] also tested for MSH6 mutations. In this

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

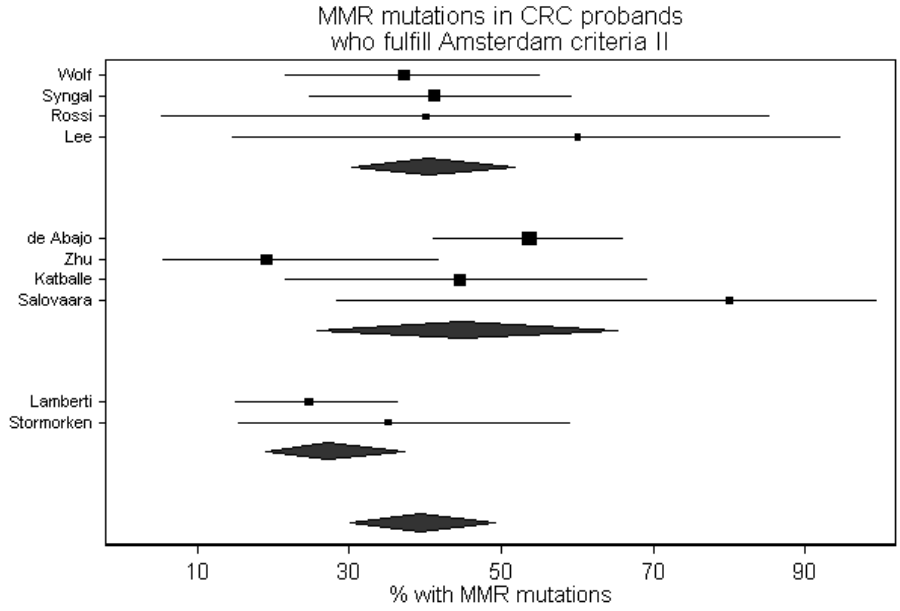
study five out of 20 Amsterdam II patients had deleterious MLH1 and MSH2 mutations (25% [95% CI: 9, 49%]) and another two had deleterious MSH6 mutations (10% [95% CI: 1, 32%]).

Analyses. As expected, the overall prevalence of MMR gene mutations was lower compared with patients who fulfilled the stricter Amsterdam I criteria (39% [95% CI: 30, 49%]; Table 14, Figure 4). Between-study heterogeneity was statistically significant.

Studies that performed sequencing in all Amsterdam II patients estimated similar prevalence of MMR mutations compared to studies that performed sequencing only after suggestive gene screening with SSCP, DHPLC, or other methods (40% versus 45%, respectively; Table 14). Studies that were rated C for their overall quality estimated significantly lower prevalence rates (27%; Table 14) compared to studies that received B for their overall quality. In the latter, the summary estimate was 43% (95% CI: 34, 54%) with no evidence for heterogeneity.

Studies that mentioned how pathogenic mutations were characterized as such, contributed fewer than 20 patients, or sampled unselected, non-referral patients with CRC, tended to provide higher estimates for the prevalence for MMR mutations. However, all of these studies were small and thus it is possible that the observed variability could have been due to chance (Table 14).

Figure 4. Prevalence of mismatch repair gene mutations among colorectal cancer patients fulfilling the Amsterdam II criteria



Three panels are shown, representing three subgroups of studies. Top: Studies that performed sequencing in all Amsterdam I patients. Middle: Studies that performed sequencing only after a suggestive gene screening method. Bottom: studies that did not perform sequencing to any patient. Within each subgroup, studies are ordered by decreasing number of patients. The bottom diamond represents the overall random effects synthesis. MMR: mismatch repair.

Table 14. Summary estimates of the prevalence of MMR mutations among CRC fulfilling Amsterdam II criteria

Summary	Number of studies (patients fulfilling AM2)	% with mutations (95% CI)	Heterogeneity P-value, (I^2 [%])	Between-subgroup heterogeneity, P-value
Overall	10 (279)	39 (30, 49)	0.03 (53)	NA
Overall quality scale				
B [a]	8 (190)	45 (38, 52)	0.16 (34)	0.01
C	2 (89)	27 (19, 37)	0.36 (0)	
Sequencing				
All samples	2 (87)	40 (30, 52)	0.82 (0)	0.02
Some [b]	4 (111)	45 (26, 65)	0.03 (66)	
None	2 (89)	27 (19, 37)	0.36 (0)	
Deletion analysis and sequencing in all samples				
Yes	0 (0)	NA	NA	NA
No	10 (279)	39 (30, 49)	0.03 (53)	
Assessment of additional MMR genes (other than MLH1 and MSH2)				
Yes	1 (20)	35 (15, 59)	NA	0.68
No	9 (259)	40 (30, 51)	0.02 (57)	
Any definition for pathogenic mutations				
Yes	7 (233)	43 (31, 55)	0.02 (60)	0.14
No	3 (46)	29 (18, 44)	0.45 (0)	
Total number of patients fulfilling Amsterdam criteria II				
≥20	5 (226)	36 (24, 49)	0.01 (73)	0.41
<20	5 (53)	44 (31, 58)	0.51 (0)	
Sampling among unselected, non-referral CRC				
Yes	2 (23)	57 (23, 85)	0.19 (43)	0.28
No	8 (256)	37 (28, 48)	0.03 (56)	

AM2: Amsterdam II criteria; CI: confidence interval; CRC: colorectal cancer patients.
[a] None of the studies was rated A in the overall quality scale.
[b] Gene sequencing was performed only to those selected by gene screening methods.

Interpretation of the MMR Prevalence Eestimates. The summary prevalence was 44% among all studies on Amsterdam I patients, and 51% for studies that sequenced all patients. For Amsterdam II the corresponding numbers were 39% and 40%. These estimates pertain mainly to MLH1 and MSH2 mutations, because most studies tested only these two genes. The true prevalence of MMR mutations among Amsterdam I (or Amsterdam II) patients may be different (possibly higher rather than lower) than these estimates for several reasons:

- **Genes other than MLH1 and MSH2:** Only a minority of studies tested for mutations in other MMR genes (especially MSH6, the gene most commonly implicated in patients without mutations in MLH1 or MSH2[88]).
  - Stormorken 2001 and Wang 1999 did not find any deleterious MSH6 mutations among 34 Amsterdam I patients in total. The upper boundary of the exact binomial 95% CI for the frequency of MSH6 mutations in these two studies is approximately 10%.
  - Stormorken 2001 found two deleterious MSH6 mutations in addition to five MLH1 and MSH2 mutations out of 20 Amsterdam II patients. Thus, MSH6 mutations resulted in an increment of 10% (95% CI: 1, 32%).

  Presumably, if other studies had assessed MSH6 routinely, the total prevalence of MMR mutations would be higher. However, there were insufficient data to provide robust estimates.

- **Comprehensiveness of genetic testing – sequencing of all samples and testing for large deletions/rearrangements:** Studies that performed sequencing in all samples estimated higher frequency of MMR mutations among Amsterdam I patients compared to other studies. This was not true among studies that focused on Amsterdam II patients. Thus, more comprehensive testing resulted in more identified MMR mutations (at least in Amsterdam I studies), as expected.

- **Role of founder mutations:** The degree to which a search for mutations other than MLH1 and MSH2 will identify mutations in other MMR genes depends upon the population being studied. The spectrum of MMR mutations is variable and some are specific to certain populations. An example of the latter is the presence of founder mutations in the MLH1 genes that are common among patients of Finnish origin, but are not prevalent in patients of different descent. The Finnish study[63] included only 4 patients fulfilling the Amsterdam criteria I, all of whom were found to have MMR mutations. An American founder mutation in the MSH2 gene has been described in one kindred, probably originating from German immigrants, but it is unlikely to have a large impact in the general population.[90,91] The Finnish founder mutations are probably not applicable to the US population. Nevertheless, the summary estimates are practically identical if the Finnish study[63] is excluded.

- **Identifying deleterious mutations:** As mentioned in the methods, only mutations that were characterized by the primary studies as deleterious or pathogenic were analyzed. Misclassifications may have occurred in both directions:
  - Some deleterious missense mutations may have been erroneously misclassified as non-pathogenic.
  - The opposite is also likely, especially when pathogenicity is inferred on the basis that the mutation was absent from cancer-free controls. This was done in five studies.[63,66,67,82,83] However, most of the studies included only small numbers of cancer-free controls (only around 100 patients were used as controls in the five studies). Absence of a mutation in such a small population of cancer-free controls does not necessarily prove that the mutation is not pathogenic.

  There were insufficient data to provide precise estimates of how many missense mutations were found among the subgroup of Amsterdam I (or Amsterdam II) patients in the studies that performed comprehensive genetic testing.

- **Sampling of tested Amsterdam I and Amsterdam II cases:** The strategy used for selecting patients with CRC can have a substantial impact on the prevalence estimates (see comments on additional studies that were suggested for consideration by the TEP, below). Only three studies on Amsterdam I patients studied unselected patients with CRC, but they identified a total of only 28 patients. By contrast, studies that identified cases that had been included in cancer databases (e.g., the ICG-HNPCC database that was used in the Park 1999 study) represent a highly selected population,[88] or may pertain almost entirely to individuals with a high probability of having mutations (e.g., because of suggestive MSI testing prior to their registration in the database).[61] Biases resulting from sampling are not only a theoretical concern (see below comments on additional studies that were suggested for consideration by the TEP).

Additional studies suggested for consideration by the TEP. Several additional studies were considered by the TEP during the peer review process as important and potentially relevant that had not been included in the draft report. Although none of these studies was eligible for these analyses according to the eligibility criteria, they provide insight and complementary data, directly supporting the analyses and their interpretation. Most importantly, the findings from these studies are in accordance with the notion that sampling of Amsterdam I or II patients from different sources may result in very different prevalence estimates, even when the genetic testing strategies are comprehensive.

- Balmana 2006[92] reported the prevalence of MLH1 and MSH2 gene mutations among 1914 unrelated people who had been tested for MMR mutations in the Myriad Genetic Laboratories. Genetic analysis was performed using a combination of sequencing and southern blotting. The cohort included 534 unrelated people fulfilling the Amsterdam II criteria (although it was unclear if all of them had CRC). Amsterdam I patients were not reported separately. Only half of the Amsterdam II patients (274/534=51%) were tested for large deletions or rearrangements.

  In total, 180 Amsterdam II participants were found to be carriers of MLH1 or MSH2 mutations (34% [9%% CI: 30, 38%]). This estimate includes large deletions or rearrangements and pathogenic and non-pathogenic mutations. About one fourth (27% [95% CI: 20, 35%] of the mutations were due to large deletions/rearrangements in a subgroup of the 1016 participants who underwent such testing. Extrapolating the proportion of large deletions to all Amsterdam II patients would result in a total prevalence of approximately 38%, very close to the summary estimate provided above. This study was excluded from the analyses because it was unclear whether all Amsterdam II participants had CRC.

- Wagner 2003[93] evaluated 49 individuals from the Henry Lynch Cohort fulfilling the Amsterdam criteria (presumably Amsterdam I criteria), at least 9 of whom were not CRC patients. They used DGGE to screen for MMR mutations in the MLH1, MSH2 and MSH6 genes, and performed sequencing on exons with altered migration patterns. They tested for large deletions, and performed mono-allelic mutation analysis (MAMA) in very few samples, mainly to demonstrate the feasibility of the technique.

  Overall, 25 out of 49 had deleterious MLH1 or MSH2 mutations, 1 had deleterious MSH6 mutations, and 14 had genomic rearrangements. Thus, the overall proportion was 82% (95% CI: 68, 91%). In addition, 5 missense mutations were found; were they to be considered pathogenic, the overall proportion would be 92% (95% CI: 80, 98%).
  The detection of large deletions/rearrangements resulted in 29% increase in the estimated prevalence of MMR mutations. The degree to which the Henry Lynch Cohort is representative of the whole Amsterdam I population is unclear.

- Liu 1996[94] assessed the MLH1, MSH2 and PMS2 genes 48 families of Amsterdam I patients who had suggestive MSI (note that more than one person per family was tested for mutations –i.e, reporting was at the family not the individual level). They found a prevalence of "drastic" (deleterious) mutations of 54%. Counting all identified mutations, drastic or not, 71% (95% CI: 56, 83%) of the families carried a mutation. Fifteen families had large genomic deletions/rearrangements (31%), but many families had more than one type of mutations.

It is unclear how many unrelated people had only large deletions or rearrangements in this study. There might be some overlap with the Wanger 2003[93] families for patients from North America. The authors note that their sample was not representative of the general Amsterdam I population.

Caveat on true overall prevalence of MMR mutation carriers. Limited data suggested that approximately one-fourth to one-third of genotypes associated with HNPCC are related to deletions or rearrangements that would be missed through sequencing alone. As a result, the prevalence of mutations in Amsterdam I (or II patients) assessed from studies that performed sequencing alone are likely to be underestimates.

Accounting for this effect, one may derive a prevalence of MMR mutations of approximately 63% to 67% in Amsterdam I patients. For Amsterdam II patients the corresponding prevalence values would be 50 to 53%.

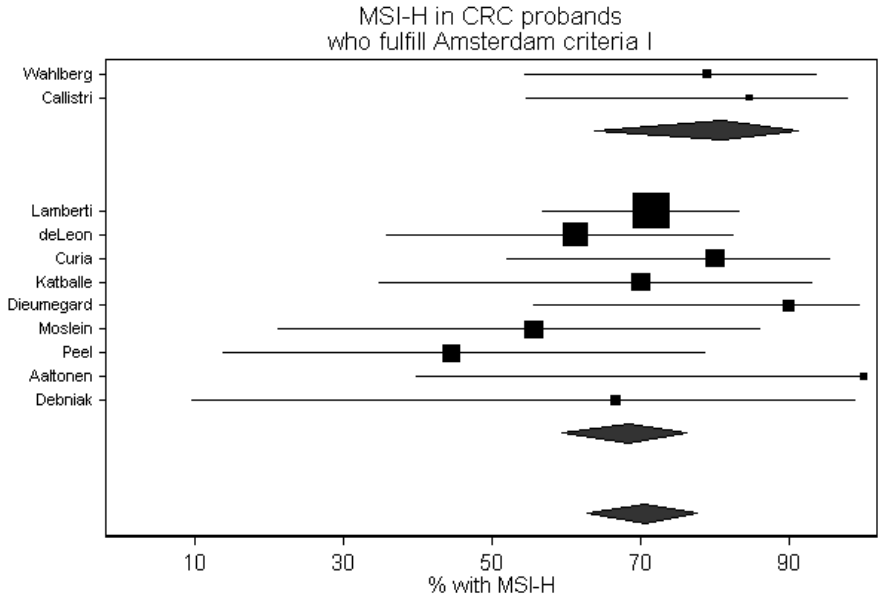
Furthermore, limited data suggest that approximately 10% of MMR genotypes involve MMR genes other than MLH1 and MSH2. Thus, assuming an additional 10% increase in mutations by assessing more genes would result in an overall prevalence of up to 70% to 75% for Amsterdam I patients, and 55 to 59% for Amsterdam II patients.

Key Question 2b: Assuming a Clinical Definition of the Lynch Syndrome, What Proportion of Patients has MSI?

Among patients fulfilling the Amsterdam I criteria (data from 11 studies, n=159 patients fulfilling the criteria), 71% (95% CI: 63, 78%) of tumors were found to be MSI-H (p=0.51 for heterogeneity; I^2=0%). Among patients who fulfilled the Amsterdam II criteria (data from four studies, n=102 patients fulfilling the criteria), the corresponding summary prevalence was 68% (95% CI: 58, 76%) without evidence for between-study heterogeneity (p=0.91, I^2=0%).

Patients Fulfilling Amsterdam I Criteria. Eleven studies with 159 patients fulfilling the Amsterdam I were criteria were included in the analysis (Figure 5). These were described in 13 papers.[63-66,68-72,75,79,80,95] Although the total number of patients with CRC included in the primary studies ranged from 11 to 509, the median number of CRC patients eligible for this analysis was 10 (interquartile range: 9, 18), and only one study assessed more than 20 CRC patients. (Lamberti 1999,[72] n=57). Five studies [63,65,66,70,71,79,80] received a grade B in the overall quality rating while the others were rated a grade C.

Figure 5. Prevalence of MSI in colorectal cancer tumors from patients fulfilling the Amsterdam I criteria



MSI-H was considered as defined in the primary studies. Two subgroups are shown. The upper subgroup shows studies that used the NCI recommended 5 marker set, the lower subgroup shows studies that used other marker sets. Within each subgroup studies are ordered by decreasing number of patients. The bottom diamond represents the overall random effects estimate.

We considered specific study features as described in Chapter 2 (i.e., whether the study reported use of microdissection and used the microsatellite marker set recommended by the National Cancer Institute). Only six studies reported that they used microdissection (which helps assure that the tissue sample that was tested for MSI consisted mainly of malignant cells).[64-66,68,71,79,80,95] Only two studies (reported in three papers[64,79,80]) used the marker set recommended by the National Cancer Institute (Appendix F-3*).

Overall, 71% (95% CI: 63, 78%) of tumors were MSI-H (p=0.51 for heterogeneity; I^2=0%). The estimate was consistent among the various subgroups that we assessed (Table 15). The prevalence rates were generally higher among studies that used the marker sets recommended by the National Cancer Institute (Table 15).

The prevalence of combined MSI-H and MSI-low was similar to that of MSI-H (Appendix F-3*).

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Table 15. Summary estimates of the prevalence of MSI-H in colorectal cancer tumors from patients fulfilling Amsterdam I criteria

Summary	Number of studies (CRC patients fulfilling AM1)	% with MSI-H (95% CI)	Heterogeneity P-value, (I^2 [%])	Between-subgroup heterogeneity, P-value
Overall	11 (159)	71 (63, 78)	0.51 (0)	NA
Overall quality scale				
B [a]	5 (58)	79 (67, 88)	0.81 (0)	0.10
C	6 (101)	66 (56, 75)	0.44 (0)	
Microdissection				
Yes	6 (84)	70 (58, 80)	0.32 (15)	0.89
No	5 (75)	71 (59, 81)	0.50 (0)	
Use of the NCI-recommended marker sets				
Yes	2 (32)	81 (64, 91)	0.69 (0)	0.17
No	9 (127)	68 (59, 76)	0. 52 (0)	
Total number of patients fulfilling Amsterdam criteria I				
≥20	1 (49)	71 (57, 83)	NA	0.89
<20	10 (110)	70 (60, 79)	0.42 (2)	
Sampling among unselected, non-referral CRC				
Yes	2 (14)	70 (62, 78)	0.39 (5)	0.81
No	9 (145)	74 (44, 91)	0.39 (0)	

AM1: Amsterdam I criteria; CI: confidence interval; CRC: colorectal cancer patients.
[a] None of the studies was rated A in the overall quality scale.

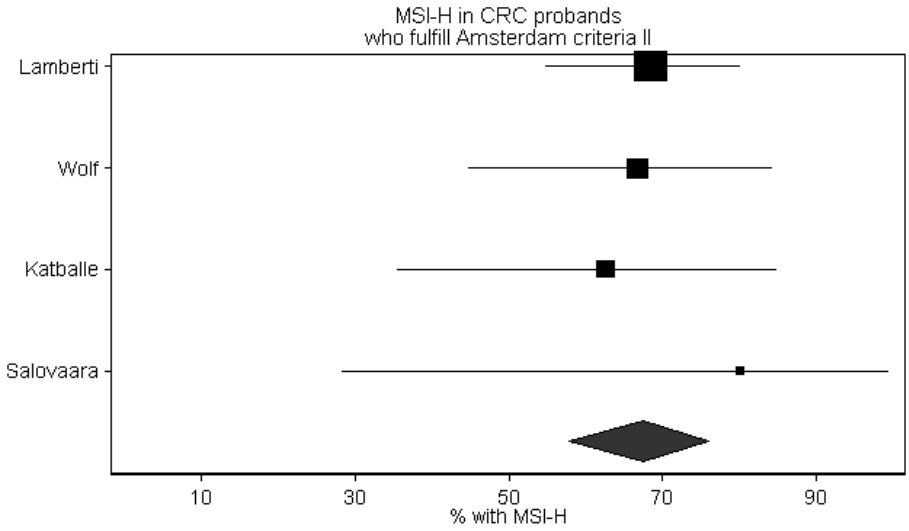
Patients Fulfilling Amsterdam II Criteria. Only four studies including 102 patients fulfilling the Amsterdam II criteria were identified (Appendix F-4*). These were described in five papers.[65,71,72,85,86] The largest study (Lamberti 1999[72]) analyzed 59 available eligible tumors, and the smallest study (Salovaara 2000[85]) had only 4 eligible tumors. The total number of patients with CRC included in these studies ranged from 45 to 535. All studies except for the one by Lamberti 1999[72] received grade B for overall quality. None of the studies used the National Cancer Institute recommended marker sets, and only two [79,80,86] used microdissection (Appendix F-4). Overall, the summary prevalence was 68% (95% CI: 58, 76%) without any evidence for between-study heterogeneity (p=0.91, I^2=0%) (Figure 6).

A pooled estimated for the three fair quality (B overall quality) studies was 66% (95% CI: 51, 79%) with no evidence for heterogeneity. Estimates from the two studies with more than 20 patients that fulfilled the Amsterdam II were similar to the overall estimate. The results were also similar for studies that used the markers sets recommended by the National Cancer Institute.

Finally, results were consistent when the MSI-L and MSI-H tumors were combined in two studies (described in three papers [65,71,72], Appendix F-4*). The overall prevalence was 71% (95%CI: 60, 81%) with no statistically significant heterogeneity.

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Figure 6. Prevalence of MSI-H in colorectal cancer tumors from patients fulfilling the Amsterdam II criteria



MSI-H was considered as defined in the primary studies. Studies are ordered by decreasing number of patients. MSI-H: MSI-H.

Interpretation of the MSI Prevalence Estimates. Some of the biases that may affect the calculated prevalence for MMR mutations are also applicable in this section, specifically biases that pertain to the selection/sampling of Amsterdam I and Amsterdam II patients who were studied. In addition, even among the selected Amsterdam I or Amsterdam II patients in each study, MSI testing often depended upon practical and logistical considerations (e.g., patient availability and consent and availability of tumor tissue). Thus, not all patients had all tests and it was unclear whether additional bias may have been introduced in selecting patients for testing.

Key Question 2c: Assuming a Clinical Definition of the Lynch Syndrome, What Proportion of Patients has Abnormal Protein Expression by Immunohistochemistry?

Among patients fulfilling the Amsterdam I criteria, the overall prevalence of tumors with loss of protein expression was 40% (95% CI: 28, 53%; n= 6 studies, 63 patients) with no evidence for between-study heterogeneity ($p=0.75$, $I^2=0\%$).

Only one eligible study provided relevant data for 20 patients fulfilling the Amsterdam II criteria. Eight out of 20 tumors had suggestive IHC for the MLH1, MSH2 or MSH6 genes (40% [95% CI: 9, 64%]).

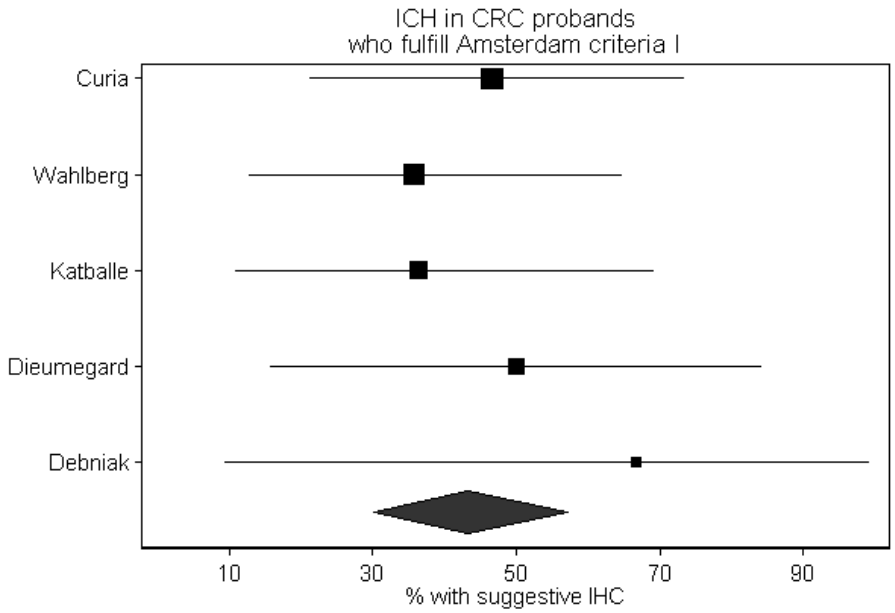
Patients Fulfilling Amsterdam I Criteria. Only six studies that included a total of 63 eligible patients (median 12 tumors in each study) provided relevant data; no study contributed more than 15 tumors in this analysis. The total number of patients included in the pertinent studies ranged from 30 to 168. These were described in eight publications.[65,66,69-71,78-80]

Five out of six studies were characterized as grade B quality while two[69,78] were rated grade C. Only Stormorken 2001[78] assessed the expression of the MSH6 genes (in addition to MLH1 and MSH2) (Appendix F-5*). However, none of the samples was positive for only MSH6.

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

The overall prevalence of tumors with loss of protein expression was 43% (95% CI: 30, 57%) with no evidence for between-study heterogeneity (p=0.85, I^2=0%) (Figure 7). The summary estimate was similar (42% [95% CI: 29, 56%] without any heterogeneity) after excluding the report by Debniak 2000,[69] which was the only one graded C for overall quality.

Figure 7. Prevalence of suggestive IHC in colorectal cancer tumors from patients fulfilling the Amsterdam I criteria



Studies are ordered by decreasing number of patients.

Patients Fulfilling Amsterdam II Criteria. Only Stormorken 2001[78] assessed the prevalence of suggestive IHC among 20 patients fulfilling Amsterdam criteria II. This study was rated C for its overall methodologic quality. The expression of three MMR genes was sought, namely MLH1, MSH2 and MSH6. Eight out of 20 Amsterdam II patients had suggestive IHC (40% [95% CI: 19, 64%]).

Tumors that have suggestive IHC for MSH2 may also have suggestive IHC for MSH6, because in the absence of functional MSH2 protein the MSH2/MSH6 heterodimer is not formed correctly. Two out of 20 Amsterdam II patients had deleterious mutations in the MSH6 gene in Stormorken 2001.[78] None had suggestive IHC only for the MSH6 antibody.

Interpretation of the IHC Prevalence Estimates. In contrast to MSI testing that detects replication errors, and is generally not MMR-gene specific, IHC uses antibodies that target specific MMR genes. Only one of the eligible studies used antibodies against MSH6.[78] The MSH6 gene is the third most common gene with pathogenic mutations in HNPCC patients (approximately 6% of the mutations in the ICG-HNPCC database are in MSH6[88]). Thus, the prevalence of suggestive IHC would probably be higher if anti-MSH6 or additional antibodies were used.

Some of the biases that may affect the calculated prevalence for MMR mutations are also applicable in this section, specifically biases that pertain to the selection/sampling of Amsterdam I and Amsterdam II patients who were studied. In addition, even among the selected Amsterdam I or Amsterdam II patients in each study, IHC testing often depended upon practical and

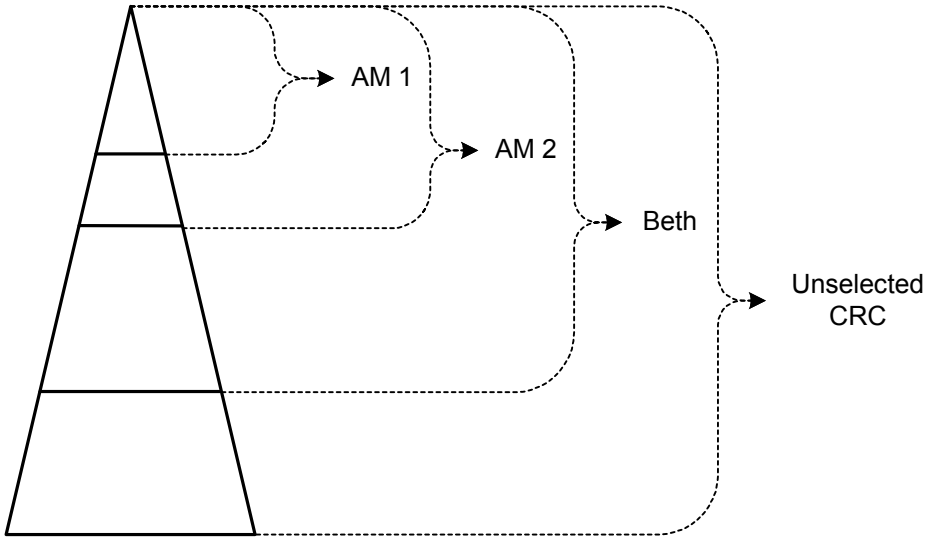
logistical considerations (e.g., patient availability and consent and availability of tumor tissue). Thus, not all patients had all tests and it was unclear whether additional bias may have been introduced in selecting patients for testing.

Key Question 2: How Accurate Are Various Predictors Assuming a Genetic Definition of the Lynch Syndrome?

We examined a variety of clinical and laboratory predictors as described in Chapters 1 and 2. A central question of this report relates to the accuracy of various predictors in unselected patients with CRC. Because testing for MMR mutations is expensive, most studies performed testing only in patients who had been pre-selected based on clinical or laboratory features. Thus, it is critically important to describe the specific CRC population when attempting to make comparisons among studies.

The various clinical predictors defined populations at different risk for HNPCC. Figure 8 illustrates the relationship among patient populations defined by Amsterdam I criteria, Amsterdam criteria II, Bethesda guidelines, and unselected CRC patients.

Figure 8. Relationship between the CRC populations defined by different clinical predictors



The whole pyramid represents all existing unselected patients with CRC. Each of the different clinical predictors (AM1: Amsterdam I criteria; AM2: Amsterdam II criteria; Beth: Bethesda guidelines) defines populations that are a subset of the immediately broader definition, as shown in the figure. The precise areas of the populations are only illustrative and not drawn to scale; thus they do not represent the actual relative proportions of these different populations.

Table 16 presents the different populations that underwent MMR testing in all studies included in this analysis. Moving to the right, columns represent increasingly selected patient populations (by analogy, moving to the top of the pyramid in Figure 8). Table 16 provides a roadmap for the analyses presented in the following sections.

Table 16. Overview of available evidence on the sensitivity and specificity of various predictors for MMR mutation rates

Predictor	All studies	Specific CRC populations defined by increasingly selective criteria					
		Unselected CRC probands	Revised Bethesda guidelines	Bethesda guidelines	Modified Amsterdam criteria	Amsterdam II criteria	Amsterdam I criteria
Amsterdam I criteria	F-6 (n=19)	F-7 (n=2)	ND	F-8 (n=2)	ND	F-9 (n=5)	
Amsterdam II criteria	F-10 (n=12)	F-11 (n=2)	F-12 (n=1)	F-13 (n=3)	ND		
Modified Amsterdam criteria	F-14 (n=2)	ND	ND	ND			
Bethesda guidelines	F-15 (n=5)	F-16 (n=1)	ND				
Revised Bethesda guidelines	F-17 (n=1)	F-17 (n=1)					
Young age of onset	F-18 (n=5)	F-19 (n=4)	ND	ND	ND	ND	ND
Family history	F-20 (n=9)	F-21 (n=4)	ND	ND	ND		
Multiple tumors in the same patient	F-22 (n=3)	F-22 (n=3)	ND	ND	ND	ND	ND
Age <50 years, family history, or multiple tumors in same patient	F-23 (n=3)	F-23 (n=3)	ND	ND	ND	ND	ND
Suggestive MSI (MSI-H; MSI-H and MSI-L)	F-24 (n=16)	F-25 (n=2)	F-26 (n=1)	ND	ND	ND	F-27 (n=3)
Suggestive IHC	F-28 (n=9)	ND	ND	ND	ND	ND	F-29 (n=2)

Table rows represent different predictors; the table columns describe the populations addressed in the summary tables. Each cell in the table refers to a corresponding Summary Table of the appendix. For example, the second cell of the second row shows that there were 19 studies that described the ability of Amsterdam I criteria to predict MMR status. The studies are described in Appendix F-6. The third cell of the second row shows that of these 19 studies, only two performed MMR testing in unselected patients; these studies are described in Appendix F-7, and so on.
ND: No data

Table 17 summarizes the overall sensitivity and specificity of the various predictors among several CRC populations. These are described in detail in the sections below.

Table 17. Sensitivity and specificity of various predictors for detecting MMR gene mutations

Predictor	Unselected CRC probands			Revised Bethesda guidelines			Bethesda guidelines			Amsterdam II criteria			Amsterdam I criteria		
	N	Sens [%] (95% CI)	Spec [%] (95% CI)	N	Sens [%] (95% CI)	Spec [%] (95% CI)	N	Sens [%] (95% CI)	Spec [%] (95% CI)	N	Sens [%] (95% CI)	Spec [%] (95% CI)	N	Sens [%] (95% CI)	Spec [%] (95% CI)
Amsterdam I criteria	2	45 (29, 63)	99 (74, 100)		ND		2	57 (46, 68)	48 (35, 62)	5	80 (70, 88)	24 (12, 43)			
Amsterdam II criteria	2	28 (15, 47)	99 (97, 100)	1	68 (43, 87)	65 (51, 76)	3	84 (74, 91)	62 (53, 69)						
Modified Amsterdam criteria		ND			ND			ND							
Bethesda guidelines	1	73 (39, 94)	82 (80, 84)		ND										
Revised Bethesda guidelines	1	91 (59, 100)	77 (75, 79)												
Age <50 years	3	31 (18, 47)	95 (94, 96)		ND			ND			ND			ND	
1st degree family history of CRC or EC	4	76 (50, 91)	87 (86, 89)		ND			ND							
Multiple CRC or EC tumors in the same patient	3	38 (25, 54)	97 (91, 99)		ND			ND			ND			ND	
Age <50 years, family history of CRC or EC, or multiple tumors in same patient	3	88 (60, 97)	77 (74, 81)		ND			ND			ND			ND	
Suggestive MSI[a]	2	100 (88, 100)	90 (88, 92)	1	100 (75, 100)	79 (63, 90)		ND			ND		3	100 (62, 100)	69 (20, 95)
Suggestive IHC		ND			ND			ND			ND		2	50 (20,80)	72 (15, 97)

CRC: colorectal cancer; EC: endometrial cancer; N: Number of studies; Sens: (summary) sensitivity; Spec: (summary) specificity.
[a] Estimates are the same for combined MSI-H and MSI-L versus MSS and for MSI-H versus MSS.

We first present an overview of the clinical predictors among unselected CRC patients. We then present each one of the clinical predictors separately.

Overview of Clinical Predictors Among Unselected Patients With CRC

In total, five studies provided information on eight clinical predictors among unselected CRC probands (Salovaara 2000,[85] Aaltonen 1998,[63] Colombino 2005,[83], Samowitz 2001,[10] and Pinol 2005[12]) (Appendices F-7, F-11, F-16, F-17, F-19, F-21, F-22, F-23, F-25[*]). All studies received a grade B in the overall quality rating. All were limited by verification bias. In the two studies from Finland[63,85] founder mutations that are unique in the Finnish population were also assessed. These specific founder mutations have not been found in the US population.

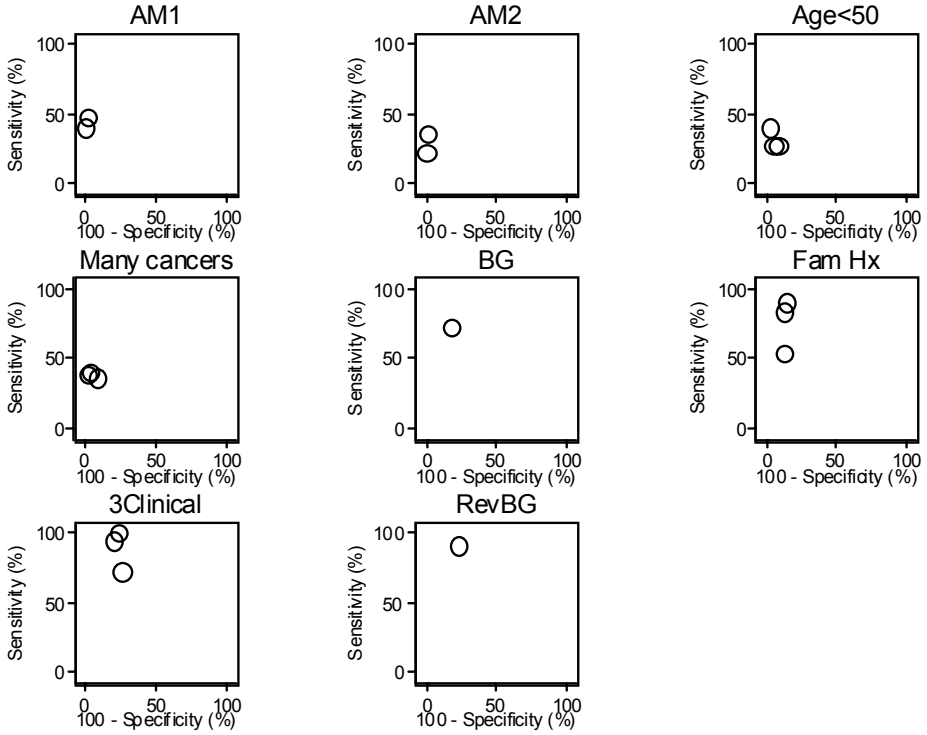
The five studies differed in their testing strategies. Aaltonen 1998[63] and Salovaara 2000[85] performed comprehensive genetic testing only among patients whose tumors exhibited MSI. The remaining patients were tested only for common founder mutations in the Finnish population (large deletions in the MLH1 gene). Samowitz 2001[10] and Pinol 2005[12] performed genetic testing only among patients with tumors that had suggestive MSI (or suggestive IHC testing for Pinol 2005[12]). Finally, Colombino 2005[83] performed comprehensive genetic testing only among patients with cancer history in 1st or 2nd degree family, and tested the remaining cases only for the mutations that were identified in the former.

As noted in the methods, we assumed that all patients who were not selected for genetic testing based on the MSI/IHC results were mutation negative.

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Figure 9 illustrates the sensitivity and specificity of the clinical criteria among unselected CRC patients.

Figure 9. Sensitivity and specificity of clinical criteria for detecting mismatch repair mutations among unselected patients with CRC



AM1/AM2: Amsterdam criteria I/II; Age<50: proband age at diagnosis <50 years; Many cancers: Multiple synchronous or metachronous colorectal or endometrial cancers in the same proband; BG: Bethesda guidelines; Fam Hx: History of colorectal cancer or endometrial cancer in 1st degree family; 3Clinical: Proband age <50y at diagnosis, history of colorectal cancer or endometrial cancer in 1st degree family, or multiple synchronous or metachronous tumors in the same proband; RevBG: Revised Bethesda guidelines. The study by Samowitz 2001 is not depicted in the graphs because it used slightly different criteria.

As shown in Figure 9 and Table 17, the revised Bethesda guidelines had the best sensitivity (91%, [95% CI: 59, 100%]). However, clinical predictors that are relatively easier to assess such as the family history of colorectal or endometrial cancer, or the fulfillment of at least one of three simple clinical criteria (proband age less than 50 years at diagnosis, history of CRC or endometrial cancer in 1st degree family, or multiple synchronous or metachronous CRC or endometrial cancer in the proband) appeared to have better test performance than the Amsterdam I and II criteria, or even the Bethesda guidelines. Specific summary estimates are presented in Table 17 and are also mentioned in the following sections.

Amsterdam I Criteria

Nineteen studies (described in 21 publications[29,61,63-68,71-77,79-83,96]) provided information needed to calculate sensitivity and specificity for this analysis (Appendix F-6*). Fifteen assessed

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

the clinical predictors among all patients, and three (Casey 2005,[61] Raedle 2001,[29] and Nakahara 1997,[96] Appendix F-6*) among patients with suggestive MSI.

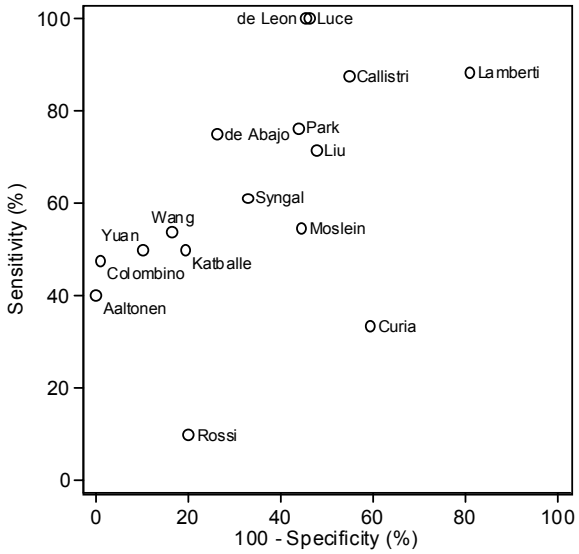
Ten studies were rated grade B for their overall methodologic quality[29,61,63,65-67,71,74,77,79-81] and nine received grade C.

Genetic Testing Irrespective of MSI or IHC Status. Sixteen studies performed some form of genetic testing in all people, irrespective of suggestive MSI or IHC (Appendix F-6*). Only four of these studies performed sequencing on all available samples,[75,77,79,81] and only Wang 1999[81] also tested for large deletions/rearrangements. Most studies tested only for mutations in MLH1 and MSH2. Wang 1999[81] also assessed the MSH6 gene, the PMS2 and PMS1 genes (no deleterious mutations were identified in non-MLH1 and non-MSH2 genes). Park 1999[76] sought mutations in additional MMR genes (PMS1 and PMS2) and in only a minority of patients (without finding any additional mutations). Eight out of the sixteen 2x2 tables had at least 40 patients in total.

There was variability in the CRC populations that were examined (Appendix F-6*). The sample selection process was considered to be transparent (based on clearly stated selection criteria applied to all available patients) in only eight studies.[63,65,67,71,74,77,79-81,83] Of these, three sampled from unselected, non-referral CRC populations.[63,65,71,83] The remaining five sampled from referral or otherwise selected CRC patients[67,74,77,79-81] (Appendix F-6*).

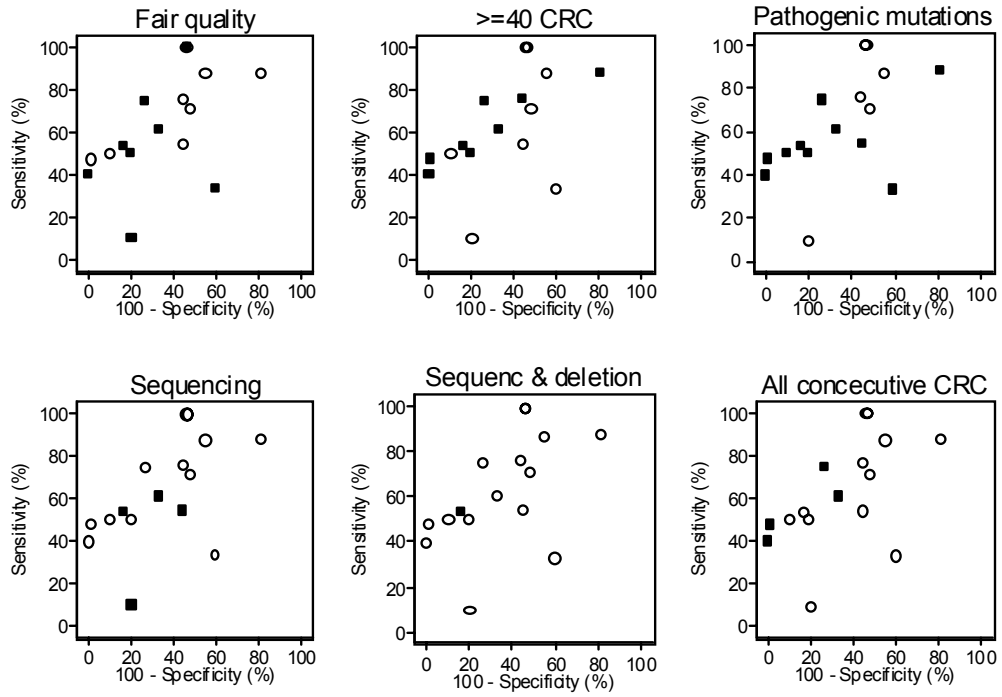
Figure 10 graphically depicts the sensitivity and specificity of the 16 studies that performed some form of genetic testing in all people irrespective of suggestive MSI/IHC. A study suggesting that the Amsterdam I criteria had perfect test characteristics (i.e., sensitivity and specificity of 100%) would appear in the upper left corner. The graph shows that the Amsterdam I criteria are not highly sensitive for detecting MMR gene mutations. In two studies that enrolled unselected patients with CRC, the summary estimate of sensitivity was only 45% (95% CI: 29, 63%), and the summary specificity was 99% (95% CI: 74, 100%).[63,83] However, both studies were limited by verification bias, as described below.

Figure 10. Sensitivity and specificity of the Amsterdam I criteria for detecting mismatch repair mutations in patients with CRC



Overall, the distribution of sensitivity and specificity of the various studies did not follow a discernable pattern. The differences may be related to variation in genetic strategies (gene screening and deletion analysis), the definition of pathogenic mutations, sample selection processes (clearly described versus not), overall study methodologic quality (B versus C), or within-study heterogeneity of "baseline risk" for HNPCC (study sample included patients at very different risks for HNPCC). However, as the graphs in Figure 11 show, none of these factors alone appears to account for the variability. The variability is most likely attributable to the very different CRC populations assessed in these studies.

Figure 11. Sensitivity and specificity of the Amsterdam I criteria for detecting mismatch repair mutations: study subgroups according to various factors



Filled squares versus empty circles imply that the pertinent characteristic is present versus absent. Fair quality: Overall quality B versus C; ≥40 CRC: total of ≥40 versus <40 patients in the 2x2 table; Pathogenic mutations: Any versus none definition for pathogenic mutations; Sequencing: Sequencing of all available samples; Sequence & Deletion: Sequencing and deletion analysis to all samples; All consecutive CRC: Studies were all available consecutive CRC were used versus studies without consecutive CRC or studies that included some but not all available consecutive CRC.

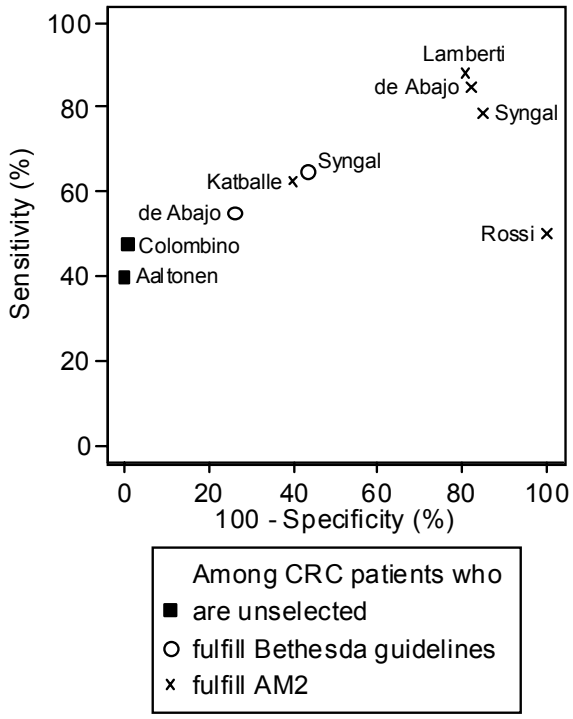
Amsterdam I Criteria Among Different Populations. As discussed in the Methods section, clinical predictors are expected to have substantially different sensitivity and specificity to detect mismatch repair gene mutations in CRC populations selected using different clinical criteria. We assessed the sensitivity and specificity of Amsterdam I criteria among CRC subpopulations that were defined consistently across studies (Appendices F-7, F-8 and F-9*).

Figure 12 depicts the change in the sensitivity and specificity of the Amsterdam I criteria among CRC populations that had an increasing likelihood of having HNPCC: unselected CRC, CRC fulfilling the Bethesda criteria, and CRC fulfilling Amsterdam II criteria. As the likelihood for HNPCC increases, the studies shift toward the upper right (i.e., better sensitivity and worse

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

specificity). Thus, there is evidence for spectrum effects in the diagnostic ability of the Amsterdam I set of criteria.

Figure 12. Sensitivity and specificity of the Amsterdam I criteria for detecting mismatch repair gene mutations among populations with an increasing likelihood of having HNPCC



The average patient risk for HNPCC is lowest for unselected colorectal cancer patients populations, higher for patients fulfilling the Bethesda guidelines and highest for patients fulfilling Amsterdam II criteria. The same studies (Syngal, de Abajo) have information both for the subpopulation of CRC that fulfills the Bethesda guidelines and the subpopulation of CRC that fulfills the Amsterdam II criteria. AM2: Amsterdam II criteria; CRC: colorectal cancer.

Amsterdam I Criteria Among Unselected Patients With CRC. Two studies (Aaltonen 1998,[63] and Colombino 2005[83]) assessed the performance of the Amsterdam I criteria among unselected CRC patients (Appendix F-7[*]). The first[63] assessed all new incident CRC cases from nine Finnish hospitals, while the second[83] was retrospective and assessed all CRC patients who were registered in a tertiary hospital during the 3 years before the study (the authors commented that their hospital registered practically all CRC cases diagnosed in Sardinia).

Both studies were limited by verification bias. Aaltonen 1998[63] performed comprehensive genetic testing only among 63 patients who had tumors with MSI, and tested the remaining 446 for a common founder mutation in the MLH1 gene. Colombino 2005[83] performed detailed genetic testing only among patients with family history and assessed patients with apparently sporadic CRC only for mutations found in the familial cases. Thus, both studies clearly overestimated the sensitivity and the specificity of Amsterdam I criteria to detect mutations. Their summary sensitivity was 45% (95% CI: 29, 63%) and their summary specificity was 99% (95% CI: 74, 100%). The wide confidence intervals of the sensitivity values reflect that few patients fulfilled Amsterdam I criteria (n=4 in Aaltonen 1998,[63] and n=21 in Colombino 2005[83]).

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Genetic Testing Only Among People Who Have MSI Positive (or IHC Negative) Tumors. In the three studies that were pertinent to this category[29,61,96] the sensitivities ranged between 55% and 78% and the specificities between 40% and 100% (Appendix F-6*).

Amsterdam II Criteria

Twelve studies described in 14 publications[12,29,65,67,71,77,79,80,84-87,97,98] provided data for determining the sensitivity and specificity of the Amsterdam II criteria for predicting the presence of mismatch repair mutations (Appendix F-10*). Eight assessed sensitivity and specificity of the Amsterdam II criteria among all available patients,[65,67,71,77,79,80,84-87] three among patients who had tumors with MSI instability,[29,97,98] and one among patients whose tumors were selected after MSI or IHC testing.[12]

Genetic Testing Irrespective of MSI or IHC Status. Eight studies provided data on genetic testing irrespective of MSI or IHC status.[65,67,71,77,79,80,84-87] The populations evaluated were heterogeneous (Appendix F-10*). One study[84] received grade C for overall quality rating while the rest were rated grade B.[65,67,71,77,79,80,85-87] All eight studies tested only for mutations in MLH1 and MSH2 genes. Seven studies had more than 40 patients in total in the pertinent 2x2 tables (all except for Rossi 2002[77]). The sampling process was described in all but one study.[87]

Figure 13 provides an overview of the eight studies while Figure 14 shows the various studies according to the presence or absence of various characteristics that may affect the sensitivity and specificity of the Amsterdam II criteria. Overall, sensitivities varied between 22% and 80% and specificities varied between 61% and 100%. The plot suggests that Amsterdam II criteria are not a good screening test for HNPCC. The seven studies did not follow any particular pattern with respect to the factors addressed in Figure 14. The variability is mostly likely due to the very different CRC populations assessed in these studies.

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Figure 13. Sensitivity and specificity of the Amsterdam II criteria for detecting mismatch repair gene mutations

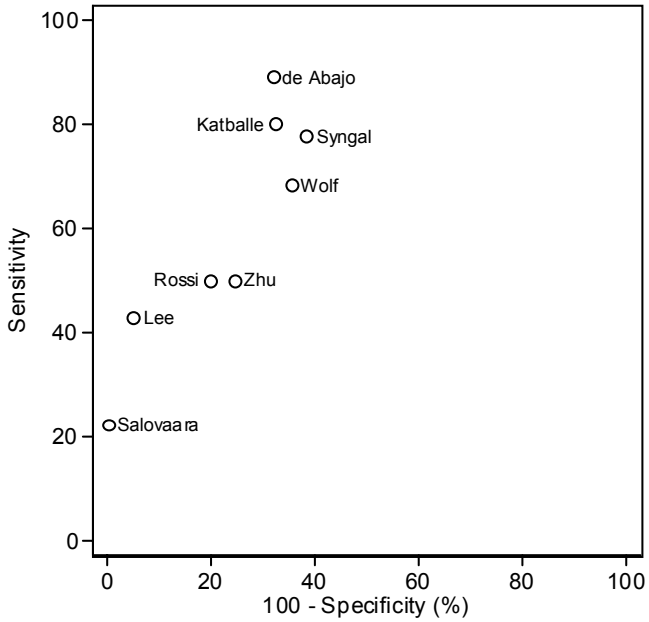
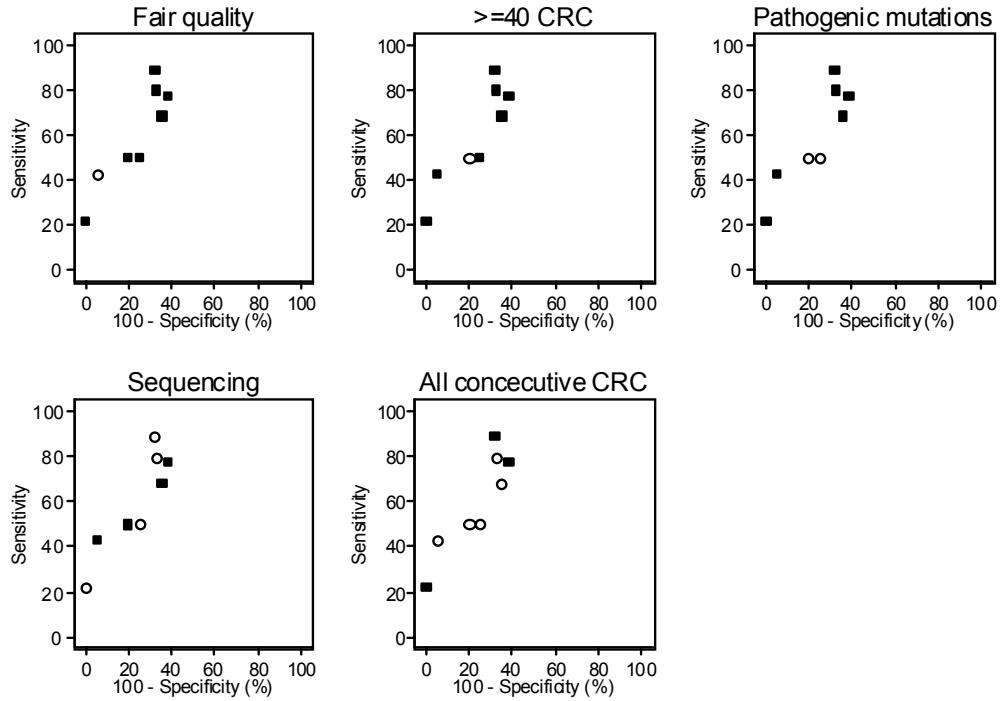


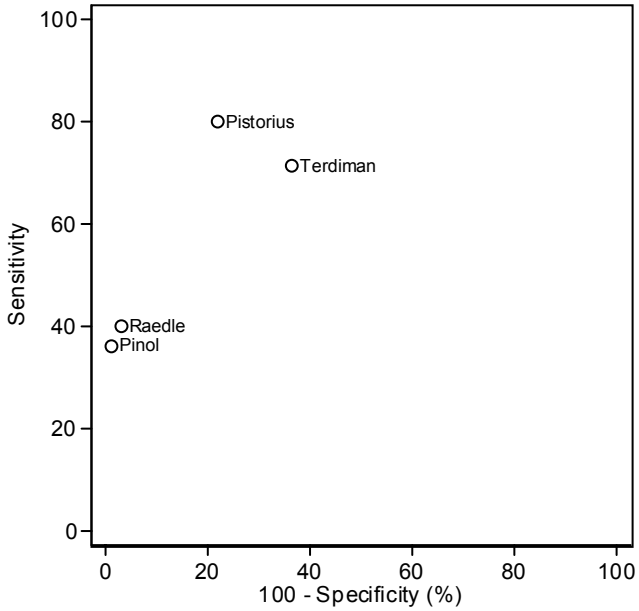
Figure 14. Sensitivity and specificity of the Amsterdam II criteria for detecting mismatch repair mutations: study subgroups according to various factors



Filled squares versus empty circles imply that the pertinent characteristic is present versus absent. Fair quality: Overall quality B versus C; ≥40 CRC: total of ≥40 versus <40 patients in the 2x2 table; Pathogenic mutations: Any versus none definition for pathogenic mutations; Sequencing: Sequencing of all available samples; All consecutive CRC: Studies where all available consecutive CRC were used versus studies without consecutive CRC or studies that included some but not all available consecutive CRC.

Genetic Testing Only Among People Who Have MSI Positive (or IHC Negative) Tumors. Three studies performed genetic testing only among patients who fulfilled the Amsterdam II criteria and had MSI high tumors,[29,97,98] and one[12] among patients selected based on the results of MSI and IHC testing (Figure 15).

Figure 15. Sensitivity and specificity of the Amsterdam II criteria for detecting mismatch repair mutations among patients selected after MSI or IHC testing



Pinol 2005[12] and Raedle 2001[29] assessed all available consecutive CRC patients. Terdiman 2001[98] selected patients who fulfilled the Bethesda guidelines and Pistorius 2000,[97] patients in whom there was increased clinical suspicion for HNPCC (at least two 1st degree relatives with CRC or young age at diagnosis or multiple tumors in the same patient). Sensitivities ranged from 36% to 80% and specificities ranged from 64% to 99% (Appendix F-10*).

Amsterdam II Criteria Among Unselected CRC Cases. As noted above, a question of central importance in this report is the accuracy of predictors in unselected patients with CRC. Only two studies assessed the accuracy of the Amsterdam II criteria in such patients (Pinol 2005,[12] and Salovaara 2000[85]) (Appendix F-11*). The latter study[85] performed comprehensive genetic testing in a subset of 66 patients who had tumors with MSI but tested the remaining 469 only for founder mutations in the MLH1 gene common in the Finnish population. The study by Pinol 2005[12] also performed comprehensive testing in only a subset of patients; however we included it by making an assumption that patients who were not selected for genetic testing based on MSI/IHC results would test negative for mutations.

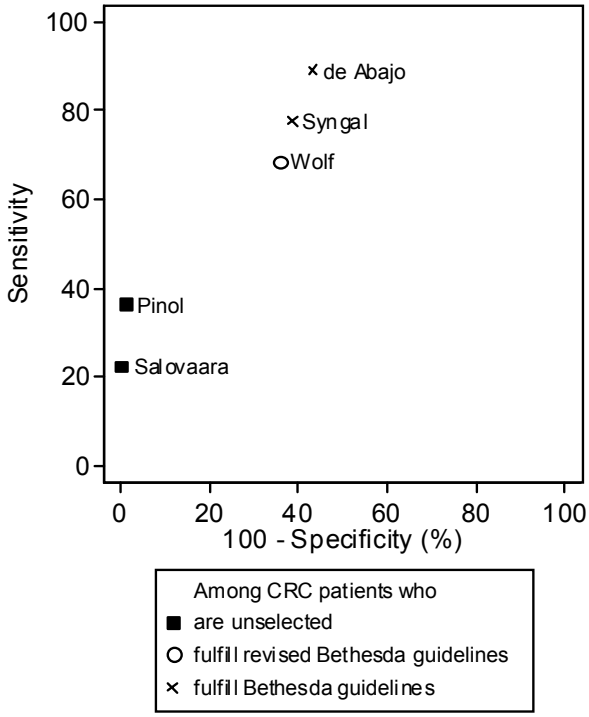
Two studies that focused on unselected CRC probands (Pinol 2005,[12] and Salovaara 2000[85]) provided a summary estimate of 28% (95% CI: 15, 47) for sensitivity and 99% (95% CI: 97, 100) for specificity. However, both studies were limited by verification bias.

Amsterdam II Criteria Among Different Populations. Figure 16 depicts sensitivity and specificity of the Amsterdam II criteria for identifying MMR mutations, among populations at

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

variable risk for HNPCC. The studies are described in Appendices F-11, F-12 and F-13*. Studies focusing on populations at increasing risk appear to be associated with better sensitivity and worse specificity, similar to the findings described above for the Amsterdam I criteria.

Figure 16. Sensitivity and specificity of the Amsterdam II criteria for detecting mismatch repair mutations among populations with an increasing likelihood for HNPCC



The average risk for HNPCC is lowest for unselected colorectal cancer patients and highest for patients fulfilling the revised Bethesda guidelines or the Bethesda guidelines. CRC: colorectal cancer patients.

Modified Amsterdam Criteria

The modified Amsterdam criteria are fulfilled when CRC has appeared in more than one generation, there are at least two CRC in 1st degree family, and at least one CRC diagnosed at age younger than 55 years; or when there are at least two 1st degree relatives affected by CRC plus another relative with an unusually early onset neoplasm or endometrial cancer. Two studies (described in three papers[79,80,86]) provided data on the modified Amsterdam criteria (Appendix F-14*). Both studies received grade B for their overall quality. Neither assessed mutations in genes other than MLH1 or MSH2. The studies differed in their inclusion criteria and neither included unselected patients. Despite these differences, the estimates of sensitivity and specificity were similar. Overall, the summary sensitivity was 78% (95% CI: 61, 89%) and the summary specificity was 46% (95% CI: 37, 55%). It is likely that the sensitivity of the modified Amsterdam criteria in an unselected CRC population would be lower.

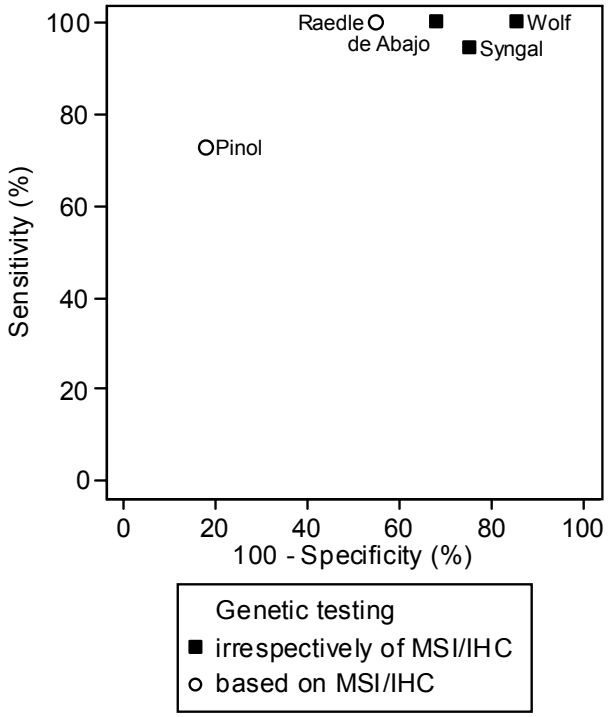
* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Bethesda Guidelines

Five studies described in six papers assessed the sensitivity and specificity of the Bethesda guidelines to detect MMR mutations: Syngal 2000,[79,80] Wolf 2005,[86] de Abajo 2005,[67] Raedle 2001,[29] and Pinol 2005.[12] Three studies performed genetic testing irrespective of tumor MSI status[67,79,80,86] while the other two performed genetic testing only among patients who were selected based on MSI testing[29] or MSI and IHC testing.[12] All four studies were rated grade B for the overall quality. All studies examined only the MLH1 and MSH2 genes. Only Syngal 2000 and Wolf 2005 performed sequencing to all available samples.

Only the study by Pinol 2005[12] assessed incident, newly diagnosed CRC cases. We included it by making an assumption that patients who were not selected for genetic testing based on MSI/IHC results would test negative for mutations. Figure 17 depicts the sensitivity and specificity of the five studies.

Figure 17. Sensitivity and specificity of the Bethesda guidelines for detecting mismatch repair gene mutations: all available studies



The study by Pinol 2005 included unselected incident CRC probands. See text about the verification bias in this study.

Based on the study by Pinol 2005,[12] the Bethesda guidelines had a sensitivity of 73% (95% CI: 39, 94%) and a specificity of 82% (95% CI: 80, 84%) for detecting MMR mutations among unselected patients with CRC (Appendix F-15*). However, these are probably overestimates because of verification bias.

Among a referral population (or otherwise selected CRC populations) the Bethesda guidelines appeared to have high sensitivity (over 90%) but low specificity (below 50%) in all

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

three studies (including the study by Raedle 2001[29] that focused on patients whose tumors exhibited MSI-H) (Appendix F-15*).

Revised Bethesda Guidelines

Only one study (Pinol 2005[12]) provided data on the sensitivity and specificity of the revised Bethesda guidelines in a unselected population of patients with CRC (Appendix F-17*). The sensitivity and specificity of the clinical criteria were calculated to be 91% (95% CI: 59, 100%) and 77% (95% CI: 75, 79%), respectively. Thus, the revised Bethesda guidelines were more sensitive and as specific as the original Bethesda guidelines (Appendices F-16 and F-17*). However, these may be overestimates because of the possibility of verification bias as discussed above.

Young Age of Onset

Appendix F-18 summarizes five studies that assessed young age of onset as a clinical predictor to identify mismatch repair gene mutations (Salovaara 2000,[85] Aaltonen 1998,[63] Colombino 2005,[83] Samowitz 2001,[10] and Pinol 2005[12]) (Appendix F-18*). Young age of onset was defined as less 50 years in all studies, with the exception of Samowitz 2001,[10] where the definition was less than 55 years. Colombino 2005[83] provided data only in patients with familial CRC (Appendix F-18*). All studies suffered from verification bias because genetic testing was not thorough[63,85] or was not performed at all[10,12] among patients without MSI (MSI or IHC for Pinol 2005[12]).

Among the three studies of unselected patients with CRC (Appendix F-19) that defined young age of onset as less than 50 years old, a summary estimate of sensitivity was 31% (95% CI: 18, 47%) and specificity was 95% (95% CI: 94, 96). Only the specificity estimate was heterogeneous ($p<0.01$, $I^2=81\%$) (Appendix F-18 and F-19*). These estimates might be inflated because of verification bias. Sensitivity and specificity were similar in the studies by Colombino 2005[83] and Samowitz 2001[10] (Appendix F-19*).

Familial Cancer History

Nine studies provided data eligible for this analysis[10,12,63,75,81,84,85,87,99] (Appendix F-20*). Six studies were rated with grade B in the overall quality scale,[10,12,63,81,85,87] and the remaining three studies were rated with grade C. Only one study had fewer than 40 patients in total in the 2x2 tables.[75]

There was variability in the populations that were examined and the definition of familial disease. A familial history of cancer was defined as presence of colorectal cancer or endometrial cancer in a first-degree family member of the proband in three prospective studies that assessed consecutive unselected CRC cases.[12,63,85] Another study on incident CRC (Samowitz 2001[10]) defined familial cancer as CRC in a first degree family. In these studies, all other CRC cases were used as a comparator.

In the remaining studies, familial cases were highly selected, based on sets of clinical criteria such as the Amsterdam I or II criteria, the Korean HNPCC criteria, the aggregation of HNPCC

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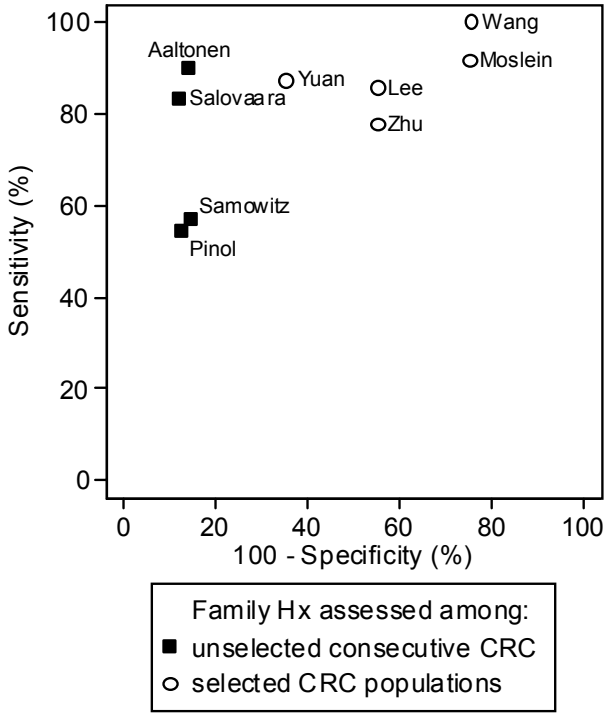
related tumors in a family, or combinations thereof. Similarly, in these studies the comparator ("sporadic CRC cases") was defined variably (Appendix F-20*), and was most often a group of "apparently sporadic" CRC that were selected based on their young age of onset or a diagnosis of multiple tumors.

Figure 18 depicts all studies according to whether they assessed unselected CRC cases.

Figure 18. Sensitivity and specificity of familial cancer history for detecting mismatch repair gene mutations: all available studies



CRC: colorectal cancer; Family Hx: family history (of cancer). Empty circles denote studies that contrasted familial CRC cases with increased likelihood for HNPCC against sporadic CRC cases who were also at a higher risk for HNPCC compared to an unselected CRC population (i.e., young age of onset or multiple tumors in the same patients). See text and Appendix F-20 for more details.

Four studies reported estimates in unselected, consecutive patients with CRC.[10,12,63,83] Three used identical definitions (CRC or endometrial cancer in first degree family),[12,63,83] and one defined familial cancer as CRC in a first degree family member[10] (Appendix F-21*).

A summary estimate of sensitivity based on the four studies that used similar definitions was 76% (95% CI: 50, 91%) with borderline between-study heterogeneity (p=0.13, I^2=50%); the summary estimate of specificity was 87% (95% CI: 86, 89%) (without between-study heterogeneity: p=0.51, I^2=0%). The estimates were similar when Samowitz 2001[10] was included in the calculations, despite the different definition of familial cases. The other studies assessed substantially different populations to allow for a meaningful synthesis (Appendix F-20*). However, they uniformly suggested high sensitivities (above 75%) and variable, but generally low, specificities (ranging from 24% to 65%).

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

Multiple CRC or Endometrial Cancer in the Same Patient

Appendix F-22* summarizes three studies that included unselected CRC and assessed the sensitivity and specificity of multiple, synchronous or metachronous CRC or endometrial cancer in the same proband to predict MMR mutations (Salovaara 2000,[85] Aaltonen 1998,[63] and Pinol 2005[12]). Their characteristics and limitations have already been discussed. The respective summary estimates of sensitivity and specificity were 38% (95% CI: 25, 54%) with no evidence for between-study heterogeneity ($p=0.98$, $I^2=0\%$) and 97% (95% CI: 91, 99%) with significant between-study heterogeneity ($p<0.01$, $I^2=94\%$).

Familial History of Cancer, Age of Onset Less Than 50 Years, or Multiple Cancers in the Same Proband

Appendix F-23* summarizes three studies that enrolled unselected CRC (Salovaara 2000,[85] Aaltonen 1998,[63] and Pinol 2005[12]). The sensitivity and specificity of the presence of either of the following three simple clinical criteria was assessed: history of colorectal or endometrial cancer in first degree family, CRC onset at less than 50 years of age, or multiple tumors in the same proband versus all other cases. Their characteristics and limitations have already been discussed. Summary estimates of sensitivity and specificity were 88% (95% CI: 60, 97%) with little evidence for between-study heterogeneity ($p=0.19$, $I^2=39\%$) and 77% (95% CI: 74, 81%) with significant between-study heterogeneity ($p=0.03$, $I^2=73\%$), respectively.

MSI

The presence of MSI is a marker for replication errors and thus a predictor of MMR mutations. We assessed the sensitivity and specificity of MSI-H versus MSS. We also analyzed combined MSI-H and MSI-L versus MSS.

Studies were considered eligible if they performed both MSI testing and MMR genetic testing in CRC patients and each test was applied irrespective of the results of the other test.

In these analyses we assumed that any genetic testing was the reference standard.

Overview of MSI Testing. Overall, MSI testing appeared to have modest to good discriminating ability to identify carriers of MLH1 and MSH2 mutations. The sensitivity of MSI-H versus MSS ranged between 56% and 100% and the specificity between 17% and 93%.

MSI-H Versus MSS. Sixteen studies described in 18 publications[11,63-66,69-71,75,79,80,84-86,95,100-102] were eligible. One received grade A in the overall quality rating,[11] nine received grade B,[63,65,66,70,71,79,80,85,86,101,102] and six received grade C (Appendix F-24*).

Eight studies performed sequencing on all available samples[11,69,75,80,84,86,95,101] and one of them (Barnetson 2006[11]) also used specific methods to detect large genomic deletions or rearrangements. All studies tested for MLH1 and MSH2 gene mutations. One study also assessed the presence of MSH6 mutations (Barnetson 2006[11]) and another tested for MSH6 and PMS2 mutations (Southey 2005[102]). It was not possible to examine the changes in the sensitivity and

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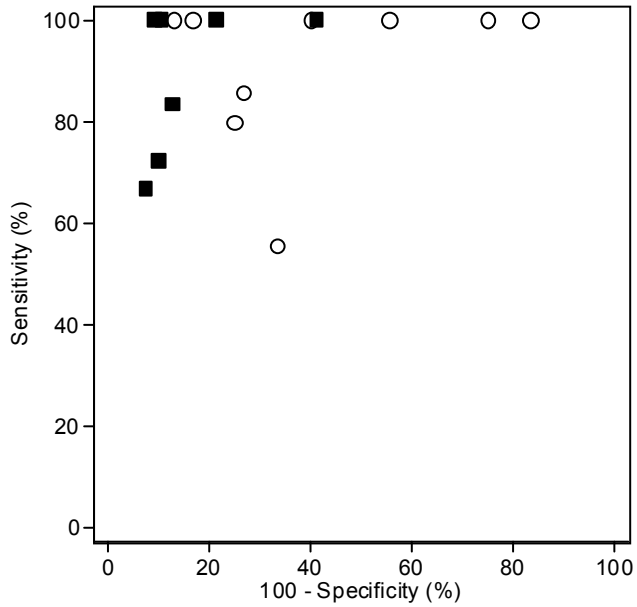
specificity of MSI with and without the non-MLH1/non-MSH2 mutations in these studies. Seven out of 16 had information in the 2x2 tables on at least 40 patients (Figure 19, Appendix F-24*).

Two studies analyzed all available incident CRC cases (Aaltonen 1998[63] and Salovaara 2000[85]), but had substantial verification bias since individuals who were negative in the MSI testing were assessed only for the presence of founder mutations in the MLH1 gene. Two other prospective studies analyzed incident CRC patients (Barnetson 2006,[11] Southey 2005[102]) who were diagnosed at young age (<55 and <45 years, respectively).

The sample selection process was based on clearly stated selection criteria that were applied to all available patients in ten studies; in five of them, cases were selected among incident CRC patients (Aaltonen 1998[63], Salovaara 2000[85], Katballe 2002[65,71], Southey 2005[102] and Barnetson 2006[11]). Twelve studies used microdissection to help assure that the analyzed tumor tissue contained a high proportion of malignant cells,[11,64-66,69,71,79,80,84,86,95,100-102] and eight used the marker sets proposed by the NCI.[11,64,69,79,80,84,100-102]

Figure 19. Sensitivity and specificity of MSI-H to identify mismatch repair gene mutations



Studies shown according to the total number of patients in the 2x2 tables. Black squares, at least 40; empty circles, 40 or less.

Overall, the sensitivity of MSI ranged between 56% and 100% and the specificity between 17% and 93%. Two small studies found a specificity of only 25% (Durno 2005[100] among only four patients without a mutation) and 17% (Curia 1999[66] among 18 patients without a mutation). Notably, the study by Curia 1999[66] found few pathogenic mismatch repair gene mutations among CRC cases with HNPCC-related cancers (3/30=10%) and thus its applicability is unclear.

We could not explain the heterogeneity among estimates based on the overall study quality, the comprehensiveness of the genetic testing, the presence of microdissection, the use of NCI-recommended marker sets, or whether the study used a transparent sample selection process (Figure 20). Larger studies (with at least 40 patients in the 2x2 tables) tended to cluster more

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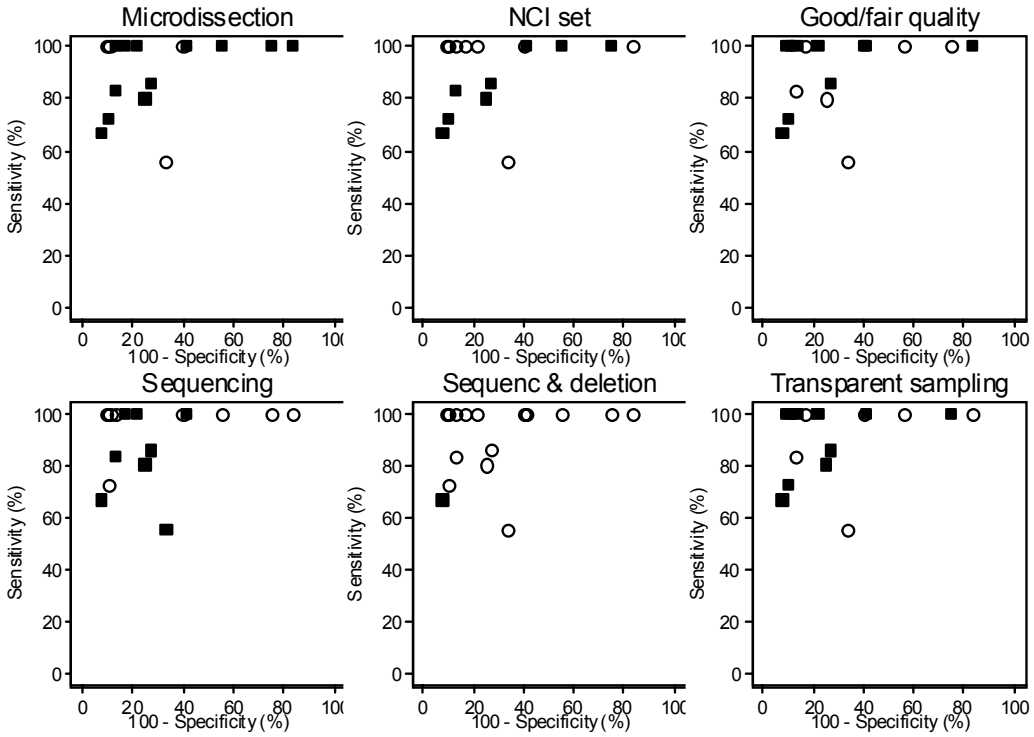
around the upper left corner of the plot (Figure 19) than studies that used deletion analysis to test for MMR mutations (all four of which had more than 40 patients in the 2x2 tables) (Figure 20).

Figure 20. Sensitivity and specificity of MSI-H to identify mismatch repair gene mutations based upon study characteristic

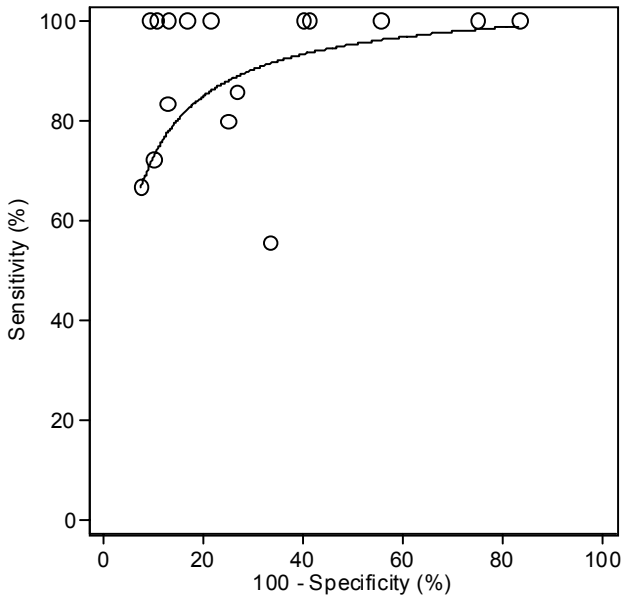


Studies are depicted with filled squares versus empty circles according to the presence or absence of the following characteristics: Microdissection: Use versus non-use/not stated use of microdissection; NCI set: Use versus non-use/not stated use of the National cancer Institute marker sets; Good/fair (A/B) versus poor (C) overall quality; Sequencing: Sequencing of all available samples; Sequenc & deletion: Sequencing of all available samples and deletion analysis for large genomic rearrangements; Transparent Sampling: Sample selected with clearly reported criteria among all available patients versus not.

Among studies with more than 40 patients in the 2x2 tables, a summary estimate of sensitivity was 83% (95% CI: 65, 92%), with little evidence for between study heterogeneity ($p=0.15$, $I^2=37\%$). A summary estimate of specificity was 87% (95% CI: 80, 91%) with high between-study heterogeneity ($p<0.01$, $I^2=83\%$). The estimates were similar after the exclusion of the two Finnish studies (Salovaara 2000[85] and Aaltonen 1998[63]) in which genetic testing was less rigorous for patients without MSI-H tumors. The latter studies also included the Finnish founder mutations in their analyses; these mutations are not found in populations of non-Finnish origin.

A summary ROC analysis is shown in Figure 21.

Figure 21. Summary ROC curve for the diagnostic ability of MSI-H and MSI-L to identify mismatch repair gene mutations



Combined MSI-H and MSI-L versus MSS.

Combined MSI-H and MSI-L Versus MSS. There were seven studies in which allowed the comparison of combined MSI-H and MSI-L versus MSS. These were described in eight publications.[11,63,65,70,71,84,85,102]

The summary estimate for sensitivity was 94% (95% CI: 86, 97) (no heterogeneity: p=0.85, I^2=0%) and for specificity was 83% (95% CI: 77 to 88%) (with substantial heterogeneity: p<0.01, I^2=80%). When analyses were limited to the four studies that had at least 40 patients in the 2x2 tables,[11,63,85,102] the corresponding results were 87% (95% CI: 82, 91%) and 95% (95% CI: 86, 98%); both estimates had substantial between-study heterogeneity.

What is the role of MSI-L in the detection of MMR mutations? We evaluated the sensitivity and specificity in the seven studies excluding the MSI-L tumors.[11,63,65,70,71,84,85,102] The summary sensitivity became smaller (80% [95% CI: 63, 90%]) and the summary specificity increased (88% [95% CI: 83, 91%]). The summary sensitivity estimate was not heterogeneous (p=0.21, I^2=28%) but the summary specificity estimate was heterogeneous (p<0.01, I^2=71%).

MSI-H and Combined MSI-H and MSI-L Versus MSS Among Unselected CRC Probands. There were only two relevant studies, both from Finland (Salovaara 2000[85] and Aaltonen 1998[63] (Appendix F-25[*]). MSI was assessed based upon the BAT26 marker set in the former, and on at least 30% of at least seven markers being unstable in the latter. Patients without MSI tumors were screened only for founder mutations in the MLH1 gene, which are common in Finland, whereas patients with MSI tumors received more comprehensive genetic testing (for both MLH1 and MSH2 genes). Thus, their estimates are likely to be biased. Summary estimates of sensitivity and specificity were 100% (95% CI: 88, 100%) and 90% (95% CI: 88, 92%), respectively.

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

MSI-H Versus MSS Among Patients Fulfilling the Revised Bethesda Guidelines. Only the study by Wolf 2005[86] provided relevant data (Table 17, Appendix F-26*). Sensitivity was 100% (95% CI: 75, 100%) and specificity was 79% (95% CI: 63, 90%).

MSI-H Versus MSS Among Patients Fulfilling Amsterdam I Criteria. There were very few patients for these analyses and thus the precision of the estimates is poor (Table 17, Appendix F-27*). Sensitivity is of MSI-H (and combined MSI-H and MSI-low) versus MSI-stable was universally excellent (100% [95% CI: 62, 100%]) in the three studies that provided the relevant information,[70,75,95] although none contributed more than 10 patients who fulfilled the Amsterdam I criteria. Overall specificity was 69% (95% CI: 20, 95%).

IHC

Nine studies (described in 11 publications[11,65,66,69-71,79,80,98,100,102]) provided information needed to calculate the sensitivity and specificity of IHC for predicting MMR mutations (Appendix F-28*). One of these (Terdiman 2001[98]) assessed the ability of IHC only in individuals with MSI-H positive tumors.

Studies That Assessed IHC Irrespective of MSI Testing. Eight studies assessed IHC in patients with available tumor tissue; one received grade A in overall quality,[11] five grade B[65,66,70,71,79,80,102] and two grade C.[69,100]

Three studies performed sequencing in all available patients,[11,69,79] and one of them (Barnetson 2006[11]) also used specific methods to detect large genomic deletions or rearrangements.

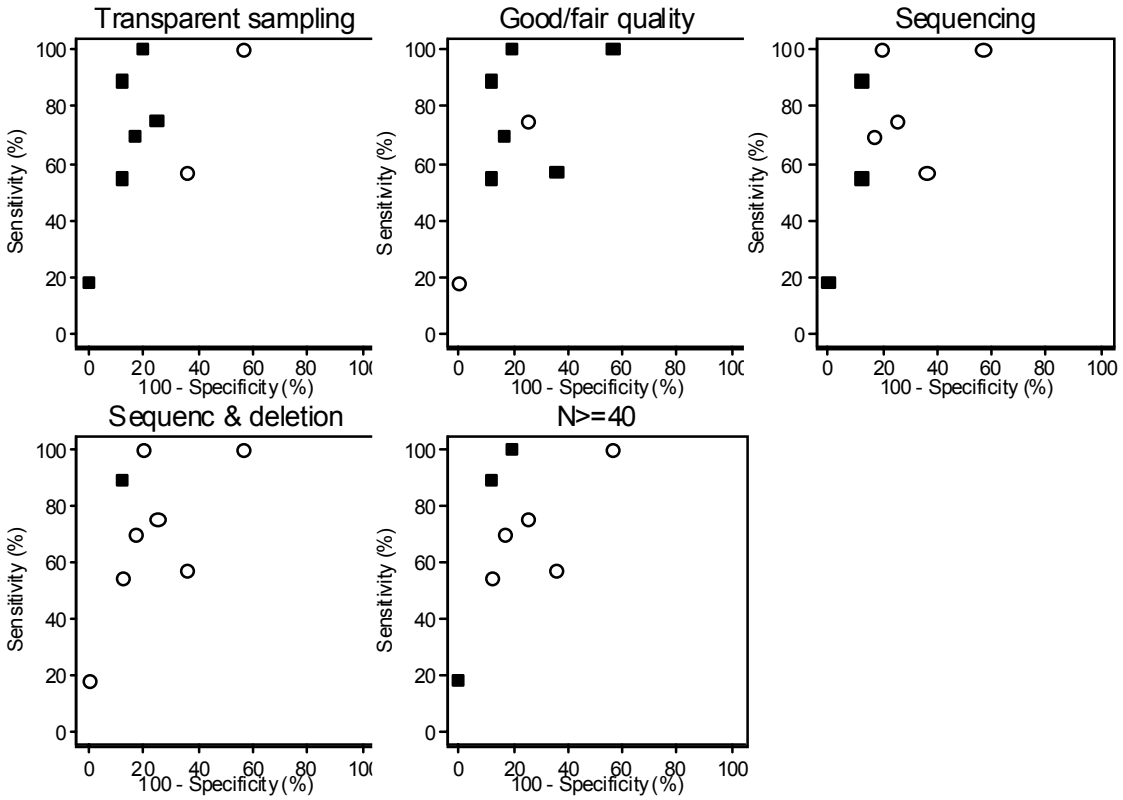
All studies tested for MLH1 and MSH2 gene mutations. One study also tested for the presence of MSH6 mutations (Barnetson 2006[11]) and another tested for MSH6 and PMS2 mutations (Southey 2005[102]). IHC testing was performed for all assessed MMR genes (MLH1, MSH2 and the additional MMR genes) in the latter two studies.[11,102] It was not possible to examine the changes in the sensitivity and specificity of IHC with and without the non-MLH1/non-MSH2 mutations in these studies.

Three studies had information in the 2x2 tables on at least 40 patients.[11,69,102] Two papers described prospective studies that analyzed incident CRC patient (Barnetson 2006[11] and Southey 2005[102]) diagnosed at young age (<55 and <45 years, respectively).

Figure 22 shows the distribution of the studies according to the presence or absence of several study characteristics. Overall, the sensitivity ranged from 27% to 100% and the specificity from 43% to 100%. The six good or fair quality studies (grade A or B respectively) had a summary sensitivity of 74% (95% CI: 54, 87%) and specificity of 77% (95% CI: 61, 88%).

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

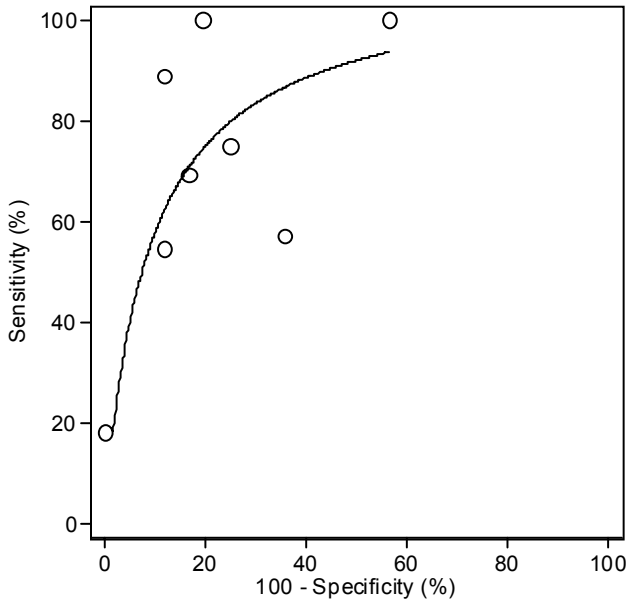
Figure 22. Sensitivity and specificity of IHC to identify mismatch repair gene mutations based on study characteristics



Studies are depicted with filled squares versus empty circles according to the presence versus absence of the following characteristics: Sample selected with clearly reported criteria among all available patients versus not; Good/fair (A/B) versus poor (C) overall methodologic quality; Sequencing: Sequencing performed in all available samples; Sequenc & deletion: Sequencing and testing for large genomic deletions/rearrangements performed in all samples; Sample size in the analyses at least 40 versus less than 40.

Figure 23 shows the summary ROC curve for the pertinent studies.

Figure 23. Summary ROC curve for the diagnostic ability of IHC to identify mismatch repair gene mutations



IHC among people fulfilling Amsterdam I criteria. Only the studies described by Katballe 2000 (another paper by Christensen 2002 reported the same patients[65,71]) and the study by Dieumegard 2000[70] provided relevant information, and they are described in Appendix F-29*. Both contributed nine or fewer patients and thus estimates of sensitivity and specificity lack precision (Table 17).

Studies That Assessed IHC After Suggestive MSI Testing. A single study by Terdiman 2001[98] assessed IHC among individuals who had tumors with MSI-H. The sensitivity of IHC was 94% (95% CI: 71, 100%) and the specificity was only 13% (95% CI: 4, 30%).

Expected Outcomes With Different Testing Strategies

Outline of the Problem

The prevalence of MMR mutations among people who are newly diagnosed with CRC is low.[7,10,12,63,85] Because of practical, economic, and logistical considerations, genetic testing would ideally be performed only in patients with high probability of HNPCC. Such patients may be selected based on heightened suspicion from the clinical history, suggestive laboratory testing of tumor tissue (in particular MSI or IHC), or complex combinations of all of the above. The various strategies differ in the number of tests (MMR, MSI or IHC) that need to be performed and the accuracy of the diagnosis.

We performed analyses using decision trees to model the expected outcomes with different testing strategies from the payers'/third party perspective. The outcomes were the number of incident CRC with positive diagnosis for HNPCC, and the number of tests (MMR, MSI, or IHC)

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

needed to detect them. We also assessed how many patients found to be mutation carriers with each strategy would be truly positive.

This analysis pertains to a cross-section in time. We used a hypothetical population of 100,000 incident cases of CRC. This number is in the order of incident cases expected annually in the US (approximately 150,000, given an annual incidence of 50/100,000 and a population of 300 million) and is a number convenient for calculations. The interested reader could multiply the reported numbers by 1.5 to extrapolate to the whole population in the United States.

We assessed nine different strategies, which were most commonly represented in the studies summarized in this review. The strategies used clinical criteria, MSI, IHC, or a combination of clinical criteria with MSI or IHC to select patients for MMR mutation testing.

Among the various clinical criteria that have been proposed, we opted to model the revised Bethesda guidelines and a set of three clinical criteria that are more easily ascertained (fulfillment of at least one of the following: age less than 50 year old at diagnosis, history of CRC or endometrial cancer in 1st degree family, or presence of multiple -synchronous or metachronous CRC or endometrial cancer in the proband). The revised Bethesda guidelines were selected because they appear to have the best sensitivity and specificity among unselected incident patients with CRC (data from Pinol 2005[12]).

The set of three clinical criteria is a simple alternative that appears to have comparably high sensitivity and specificity, but is much simpler to assess. Interestingly, in the predictive models that have been developed in the literature, the aforementioned three simple clinical criteria have been the most influential predictors of HNPCC status (based on the coefficients from the logistic regression models from Barnetson 2006,[11] Wijnen 1998[103] and Balmana 2006[92]).

The selected strategies are not exhaustive. However, these strategies have been discussed in the literature as possible options, and some of them have been evaluated in previous decision analyses.[19] It would be impractical to provide analyses on a more extensive list of strategies, especially given the paucity of relevant data in the literature.

Brief Description of Strategies and Values Used in the Decision Tree

Nine strategies were modeled as described briefly below.

- **MMR-All:** Perform MMR testing on all patients with CRC.

- **BethR-All:** Perform MMR testing only among those fulfilling the revised Bethesda guidelines.

- **Clinical-All:** Perform MMR testing only among those fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis; 1st degree family history of CRC or endometrial cancer; or multiple, synchronous or metachronous, CRC or endometrial cancer in the same patient).

- **MSI-All:** Perform MSI testing on all patients; followed by MMR testing only among those with suggestive MSI test.

- **IHC-All:** Perform IHC testing on all patients; followed by MMR testing only among those with suggestive IHC test.

- **BethR-MSI:** Perform MSI testing on patients fulfilling the revised Bethesda guidelines; perform MMR only among those with suggestive MSI test.

- **3Clinical-MSI:** Perform MSI testing on patients fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis; 1st degree family history of CRC or endometrial cancer; or multiple, synchronous or metachronous, CRC or endometrial cancer in the same patient); perform MMR only among those with suggestive MSI test.

- **BethR-IHC:** Perform IHC testing on patients fulfilling the revised Bethesda guidelines; perform MMR only among those with suggestive IHC test.

- **3Clinical-IHC:** Perform IHC testing on patients fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis; 1st degree family history of CRC or endometrial cancer; or multiple, synchronous or metachronous, CRC or endometrial cancer in the same patient); perform MMR only among those with suggestive IHC test.

Figure 24 illustrates the general concepts in the architecture of these decision trees.

The strategies are conceptually organized in similar groups depending upon the pattern they follow in selecting who would be sequenced for MMR mutations.

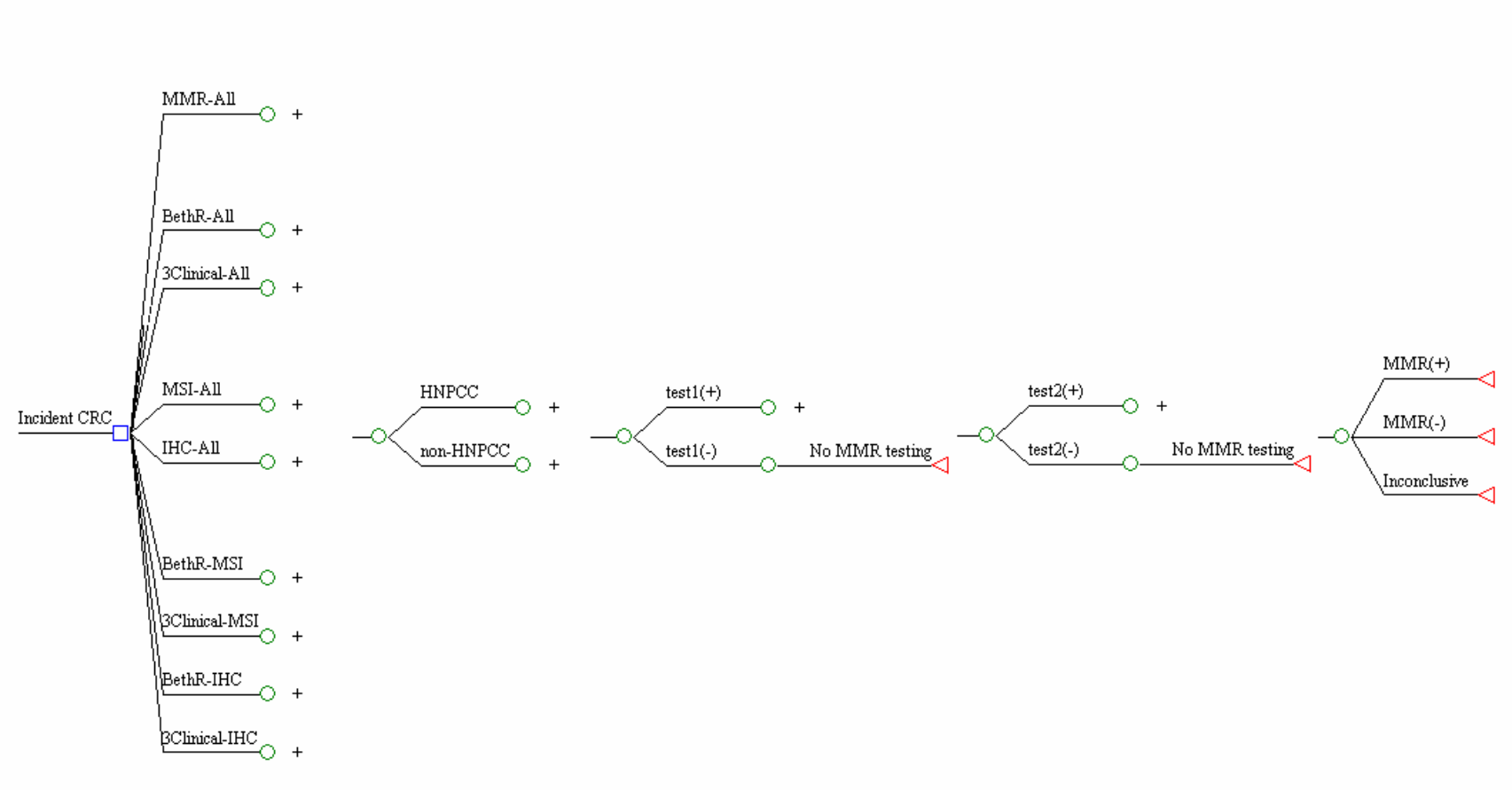
- Group 1: Test everyone (MMR-All)

- Group 2: Screen with a set of clinical criteria (BethR-All, 3Clinical-All)

- Group 3: Screen with a laboratory test (MSI-All, IHC-All)

- Group 4: Screen using two serial tests: a set of clinical criteria first and then a laboratory test (BethR-MSI, BethR-IHC, 3Clinical-MSI, 3Clinical-IHC).

Figure 24. Decision tree model used to calculate the impact of different testing strategies



The figure consists of 5 panels arranged horizontally; these correspond to different levels/nesting in the decision tree.
The nine branches in the first level stand for the modeled strategies (abbreviations are explained in the text). These may be clustered/organized in 4 conceptually similar groups. The second level models the prevalence of HNPCC. The third and fourth levels stand for the screening tests that may be applied. The fifth level represents genetic testing for MMR mutations. Depending on the strategy, all patients may receive genetic testing (i.e., reach the fifth level) (strategy MMR-All), all may be screened with only one test (clinical criteria for BethR-All and 3Clinical-All, MSI for MSI-All and IHC for IHC-All), or all may be screened with two serially applied tests (the first based on clinical criteria and the second on MSI or IHC) to select the ones who will reach the fifth level (BethR-MSI, 3Clinical-MSI, BethR-IHC, 3Clinical-IHC).

Table 18 shows the selected baseline values for the decision tree parameters, as well as the ranges of the sensitivity analyses. It should be noted that only some of the conditional probabilities that were presented in the previous sections are applicable to the following modeling approach; furthermore some conditional probabilities are imputed based on realistic assumptions because they were not extractable from the reviewed literature. All probabilities, their derivation and the rationale for any assumptions are described in more detail in the sections that follow.

Prevalence of Mutations Among Incident Unselected CRC Patients (*Prev*). The prevalence of mutations among incident unselected CRC is the most influential variable in the analyses (see Appendix G[*]). Based on the following considerations we present here two sets of results; one using a low prevalence estimate (0.9%) and one using a higher prevalence estimate (2.7%). The rationale for these values is given below. The prevalence value ranged from 0.6% to 5.1% in the sensitivity analyses.

Relevant studies. Among the 40 studies that were assessed for Key Question 2, four (3,332 analyzed patients in total) evaluated incident unselected CRC patients (Aaltonen 1998,[63] Salovaara 2000,[85] Pinol 2005,[12] and Samowitz 2001[10]). All were limited by verification bias, in that comprehensive genetic testing was performed only in patients with suggestive MSI results,[10,63,85] or MSI and IHC results.[12]

We also considered the study by Hampel 2005,[8] although it was not eligible (did not provide needed data) for the quantitative analyses described in the previous sections. Patients with suggestive MSI were sequenced for MLH1, MSH2, MSH6 and PMS2 mutations; analyses for large genomic deletions/rearrangements were also performed.

Low estimate for mutation prevalence (0.9%). The two Finnish studies[63,85] reported founder mutations (large genomic deletions) in the MLH1 gene of several CRC patients; however the Finnish founder mutations have not been identified in patients of non-Finnish origin.[12] Thus, they are not applicable to the US population. Excluding the founder mutations from the analyses, the proportion of mutation carriers among unselected patients with CRC was estimated at 0.9% (95% CI: 0.6 to 1.3%).

An American Founder Mutation (AFM) in the MSH2 gene has been described in one kindred probably originating from German immigrants, but it is unlikely to have a large impact in the general population.[90,91] AFM is expected to account for very few HNPCC incident cases (between 51 and 290 CRC in the US for AMF) annually.[90,91] Thus, we did not model the AFM.

Higher estimate for mutation prevalence (2.7%). In the Hampel 2005 study twenty-three out of 1066 CRC were identified as carriers (prevalence 2.2% [95% CI: 1.4, 3.2%]), after suggestive MSI. Conservative accounting for carriers who did not have suggestive MSI (using the lower 95% CI boundary for the sensitivity of MSI among unselected CRC) raises the prevalence value to 2.7%.

Range of sensitivity analyses. We used a wide range of prevalence values in the sensitivity analyses from 0.6% to 5.1%. 0.6% is the lower 95% CI boundary for the low prevalence estimate. 5.1% is 20% more than the upper 95% CI of the higher prevalence estimate and was

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/hnpcctp.htm

deemed to be high enough to encompass practically all realistic prevalence estimates that have been suggested by experts.[7]

Sensitivity and Specificity of Genetic Testing (*S_MMR, C_MMR*). We assumed:

- That complete gene sequencing for MMR testing would be done.
- Near perfect sensitivity and specificity for genetic testing.
- The proportion of inconclusive genetic test results (that is mutations of unknown clinical significance) was not incorporated in the estimates of sensitivity and specificity of genetic testing; it is rather modeled separately (see below).

Because the available estimates for the diagnostic performance of all clinical predictors were based on MLH1 and MSH2 gene mutation testing in the primary studies, we "penalized" the maximum sensitivity of genetic testing to account for HNPCC cases with mutations other than MLH1 and MSH2. There were limited data on this proportion in the eligible studies. We therefore assumed that 5% of patients with HNPCC would have mutation in MMR genes other than MLH1 and MSH2 (thus we set sensitivity to 95% in the base case). In further analyses, we examined sensitivity as low as 72% based on data from Southey 2005,[102] where 28% of the detected mutations among CRC aged less than 45 years of age (a selected population) were due to MSH6 and PMS2 mutations. Thus, the modeling accounts for the incremental value of testing for additional MMR genes other than MLH1 and MSH2.

Proportion of Inconclusive Results Among HNPCC With Non-Positive Test Results (*Pinconcl1*), and Proportion of Inconclusive Results Among Patients Without HNPCC With Non-Positive Test Results (*Pinconcl2*). These estimates are associated with considerable uncertainty. Syngal 1999[55] found that 2/18 patients with pathogenic mutations also had inconclusive mismatch mutations. Thus we assumed that 11% of HNPCC carriers also have inconclusive mutations.

The proportion of patients with CRC without HNPCC who carry mismatch mutations is more difficult to estimate. We based the estimate on the only study on incident, unselected CRC that provided the relevant information.[12] This study was published recently and thus the authors presumably had access to the latest knowledge on which mutations were pathogenic; the corresponding estimate was 0.25%.

Both the aforementioned proportions were varied from 0% to their upper 95% CI values in sensitivity analyses.

Sensitivity and Specificity of MSI (*S_MSI, C_MSI*) and IHC (*S_IHC, C_IHC*) Among Unselected Incident CRC. These values were based on estimates from population-based studies that performed some form of genetic testing in all available patients. Because such IHC estimates were not available in unselected CRC, we assumed that two studies on patients with young age of onset for CRC (Barnetson 2006[11] and Southey 2005[102]) provided reliable approximations. This is based on the assumption that the diagnostic ability of laboratory predictors is relatively independent of clinical criteria that were used to select a population.

Similarly, for MSI we used a synthesis from the estimates obtained from four studies on incident CRC (Salovaara 2000[85], Aaltonen 1998[63], Barnetson 2006[11] and Southey 2005[102]). The

values for MSI refer to combined MSI high and MSI low. The range of the sensitivity analyses includes the estimates for MSI high only.

Sensitivity and Specificity of the Revised Bethesda Guidelines Among Unselected CRC (*S_BethR, C_BethR*). These were obtained from the study by Pinol 2005.[12]

Sensitivity and Specificity of the Three Clinical Criteria (Young Age at Diagnosis, CRC in Family or Multiple Tumors in Proband) Among Unselected Patients With CRC (*S_3Clinical, C_3Clinical*). These were obtained from a meta-analysis of pertinent studies (Aaltonen 1998,[63] Salovaara 2000,[85] and Pinol 2005[12]). The estimates for the two Finnish studies[63,85] were calculated accounting for the presence of founder mutations.

Sensitivity and Specificity of MSI (*S_MSI_3Clinical, C_MSI_3Clinical*) and IHC (*S_IHC_3Clinical, C_IHC_3Clinical*) Among People Fulfilling the Three Clinical Criteria (Young Age at Diagnosis, CRC in Family or Multiple Tumors in Proband). The values for MSI were based on the studies by Aaltonen 1998 and Salovaara 2000.[63,85] Values include both MSI-H and MSI-low, as discussed above.

Similar values for IHC were not available. We assumed that these could be substituted by the corresponding estimates for the unselected CRC (which were based on data from Barnetson 2006 and Southey 2005, two studies[11,102] that assessed people at young age of CRC diagnosis).

Sensitivity and Specificity of MSI (*S_MSI_BethR, C_MSI_BethR*) and IHC (*S_IHC_BethR, C_IHC_BethR*) Among People Fulfilling the Revised Bethesda Guidelines. A small study on 81 patients by Wolf 2005[86] provided the relevant information for the accuracy of MSI. However, the study reported perfect sensitivity (100%) for MSI, which was considered improbable. We therefore used the same estimates as for patients fulfilling at least one of the three simple clinical criteria (<50 years of age at diagnosis, CRC or endometrial cancer in family or multiple colorectal or endometrial cancers in proband).

There were no available data for the diagnostic accuracy of IHC among people fulfilling the revised Bethesda guidelines. Therefore, we assumed that Southey 2005[102] (a study that assessed incident CRC aged less that 45 at diagnosis) provided suitable corresponding estimates.

Table 18. Probabilities and parameters used in the decision trees for different strategies to detect MMR mutations

Variable	Baseline	Range for sensitivity analysis			Reference
		Low	High	Rationale	
Prevalence of HNPCC among unselected CRC assuming no founder mutations in non-Finnish populations, (%) *Prev*	Low=0.90 Higher=2.75	0.6	5.1	Lower limit is the lower 95% CI boundary excluding founder mutations in MLH1; Upper limit is more than the upper 95% CI boundary of the higher prevalence estimate The range was intentionally broad.	Aaltonen 1998, Salovaara 2000, Pinol 2005, Samowitz 2001, Hampel 2005
Sensitivity of genetic testing, (%) *S_MMR*	95	72	100	Assumed to be the same in every subset of CRC	Assumption, 5% are not detected by MLH1 or MSH2
Specificity of genetic testing, (%) *C_MMR*	99.5	98	100	Assumed to be the same in every subset of CRC	Assumption
Proportion with inconclusive results among those with HNPCC whose MMR test results were not positive, (%) *Pinconcl1*	11	0	35	Upper limit based on 95% CI	Syngal 1999
Proportion with inconclusive results among those without HNPCC whose MMR test results were not positive, (%) *Pinconcl2*	0.25	0	0.7	Upper limit based on 95% CI	Pinol 2005
Unselected incident CRC probands					
Sensitivity of MSI testing, (%) *S_MSI*	95	81	99	Assuming combined MSI-H and MSI-L is “positive”; limits based on 95% CI	Aaltonen 1988, Salovaara 2000, Barnetson 2006, Southey 2005
Specificity of MSI testing, (%) *C_MSI*	87	81	91	Assuming combined MSI-H and MSI-L is “positive”; Limits based on 95% CI	Aaltonen 1988, Salovaara 2000, Barnetson 2006, Southey 2005
Sensitivity of IHC testing, (%) *S_IHC*	91	75	97	among <45 and <55y of age; Limits based on 95% CI	Southey 2005, Barnetson 2006
Specificity of IHC testing, (%) *C_IHC*	87	83	90	among <45 & <55y of age; Limits based on 95% CI	Southey 2005, Barnetson 2006
Sensitivity of Revised Bethesda Guidelines, (%) *S_BethR*	91	59	100	Limits based on 95% CI	Pinol 2005
Specificity of Revised Bethesda Guidelines, (%) *C_BethR*	77	75	79	Limits based on 95% CI	Pinol 2005

Table 18. Probabilities and parameters used in the decision trees for different strategies to detect MMR mutations (continued)

Variable	Baseline	Range for sensitivity analysis			Reference
		Low	High	Rationale	
Sensitivity of 3 clinical criteria, (%) *S_3Clinical*	88	60	97	Limits based on 95% CI	Aaltonen 1998, Salovaara 2000, Pinol 2005
Specificity of composite clinical criteria, (%) *C_3Clinical*	77	74	81	Limits based on 95% CI	Aaltonen 1998, Salovaara 2000, Pinol 2005
Among CRC fulfilling the composite clinical criteria					
Sensitivity of MSI testing, (%) *S_MSI_3Clinical*	95	65	100	10/10 and 17/18; limits based on 95% CI	Aaltonen 1998, Salovaara 2000
Specificity of MSI testing, (%) *C_MSI_3Clinical*	87	73	94	98/118 and 82/99; Limits based on 95% CI	Aaltonen 1998, Salovaara 2000
Sensitivity of IHC testing, (%) *S_IHC_3Clinical*	91	75	97	Assumption, see text	[Southey 2005, Barnetson 2006]
Specificity of IHC testing, (%) *C_IHC_3Clinical*	87	83	90	Assumption, see text	[Southey 2005, Barnetson 2006]
Among CRC fulfilling the Revised Bethesda guidelines					
Sensitivity of MSI testing, (%) *S_MSI_BethR*	95	65	100	Assumption, see text	[Aaltonen 1998, Salovaara 2000]
Specificity of MSI testing, (%) *C_MSI_BethR*	87	73	94	Assumption, see text	[Aaltonen 1998, Salovaara 2000]
Sensitivity of IHC testing, (%) *S_IHC_BethR*	84	73	96	Assumption, see text; Limits based on 95% CI	[Southey 2005]
Specificity of IHC testing, (%) *C_IHC_BethR*	95	94	97	Assumption, see text; Limits based on 95% CI	[Southey 2005]

In the first column the italicized word is the name of the pertinent variable in the decision trees. For references that are in brackets: The variable values are based on assumptions that the studies referenced within brackets provide suitable estimates.
CI: confidence interval; CRC: colorectal cancer; IHC: immunohistochemistry; MSI: MSI.

Results With the Different Strategies

Testing for MSI (or even IHC) among CRC patients with high clinical suspicion for HNPCC may be a reasonable compromise between the number of laboratory tests that would be required and an accurate diagnosis in the majority of people with HNPCC (i.e., strategies in group 4).

More specifically, group 4 strategies selected relatively fewer patients for genetic testing (6% or less) and missed at most 27% of patients with HNPCC. In contrast, for strategies in groups 1 to 3, more than 13% and up to 100% of newly diagnosed CRC patients would be genetically tested, and 5% to 16% of patients with HNPCC would be missed. These descriptives are true for both the low (0.9%) and the higher estimate for the prevalence of mutation carriers among incident unselected CRC (Table 19).

Because the revised Bethesda guidelines are more difficult to ascertain compared to the fulfillment of at least one of the three simple clinical criteria, it might be easier to use the combination of the three clinical criteria. Although it seemed that MSI was preferable over IHC as a screening tool among people with high clinical suspicion, estimates were based on several assumptions due to missing information, compromising the validity of this observation.

We decided not to highlight the relative ranking of the strategies within each group because of the many assumptions in the used probabilities. We therefore focus more on the differences across the four groups of strategies. The relative ranking of the 4 strategy groups with respect to each outcome was robust in all one-way sensitivity analyses.

Baseline Analyses.

Number of patients identified as positive – low estimate for prevalence of mutation carriers. In the hypothetical population of 100,000 CRC, 900 individuals had MMR mutations. The number of positive diagnoses with each strategy ranged from 1,351 (MMR-All) to 659 (Beth-IHC) (Table 19). The number of HNPCC patients who were truly identified was highest in the MMR-All strategy (n=855 out of 900) and lowest in group 4 strategies (ranging between 654 and 739, depending on the strategy). Reciprocally, the number of HNPCC patients who were missed with each strategy increased for strategies that performed fewer MMR tests. While only 45 patients would be missed if all CRC were to be genetically tested, up to one out of five (between 161 and 246 out of 900) would be missed if MSI or IHC were performed only on people with a high clinical suspicion for HNPCC.

As shown in Table 19, the number of inconclusive MMR tests increases with the number of total MMR tests. In group 4 strategies it ranged between 6 and 12, and in the MMR-All strategy it was approximately 250.

Number of patients identified as positive – higher estimate for prevalence of mutation carriers. In the hypothetical population of 100,000 CRC, 2,750 individuals had MMR mutations. The number of positive diagnoses with each strategy ranged from 3,098 (MMR-All) to 2,002 (Beth-IHC) (Table 19). The number of HNPCC patients who were truly identified was highest in the MMR-All strategy (n=2,612 out of 2,750) and lowest in group 4 strategies (ranging between 1,997 and 2,258, depending on the strategy). The number of HNPCC patients who were missed with each strategy increased for strategies that performed fewer MMR tests. While only 138 patients would be missed if all CRC were to be genetically tested, up to one out of four (between 492 and 753 out of 2,750) would be missed if MSI or IHC were performed only on people with high clinical suspicion for HNPCC.

As shown in Table 19, the number of inconclusive MMR tests increases with the number of total MMR tests. In group 4 strategies it ranged between 14 and 20, and in the MMR-All strategy it was a bit over 260.

Number of tests performed for assessments – low estimate for prevalence of mutation carriers. The expected percentage of the CRC cohort receiving MMR tests ranged from 100% (MMR-All) to 1.83% (BethR-IHC) (Table 19).

Generally, strategies that used clinical criteria to select patients to be tested with MSI or IHC (BethR-MSI, BethR-IHC, 3Clinical-MSI, 3Clinical-IHC) required fewer MMR genetic tests. Fewer than 4% of newly diagnosed patients with CRC would be expected to need MMR genetic testing based on this approach. Furthermore, for the strategies in group 4, approximately one out of four patients would receive MSI or IHC testing, based upon suggestive clinical criteria.

The use of clinical criteria alone to screen cases (BethR-All, 3 Clinical-All) would require MMR genetic testing in almost a quarter of the incident cases of CRC. Using laboratory tests as a first screening would result in approximately one out of seven patients requiring genetic testing (Table 19). By definition, all patients in the strategies that first screen with MSI or IHC would receive MSI or IHC testing (MSI-All or IHC-All, respectively), but fewer than one out of four patients would need to be screened with MSI or IHC based upon suggestive clinical criteria (Table 19).

Number of tests performed for assessments – higher estimate for prevalence of mutation carriers. The expected percentage of the CRC cohort receiving MMR tests ranged from 100% (MMR-All) to 3.22% (BethR-IHC) (Table 19). The overall percentages of people receiving genetic testing, MSI testing or IHC testing with the various strategies was similar to what was calculated for the low prevalence estimates (Table 19).

Table 19. Expected number of MMR, MSI or IHC tests and expected MMR testing results with the nine strategies, assuming a population of 100,000 incident cases of CRC

Strategy	Received tests			Number of MMR tests that were			Unidentified MMR mutation carriers
	MMR	MSI	IHC	Positive	True positive	Inconclusive	
Low prevalence estimate for MMR mutation carriers (0.90%)							
MMR-All	100,000	0	0	1,351	855	252	45
BethR-All	23,612	0	0	892	778	61	122
3Clinical-All	23,585	0	0	866	752	61	148
MSI-All	13,738	100,000	0	877	812	37	88
IHC-All	13,702	0	100,000	843	778	37	122
BethR-MSI	3,741	23,612	0	754	739	12	161
3Clinical-MSI	3,716	23,585	0	730	715	11	185
BethR-IHC	1,828	0	23,612	659	654	6	246
3Clinical-IHC	3,684	0	23,585	700	685	11	215
Higher prevalence estimate for MMR mutation carriers (2.75%)							
MMR-All	100,000	0	0	3,098	2,612	257	138
BethR-All	24,870	0	0	2,489	2,377	69	373
3Clinical-All	24,787	0	0	2,410	2,299	69	451
MSI-All	15,255	100,000	0	2,545	2,481	46	269
IHC-All	15,145	0	100,000	2,440	2,377	45	373
BethR-MSI	5,285	24,870	0	2,273	2,258	20	492
3Clinical-MSI	5,206	24,787	0	2,198	2,184	20	566
BethR-IHC	3,220	0	24,870	2,002	1,997	14	753
3Clinical-IHC	5,110	0	24,787	2,106	2,092	20	658

In the hypothetical population, for the low prevalence estimate 900/100,000 patients are assumed to carry MMR mutations; for the high prevalence estimate 2750 people are assumed to carry MMR mutations. Strategies are presented with respect to the group (1 to 4) to which they belong.

One may consider the nine strategies as nine (composite) diagnostic tests, and thus calculate their overall (strategy-level) sensitivity and specificity (Table 20). In these calculations, inconclusive tests were assumed to be false negative (for the calculation of overall sensitivity for each strategy) or false positive (for the calculation of overall specificity for each strategy). The overall specificity was high in all strategies. Group 4 strategies had the lowest overall sensitivity (ranging between 73% and 82%). The overall sensitivity and specificity values were similar for both the low and the higher estimates for the prevalence of MMR mutation carriers.

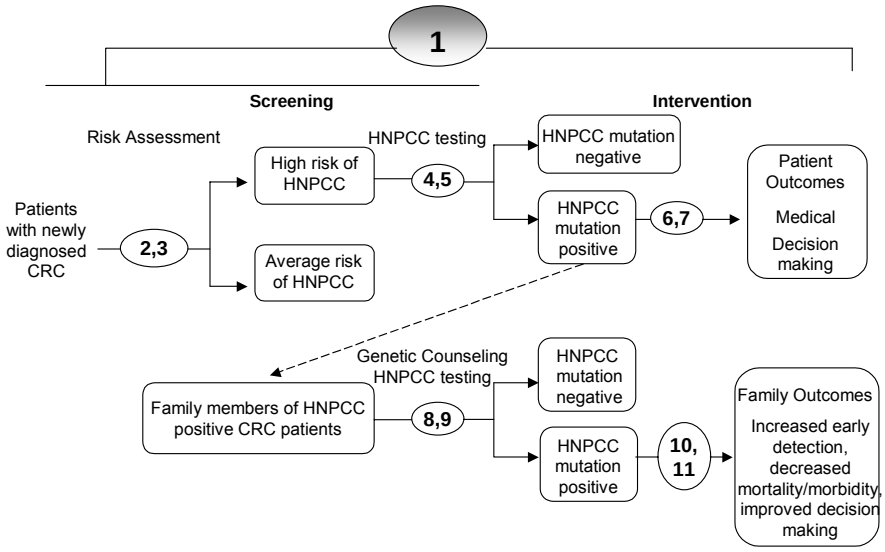
Table 20. Overall sensitivity and specificity for each of the nine strategies

Strategy	Sensitivity of strategy (%)	Specificity of strategy (%)
MMR-All	95.0	99.3
BethR-All	86.5	99.8
3Clinical-All	83.6	99.8
MSI-All	90.3	99.9
IHC-All	86.5	99.9
BethR-MSI	82.1	100.0
3Clinical-MSI	79.4	100.0
BethR-IHC	72.6	100.0
3Clinical-IHC	76.1	100.0

The overall sensitivity and specificity values were very similar for both the low and the higher estimates for the prevalence of MMR mutation carriers.
Strategies are presented with respect to the group (1 to 4) to which they belong.

Benefits and Harms

Key Question 1: Does Risk Assessment and HNPCC Mutation Testing in Patients With Newly Diagnosed CRC Lead to Improved Outcomes for the Patient or Family Members, or is it Useful in Medical, Personal, or Public Health Decision Making? (Overarching Question)

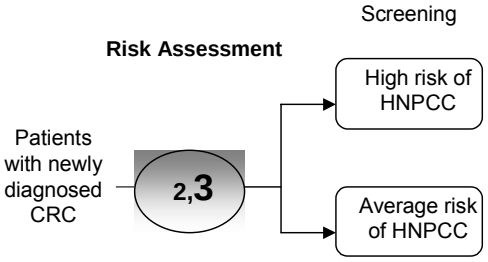


Key Question 1 is a general question that includes all other Key Questions. An ideal study addressing Key Question 1 would enroll patients with CRC patients and/or their family members and randomize them to risk assessment and mutation testing or a control intervention, and follow them up prospectively. The study would compare a spectrum of health outcomes among those who received screening for HNPCC to those who did not, while considering subsequent treatments or interventions in each group.

Summary of Findings. No study directly addressed Key Question 1 based on the ideal framework described above.

Gaps in the Literature. While our literature review did not identify a comprehensive study that directly answered Key Question 1, it is unlikely that such an "ideal" study is feasible. Such a study would likely be prohibitively complex, require multiple years of follow-up and randomization of patients or family members to care that is no longer considered to reflect contemporary practices. However, studies addressing specific components of this general question (as described in the remaining Key Questions) provide some of necessary pieces to better understand this overarching question.

Key Question 3: What Are the Harms Associated With Screening High-Risk Individuals for HNPCC?



Studies were considered eligible for Key Question 3 if they reported harms of a risk assessment process (e.g., Amsterdam, Bethesda and/or MSI, IHC) used to identify CRC patients at increased risk for HNPCC.

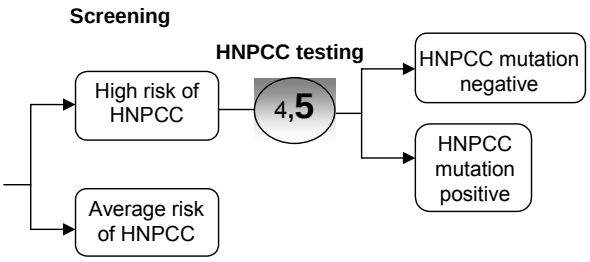
Summary of Findings. No study described any harms of the risk assessment process in CRC patients at increased risk for HNPCC.

Gaps in the Literature. The goal of the risk assessment process is to identify individuals suspected of having HNPCC who might benefit from subsequent genetic testing. It is possible that such a process could lead to harm, such as stigmatization or worry, but no study directly assessed such associations.

The risk assessment tools also have the potential to misclassify patients. The test characteristics (i.e., sensitivity, specificity and predictive values) of these methods are summarized in the "Clinical Validity" section. We did not consider misclassified patients (i.e., false positive or negatives) as having been harmed. However, it is possible that subsequent genetic testing and/or management options could harm patients who had been misclassified. The harms associated with genetic testing and/or management options are described later in this report.

Future research is needed to identify the best strategy of the risk assessment process that increases the accuracy, feasibility and applicability of diagnostic methodologies to determine HNPCC so the potential harms can be minimized.

Key Question 5: What Are the Harms Associated With Screening for High-Risk Individuals?



Studies were considered eligible for Key Question 5 if they reported the harms associated with testing CRC patients for MMR mutations. Common harms that are thought to be associated with genetic testing are labeling, discrimination in health coverage, and emotional distress.

Three quantitative, comparative studies of quality A and B, and one qualitative study of B quality reported harms associated with MMR mutation testing in CRC patients[104-106] (Table 21).

Summary of Findings. One 1-year prospective study (Grade A) compared the psychological impact of MMR mutation testing between mutation carriers and non-carriers.[104] Results for non-carriers are summarized below and also in Table 21. Subjects in this study were CRC probands or relatives from HNPCC families with a prior diagnosis of any cancer (excluding non-melanoma skin cancer). Anxiety, depression, and quality of life measures did not change over time, and there were no differences in these measures between mutation carriers and non-carriers. Distress levels were significantly decreased 2 weeks and 6 months after revealing the genetic testing results, but were not significantly different from the baseline at 1-year follow-up. There was no difference in the distress levels between mutation carriers and non-carriers.

Another 1-month prospective study (Grade B) found that three of the 27 probands (11%) had minor depression at 1 month after revealing the genetic testing results, but the prevalence of minor depression was not significantly different compared to the prevalence at baseline or between mutation carriers and non-carriers.[105] Of the six probands who received a positive result, two (33%) felt severe guilt regarding their children.

One prospective study (Grade B) reported changes in the psychological outcomes of CRC patients from self-completed questionnaires pre- and 4-6 weeks post-genetic counseling.[107] There was no genetic testing performed in this study. There was a trend toward greater anticipated ability to cope with a positive gene test after counseling, as reflected by a decreased in anxiety and cancer-specific distress.

The qualitative study of 111 newly diagnosed CRC patients reported a high acceptance and understanding about information on HNPCC.[106] Nineteen percent of participants rated their current level of worry caused by the genetics information at or above the midpoint of 4 on a 1 (not at all) to 7 (all the time) scale.

Gaps in the Literature. Most research on psychosocial aspects of genetic counseling and testing for cancer risk has focused on hereditary breast and ovarian cancer. Genetic testing for hereditary colorectal cancer has become available only in the past decade. As a result, only few studies with small number of CRC patients have evaluated the acceptability and psychosocial sequelae of genetic counseling and testing. Some of the findings from studies of counseling and testing in other forms of cancer may be applicable to the CRC setting.

Table 21. Key Question 5. What are the harms associated with genetic testing for HNPCC mutations?

Author, year Country	Study design (follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Measures of psychological impact after revealing genetic testing results	Observed impact: Test receivers vs. normal ranges[a]	Observed impact: Follow-up vs. baseline (changes over time)	Observed impact: Mutation positive vs. negative	Quality
Quantitative, Comparative Studies									
Gritz, 2005 US	Prosp (2 wk, 6 mo & 1 yr)	Affected[b] persons at least 18 years old with HNPCC defined by Amsterdam or suggestive family history followed by mutation testing (126/89)	89	37%	Anxiety (State-Trait)		⇔ at all f/up	⇔ at all f/up	A
					Depression (CES-D)		⇔ at all f/up	⇔ at all f/up	
					Quality of life (SF-36)		⇔ at all f/up	⇔ at all f/up	
					Distress from receiving testing results (RIES)		↓ at 2 wk & 6 mo; ⇔ 1 yr	⇔ at all f/up	
Keller, 2002 Germany	Prosp (4-6 wk)	Patients with HNPCC-related cancer from families at risk for HNPCC (ND/31)	31	N/A[c]	Anxiety (HAD)		↓		B
					Depression (HAD)		⇔		
					Cancer-specific distress (IES scales)		↓⇓		
Murakami, 2004 Japan	Prosp (1 mo)	Probands whose family members had been identified as carrying the MLH1/MSH2 mutation, age ≥20 years, and opted for genetic testing (31/27)	27	22%	Minor depression (DSM-III-R and DSM-IV scales)		⇔	⇔	B
					Guilt (mutation carriers only)			2/6 (33%)	

↑ Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
↓ Statistically decreased ⇓ Decreased, but not statistically significant

[a] Published population levels or the normal ranges
[b] "Affected" persons included any index patients or relatives with a prior diagnosis of any cancer excluding non-melanoma skin cancer
[c] Patients received only pre-test genetic counseling. Psychological measures were done immediately after counseling without performing genetic testing

Table 21. Key Question 5. What are the harms associated with genetic testing for HNPCC mutations? (continued)

Author, year Country	Study Design (Follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Results	Quality
Qualitative, Non-Comparative Studies						
Porteous, 2003 UK	Survey (N/A)	Newly diagnosed CRC patients under the age of 55 years old (160/111)	111	N/A	The majority of participants found it highly acceptable to have information about HNPCC brought to their attention at a time when they were coping with a new diagnosis of colorectal cancer, despite a lack of prior awareness that the disease could run in families. The vast majority reported high levels of subjective understanding concerning genetic testing. 19% of participants (n=21) rated their current level of worry caused by the genetics information at or above the midpoint of 4 on a 1 (not at all) to 7 (all the time) scale. 1 patient (1%) not opting for genetic testing.	B

References for standardized instruments:

CES-D: Center for Epidemiologic Studies Depression.

DSM-III-R: Association Psychiatric Association. Diagnostic and statistical manual of mental disorders. 3rd edition, revised. Washington, DC: American Psychiatric Press, 1987.

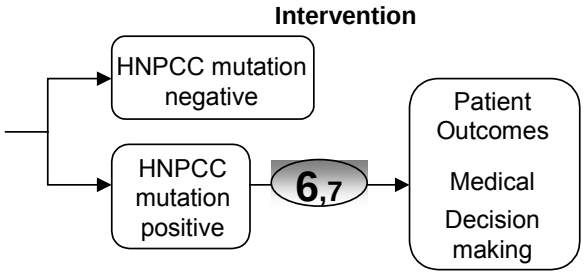
DSM-IV: Association Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Washington, DC: American Psychiatric Press, 1994.

State-Trait Anxiety: Van der Ploeg HM, Defares PB, Spielberger CD. Handleiding bij de Zelfbeoordelingsvragenlijst: een Nederlandstalige bewerking van de Spielberger State-Trait Anxiety Inventory. Lisse: Swets and Zeitlinger, 1980.

Cancer-specific distress (IES scales): Horowitz MJ, Wilner N, Alvarez W. Impact of Event Scale: a measure of subjective stress. Psychosom Med 1979;41:209–18.

Hospital anxiety and depression scale (HAD): Goldberg D, Williams P. A User's Guide to the General Health Questionnaire. Windsor: NFER-Nelson, 1988.

Key Question 6a: What Are the Management Options for CRC Patients Who Are HNPCC Positive? b: Does the Identification of HNPCC Mutations Lead to Improved Patient Outcomes in Terms of Early Detection, Mortality/Morbidity, or Management Decisions (e.g., Counseling, Surveillance, Treatment, Other Decision Making) by Patients and Providers?



Key Question 6 examines the outcomes of using MMR mutation status to make management decisions compared to conventional management without MMR mutation status. There are three aspects to Key Question 6: 1) Are management options for patients with CRC with a MMR mutation different from those without a MMR mutation? 2) Does the knowledge of MMR mutation status change management decisions by patients and providers? 3) Does changing management options for MMR positive patients with CRC improve outcomes (e.g., prognosis and survival) compared to standard approaches for patients with CRC?

We encountered a variety of surgical and medical management options in patients with CRC who were MMR positive. These included various forms of colonic resection[108-110] and chemotherapy for patients with stage III tumors.[111] However, these studies did not directly address the components of Key Question 6 described above.

Because of the limited data, we broadened the scope of this question to include studies evaluating all forms of cancer related to HNPCC, since patients with CRC from HNPCC families are potentially at increased risk for all of these cancers. We also included studies using clinical criteria and laboratory testing (such as MSI and IHC) as a surrogate for identification of HNPCC mutations.

Despite having broadened the inclusion criteria, we did not identify any prospective, comparative study (direct evidence) addressing any aspect of Key Question 6. However, two retrospective cohort studies indirectly addressed the prognosis of CRC and the survival outcome of patients with endometrial cancer from HNPCC families, in relation to MMR mutation testing.[112,113] Both were of C quality, due to potential selection bias and unclear effects of treatments or interventions on the outcomes.

In addition, five retrospective cohort studies indirectly addressed mortality or morbidity in relation to risk assessments (clinical criteria and laboratory testing) for HNPCC mutations[108,109,111,114,115] (Table 22). Of these, three studies of B quality described clinical outcomes in patients with CRC who were screened for HNPCC using clinical criteria.[108,109,115] Another two described clinical outcomes in patients with CRC who were screened for HNPCC with laboratory testing.[111,114] Both were of C quality and limited by the potential selection bias, and/or unclear effects of treatments or interventions on the outcomes.

Summary of Findings. Indirect evidence from one study suggested that identification of HNPCC mutations was associated with better prognosis of CRC. However, there was no data on whether management options for CRC differed based on MMR mutation status.

Indirect evidence from one study showed no difference in survival of patients with endometrial cancer, comparing those who were mutation positive to those who were mutation negative.

In five studies with indirect evidence, there was no evidence in favor or against differences in survival, when comparing CRC patients who fulfilled different clinical criteria for HNPCC or screened positive for HNPCC by laboratory testing with those who did not (Overview Table 1; Table 22).

Overview Table 1: Key Question 6b

Reported Outcomes	Body of evidence (study duration)	Summary
Prognosis of CRC	1 Retro study (~8 yr)	• ⇔ Comparing mutation carriers to non-carriers with MSI • Better, comparing mutation carriers to non-carriers with MSS
Survival of patients with endometrial cancer	1 Retro study (ND)	• ⇔ Comparing MMR mutation carriers to non-carriers
Survival of CRC patients	5 Retro studies (12-153 mo)[a]	• Results were inconsistent among studies that compared survival in patients with CRC who fulfilled Amsterdam I criteria with those considered to have sporadic CRC • ⇔ Comparing Amsterdam I to Japanese A criteria • ⇑ Comparing Amsterdam I to Japanese B criteria • ⇔ Comparing MSH2 and/or MLH1 status by IHC in patients with sporadic CRC • ⇔ Comparing MSI-H to MSI-L/MSS CRC • ⇔ 5-year cumulative survival comparing patients with endometrial cancer who were mutation positive to those who were mutation negative. • ⇔ Comparing stage III CRC patients receiving adjuvant chemotherapy with MSI tumors to those with MSS/MSI-L.

⇑ Statistically increased ⇧ Increased, but not statistically significant
⇔ No statistically significant differences
⇓ Statistically decreased ⇩ Decreased, but not statistically significant

[a] Only two studies reported the study duration

Gaps in the Literature. Some studies described the management of patients with HNPCC but surprisingly, there were no data directly exploring how MMR mutation status influenced the choice among these management options or their outcomes. As a result, the available data do not directly answer whether knowledge of MMR mutation status changes management decisions by patients and providers or whether changes in management for MMR positive patients improves their outcomes compared to standard CRC management. Issues related to MMR mutation testing in influencing decisions to undergo cancer screening procedures is discussed below with Key Question 10.

Table 22. Key Question 6b. Does the identification of HNPCC mutations lead to improved patient outcomes in terms of early detection, mortality/morbidity or management decisions (e.g., counseling, surveillance, treatment, other decision making) by patients and providers?

Author, year Country	Study Design (f/up duration)	Target population for genetic testing (Eligible/Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	% Received interventions or treatments	Outcomes		Quality
Mutation testing									
Benatti, 2001 Italy	Retro (8.2, 6.7, and 9.4 yrs for group A, B, and C respectively)	29 HNPCC families met inclusion criteria: 10 carried MMR gene mutations (Group A), 10 were characterized by MSI phenotype but not by MMR gene mutation (Group B), and 9 did not show mutations or MSI (Group C)	Group A: 361 Group B: 241 Group C: 355	100% 0% 0%	MMR gene mutation	ND	Prognosis of CRC	⇔ Compared Group B (or MSI) Better compared Group C (or MSS) (p=0.001)	C
Boks, 2002 Netherlands	Retro (ND)	Patients with endometrial cancer from 46 HNPCC families with a mutation or that met revised Amsterdam criteria (ND/66)	50	75%	MMR gene mutation	ND	⇔ 5-year cumulative survival		C
Clinical criteria and laboratory testing									
Bertario, 1999 Italy	Retro (60 mo)	3 CRC groups: 144 HNPCC 161 FAP 2035 sporadic (ND/2,340)	2,340	5%	Amsterdam criteria	"Adjuvant-treatment protocols were the same b/w the 3 groups" HNPCC and FAP patients underwent periodic examinations for different "spectrum of the diseases"	⇔ Survival b/w HNPCC, FAP, and sporadic CRC groups adjusting for age, gender, stage and tumor location. HR for HNPCC was 1.01 (95% CI 0.72-1.39) compared with sporadic.		B

Table 22. Key Question 6b. Does the identification of HNPCC mutations lead to improved patient outcomes in terms of early detection, mortality/morbidity or management decisions (e.g., counseling, surveillance, treatment, other decision making) by patients and providers? (continued)

Author, year Country	Study Design (f/up duration)	Target population for genetic testing (Eligible/Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	% Received interventions or treatments	Outcomes	Quality
Fujita, 1996 Japan	Retro (ND)	CRC patients who underwent surgery (ND/3,356)	1785	8 /1000	Amsterdam I criteria	100% surgery	5-year survival rate (30 day mortality excluded): Amsterdam I=92.3% Japanese A=81.2% Japanese B=66.5% Sporadic=60% Better survival for Amsterdam and Japanese A vs. Japanese B and Sporadic groups (p<0.05)	B
				17 /1000	Japanese criteria A			
				56 /1000	Japanese criteria B			
Tomoda 1996 Japan	Retro (12-153 mo)	Nonpolyposis CRC patients who underwent resection (ND/1042)	1042	3.7%	Japanese criteria B	100% resection	Mean age was significantly younger for cases meeting Japanese criteria (or with HNPCC) than cases who didn't (55.9 yr vs. 61.1 yr, p=0.01) ↑ Metachronous (postoperative) CRC, compared cases with HNPCC to those without HNPCC (10.2% vs. 3.5%, p=0.0001) ↑ Survival, compared cases with HNPCC to those without HNPCC (p=0.02) ⇑ Survival, compared cases with HNPCC to those without HNPCC with stage III cancers (p=0.06)	B
Perrin, 2001 France	Retro (ND)	Patients diagnosed with sporadic CRC (ND/225)	208	13%	Lack of expression of MSH2 and/or MLH1 by IHC	ND	⇔ Disease-free survival compared MSH2 and/or MLH1 status by IHC. Subpopulation of proximal tumors, MSH2 and/or MLH1 negativity was associated with longer disease-free survival	C
Kruhoffer, 2005 Denmark	Retro (ND)	Stages II and III CRC patients with sporadic MSI, hereditary MSI, or MSS [a] tumors (151/101)	19	100%	Sporadic MSI-H tumors	65 patients with Stage III tumors receiving adjuvant chemotherapy	Overall survival highly significantly related to classification in 36 Stage II patients as 10 of 11 patients who died ≤ 5 years belonged to MSS/MSI-L group (p=0.0014) 16 classified as MSI and 49 as MSS/MSI-L tumors for 65 Stage III patients receiving adjuvant chemotherapy. ⇔ overall survival b/w groups (6 MSI and 30 MSS/MSI-L patients died ≤ 5 years f/up (p=0.55)	C
			15	100%	Hereditary MSI-H tumors [b]			
			67	0%	MSS or MSI-L tumors			

FAP=familial adenomatous polyposis

↑ Statistically increased ⇑ Increased, but not statistically significant

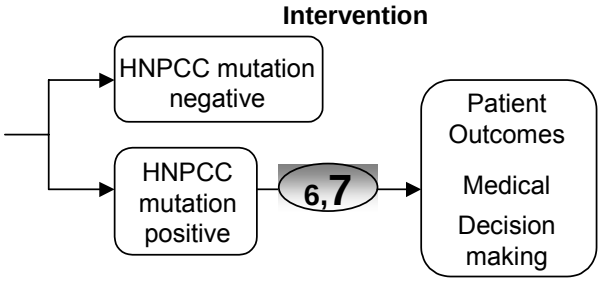
⇔ No statistically significant differences

↓ Statistically decreased ⇓ Decreased, but not statistically significant

[a] The definition of MSS in the study was Including MSI-L.

[b] MSI tumors with HNPCC origin were classified by a maximum likelihood MSI classifier with a "leave-one-out" cross validation scheme basically as described (Dyrskjot et al, 2003).

Key Question 7: What Are the Harms Associated With Subsequent Management Options After Identification of HNPCC Mutations in CRC Patients?



Due to limited data on harms associated with subsequent management options or interventions, we broadened the scope of this question to include studies evaluating all forms of cancer related to HNPCC, since patients with CRC from HNPCC families are potentially at risk for all forms of HNPCC-related cancers. We also included studies that reported any outcome relating to subsequent management options or interventions in these patients.

Two retrospective studies of B and one of C quality reported outcomes related to subsequent actions or interventions in patients with CRC and gastric cancers, respectively (Table 23).[110,116] The validity of the study of C quality (gastric cancers) is questionable because of unclear participation rates and an unclear description of how survival/death was ascertained.

Summary of Findings. No study described harms associated with subsequent management options after identification of HNPCC mutations in patients with CRC or other forms of HNPCC-related cancers.

One study (involving two centers) described the types of colorectal surgery performed on CRC patients who were part of an Amsterdam criteria-positive family, and compared rates of metachronous cancers that followed each type of index operation.[110] The overall rate of second surgeries for metachronous cancer were 23% in patients who underwent right colectomy, 17% in patients who underwent left/sigmoid colectomy or proctosigmoidectomy, 0% in patients who underwent total/subtotal colectomy, and 44% in patients who underwent segmental colectomy. The two centers had significantly different second resection rates for metachronous cancer.

One study described the survival rate of 45 patients with gastric cancer from HNPCC families with MMR mutations.[116] Many of these patients had already had treatments for other HNPCC-related cancers, including CRC. The 5-year survival was higher in patients in whom radical surgery was performed (48%) than in patients in whom radical palliative surgery or explorative laparotomy alone was performed (15%).

Gaps in the Literature. There are no data on harms associated with surgical procedures or chemotherapy in CRC patients with HNPCC, although it is likely that such patients encounter the same spectrum of harms associated with chemotherapy and surgery as patients with CRC (or other forms of HNPCC-related cancer) without HNPCC. One cannot assume "no harm" when a study did not report data on harms.

There were also no data on whether cancer patients with MMR mutations derive a greater benefit from specific management options compared with patients without MMR mutations.

However, several studies have suggested a relatively favorable prognosis of colorectal cancer in patients with HNPCC or those who have clinical features associated with HNPCC such as tumors that are MSI-H.[117-120] These data have also suggested that patients with colorectal cancer that demonstrate MSI may not derive a benefit from adjuvant chemotherapy with 5-fluorouracil (5-FU), possibly because of a higher cure rate with surgery alone and/or intrinsic resistance to 5-FU.[120]

However, a 2004 consensus statement issued by the American Society of Clinical Oncology[121] found insufficient evidence to recommend modification of overall treatment recommendations in patients with stage II colorectal cancer with a known mismatch repair mutation or tumors that demonstrate MSI, noting the retrospective and inconclusive nature of the existing evidence. The report cites an ongoing European trial (FOLFIRI), which is comparing irinotecan and 5-FU and is stratifying patients based on tumor MSI status, and a United States Intergroup trial that is also incorporating MSI status as a prognostic factor. The final results of these trials have not been published.

Our review of the literature did not provide additional strong evidence in favor or against the use of adjuvant chemotherapy in patients with HNPCC of any stage. However, our literature search strategy excluded studies with patients who had tumors that demonstrated MSI unless the study performed additional testing for HNPCC (either by clinical criteria, IHC, or genetic testing). Thus, the literature search was not comprehensive for establishing the relationship between tumor MSI status and the outcome of adjuvant chemotherapy. The ongoing trials described above will help clarify this issue (as well as the role of other biologic markers proposed for guiding adjuvant chemotherapy in patients with colorectal cancer).

Table 23. Key Question 7. What are the harms associated with subsequent management options after identification of HNPCC mutations in CRC patients?

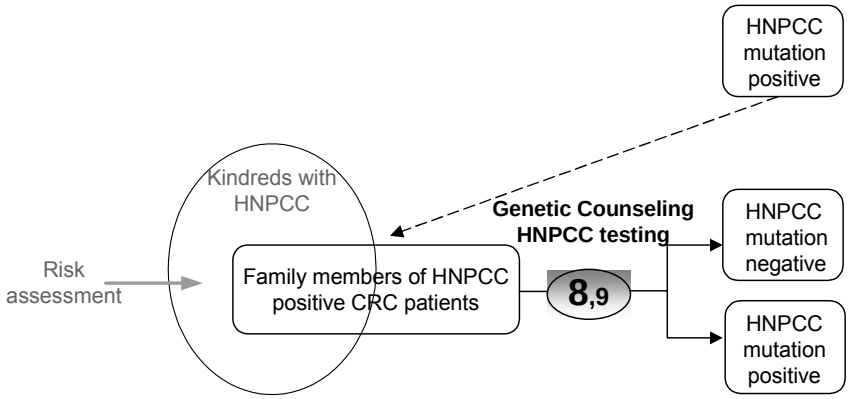
Author, year Country	Study design (Follow-up duration)	Target population (Eligible/Enrolled N)	% with CRC at baseline	N evaluated	% who took actions	Description of subsequent actions or interventions	Harms	Outcomes		Quality
Van Dalen 2003 US	Retro (1-49 yr)	CRC patients from 39 HNPCC families met Amsterdam I criteria (ND/93)	100%	93	100%	33% received RC, 14% L/SC, 18% PSC, 25% TC, and 10% SGC	ND	2nd resections rate	7/31 patients received RC; 5/30 patients received L/SC or PSC; 0/23 patients received TC; 4/9 patients receive SGC	B
Aarnio 1997 Finland	Retro (ND)	Patients with gastric cancers from 51 HNPCC families by Amsterdam I or with a known MMR mutation (ND/45)	40%[a]	45	36% 63%	Radical surgery Palliative surgery or explorative laparotomy alone with no surgery	ND	5-year survival	48% 15%	C

RC=Right colectomy; L/SC=Left/sigmoid colectomy; PSC=Proctosigmoidectomy; TC=Total/subtotal colectomy; SGC=Segmental colectomy.
[a] 18 (40%) already had been treated for CRC (13 cases), endometrial cancer (2 cases), ovarian cancer (1 case), urinary-tract cancer (1 case) and testicular cancer (1 case).

Key Questions 8 to 11 summarize what is known about the processes and benefits of bringing family members into the testing process. Knowing that a CRC patient is MMR mutation positive has implications for family members. As a general rule, family members receive counseling and, subsequently, genetic testing for MMR mutations ideally based upon the results of MMR testing in the proband.

Key Question 8b: What is the Accuracy of HNPCC Testing in Family Members in Predicting the Risk of CRC?

Key Question 8c: Do Other Factors, Such as Race/Ethnicity, Age, Gender, or Co-Morbidities Affect the Accuracy of the Testing?



The analytic framework proposed above greatly restricted the pool of potentially eligible studies since it required that CRC probands were proven to have a MMR mutation. As a result, we broadened the scope of our search to include studies using clinical criteria, laboratory testing or mutation testing in predicting the risk of CRC or extracolonic cancers in family members of CRC probands with HNPCC (based on clinical or genetic criteria) and/or kindreds with HNPCC (based on clinical or genetic criteria). We considered the findings in such studies to be applicable to the Key Question. Any measure of relative risk (e.g., the standardized incidence ratio) was included as indirect evidence for the accuracy of the testing.

We summarize the findings for family members of CRC probands with HNPCC (in Table 24) and for kindreds with HNPCC (in Table 25) separately, because the risk of CRC and/or extracolonic cancers is different between the two groups.

Six studies reported the accuracy of HNPCC testing in family members of CRC probands (based on clinical or genetic criteria) in predicting the risk of CRC[20,122-126] (Table 24). Of these, only two (both of quality B) reported the accuracy of MMR mutation testing in family members of CRC probands with a positive MMR mutation.[20,126]The other four reported the accuracy of HNPCC testing in family members of CRC probands with HNPCC based on clinical criteria[122-125] Half studies were of B quality and half were of C quality. These studies were limited by verification bias, selection bias, unclear follow-up duration, and/or bias from post-hoc analyses.

Six studies indirectly reported the accuracy of the HNPCC testing in kindreds with HNPCC (based on clinical or genetic criteria)[127-132] (Table 25). Of these, two were of B quality and four

were of C quality. These studies were limited by selection and verification bias. Most studies did not adjust for familial clustering of cancers.

Summary of Findings. Only two studies of quality B reported the risk of CRC in family members of probands with positive MMR mutations (the proposed framework). The lifetime risk of CRC was 68.7% for men and 52.2% for women with MMR mutations in one study,[20] and it was 74% and 30% respectively in the other study.[126] Men had a higher lifetime risk of CRC in both studies.

In a study that reported the risk of CRC in family members of probands with HNPCC based on clinical criteria, the cumulative risk of CRC by age 75 years old was 57% and 41% in families that fulfilled the Amsterdam I and II criteria, respectively.[122] The cumulative risk of CRC by age 75 years old was 42% and 23% for men and women, respectively, from families that fulfilled Bethesda criteria. In another study[123] family members of CRC probands who were younger than 50 years old at cancer diagnosis had a higher risk of CRC, compared to family members of CRC probands who were 50 years old and older. The risk of CRC was increased three-fold, comparing first-degree relatives of CRC probands who developed a second primary in the HNPCC spectrum with the single primary group in a third report[124] (Overview Table 2; Table 24).

In a study that reported the risk of CRC in kindreds with HNPCC based on genetic criteria, the cumulative risk of CRC by age 70 yr was 82% in MLH1/MSH2 mutation carriers.[132] The cumulative incidence of CRC was 100% in men and 54% in women. There was overall a higher risk of CRC in men but the sex difference was not consistent among HNPCC kindreds with different MMR mutations (Overview Table 2; Table 25).

Overview Table 2. Key Questions 8b and 8c

Reported Outcomes	Body of evidence (study duration)	Summary
CRC risks in family members of probands with positive MMR mutations; factors that influenced the accuracy of testing	2 Retro studies (ND)	• In a Finland population, lifetime risk of CRC was 68.7% for men and 52.2% for women with MMR mutations. Mean age of diagnosis of CRC 55.1 for men and 60.3 for women. • In a Scottish population, cumulative risk of CRC (95% CI) by age 70 yr was 74% for men and 30% for women with MMR mutations (P=0.0066). The risk of CRC was significantly higher at all ages in men than in women.
CRC risks in family members of probands with HNPCC based on clinical criteria; factors that influenced the accuracy of testing	1 Prosp study (2 yr) and 3 Retro studies (8 yr)[a]	• Cumulative risk of CRC (95% CI) by age 75 yr: Am I families=57.1% (46-68.8%) Am II families=41.2% (33.3-50.1%) Bethesda families= Men 41.7% (39.4-44.1%) Women 23.3% (22.2-24.4%) • SIR of CRC (95% CI): ↑ Proband<50 yr; MSI = 21(12-35) ↑ Proband<50 yr; MSS = 7 (3-14) ⇔ Proband>50 yr = 1.07 (0.74-1.40) • 8% in the group fulfilled Amsterdam I criteria had adenomas, while 2% in the group with family history of CRC only had adenomas. • ↑ CRC risk, compared 1st degree relatives of CRC probands who developed a second primary in the HNPCC spectrum with the single primary group (RR=3.2, p<0.00001)

Overview Table 2. Key Questions 8b and 8c (continued)

Reported Outcomes	Body of evidence (study duration)	Summary
CRC risks in kindreds with HNPCC based on clinical criteria and/or genetic criteria; factors that influenced the accuracy of testing	6 Retro studies (ND)	• Cumulative risk of CRC by age 70 yr was 82% in MLH1/MSH2 mutation carriers, compared with only 1.6% in the Finnish population as a whole. • Cumulative risk of CRC by age 60 yr: MLH1 mutation (+): 84% - 94% in men compared to 63% in women (p=NS) MSH2 mutation (+): 71% - 96% in males compared to 39% in women (p=0.034) • SIR 95%CI of CRC in HNPCC families based on genetic criteria: MLH1/MSH2 mutation (+): 68 (56–81) - SIR was higher in men (83) than in women (48) • SIR and 95%CI of CRC in HNPCC families meeting either Amsterdam or Bethesda criteria: Mutation (-): 158.61 (132.8–189.4) MLH1 mutation (+): 196.76 (143.0–270.7) MSH2 mutation (+): 134.24 (99.1–181.8) • Comparing family members with pathogenic MSH6 mutations to those with MLH1/MSH2 mutations: 🡻 Prevalence of CRC 🡻 Cumulative risk by age to develop CRC or any tumor • Compared MSH2 carriers to MLH1 carriers: 🡹 Risk of CRC (p=0.13) • Relative risk of developing adenoma before age 54 yr compared to reference group[b]: 🡹 MMR mutation carriers (MSH2, MLH1, or hMSH6) (RR=4.4. p<0.001) • 🡹 Risk of CRC comparing male to female MSH2 mutation carriers (p<0.01) • ⇔ Risk of CRC comparing male to female MLH1 or MSH6 mutation carriers • Comparing family members with pathogenic MSH6 mutations to those with MLH1/MSH2 mutations: 🡹 Median age of CRC onset (54 vs. 44 yr) • 🡹 Age at diagnosis compared MMR mutation (+) to mutation (-) (+ 5 yr) • ⇔ Age at diagnosis compared MSH2 mutation (+) to MLH1 mutation (+), adjusted for familial clustering. • Proportion of patients younger than 50 found to have an adenoma: 🡹 Amsterdam I

SIR=standardized incidence rate.
🡹 Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
🡻 Statistically decreased ⇓ Decreased, but not statistically significant
[a] Only one study reported the duration of follow-up.
[b] Reference group was based on three published forensic autopsy studies used for estimates of adenoma prevalence in the normal population.

Gaps in the Literature. We found only limited evidence on the accuracy of MMR mutation testing in predicting the risk of HNPCC-related cancers in family members of CRC probands. Only two studies based such predictions on proven MMR mutations in the proband.[20,126] The study by Hampel[20] found that cancers in family members developed at a later age compared with

previous reports that had used clinical criteria to establish the diagnosis of HNPCC, suggesting that additional studies are required to fully understand penetrance in family members of CRC probands who carry MMR mutations.

No study directly examined factors that influenced the accuracy of the HNPCC testing, although some found significant differences in the risks of CRC among HNPCC kindreds with different MMR mutations. More studies are needed to fully understand the relationships among specific MMR mutations and cancer risk in probands with HNPCC-related cancers and their family members.

Table 24. Key Question 8b. What is the accuracy of HNPCC testing in family members in predicting the risk of CRC? Key Question 8c. Do other factors, such as race/ethnicity, age, gender, or co-morbidities affect the accuracy of the testing?

Author, year Country	Study design (follow-up duration)	Target population for HNPCC test (Eligible /Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	Accuracy of HNPCC testing in predicting risk of CRC or other HNPCC-related cancers / Factors that affected the accuracy of the testing	Quality
Family members of CRC probands with HNPCC based on genetic criteria							
Hampel 2005 Finland	Retro (ND)	Probands and 373 mutation-positive family members from 70 HNPCC families (ND/461)	461	100%	Mutations (MLH1 or MSH2) and clinical criteria, mostly Amsterdam I or II	Lifetime risk of CRC was 68.7% for men and 52.2% for women. Lifetime risk of endometrial cancer was approximately 54% Mean age of diagnosis of CRC 55.1 (95% CI 52.6-57.6)) for men and 60.3 (95% CI 58.0-62.6) for women; approximately 10-15 years older than previous estimates of age at onset for CRC among HNPCC patients.	B
Dunlop 1997 Scotland	Retro (ND)	Relatives of 6 probands with MMR gene mutations (from the Scottish National Cancer Registry) (156/156)	156	43%	Mutations (MLH1 or MSH2)	By 70 years of age, the male risk of CRC was 74% while the female risk was only 30% (p=0.0066). The risk of CRC was significantly higher at all ages in men than in women. In females, the risk of uterine cancer exceeded the risk of CRC by age 58 years, giving an estimate of 42% by age 70 years.	B
Family members of CRC probands with HNPCC based on clinical criteria							
Bermejo 2005 Sweden	Retro (ND)	Families from Swedish Family-Cancer Database with ≥4 generations of cancer. No age restrictions for parents but maximum age in 2nd generation was 70 year. (ND/566,877)	566,877 families	0.04 /1000	Amsterdam I	Cumulative risk of CRC (95% CI) by age 75 yr: Am I families=57.1% (46-68.8%) Am II families=41.2% (33.3-50.1%) Bethesda families= Men 41.7% (39.4-44.1%) Women 23.3% (22.2-24.4%) Cumulative risk of endometrial cancer (95% CI) by age 75 yr: Am II families=45.4% (34.1-58.5%) Bethesda families=8.7% (8-9.5%) Cumulative risk for ovarian cancer (95% CI) by age 75 yr: Bethesda group=4.7% (4.2-5.3%) Gastric cancer= 2.3% (2-2.7%)	B
				0.07 /1000	Amsterdam II		
				8.99 /1000	Bethesda 1-5		

Table 24. Key Question 8b. What is the accuracy of HNPCC testing in family members in predicting the risk of CRC? Key Question 8c. Do other factors, such as race/ethnicity, age, gender, or co-morbidities affect the accuracy of the testing? (continued)

Author, year Country	Study design (follow-up duration)	Target population for HNPCC test (Eligible /Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	Accuracy of HNPCC testing in predicting risk of CRC or other HNPCC-related cancers / Factors that affected the accuracy of the testing	Quality
Cederquist, 2001 Sweden	Retro (8 yr)	1st degree family members of 36 colon-endometrial caner probands and 43 colon-colon cancer probands. (ND/649)	649	23%	Proband<50 yr; MSI+	↑ CRC risk (SIR =21; 95%CI 12-35) ↑ All cancer risk (SIR =3.2; 95%CI 2.2-4.5)	B
				24%	Proband<50 yr; MSS	↑ CRC risk (SIR =7; 95%CI 3-14) ↑ All cancer risk (SIR =2.3; 95%CI 1.6-3.2)	
				18%	Proband>50 yr; MSI+	⇔ CRC risk ⇔ All cancer risk	
				34%	Proband>50 yr; MSS	⇔ CRC risk ⇔ All cancer risk	
Brown 1998 UK	Retro (ND)	Multiple primary group: 1st degree relatives of CRC probands, who developed a second primary in the HNPCC spectrum[1] (166/157)	128	8%	Amsterdam I and modified criteria	↑ CRC risk, compared to the single primary group (RR=3.2, p<0.00001) Single primary group: both HNPCC associated and non-HNPCC associated extracolonic cancers had expected frequency. Multiple primary group: extracolonic non-HNPCC cancers had expected frequency however, HNPCC associated extracolonic cancers had twice the frequency of general population.	C
		Single primary group: 1st degree relatives of single CRC patients (595/444)	444	0.7%			
Bradshaw, 2003 UK	Prosp (2 yr)	Asymptomatic individuals with a family history of CRC at a young age who received colonoscopy (448/190)	163	28%	Amsterdam I (or high risk)	Colonoscopy was complete to the cecum in 92%. No cases of CRC were detected. 4 patients (8%) in high-risk group had adenomas. In moderate group, overall five individuals had an adenoma (2%). Proportion of patients younger than 50 found to have an adenoma was significantly greater in the high-risk group than those in the moderate risk group (p=0.05).	C
				64%	Family history of CRC only (moderate risk)		
				8%	Low risk		

[1] CRC, stomach, urinary, ovary, or endometrial cancers

Table 25. Key Questions 8b and 8c in kindreds with HNPCC based on clinical and/or genetic criteria

Author, year Country	Study design (follow-up duration)	Target population for HNPCC test (Eligible /Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	Accuracy of HNPCC testing in predicting risk of CRC or other HNPCC-related cancers / Factors that affected the accuracy of the testing	Quality
Mutation testing							
Aarnio, 1998 Finland	Retro (43 yr)	50 HNPCC families in which a MLH1 gene mutation (47 families) or MSH2 gene mutation (3 families) had been detected (1,763/1,763)	1,763	100%	MLH1/MSH2	In mutation carriers, cumulative incidence rate at 70 years of age were 82% for CRC and 60% for endometrial cancer, compared with only 1.6% and 1.3%, respectively, in the Finnish population as a whole. The cumulative incidence of CRC was 100% in men and 54% in women. The cumulative incidence for gastric cancer was 13% and, for ovarian cancer 12%. For uroepithelial, kidney and bile-duct cancer and for brain tumors, the cumulative incidences ranged from 2 to 4% by 70 years of age.	B
Scott, 2001 Australia	Retro/ Prosp (ND)	HNPCC families meeting either Amsterdam (34%) or Bethesda (66%) criteria (ND/95)	95 families	18%	MLH1[2]	Relative standardized incidence rates and 95%CI of CRC: Mutation (-): 158.61 (132.8–189.4) MLH1 mutation (+): 196.76 (143.0–270.7) MSH2 mutation (+): 134.24 (99.1–181.8) Compared MSH2 mutation–positive group to MLH1 mutation–positive group: ⇔ Trend for lower CRC rate (p=0.087) ⇔ Frequency of extracolonic cancers Malignancies were overrepresented in all 3 groups compared with expected frequency in general population ⇔ For age at diagnosis compared MSH2 mutation–positive group to MLH1 mutation–positive group, adjusted for familial clustering. (MSH2 mutation-positive families=45.77 yr; MLH1 mutation-positive families=47.16 yr)	B
				16%	MSH2[f]	↑ For age at diagnosis compared mutation–positive group to mutation-negative group (+ 5 yr)	

[2] Genetic analysis was performed on a fresh blood specimen from the youngest living affected proband in each family.

Table 25. Key Questions 8b and 8c in kindreds with HNPCC based on clinical and/or genetic criteria (continued)

Author, year Country	Study design (follow-up duration)	Target population for HNPCC test (Eligible /Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	Accuracy of HNPCC testing in predicting risk of CRC or other HNPCC-related cancers / Factors that affected the accuracy of the testing	Quality
Plaschke, 2004 Germany	Retro (ND)	HNPCC families meeting Amsterdam I or II, plus Bethesda criteria. Selected members with MSI-low or -high, and abnormal IHC, plus MLH1/MSH2 mutation-positive, or MSH6 mutation-positive (706/183 families)	1,974	80% 20%	MLH1/MSH2 MSH6	Compared family members with pathogenic MSH6 mutations to those with MLH1/MSH2 mutations: ↓ Prevalence of malignant disease (29% vs. 37.5%) ↓ Cumulative risk by age to develop CRC or any tumor Compared family members with pathogenic MSH6 mutations to those with MLH1/MSH2 mutations: ↑ Median age of CRC onset (54 vs. 44) ↑ Median age of any tumor onset (51 vs. 43)	C
Vasen, 2001 Netherlands & Norway	Retro (ND)	Families with HNPCC meeting Amsterdam I or II or high suspicion of HNPCC (193/193)	138 families	25%	MLH1	Compared MSH2 carriers to MLH1 carriers: ↑ Lifetime risk of developing cancer at any age ($p<0.01$) ↑ Risk of CRC ($p=0.13$) ↑ Risk of developing endometrial cancer ($p=0.057$) ↑ Risk of developing cancer of the urinary tract by age 70 (12%, $p<0.05$) ↑ Risk of CRC comparing male to female MSH2 mutation carriers ($p<0.01$), ⇔ for MLH1 or MSH6 mutation carriers Mean age of CRC diagnosis higher in carriers of MSH6 compared with MLH1 or MSH2 (50 versus 43 and 44, respectively), statistical significance not reported	C

Table 25. Key Questions 8b and 8c in kindreds with HNPCC based on clinical and/or genetic criteria (continued)

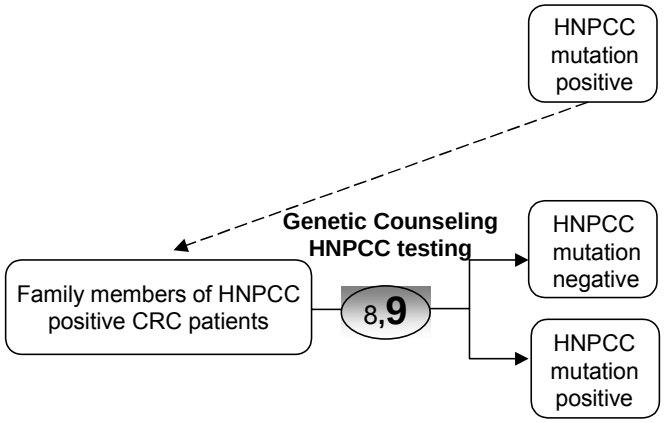
Author, year Country	Study design (follow-up duration)	Target population for HNPCC test (Eligible /Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	Accuracy of HNPCC testing in predicting risk of CRC or other HNPCC-related cancers / Factors that affected the accuracy of the testing	Quality
Lin, 1998 US	Retro (ND)	22 females and 27 males from 2 kindreds were identified with MSH2 mutation; 28 females and 28 males from 2 kindreds were identified with MLH1 mutations (ND/105)	105	100%	MLH1/MSH2	The cumulative incidence of CRC by age 60 was 84% in MLH1 and 71% in MSH2 carriers (p=NS) The cumulative incidence of CRC by age 60 was 96% in MSH2 males compared to 39% in MSH2 females (p=0.034), and 94% in MLH1 males compared to 63% in MLH1 females (p=NS) The cumulative incidence of extrocolonic cancers by age 60 was 11% in MLH1 and 48% in MSH2 carriers (p=0.016)	C
Other HNPCC testing							
Lindgren, 2002 Sweden	Retro (ND)	Patients who were undergoing colonoscopy surveillance were classified as having HNPCC, defined as meeting Amsterdam criteria, germline mutation, or MSI, HCC, TCR, or OCR (ND/304)	304	15%	HNPCC, at 70% risk[3]	Relative risk of developing adenoma before age 54 yr compared to reference group[4]: ⬆ HNPCC, at 70% risk group (RR=4.4. p<0.001) ⬆ TCR group (RR=3.1. p<0.001) No influence of sex on relative risk of developing adenoma before age 54 years	C
				16%	HNPCC, at 35% risk[5]		
				2%	HNPCC, at 17% risk[6]		
				8%	HNPCC, at 5% risk[7]		
				60%	HCC, TCR, or OCR		

⬆ Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
⬇ Statistically decreased ⇓ Decreased, but not statistically significant

[3] Germline mutation carriers (MSH2, MLH1, or hMSH6).
[4] Reference group was based on three published forensic autopsy studies used for estimates of adenoma prevalence in the normal population.
[5] Fist generation of HNPCC families.
[6] Second generation of HNPCC families.
[7] Subjects in HNPCC families who were under surveillance before testing but tested negative for mutation.

Key Question 9: What Are the Harms Associated With Informing/ Counseling Family Members or With Subsequent Testing for HNPCC Mutations?

Key Question 8a: What is the Efficacy of Pre-Test Genetic Counseling for Informing Family Members of Potential Risks and Benefits of Testing?



Studies were considered eligible for Key Question 8a if they summarized the efficacy of pre-test genetic counseling immediately after counseling and before performing genetic testing. Studies addressing Key Question 9 were considered together with those addressing Key Question 8a because there was substantial overlap in the studies addressing these questions. The studies addressed the long-term efficacy of pre-test genetic counseling or harms associated with screening high-risk individuals (such as by using clinical criteria, MSI, or IHC), genetic testing, and informing/counseling family members or with subsequent testing for HNPCC mutations.

Four studies addressed the efficacy of pre-test genetic counseling immediately after counseling and before performing MMR genetic testing[107,133-138] (Table 26). Of these, three were of B quality and one was of C quality.

Eight comparative and four qualitative studies addressed the harms associated with genetic testing for HNPCC mutation or with informing/counseling family members or with subsequent testing for HNPCC mutations[4,104-106,133-144] (Table 27). Of these, one study was of A quality, six of B quality, and five of C quality.

Summary of Findings. Overall pre-test genetic counseling had good efficacy in improving knowledge about HNPCC, and resulted in high likelihood of proceeding with genetic testing, satisfaction in decision to undergo genetic testing, and decreasing in depression and distress levels among family members of HNPCC probands or among asymptomatic individuals from HNPCC families. Family members of HNPCC who received positive MMR mutation test results had higher psychological distress levels and anxiety compared to those who received negative test results, but this difference generally disappeared with time. However, all of these psychological measures were within the normal ranges for the general populations. Available

evidence did not show differences in quality of life, comparing mutation carriers to non-carriers or the general population (Overview Table 3; Table 26-27).

Overview Table 3. Key Questions 8a and 9

Reported Outcomes	Body of evidence (study duration)	Summary
Decision to genetic testing	2 Prosp studies (1 mo to 1 yr)	• 1%-2% family members of HNPCC enrolled in the studies opted not to undergo genetic testing.
Shortened vs. extended pre-test counseling	1 RCT (3 mo)	• High satisfaction in both groups. • ⇔ Decision making outcomes or knowledge about HNPCC between groups. • Mutation carriers had higher psychological distress in extended pre-test counseling group but lower psychological distress in shortened pre-test counseling group, compared to non-carriers.
Satisfaction in decision to undergo testing	1 Prosp study & 1 RCT (1 mo to 1 yr)	• No short-term difference in satisfaction in decision to undergo genetic testing between carriers and noncarriers, but MMR mutation carriers were less satisfied in decision to undergo testing 1 year after testing.
Anxiety	6 Prosp studies (2 wk to 1 yr)	• ⇓ Anxiety levels immediately after genetic counseling • Anxiety levels of HNPCC family members were within normal ranges compared to the general population. • Generally there was no change or decrease in anxiety level over-time among non-carriers, while there was no change or increase in anxiety level among mutation carriers over-time. • MMR mutation carriers had higher anxiety levels than non-carriers shortly after genetic test results were revealed, but this difference generally disappeared with time.
Depression	5 Prosp studies (2 wk to 1 yr)	• ⇓ Depression levels immediately after genetic counseling • Depression levels of HNPCC family members were within normal ranges compared to the general population. • Conflicting results on changes in depression levels over-time • Conflicting results comparing MMR mutation carriers to non-carriers
Distress	5 Prosp studies & 1 RCT (2 wk to 1 yr)	• Psychological distress, cancer-specific distress (including Intrusive and avoidant thoughts about CRC or cancer worries), and distress from receiving test results generally decreased with time. • In general, MMR mutation carriers had higher cancer-specific distress levels than non-carriers.
Quality of life	2 Prosp studies (2 wk to 1 yr)	• QOL of HNPCC family members were within normal ranges compared to the general population. • ⇔ QOL compared MMR mutation carriers to non-carriers

⇑ Statistically increased ⇗ Increased, but not statistically significant
⇔ No statistically significant differences
⇓ Statistically decreased ⇘ Decreased, but not statistically significant

Gaps in the Literature. The results described above apply only to individuals who agreed to participate in the genetic studies and opted for genetic testing. Many individuals from HNPCC families chose not to participate in the study or pre-test genetic counseling. The reasons were often not described; however, they included fear of knowing the results, fear of insurance discrimination, survivor's guilt and disinterest.

The literature did not provide what key elements should be included in genetic counseling, and the effectiveness of different counseling strategies. Only a small RCT compared the efficacy of shortened to extended pre-test counseling and found no differences in psychological and decision making outcomes or knowledge about HNPCC between groups immediately after

counseling and before revealing the results of genetic testing.[133] At 3-month follow-up, mutation carriers in the shortened group experienced the least distress along with a high level of decision satisfaction, in contrast to carriers in the extended group who reported the most distress and least decision satisfaction. However, there were no details regarding baseline familiarity, education level and other potentially relevant characteristics across study groups to assure randomization was successful.

Some studies examined the factors that influenced decisions regarding genetic testing or the reasons for pursuing or for not pursuing genetic testing.[134-138,145-151] Although these studies (most of which were descriptive) were not specifically relevant to any of the Key Questions, they were clinically relevant and thus we attempted to summarize them in ancillary tables (Extra Table 1). People who were employed, who had higher education levels, who had CRC screening, and who had familial cancers were more likely to accept genetic testing.

Common reasons for not pursuing genetic testing include worry about losing health insurance, and concerns about coping with the emotional reactions if the test were positive. Women appeared to need more professional psychological support for coping with the results of genetic testing. These findings are important considerations for genetic counseling programs and support systems to minimize the harms associated with genetic testing for HNPCC mutations.

Findings from two surveys of genetic centers or insurance providers suggested the need for more empirical research on which components of pretest counseling are effective in promoting good psychological outcomes and the need to prevent insurance discriminations to the persons with genetic risk of CRC[152,153] (Extra Table 2).

Table 26. Key Question 8a. What is the efficacy of pre-test genetic counseling for informing family members of potential risks and benefits of testing?

Author, year Country	Study design (follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	Efficacy of pre-test genetic counseling	Quality
Atkan-Collan 2000; 2001a; 2001b; 2001c Finland	Prosp (1 mo & 1 yr)	Family members from 36 HNPCC families with known mutations at 50% risk (446/401)	334	At 1 year, pretest counseling was considered: Very useful: 49% Fairly useful: 40% Slightly useful: 10% 7 subjects (2%) not opting for genetic testing	B
Arver, 2004 Sweden	Prosp (1 yr)	Healthy women from 73 of 80 families with an identified HNPCC or BRCA1, BRCA2 mutation (114/90)	87	1 subject (1%) refused genetic testing because of worries about insurance and employment	B
Keller, 2002 Germany	Prosp (4-6 wk)	Unaffected individuals from families at risk for HNPCC (ND/34)	34	↓ Anxiety (HAD) ↓ Depression (HAD) ↓↓ Cancer-specific distress (IES scales)	B
Brain, 2005 UK	RCT (3 mo)	Unaffected individuals from families with a known HNPCC mutation (51/34)	All: 27 15 in shortened; 11 in extended pre-test counseling	High satisfaction in both groups. ⇔ In psychological & decision making outcomes or knowledge about HNPCC between groups.	C

↑ Statistically increased ⇑ Increased, but not statistically significant (or statistical analysis was not performed)
⇔ No statistically significant differences
↓ Statistically decreased ⇓ Decreased, but not statistically significant (or statistical analysis was not performed)

Table 27. Key Question 9. What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations?

Author, year Country	Study design (follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Measures of psychological impact after revealing genetic testing results	Observed impact (Key Question 9)			Quality
						Test receivers vs. normal ranges[8]	Follow-up vs. baseline (changes over time)	Mutation positive vs. negative	
Quantitative, Comparative Studies									
Gritz, 2005 US	Prosp (2 wk, 6 mo & 1 yr)	Unaffected[9] persons at least 18 years old with HNCC defined by Amsterdam or suggestive family history followed by mutation testing (178/66)	66	28%	Anxiety (State-Trait)		Mutation (+): ⇑ 1 yr Mutation (-): ⇔ at all f/up	⇑ at 2 wk; ⇔ at 6 mo; ⇑ 1 yr	A
					Depression (CES-D)		Mutation (+): ⇑ 1 yr Mutation (-): ⇔ at all f/up	⇑ at 2 wk; ⇓ at 6 mo; ⇑ 1 yr	
					Quality of life (SF-36)		⇔ at all f/up	⇔ at all f/up	
					Cancer worries[10]		Mutation (+): ⇔ 1 yr Mutation (-): ⇓ at all f/up	⇑ at all f/up	
					Distress from receiving test results (RIES)		⇓	⇑ at 2 wk & 6 mo; ⇔ 1 yr	
Atkan-Collan 2000; 2001a; 2001b; 2001c Finland	Prosp (1 mo & 1 yr)	Family members from 36 HNPCC families with known mutations at 50% risk (446/401)	334	30%	Need for support			⇑ at 1 mo	B
					Satisfaction in decision to undergo testing			⇔ at 1 mo; ⇓ at 1 yr	
					Fear of cancer, or fear of dying (n=271)[11]		⇓	⇑ at 1 mo; ⇑ at 1 yr	

⇑ Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
⇓ Statistically decreased ⇓ Decreased, but not statistically significant

[8] Published population levels or the normal ranges.
[9] "Unaffected" persons included relatives with no personal history of cancer.
[10] Lerman C, Trock B, Rimer BK, et al: Psychological and behavioral implications of abnormal mammograms. Ann Intern Med 114:657-661, 1991.
[11] Subjects who had received genetic testing and returned all 3 questionnaires (n=271, 61% of the whole group).

Table 27. Key Question 9. What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations? (continued)

Author, year Country	Study design (follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Measures of psychological impact after revealing genetic testing results	Observed impact (Q9)			Quality
						Test receivers vs. normal ranges[12]	Follow-up vs. baseline (changes over time)	Mutation positive vs. negative	
Meiser 2004 Australia	Prosp (2 wk, 4 mo & 1 yr)	Individuals from families with known HNPCC mutation who chose to receive predictive genetic testing and had no personal history of an HNPCC-related cancer (134/114)	114[13]	28%	Intrusive and avoidant thoughts about CRC (Miller behavioral style scale)		Mutation (+): ↑ at 2 wk; ⇔ at 4 mo & 1 yr Mutation (-): ↓ at all f/up	↑ at all f/up	B
					Depression (HAD)		Mutation (+): ↓ at 2 wk & 4 mo; ⇔ at 1 yr Mutation (-): ↓ at all f/up	⇔ at all f/up	
					Anxiety (State-Trait)		Mutation (+): ⇔ at all f/up Mutation (-): ↓ at 2 wk; ⇔ at 4 mo & 1 yr	↑ at 2 wk; ⇔ at 4 mo & 1 yr	
Arver, 2004 Sweden	Prosp (1 yr)	Healthy women from 73 of 80 families with an identified HNPCC or BRCA1, BRCA2 mutation (114/90)	87	36%	Quality of life (SF-36)	⇔		↓ Vitality scores; ⇔ Other SF-36 subscales	B
					Anxiety (HAD)	⇔	↓	⇔	
					Depression (HAD)	⇔	↓ Mutation positive; ↑ Mutation negative	↓	

[12] Published population levels or the normal ranges.

[13] N=97 returned 2-week-followup questionnaire, N=98 returned 4-month-followup questionnaire; N=95 returned 1-yr-followup questionnaire.

Table 27. Key Question 9. What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations? (continued)

Author, year Country	Study design (follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Measures of psychological impact after revealing genetic testing results	Observed impact (Q9)			Quality
						Test receivers vs. normal ranges[14]	Follow-up vs. baseline (changes over time)	Mutation positive vs. negative	
Claes, 2005 Belgium	Prosp (1 yr)	Self-referred unaffected persons who opted for genetic testing for HNPCC (79/79)[15]	72	50%	Anxiety (State-Trait)		↓	⇔	B
					Cancer-specific distress (IES scales)		↓	↑	
					Reported >1 disadvantages of knowing genetic test results			50% vs. 20%	
Murakami, 2004 Japan	Prosp (1 mo)	Unaffected relatives whose family members had been identified as carrying the MLH1/MSH2 mutation, age ≥20 years, and opted for genetic testing (16/15)	15	33%	Minor depression (DSM-III-R and DSM-IV scales)			0%	B
					Guilt (mutation carriers only)			1/5 (20%)	
Claes, 2004 Belgium	Prosp (1 mo)	Self-referred unaffected persons who opted for genetic testing for HNPCC (48/48)	40	48%	Anxiety (State-Trait)		↓	⇔	C
					Cancer-specific distress (IES scales)		⇓	⇔	

[14] Published population levels or the normal ranges.
[15] Might have some subjects overlapped with Claes, 2004 (337) study.

Table 27. Key Question 9. What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations? (continued)

Author, year Country	Study Design (Follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Results		Quality
Brain, 2005 UK	RCT (3 mo)	Unaffected individuals from families with a known HNPCC mutation (51/34)	All: 27 15 in shortened; 11 in extended pre-test counseling	30% 33% 27%	Psychological distress[16] Satisfaction in decision to undergo testing[17]	↓ Shortened group; ↑ Extended group ⇔ in both intervention groups	C
Qualitative, Non-Comparative Studies							
Wagner, 2005 Netherlands	Prosp (6 mo to ~8 yr)	1st and 2nd degree relatives who were germline mutation carriers from 24 HNPCC families (115/94)	70	100%	84% approved of how they were informed of HNPCC genetic testing 97% approved information given during counseling 4/10 had life, disability or mortgage insurance problems, none had health insurance		B
Lynch 1997 US	Prosp (ND)	High-risk members from 4 extended HNPCC families that were selected from Creighton's HNPCC resource, which comprised about 100 HNPCC extended kindreds (219/130)	130	36%	Of the 89 members who did not enroll in the study, 67% did not respond to the invitation, 22% had their counseling postponed, and 10% refused, citing fear of knowing, fear of insurance discrimination, survivor's guilt and disinterest. When mutation positive, 20% exhibited sadness, particularly because of concern of the potential risk of transmission of the mutation to their children. Others were concerned about their personal cancer destiny, particularly the possibility of an unfavorable prognosis that could lead to their early death and may put their children in jeopardy of not having a biological parent to care for them. 18/130 were concerned about sharing information with their insurance company.		C
Lynch, 1996 US	Retro (ND)	Family members of Native American CRC patients who received MLH1 genetic testing (ND/51)	44	ND	Many family members believed the family's fate was influenced by factors such as fear, a taboo, or a curse. 4 family members denied their heightened cancer genetic risk. 23 individual (52%) chose to receive genetic test results and DNA-base counseling. All but 1 of the 23 individual counseled appeared to understand what was told them. The family who were given negative results exhibited profound relief and happiness. Individuals with positive results were for the most part stoic.		C

[16] Goldberg D, Williams P. A User's Guide to the General Health Questionnaire. Windsor: NFER-Nelson, 1988.
[17] Holmes-Rovner M, Schmitt N, Rothert ML et al. Patient satisfaction with health care decisions: the satisfaction with decision scale. Med Decis Making 1996: 16: 58–64.

Table 27. Key Question 9. What are the harms associated with informing/counseling family members or with subsequent testing for HNPCC mutations? (continued)

Author, year Country	Study Design (Follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	Results	Quality
Mesters, 2005 Netherlands	Survey (N/A)	Persons from the database of the Netherlands Foundation for the Detection of Hereditary Tumors (44/30)	30	37%	Disclosure was stimulated if people felt morally obliged to do so or when they anticipated regret if something preventable would happen. Presence of external cues (e.g., professionals) appeared important for disclosure as well. Disrupted and tense family relations were reasons not to disclose, as well as young age of the message recipients and negative experience at their first attempt to disclose. Disclosure was restricted to the nuclear family. A personal approach was preferred. Participant disclosed only the presence of the hereditary defect and the possibility of testing. It was mostly considered the recipients' responsibility and own choice to obtain further information.	C
Peterson 2003 US	Props (15 mo)	A proband with HNPCC mutation and family members with >50% risk of carrying a mutation from 5 HNPCC families (ND/39)	39/25[18]	52%	The identification of a cancer-predisposing gene mutation was new information that confirmed their beliefs, and families were not shocked or surprised by the news. This information was not viewed as a medical crisis. Compared with mutation carriers, those who tested negative for a mutation and those who did not test perceived those topics as less personally relevant and were less involved in discussing the mutation and the need for genetic testing.	C

⬆ Statistically increased ⇑ Increased, but not statistically significant ⇔ No statistically significant differences ⬇ Statistically decreased ⇓ Decreased, but not statistically significant

References for standardized instruments:

Cancer-specific distress (IES scales): Horowitz MJ, Wilner N, Alvarez W. Impact of Event Scale: a measure of subjective stress. Psychosom Med 1979;41:209–18.
CES-D: Center for Epidemiologic Studies Depression.
DSM-III-R: Association Psychiatric Association. Diagnostic and statistical manual of mental disorders. 3rd edition, revised. Washington, DC: American Psychiatric Press, 1987.
DSM-IV: Association Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Washington, DC: American Psychiatric Press, 1994.
Hospital anxiety and depression scale (HAD): Goldberg D, Williams P. A User's Guide to the General Health Questionnaire. Windsor: NFER-Nelson, 1988.
Miller behavioral style scale: Horowitz MJ, Wilner N, Alvarez W. Impact of Event Scale: a measure of subjective stress. PsychosomMed1979: 41: 209–218. Zilberg NJ, Weiss DS, Horowitz MJ. Impact of Event Scale: a cross-validation study and some empirical evidence supporting a conceptual model of stress response syndromes. J Consult Clin Psychol 1982: 50 (3): 407–414.
SF-36: Ware JE Jr, Gandek B. Overview of the SF-36 Health Survey and the International Quality of Life Assessment (IQOLA) Project. J Clin Epidemiol 1998; 51: 903–12.State-Trait Anxiety: Van der Ploeg HM, Defares PB, Spielberger CD. Handleiding bij de Zelfbeoordelingsvragenlijst: een Nederlandstalige bewerking van de Spielberger State-Trait Anxiety Inventory. Lisse: Swets and Zeitlinger, 1980.

[18] Of the 39 interviewed members, 25 donated blood for genetic testing.

Extra Table 1. Studies examined the factors that might affect the acceptance of genetic testing

Author, year	Findings
Atkan-Collan 2000; 2001a; 2001b; 2001c	Acceptors differ from decliners in more often having a spouse or partner, being employed and having higher education. On multivariate analysis, the only significant factor for accepting genetic testing was employment status. Most common reasons cited against undergoing testing were: being old already and having no children (23%), would not trust result (15%), timing found unsuitable because of pregnancy 1 (8%), no reason given (54%) 53% indicated that they might have used professional psychological support along with counseling if it had been provided. The support might have been used by women with children more than by others.
Berth, 2002	Survey sent to 2076 persons of the general population and 36 patients at high risk for HNPCC or FAP (familial adenomatous polyposis) "Overall, both the general population and at-risk persons hold a favorable attitude toward genetic testing".
Brodersen, 2004	Cross sectional study to examine anticipated reactions to genetic testing for HNPCC in 437 asymptomatic people with a family history of colorectal cancer. More women anticipated feeling fairly or extremely worried if they did not have the test (36 versus 23 percent, p =0.009) or if the test was positive (64 versus 40 percent, p =0.008). More women anticipated that they would feel fairly or extremely regretful if the test showed that they were at high risk (30 versus 20 percent, p =0.03). More women thought that they would feel fairly or extremely angry if the test showed that they were at high risk (33 versus 13, p <0.001). There was only one significant difference between men and women in anticipated reaction following genetic testing: if at low risk more women then men would try to adopt a healthier lifestyle (p=0.007). When asked if they would have more screening if found to be at high risk, 416/432 (96%) said that they probably (23%) or definitely (74%) would.
Codori, 1999	Evaluated uptake of genetic testing for HNPCC among first-degree relatives of CRC by comparing test acceptors (N=77) and decliners (N=181). Significant predictors of acceptance on multivariate analysis were: perceived ability to cope with a positive gene test result, risk perception, frequency of thoughts about CRC (not at all or rarely versus sometimes or more) and CRC screening history. Coping with positive test results: ≥90% confident OR 4.0 (95% CI 1.53-10.3, p=0.005) CRC screening: never had a colonoscopy (OR 0.45, 95% CI 0.21-0.93, p=0.030) Cancer thoughts: Rarely/never versus sometimes, often, a lot: OR 0.44 95% CI 0.17-1.13, p =0.088)
Graff, 2005	Investigated experiences of 12 individuals with HNPCC (pathogenic mutation) in a semi structured telephone interview. All patients had been asked to inform family members. 12 subjects (4 men and 3 women). Respondents older than nonrespondents (55 versus 49) had more cancer diagnoses per individual and were more likely to be female (no statistical comparisons made). All had told some (mainly immediate family) at-risk family members about predictive testing. Family reactions ranged form interest to disinterest. Men expressed a need for guidance and support in communicating to relatives more than women. Letters and booklets were thought to enhance the quality of information but further aids were unlikely to increase the number of relatives made aware of predictive testing by the probands.
Hadley, 2003	Elucidate factors affecting decisions regarding genetic testing in individuals form families with newly identified HNPCC. 104/165 (63%) eligible patients (including index cases and 1st degree relatives agreed to participate. This included 54 probands (of whom 87% agreed to participate) and 111 family members of whom 51% agreed to participate. Awareness of genetic testing: those at higher household income levels were more aware of genetic testing for cancer (p=0.001) and colon cancer (p=0.009) than those at lower household income levels. There was no significant association between participants' awareness and age, sex, personal cancer history or number of 1s-degree relatives. Perceived risk of being a carrier: Participant's feelings about their chances of getting colon cancer were significantly associated with their beliefs about the likelihood that they carry a mutation (p<0.001)

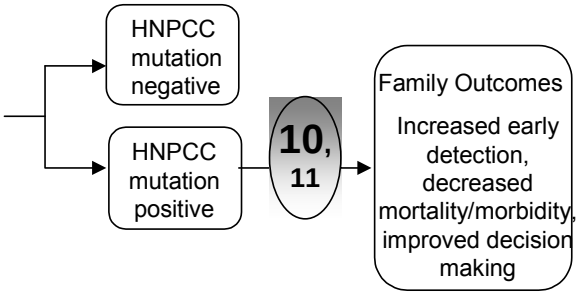
Extra Table 1. Studies examined the factors that might affect the acceptance of genetic testing (continued)

Author, year	Findings
	Intentions toward genetic testing: The intention to pursue genetic testing was found to have a positive association with participant's beliefs that cancer may be explained by family heredity (p=0.006). Concern about the psychosocial effect of genetic testing on the family demonstrated a negative association with their intention to pursue testing (p=0.001). Participant's concerns about their ability to handle the emotional aspects of genetic test results demonstrated a negative association with their intentions to pursue testing (p<0.001). No association between age, sex or cancer status in regard to their intentions toward genetic testing. Reasons for pursuing genetic tests: 50% of respondents believe that the most important reason for undergoing genetic testing was to learn about their children's risk. Second most important reason (17%) was to guide cancer screening; third (13%) as to confirm their belief that they carry a mutation. With respect to the importance of genetic testing for reproductive decision making, a statistically significant difference was detected between those younger than the median age of 43 or older (p=0.002). Reasons for not pursuing testing: Worry about losing health insurance (39%), concerns about how it might affect the family (27%), concerns about handling the results emotionally (10%). A statistically significant difference was detected between those younger than the median age of 43 compared with those older with respect to concerns about handling the emotional aspects of genetic testing (p=0.006). Testing decisions: 44/54 (81%) of eligible probands eventually chose to undergo genetic testing for HNPCC. 56/111 eligible first-degree relatives chose to pursue genetic testing (intention-to-treat analysis). Of those agreeing to participate in the study, 44/47 (94%) probands agreed to undergo genetic testing and 56/57 (98%) of family members agreed.
Keller, 2004	Of 140 eligible patients and their families, 36 families participated in the information session, 25 participants were patients, and 11 were spouses or relatives of patients who had died or were too ill to attend. Participation rate 26%. Of the 104 nonparticipating patients addressed by mail, 61 responded (59%), 12 died, 48 returned a completed questionnaire. Sample here consisted of 73 patients: 25 participants and 48 nonparticipants. Among participants, 60% reported being very worried because of familial cancer; among non-participants 35% reported being very worry because of familial cancer (P<0.05). 28% of participants reported impairment of daily life because of these worries compared to 19% of nonparticipants (difference not significant). Similarly, perceived distress of spouse and family from patient's point of view was higher in participants than in nonparticipants (difference not significant). Regardless of whether patients participated in the information session, most were in favor of genetic testing. Twice as many nonparticipants (25%) as participants (13%) were undecided about whether to accept genetic testing. All participants definitely expressed the wish to know whether a mutation is present in the family, compared to 81% of nonparticipants.
Lerman, 1996	This was a pilot study in preparation for a prospective study of genetic testing for HNPCC-associated mutations. 24 consecutive index colon cancer patients (who did not fulfill the Amsterdam criteria) identified 61 first-degree relatives for the study (index patient consent rate = 83%). Of these first degree relatives, 44 (72%) completed the interview, 2 (2%) declined, 16 (26%) could not be reached. Anticipated reactions to positive and negative results of a genetic test: Over ½ of respondents indicated that they would become depressed or very anxious if they tested positive. Most subjects reported that they would react by changing their health habits, including diet and screening behaviors. In response to negative test result, about 1/3 of respondents anticipated they would feel guilty and ½ would still worry because the test might be wrong. About ½ of respondents expected that they would decrease their use of screening tests and make fewer attempts to reduce dietary fat if they tested negative.

Extra table 2. Surveys of genetic centers or insurance providers

Author, year	Findings
Brain, 2003	Survey obtained from 16 regional genetic centers involved in predictive testing for HNPCC. Purpose was to gain evidence regarding core elements of the pre-test counseling protocol for HNPCC. 16/20 centers participated. One declined because of few patients counseled. No other details provided. Average number of pre-test counseling sessions was 2 (range 1-3) with an average of 4 weeks between sessions. Minimum number of sessions was 1 (range 1-2). Counselors included clinical geneticists and genetic nurse and gastroenterologists. Some included home visits, psychiatric consultation, explicit circumstances in which a client would be excluded or postponed from testing. Four centers reported that they were considered shortening the counseling protocol. Centers described a total of 144 topics as being core topics of pre-test counseling.
Norum 2000	11 health insurers in Norway were mailed a questionnaire to evaluate two hypothetical individuals' requests for insurance; one has HNPCC mutation, the other has BRCA1/BRCA2. 9/11 insurers responded. 2 companies reported results on personal indemnity insurance. There were no restrictions for individuals with a genetic risk of breast CA when seeking life insurance or disability pension. One company raised premium on the one with genetic risk of CRC. Another company offered standard or higher rate of premium on personal indemnity insurance in the person with genetic risk of CRC.

Key Question 10a: What Are the Management Options for Family Members of CRC Patients Who Have a Positive HNPCC Test? b1: Does the Identification of HNPCC Mutations Lead to Improved Outcomes in Terms of Decision Making by Patients, Family Members and Providers, or Public Health Policy? b2: Does the Identification of HNPCC Mutations Lead to Improved Outcomes in Terms of Early Detection and Mortality/Morbidity of Patients, and Family Members?



Below we summarize management options for family members of CRC patients or asymptomatic individuals from HNPCC families (based on clinical or genetic definitions) that were reported in the literature. This is not necessarily a complete list of management options.

Many studies described management options for family members of CRC patients or asymptomatic individuals from HNPCC families. We encountered the following in the studies included in this report.

- Annual colonoscopy beginning at age 20 to 25 or 5 years earlier than the youngest affected family members.[140,154-159]

- Colonoscopy or double contrast barium enema and sigmoidoscopy at two- or three-year intervals.[42,155,160-162]

- Endometrial screening from age 30 to 35 generally with annual or biennial transvaginal ultrasound (TVU).[44,45,154,157,158]

- Endometrial sampling in premenopausal women and annual TVU and CA 125 testing for postmenopausal women.[45,154]

- Prophylactic oophorectomy and hysterectomy (TAH) from ages 30 to 35 or when childbearing was complete.[154]

- Screening for stomach, duodenum or urinary tract cancers.[157]

- Prophylactic hysterectomy with or without bilateral salpingo-oophorectomy.[163]

- 1.5 g calcium daily in a form of calcium carbonate tablet.[164]

- Biennial breast mammography starting at age 35.[158]

We included studies evaluating all forms of cancer related to HNPCC since family members of CRC probands are potentially at risk for all such cancers.

Six studies examined the impact of mutation testing on the decision to undergo specific management recommendations among family members of CRC patients or asymptomatic individuals from HNPCC families[139-141,154,155,165] (Table 28). Of these, four were of B quality and two were of C quality. No study examined the impact of HNPCC mutation testing on public health policy or decision making by insurance providers.

Two studies (in three publications) of B and C quality indirectly addressed outcomes of early detection and mortality/morbidity in relation to the identification of MMR mutations in family members of CRC probands or asymptomatic individuals from HNPCC families[42,112,160] (Table 29). These studies were limited by potential selection bias, and/or unclear effects from treatments or subsequent interventions.

Summary of Findings. Identification of HNPCC mutations was associated with improved outcomes in terms of decision making to undergo screening for cancers in family members of HNPCC.

Survival was increased among asymptomatic HNPCC members who received colonoscopy screening regularly, regardless of their mutation status (Overview Table 4; Table 28-29).

Overview table 4. Key Question 10

Reported Outcomes	Body of evidence (study duration)	Summary
Decision making	6 Prosp studies (1 mo & 1 yr)	• Compared mutation carriers with non-carriers: ↑ Intention to undergo colonoscopy & colonoscopy within 1 yr after genetic testing ↑ TVU within 1 yr after genetic testing ↑ Endometrial sampling within 1 yr after genetic testing • Following a positive test result, 67% were considering prophylactic colectomy. 87% were considering prophylactic total abdominal hysterectomy and bilateral salpingo-oophorectomy
Age at onset CRC	1 Retro study (~8 yr)	• ⇔ Comparing mutation carriers to non-carriers with MSI • Younger, comparing mutation carriers to non-carriers with MSS
Frequency of CRC	1 Prosp study (15 yr)	• ↓ HNPCC members who received colonoscopy screening regularly, regardless of mutation status
Survival of family members	1 Prosp (15 yr)	• ↑ Among asymptomatic HNPCC members who received colonoscopy screening regularly, regardless of mutation status

↑ Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
↓ Statistically decreased ⇓ Decreased, but not statistically significant

Gaps in the Literature. Identification of HNPCC mutation alone cannot lead to improved outcomes in terms of mortality or morbidity unless persons are willing to take subsequent actions or interventions. Thus, the Key Questions above have two main components: 1) whether the identification of HNPCC mutations led to improved outcomes in terms of decision making by patients, family members and providers, or public health policy, and 2) whether the decision making subsequently leads to improved outcomes in terms of mortality or morbidity. Studies that reported outcomes relating to subsequent management options or interventions in HNPCC family members are summarized later with Key Question 11.

No study examined the impact of HNPCC mutation testing on public health policy or decision making by insurance providers on an actual cohort of patients. However, a survey of 11 health insurers in Norway evaluated two hypothetical individuals' requests (one who had an HNPCC mutation; the other who had a BRCA1/BRCA2 mutation) for insurance[153] (Extra Table 2). The insurers raised premiums on the one with genetic risk of CRC but not in the patient with a genetic risk of breast cancer.

HNPCC family members who were mutation carriers were more likely undergo screening and treatment for preventing cancers than non-carriers. Thus, the data presented above suggest that screening and testing for HNPCC mutations improves acceptance of screening procedures, at least in individuals who agree to undergo screening and testing.

Whether this benefit translates into improved morbidity or mortality is less clear from the available literature. A study that attempts to examine whether the identification of HNPCC mutations leads to improved mortality or morbidity should ideally control for the differences in the rates and intensity of screening or treatments for cancers. However, most studies did not provide such details. Furthermore, there may be complex interactions with age, gender, and time,since identification of HNPCC mutation, compliance with planed screening programs or treatments, and mutation status. No study considered all of these factors together. Large HNPCC registries with active data collection for the elements described above are needed.

Although not specifically asked in the Key Questions, a few studies reported factors that influenced compliance with screening (such as socioeconomic status and perceived barriers to screening).[166,167] We considered such observations clinically important and thus summarize them in ancillary tables. However, there were few such studies (Extra Table 3).

Table 28. Key Question 10b1. Does the identification of HNPCC mutations lead to improved outcomes in terms of decision making by patients, family members and providers, or public health policy?

Author, year Country	Study design (Follow-up duration)	Target population for genetic testing (Eligible /Enrolled N)	N evaluated	% Mutation positive	% Colonoscopy before genetic testing	Management options recommended when mutation positive	Outcome measures	Effects: Mutation positive vs. negative	Quality
Collins, 2005 Australia	Prosp (1 yr)	Individuals who had never had CRC or any of the cancers associated with HNPCC (134/114)	114/98[19]	28%	3/12 (25%) <25 yr old and 74/101 (73%) ≥25 yr old reported a colonoscopy	Annual colonoscopy beginning at age 25 or 5 years earlier than youngest affected family members Endometrial screening considered from age 30-35 or even 25 generally with annual TVU and endometrial sampling, in addition to CA 125 testing for postmenopausal women Prophylactic oophorectomy and TAH also considered for ages 30 to 35 or when childbearing is complete	Colonoscopy within 1 yr after genetic testing	↑ (71% vs. 12%) $_{adj}$OR: 20 (95%CI 5.8-68)	B
							Transvaginal ultrasound (TVU) within 1 yr after genetic testing	↑ (47% vs. 10%, p=0.004)	
							Endometrial sampling within 1 yr after genetic testing	↑ (53% vs. 5%, p<0.001)	
Halbert, 2004, US	Prosp (1 yr)	Family members with a 25% risk of having HNPCC mutation (ND/222)	98	22%	Overall: 62% 36% (8/22) of carriers, 27% (13/49) of noncarriers, and 26% (7/27) testing decliners)	ND	Colonoscopy within 1 yr after genetic testing	↑ (73% (16/22) of mutation carriers and 16% (8/49) noncarriers and 22% (6/27) testing decliners	B

[19] Baseline/follow-up N.

Table 28. Key Question 10b1. Does the identification of HNPCC mutations lead to improved outcomes in terms of decision making by patients, family members and providers, or public health policy? (continued)

Author, year Country	Study design (Follow-up duration)	Target population for genetic testing (Eligible/Enrolled N)	N evaluated	% Mutation positive	% Colonoscopy before genetic testing	Management options recommended when mutation positive	Outcome measures	Effects: Mutation positive vs. negative	Quality
Claes, 2005 Belgium	Prosp (1 yr)	Self-referred unaffected persons who opted for genetic testing for HNPCC (79/79)[20]	72	50%	65% (69% for mutation positive; 61% for mutation negative)	Colonoscopy within 1 year if not done in previous year	Colonoscopy within 1 yr after genetic testing	⇑ (75% vs. 0%)	B
							Compliance to recommendation for management options	100% in mutation positive	
Hadley, 2004 US[21]	Prosp (1 yr)	Members of families with newly identified HNPCC with any of the following features: HNPCC-associated cancer demonstrating MSI or a family history suggestive of HNPCC and 1st-degree relatives at 50% risk of inheriting the mutation (111/57)	56	30%	54% (41% for mutation positive; 59% for mutation negative)	Colonoscopy every 1 to 3 years	>1 colonoscopy within 1 yr after genetic testing	⇑ (53% vs. 8%)	B
							Colonoscopy use	⇑ (OR=62, 95%CI 5.8-653)	
							Nonadherent with colonoscopy recommendations[22]	⇑ (OR=7.5, 95%CI 1.3-42)	
Lynch 1997 US	Prosp (ND)	High-risk members from 4 extended HNPCC families that were selected from Creighton's HNPCC resource, which comprised about 100 HNPCC extended kindreds (219/130)	130	36%	ND	ND	Following a positive test result, 67% were considering prophylactic colectomy. 87% were considering prophylactic total abdominal hysterectomy and bilateral salpingo-oophorectomy. Those who were mutation negative were not asked.		C
Claes, 2004 Belgium	Prosp (1 mo)	Self-referred unaffected persons who opted for genetic testing for HNPCC (48/48)	40	48%	65%	ND	Intension to undergo colonoscopy	⇑ (100% vs. 0%)	C
							Intension to undergo endometrium examination	⇔ (100% in both groups)	

⇑ Statistically increased ⇑ Increased, but not statistically significant ⇔ No statistically significant differences

[20] Some subjects may have overlapped with Claes, 2004 (337).
[21] Same population as Hadley, 2003 (946).
[22] The recommendations were colonoscopy every 1 to 3 years for mutation-positive persons and general population screening for mutation-negative persons (i.e., flexible sigmoidoscopy every 3 to 5 years after the age of 50 years).

Table 29. Key Question 10b2. Does the identification of HNPCC mutations lead to improved outcomes in terms of early detection and mortality/morbidity, of patients, family members?

Author, year Country	Study Design (Follow-up duration)	Target population for genetic testing (Eligible/Enrolled N)	N evaluated	% Positive HNPCC test	Description of HNPCC tests	% Received interventions or treatments	Outcome measures	Effects: Mutation positive vs. negative	Quality
Mutation testing									
Jarvinen, 1995; 2000 Finland	Prosp (15 yr)	Asymptomatic member of 22 HNPCC families at 50% risk, meeting Amsterdam I criteria or other suggestive family history (ND/252)	"Study group": 133	33%	MMR gene mutation	Study group: Initial colonoscopy or double contrast barium enema and sigmoidoscopy and then repeated examinations at 3-year intervals. 4 subjects underwent total abdominal colectomy (3 in study group; 1 in control group), and 1 subject (study group) received a segmental resection of the sigmoid colon.	Frequency of CRC	⇓ 8 (6%) in study group vs. 19 (16%) in control group (p=0.01) ⇓ Among mutation-positive subjects, 18% (8 of 44) in study group and 41% (19 of 46) in control group (p=0.02)	B
			"Control group": 118[a]	40%			Mortality	0/133 in study group vs. 9/119 (8%) in control group (p<0.001) ⇓ (RR=0.35; 95%CI 0.12-0.99)	
Benatti, 2001 Italy	Retro (8.2, 6.7, and 9.4 yrs for group A, B, and C respectively)	29 HNPCC families met inclusion criteria: 10 carried MMR gene mutations (Group A), 10 were characterized by MSI phenotype but not by MMR gene mutation (Group B), and 9 did not show mutations or MSI (Group C)	Group A: 361	100%	MMR gene mutation	ND	Age at onset CRC	⇔ Compared Group B (or MSI) Younger compared Group C (or MSS) (p=0.001)	C
			Group B: 241	0%					
			Group C: 355	0%					

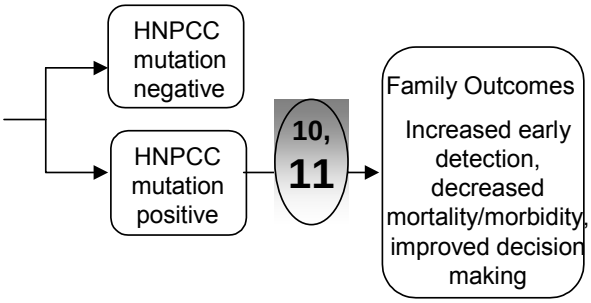
⇑ Statistically increased ⇑ Increased, but not statistically significant ⇔ No statistically significant differences ⇓ Statistically decreased ⇓ Decreased, but not statistically significant

[a] Of those invited to participate, 78 eligible declined colonoscopy screening and 40 could not be traced; these patients constituted the control group. After genetic testing, all mutation-positive subjects were recommended the screening program, whereas endoscopies were discontinued in mutation-negative subjects in whom no adenomas were found 20% received screening when requested.

Extra Table 3. Factors that might affect the compliance with CRC screening

Author, year	Findings
Adams, 2003	Secondary analysis of data recorded for a study on polyp prevention (a database) on chemopreventive effects of aspirin. Study had included 600 patients with HNPCC; of these 154 were available for analysis and full data were available on 134. Of these 134, 49 had a personal history of CRC. Socioeconomic status assessed using the Town-Deprivation Score (TDS) of the current enumeration district of residence. This score is based on relative material deprivation based on census data. There was a statistically non-significant correlation between the TDS and the age of first resection indicating that individuals in less deprived areas tended to have their colorectal cancer resected at an earlier age than those living in more deprived areas.
Bleiker, 2005	178 patients at high risk for CRC (27 Amsterdam I criteria; 43 familial CRC occurrence). All had received counseling from a geneticist and were advised to have periodic colonoscopy. Objective and self-reported compliance. 149/178 returned a completed questionnaire. Respondents significantly older than nonrespondents. Average f/u 5.8 years 61% of study sample fulfilled Amsterdam I Objective compliance data available for 132/149. Of these 95 (72%) were found to be compliant with screening advice. Of 37 (28%) noncompliant, 20 had postponed screening for at least 1 year, 13 were screened only once, and 4 never underwent screening. Compliers were significantly younger than noncompliers and there was a nonsignificant trend to increased level of education, mutation status, and type of colon screening. No difference between perceived colon cancer risk. Noncompliers significantly more likely to perceive barriers to screening. Noncompliers rated screening as being more embarrassing. On multivariate analysis only the perceived number of barriers to screening was associated significantly with noncompliance (OR 1.2, 95% CI 1.1-1.3). Of self-reported data, noncompliance was associated with embarrassment with screening procedure and sigmoidoscopy rather than colonoscopy. Compliers were significantly more likely to have remembered receiving a reminder letter.

Key Question 11: What Are the Harms Associated With Subsequent Actions or Interventions for Family Members?



Due to limited data on harms associated with subsequent management options or interventions, we broadened the scope of this question to include studies that reported any outcome relating to subsequent management options or interventions in HNPCC family members.

Nine studies reported outcomes related to subsequent actions or interventions among family members. Of these, six were of B quality and three were of C quality. Four of these nine studies reported harms or adverse events associated with subsequent actions or interventions for family

members. In addition, one study of C quality examined the psychological impacts associated with colonoscopies[44,45,156-159,161-164] (Table 30). Some of these studies did not have a control group of subjects who declined to undergo surveillance, and most results did not adjust for potential confounders such as age, personal history of cancer, and educational levels.

Summary of Findings. Fewer than 0.5 percent of family members experienced harms associated with screening or surveillance examination or surgical procedures. There was some negative psychological impact associated with colonoscopies.

HNPCC family members who took subsequent actions or interventions had a lower risk of developing HNPCC-related cancers and lower mortality rates, compared to those who did not take actions (Overview Table 5; Table 30).

Overview Table 5. Key Question 11

Reported Outcomes	Body of evidence (study duration)	Summary
Harms	3 Retro studies (0.1-35 yr), 1 Prosp study (6 mo to ~8 yr), & 1 RCT (12 wk)	• 1 patient (0.25%) had a cardiac arrest due to screening exams. • 1 patient (0.3%) had surgical complications from hysterectomy. • ⇔ Anxiety but ⇑ depression and ⇓ metal health & vitality scores associated with colonoscopies. These scores were within the normal ranges for the general population. • Colonoscopy unpleasant 57%, fearful 32%, painful 51%, shameful 16%. Worry about complications of colonoscopy 14% • No side effect was reported for 1.5 g calcium supplementation daily
Outcomes relating to subsequent actions or interventions	6 Retro (0.1-82 yr), 1 Prosp study (6 mo to ~8 yr), & & 1 RCT (12 wk)	• Colonoscopy and/or sigmoidoscopy: ⇓ Mortality ⇓ CRC mortality ⇔ Risk of developing CRC • TVU, TAH, and/or breast mammography: ⇓ Risk of extracolonic cancer ⇓ Risk of endometrial cancer ⇓ Risk of ovarian cancer • 1.5 g calcium supplementation daily: Cytolytic activity decreased with calcium but difference was not statistically significant. • ⇔ Survival b/w HNPCC, FAP, and sporadic CRC groups adjusting for age, gender, stage and tumor location • 5-year survival rate in patients with gastric cancers (40% also had CRC) was 48% vs. 15%, in those who received radical surgery and those who received palliative surgery or explorative laparotomy alone, respectively. • No cases of endometrial carcinoma detected by ultrasound but one case of endometrial carcinoma was found (in a woman with an MLH1 mutation)

TVU=transvaginal ultrasound; TAH= Prophylactic hysterectomy
⇑ Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
⇓ Statistically decreased ⇓ Decreased, but not statistically significant

Gaps in the Literature. There was little information on harms associated with screening and surveillance examinations or surgical procedures in CRC patients and their family members. However, the risks of these procedures are likely to be similar to the non-HNPCC setting. Only five studies reported data on harms or adverse events. One cannot assume "no harm" when a study did not report data on harms; therefore harms associated with the screening procedures may be underreported.

Table 30. Key Question 11. What are the harms associated with subsequent actions or interventions for family members?

Author, year Country	Study design (Follow-up duration)	Target population (Eligible/Enrolled N)	N evaluated	% who took actions	Description of subsequent actions or interventions	Harms	Outcomes, those who received intervention vs. those who did not		Quality
Vasen, 1995 Netherlands	Retro (1-20 yr)	"Cases": high-risk relatives of 50 HNPCC families who met Amsterdam I criteria and accepted surveillance (579/493)	388[a]	100%	Surveillance program included colonoscopy or sigmoidoscopy with barium enema every 2-3 years	1 patient (0.25%) had a cardiac arrest due to screening exams	CRC mortality	⇓ (1/11 vs. 108/238)[b]	B
							5-yr survival	⇑ (87% vs. 63%)[b]	
Schmeler, 2006 US	Retro (0.1-35 yr)	Women with documented MLH1, MSH2 or MSH6 gemline mutations (380/315)	315	19% TAH alone; 15% TAH&BSO	Prophylactic hysterectomy with or without bilateral salpingo-oophorectomy	1 patient (0.3%) had surgical complications from hysterectomy[c]	Risk of endometrial cancer	↓ (0/61 vs. 69/210)[d]	B
							Risk of ovarian cancer	↓ (0/47 vs. 12/223)[d]	
Renkonen-Sinisalo, 2000 Finland	Retro (Median 3 yr 8 mo in surveillance group; 5 yr 3 mo in non-surveillance group)	All new CRC cases occurring in known HNPCC families with mutation or met Amsterdam criteria (137/137)	137	24%	Surveillance program involved colonoscopy or double-contrast barium emema and sigmoidoscopy. Non-surveillance group underwent colonic examinations due to symptoms of cancer.	ND	Cumulative CRC-specific survival 10-yr after surgery	↑ (93% vs. 68%	B
							Cumulative overall survival	⇑ (85% vs. 62%	

Table 30. Key Question 11. What are the harms associated with subsequent actions or interventions for family members? (continued)

Author, year Country	Study design (Follow-up duration)	Target population (Eligible/Enrolled N)	N evaluated	% who took actions	Description of subsequent actions or interventions	Harms	Outcomes, those who received intervention vs. those who did not	Quality
Wagner, 2005 Netherlands	Prosp (6 mo to ~8 yr)	1st and 2nd degree relatives who were germline mutation carriers from 24 HNPCC families (115/94)	70	100%	Colonoscopy every 1-2 years from age 20-25 years Female carriers offered gynecological screening by US and CA125-measurements of blood from age 30-35 years and on Screening for stomach, duodenum or urinary tract as advised	Colonoscopy unpleasant 57%, fearful 32%, painful 51%, shameful 16% Worry about complications of colonoscopy 14%	88% (n=37) healthy mutation carriers had colonoscopic screening every 1-2 years	B
Rijcken, 2003 Netherlands	Retro (5 mo to 11 yr)	Women from HNPCC families (MMR gene mutation or Amsterdam II criteria) identified by physicians and national registry (ND/103)	41	100%	Annual screening started between 30 and 35 years old consisting of gynecological exam, TVU, serum levels of CA125 (normal ≤35 kU/L). Endometrial sampling as indicated.	ND	179 TVU performed over 197 patient years, Endometrial sampling for 11 from 17 TVU results, 4 from clinical symptoms. 14/17 endometrial samplings showed no severe pathology. 115 serum CA125 levels ranged from 1–24 kU/L with median of 7 kU/L No ovarian cancers detected by screening or outside of annual screening	B
Cats, 1995 Netherlands	RCT (12 wk)	First-degree relatives of patients with HNPCC participating in regular screening program. At 50% lifetime risk of developing CRC. (53/30)	30	50%	1.5 g calcium daily in a form of calcium carbonate tablet	No side effect was reported	Cytolytic activity decreased with calcium but difference was not statistically significant. No difference in sigmoid colon or rectum.	B

Table 30. Key Question 11. What are the harms associated with subsequent actions or interventions for family members? (continued)

Author, year Country	Study design (Follow-up duration)	Target population (Eligible/Enrolled N)	N evaluated	% who took actions	Description of subsequent actions or interventions	Harms	Outcomes, those who received intervention vs. those who did not		Quality
Arrigoni, 2005 Italy	Retro (21-82 yr)	Individuals from 22 HNPCC families that met Amsterdam I criteria (ND/331)	331	60%	Surveillance program included colonoscopy starting at age 25; GYN exam, and transvaginal US starting at age 30; Urinary ultrasonography and urine analysis starting at age 30; Endoscopy starting at age 30; Thyroid ultrasonography starting at age 35; Breast mammography starting at age 35. All tests repeated every 2 years.	ND	Risk of developing CRC	⇔ (3.5% vs. 3.8%)	C
							Risk of developing extracolonic cancer	↓ (0.5% vs. 4.5%)	
Liljegren 2004 Sweden	Retro (10 yr)	Individuals at high-risk for CRC (>10% lifetime risk) who were either mutation positive, suggestive family history but not fulfill Amsterdam I, or having 2 close relatives affected by CRC (304/265)	240	100%	Colonoscopies were performed every 2-3 years	⇔ Anxiety (HAD) but ↑ depression (HAD) and ↓ metal health & vitality scores (SF-36), compared to general population			C
Dove-Edwin, 2002 Netherlands	Retro (ND)	Women from 38 HNPCC families (Amsterdam I or II criteria, or mutation testing) (ND/118)	118	100%	Annual or biennial TVU from age 30-35 years.	ND	No cases of endometrial carcinoma detected by ultrasound but one case of endometrial carcinoma was found (in a woman with an MLH1 mutation)		C

Table 30. Key Question 11. What are the harms associated with subsequent actions or interventions for family members? (continued)

Author, year Country	Study design (Follow-up duration)	Target population (Eligible/Enrolled N)	N evaluated	% who took actions	Description of subsequent actions or interventions	Harms	Outcomes, those who received intervention vs. those who did not	Quality
Green, 1995 UK	Retro (ND)	HNPCC family members fulfilling Amsterdam I criteria and undergoing a colonoscopy (ND/61)	61	100%	Colonoscopy screening	ND	2 patients (3 %) had a malignant neoplasm (one Dukes B and the other Dukes C) at ages 66 and 41, respectively. Another patient presented with an obstructing sigmoid carcinoma. Seven patients had adenomas (mean age 45.3, range 26-63).	C

⬆ Statistically increased ⇑ Increased, but not statistically significant
⇔ No statistically significant differences
⬇ Statistically decreased ⇓ Decreased, but not statistically significant

[a] Surveillance detected 11 CRC cases (out of 388 high-risk individuals) during follow-up. Mortality and 5-yr survival of the surveillance-detected CRCs was compared to the controls (or symptomatic CRC patients who were not under surveillance).
[b] The 11 surveillance detected CRC cases were compared to 238 "controls" subjects, who were symptomatic CRC patients who were not under surveillance).
[c] Ureteral injury, ureterovaginal and ureteroeneteric, and then rectovaginal fistula.
[d] Compared with age-matched, mutation positive women who had not undergone either procedure (n=210 for endometrial cancer and n=223 for ovarian cancer).

Chapter 4. Discussion

Our report was based upon an analytic framework that begins with a patient with CRC and explores the spectrum of implications of screening, testing, and management of the patient and their family members considering the perspectives of the patient, provider, family member and the public health. The Key Questions we addressed reflected the main considerations along this pathway. We have explored a large and varied literature and have found a wide range of quality of the evidence across these areas.

Analytic Validity

A previous review sponsored by the CDC and EGAPP found that 13 commercial laboratories in the United States offered some form of laboratory testing for HNPCC. (The report is available at http://www.cdc.gov/genomics/gtesting/file/print/FBR/CC_AnalyticValidity.pdf, accessed on August 5th, 2006). The report noted that there were no data from which to assess the analytic sensitivity, specificity, or reliability of genetic tests used to diagnose HNPCC. We also summarized commercial laboratories performing HNPCC-related tests that had been included on a federally sponsored website (www.genetests.org). This likely represents only a partial list, but highlights the variability in the testing available at the participating centers.

The published literature regarding the analytic validity of laboratory testing in HNPCC is extremely limited. Most of the studies we reviewed were only indirectly related to analytic validity and had major methodologic limitations, particularly in use and definitions of reference standards. However, there was some evidence that there may be variability across testing facilities at least for immunohistochemistry (IHC).[56] Furthemore, a study published after our literature review found a "learning curve" in laboratories performing MSI testing.[168] Thus, analytic validity is not merely a theoretical concern but may have important clinical impact in the care of probands with HNPCC-related cancer or their families.

A better understanding of analytic validity could be achieved by incorporating non-literature based methods. This might include contact of commercial laboratories performing such testing. In addition, an external proficiency-testing program for MSI was introduced in 2006 (by the College of American Pathologists, information accessible at http://www.cap.org/apps/cap.portal) but results have not been published. Finally, some of the laboratory tests used in evaluation of HNPCC are also used for other disorders. A literature review based upon a search for these methods would not be practical. However, a committee of experts involved in these laboratory procedures could help evaluate their strengths and limitations in detecting the spectrum of genotypes/phenotypes associated with HNPCC.

Clinical Validity

We summarized the literature describing the relationships among clinical predictors of HNPCC using a clinical or genetic definition of the disorder, depending upon the parameter that we were estimating. Our analysis underscored the difficulty in comparing studies with one another because of important differences in study characteristics such as inclusion criteria, the quality and extent of laboratory testing performed, and the degree to which the diagnosis was

verified among participants. Despite these difficulties, we have clarified the accuracy of the clinical predictors and critically evaluated study characteristics that may have a bearing upon them.

We used the estimates of test accuracy to develop a decision-tree model to understand how these predictors can be used to identify MMR mutations among unselected patients with CRC. We performed sensitivity testing across the range of estimates for key parameters. The point estimates (and ranges) tested were derived from our systematic review of the data and thus confidently demonstrate the range of possibilities based upon the best available evidence.

We found that a set of three simple criteria i.e., 1) age less than 50 years old at diagnosis 2) or a history of colorectal or endometrial cancer in a first degree family member, 3) or the presence of multiple, synchronous or metachronous colorectal or endometrial cancers) performed as well as the more complex criteria (such as the Bethesda guidelines) when combined with MSI or IHC testing of tumor tissue. However, our analysis did not consider costs or utilities and thus a more formal cost-effectiveness analysis would be required to more fully understand the implications of the various strategies.

Our model was based upon parameters obtained from the published literature. As a result, it may not fully account for practical considerations that are encountered in the care of patients with CRC and family members with suspected HNPCC. For example, it may not be possible to obtain tumor tissue to perform laboratory testing. In addition, there may not be uniform access to expert laboratory and pathology facilities or qualified genetic counselors. Economic and other barriers may prevent access to care and surveillance in some settings. Obtaining a family history, even if abbreviated, may not always be feasible. As noted in Chapter 1 and 3, the patients' report of the family history may not be accurate, particularly for cancers other than colorectal that are potentially related to HNPCC.[30] Issues of uncertain paternity may also be relevant in some families while some families may be too small (or have insufficient contact among family members) to obtain a clinically meaningful family history.

Furthermore, testing for HNPCC in patients with CRC represents only one of the diagnostic considerations when evaluating patients for a genetically susceptibility to cancer. To fully understand the implications, diagnostic strategies for other forms of hereditary cancer (such as MUTYH-associated polyposis and attenuated familial adenomatous polyposis) also need to be considered. For example, it is possible that certain combination of clinical history and laboratory testing of tumor tissue may be superior to the strategy proposed above when attempting to optimize the yield in uncovering all recognizable forms of genetically-based CRC predisposition.

We identified many gaps in the literature. For example, there were relatively more studies demonstrating test characteristics of MSI testing compared with IHC and thus greater precision in the estimates of sensitivity and specificity of MSI testing. There were insufficient data to understand confidently whether MSI testing had better sensitivity and specificity compared with IHC and even fewer studies that addressed benefits of using both approaches concurrently. An advantage of IHC compared with MSI testing is that it is easier to perform and less expensive, both features offering important benefits from a public health perspective. Nevertheless, more studies are needed to clarify whether IHC should be preferentially adopted based upon these considerations.

The majority of studies provided data on patients with CRC who were implicitly or explicitly selected based upon heightened suspicion for HNPCC while there were few studies providing estimates on characteristics of these tests in an unselected population-based sample of patients with CRC. More studies on such patients are needed.

Benefits and Harms

We identified and critically evaluated studies that considered the range of outcomes (harms and benefits) in probands with HNPCC-related cancers and their family members from the perspectives of the patient, their family members, providers and the public health. Where there was limited evidence, we expanded the scope of the Key Questions to include indirect evidence, and variable definitions of HNPCC (e.g., clinical or genetic) to provide as comprehensive an appraisal of the knowledge base as possible. The major conclusions and gaps in the literature are summarized in the Executive Summary, Chapter 3, and (more briefly) below.

We found only limited data on harms associated with testing for MMR mutations in probands with CRC but the available data do not suggest severe psychological impact in most patients. In addition, pre-test genetic counseling had good efficacy in improving knowledge about HNPCC and resulted in a high likelihood of proceeding with genetic testing, satisfaction in the decision to undergo genetic testing and decreasing depression and distress levels among family members of HNPCC probands and among asymptomatic individuals form HNPCC families. Although there was little information regarding how pre-test counseling should optimally be performed, the consistent benefits observed across studies underscore the effectiveness of current programs. Testing of family members also had the potential to exclude the presence of HNPCC and thereby excuse family members from the need for intensive cancer surveillance and the psychosocial concerns related to a cancer predisposition.

There was limited direct evidence regarding whether the identification of MMR mutations led to improved outcomes for the proband in terms of early detection, mortality/morbidity or management decisions by patients or providers. However, identification of HNPCC mutations was associated with an increase in the likelihood that family members of probands with CRC would undergo cancer-screening procedures. Survival was increased among asymptomatic HNPCC family members who received colonoscopy screening, regardless of their mutation status. Family members who underwent cancer-screening procedures had a lower risk of developing HNPCC-related cancers and lower mortality rates than those who did not take actions. The literature most strongly supported surveillance for CRC while there were only a few studies that focused on prevention of other HNPCC-related tumors. However, all of the relevant studies suggesting these benefits had important limitations.

There are a multitude of ethical, social,and medical issues that require clarification. There was very little information regarding surveillance approaches in patients who were considered at risk for HNPCC, but had a negative or equivocal genetic test result. We found very few moderate or high quality studies from which to confidently understand the clinical expression (penetrance) of HNPCC among family members of CRC probands found to have MMR mutations through predictive genetic testing programs. There was also limited information regarding the relationship of specific genotypes to phenotypes.

Summary

- There is very little published information related to the analytic validity of laboratory testing in HNPCC. However, the available data suggest there may be important variability among testing facilities. Additional unpublished information about analytic validity is available through an external proficiency-testing program for MSI conducted by the College of

American Pathologists, and through internal testing performed by individual laboratories. In addition, the analytic validity of the specific testing methods used for other genetic disorders may be applicable to HNPCC.

- Among CRC fulfilling Amsterdam I criteria, approximately 44% (95% CI: 35, 52%) had a MMR (MLH1 or MSH2 in most studies) gene mutation when considering all studies together. The prevalence was 51% (95% CI: 35, 66%) among studies performing relatively comprehensive genetic testing. However, the true prevalence of MMR mutations among Amsterdam I patients may be different (possibly higher) than these estimates for several reasons:
    1. Testing for MMR genes other than MLH1 and MSH2 (particularly MSH6) may increase the proportion of patients with MMR by around 10% (this is an uncertain estimate).
    2. Genomic rearrangements and large deletions are missed when only sequencing and gene screening is performed. Limited evidence suggests that approximately one-fourth to one-third of the identified MMR mutations are large genomic deletions/rearrangements.
    3. The prevalence estimates are influenced by the certainty that identified genotypes are classified as being pathogenic.
    4. The strategy used for selecting patients with CRC can have a substantial impact on the prevalence estimates. Very few studies focused on unselected patients with CRC. Most identified cases from cancer registries or used other selection strategies that may have increased the proportion of patients with MMR mutation included in the study. This report examined various features of studies evaluating these estimates (such as the selection criteria, specific type of laboratory and genetic testing, sample size and methodologic quality), but did not identify consistent associations among these features and the prevalence estimates.

  Accounting for MMR genes other than MLH1 and MSH2 and for the routine use of methods to detect large genomic deletions/rearrangements, one may calculate that approximately 70% to 75% for Amsterdam I patients are MMR mutation carriers.

- The same considerations apply to other clinical criteria such as the Amsterdam II criteria or the Bethesda Guidelines (original and revised). Among CRC patients who fulfilled the Amsterdam II criteria, 39% (95% CI: 30, 49%) had a MLH1 or MSH2 gene mutation when considering all studies together. The prevalence was 40% (95% CI: 30, 52%) when considering two studies that performed gene sequencing in all patients. Accounting for MMR genes other than MLH1 and MSH2 and for the routine use of methods to detect large genomic deletions/rearrangements, one may calculate that approximately 55% to 59% for Amsterdam II patients are MMR mutation carriers.

- 71% (95% CI 63, 78%) of tumors from patients who fulfilled the Amsterdam I criteria were MSI-H. The corresponding prevalence for Amsterdam II was 68% (95% CI: 58, 76%). However, these estimates may be influenced by selection bias (both in selecting patients for study and in the availability of tumor tissue for testing). We examined several other study-related features but did not observe consistent associations with the prevalence estimates.

- Approximately 40% of colorectal cancers (95% CI: 28, 53%) from patients who fulfill the Amsterdam I criteria demonstrate loss of protein expression for MLH1 or MSH2 by IHC. Testing for loss of expression of MSH6 may increase the proportion by about 6% but the data were limited. The potential biases described above for the prevalence of MMR mutations may also apply to these estimates.

- The sensitivity and specificity of clinical criteria for predicting the presence of MMR mutations is described in Chapter 3. A decision model based upon these estimates characterizes the yield of various strategies in identifying MMR mutations among unselected patients with CRC. An abbreviated family history plus tumor tissue testing for MSI or IHC appeared to identify a similar number of patients with MMR mutations as strategies involving a more complex clinical history.

- No studies described harms of the risk assessment process (e.g., using the Amsterdam criteria, Bethesda guidelines, MSI or IHC testing) in identifying CRC patients at increased risk for CRC.

- There was limited data on harms associated with testing for MMR mutations in probands with CRC. However, the available data do not suggest severe psychological impact in most patients.

- There was limited direct evidence regarding whether the identification of MMR mutations led to improved outcomes for the proband in terms of early detection, mortality/morbidity or management decisions by patients or providers.

- No study described harms associated with the subsequent management options after identification of MMR mutations in patients with CRC or other forms of HNPCC-related cancers.

- Several studies described the accuracy of HNPCC testing in predicting the risk of CRC in family members of probands with HNPCC. However, in most, the diagnosis of HNPCC was not based upon genetic testing. There was limited evidence regarding variables associated with penetrance in family members.

- Pre-test genetic counseling had good efficacy in improving knowledge about HNPCC and resulted in a high likelihood of proceeding with genetic testing, satisfaction in the decision to undergo genetic testing and decreasing depression and distress levels among family members of HNPCC probands or among asymptomatic individuals form HNPCC families.

- Identification of HNPCC mutations was associated with an increase in the likelihood that family members of probands with HNPCC would undergo cancer-screening procedures. However, such patients were self-selected based upon their willingness to undergo predictive genetic testing and thus, this observation may not be applicable to all family members. Survival was increased among asymptomatic HNPCC family members who received colonoscopy screening, regardless of their mutation status.

- There was limited direct evidence related to harms of the cancer-screening procedures in family members of probands with HNPCC. Complication rates associated with these procedures in other settings are probably similar.

- HNPCC family members who underwent cancer-screening procedures had a lower risk of developing HNPCC-related cancers and lower mortality rates than those who did not take actions. However, all of the relevant studies suggesting these benefits had important limitations.

Implications for Future Research

Our report identified several areas for future research; we considered the following to be particularly important priorities:

- There is very little information regarding the analytic validity of tests used in the diagnosis of HNPCC. Studies specifically addressing sensitivity, specificity, and reliability of all of the laboratory and genetic testing methods in HNPCC are needed. Such studies should focus on contemporary testing methods and compare them against well-defined reference standards in tissue samples representative of the spectrum of genotypes associated with HNPCC. Unpublished information regarding analytic validity is also available; it may be feasible to obtain information from commercial or private laboratories performing such testing. It may also be possible to obtain data from the College of American Pathologists regarding their MSI proficiency program once experience has accumulated. Experience with genetic testing methods in other conditions is likely to be relevant to HNPCC; a review of such information could be conducted by groups of experts on genetic testing techniques.

- Additional studies are needed to clarify the validity of specific clinical and laboratory predictors of HNPCC in patients with CRC who are representative of the general population of patients with CRC.

- Future studies should consider all forms of genetically based CRC cancer predisposition to fully understand the effectiveness of various diagnostic strategies. Such studies should consider all known genetic causes of cancer predisposition and the accuracy of clinical and laboratory testing in identifying these disorders in individuals who are representative of the general population.

- Additional studies are needed to establish the availability of genetic testing centers that can provide adequate counseling and whether there are barriers to access them. Such studies may involve electronic, mail, or telephone surveys.

- More studies are needed to understand what forms of surveillance should be offered to MMR mutation carriers for HNPCC-related cancers other than CRC. Well-designed controlled trials comparing various surveillance (or other management) strategies could be helpful.

- More studies are needed to clarify the risk of cancer in family members of probands with an HNPCC-related cancer who are found to carry MMR mutations. Such studies would ideally be prospective, fully account for interventions (such as cancer screening procedures) in those at-risk, and have a well-defined control population of individuals at average risk for cancer.

- Standards for reporting studies of genetically based diseases (including those addressing all aspects of the ACCE model) should be developed. A consensus development process with publication of a guideline(s) could be helpful.

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List of Acronyms and Abbreviations

Acronym/Abbreviation	Meaning
ACCE	Four components of test evaluation—analytic validity, clinical validity, clinical utility and associated ethical, legal and social implications.
AM1, AM2	Amsterdam I criteria, Amsterdam II criteria
AHRQ	Agency for Healthcare Research and Quality
BethR-All	*Testing strategy* - Perform MMR testing only among those fulfilling the revised Bethesda guidelines
BethR-IHC	*Testing strategy* - Perform IHC testing on patients fulfilling the revised Bethesda guidelines; perform MMR only among those with suggestive IHC test
BethR-MSI	*Testing strategy* - Perform MSI testing on patients fulfilling the revised Bethesda guidelines; perform MMR only among those with suggestive MSI test
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
3Clinical-All	*Testing strategy* - Perform MMR testing only among those fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis, 1st degree family history of CRC or endometrial cancer or multiple, synchronous or metachronous, CRC or endometrial cancer in the same patient)
3Clinical-IHC	*Testing strategy* - Perform IHC testing on patients fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis, 1st degree family history of CRC or endometrial cancer or multiple, synchronous or metachronous, CRC or endometrial cancer in the same patient); perform MMR only among those with suggestive IHC test
3Clinical-MSI	*Testing strategy* - perform MSI testing on patients fulfilling at least one of the three simple clinical criteria (age <50y at diagnosis, 1st degree family history of CRC or endometrial cancer or multiple, synchronous or metachronous, CRC or endometrial cancer in the same patient); perform MMR only among those with suggestive MSI test
CRC	Colorectal cancer
CSGE	Conformation sensitive gel electrophoresis
DGGE	Denaturing gradient gel electrophoresis
DHPLC	Denaturing high-pressure liquid chromatography
DNA	Deoxyribonucleic acid
EGAPP	Evaluation of Genomic Applications in Practice and Prevention

Acronym/Abbreviation	Meaning
	(EGAPP) Project, Office of Genomics and Disease Prevention, Centers for Disease Control and Prevention
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
hMLH1	MMR gene mutation in the human mutL homolog 1 gene
hMSH2	MMR gene mutation in the human mutS homolog 2 gene
hMSH6	MMR gene mutation in the human mutS homolog 6 gene
hPMS2	MMR gene mutation in the human postmeiotic segregation 2
IHC	Immunohistochemistry
IHC-All	*Testing strategy* - Perform IHC testing on all patients; followed by MMR testing only among those with suggestive IHC test
MLPA	Multiplex ligation-dependent probe amplification
MMR	Mismatch repair
MMR-All	*Testing strategy* – perform MMR testing on all colorectal cancer patients
MSI	Microsatellite instability
MSI-All	*Testing strategy* - Perform MSI testing on all patients; followed by MMR testing only among those with suggestive MSI test
MSI-high	High microsatellite instability
MSI-low	Low microsatellite instability
MSS	Microsatellite stable
NCI	National Cancer Institute
ND	No data
ROC	Receiver operating curve
SSCP	Single-stranded conformation polymorphism
SROC	Summary receiver operating curve
TEP	Technical Expert Panel

APPENDIXES:

to

**“Hereditary Nonpolyposis Colorectal Cancer:
Diagnostic Strategies and Their Implications”**

**Prepared by the Tufts New England Medical Center
Evidence-based Practice Center**
(Contract #290-02-0022)

Appendix A. Search Strategy

"colorectal neoplasms, hereditary nonpolyposis"[MeSH Terms]
hereditary non-polyposis[tw]
(HNPCC[tw]
(MLH1[tw] OR MSH2[tw] OR MSH6[tw]
(hMSH2[tw] OR hMLH1[tw] OR hPMS1[tw]
hPMS2[tw] OR hMSH6[tw] OR hMLH3[tw]

English[Lang] AND "humans"[MeSH Terms]

Appendix B. Sample Data Abstraction Forms

Analytic Validity

Data Extraction for Analytic Validity

Study: (Author, year, UI)

Inclusion criteria (all must be yes)

	Yes	No
Did study evaluate biological material from patients with CRC considered to be at risk for HNPCC?		
Did the study report ANY of the following? (check which one below)		
1) Proportion MSI-H with NIH markers versus other markers		
2) Sensitivity or specificity of MSI-H using NIH markers compared with a reference standard that the study claims is better		
3) Sensitivity or specificity of IHC compared with an immunohistochemical standard that study claims is better		
4) Sensitivity or specificity of a genetic technique compared with a reference standard (or combination of standards)		
5) Reliability of MSI/IHC/genetic method across laboratories or within a laboratory		
Are data (proportions or 2 X 2 tables) extractable or reliability data extractable?		

*NIH markers are BAT-25, BAT-26 D2S123, DS346 and D17S250

Exclusion criteria (exclude if yes)

	Yes	No
Did the study include the index test in the reference standard?		

Describe source of biological materials (and whether patients were known to have an HNPCC phenotype)	Summarize how materials were processed and analyzed

MSI Proportion (add additional 2 X 2 tables where relevant)

Proportion MSI-H using NIH markers (≥2 markers)	Proportion MSI-H using other markers

MSI with a reference standard

		MSI-H using another reference standard	Describe
		Positive	**Negative**
MSI-H using NIH markers	**MSI-H (≥2 markers)**		
	MSI-S or MSI-L		

IHC with a reference standard

		IHC using another immunohistochemical reference standard	Describe
		Positive	**Negative**
IHC	**Positive**		
Describe	**Negative**		

Genetic technique with a reference standard

		Reference standard genetic technique	Describe
		Positive	**Negative**
Index genetic technique	**Positive**		
Descirbe	**Negative**		

Intra or inter-hospital reliability data	Describe
	Of 18 participating centers 2 were excluded: one because slides were damaged in transit and the other because of insufficient staining. Sensitivity for detecting loss of hMSH2 2 expression ranged from 84 to 100%; 10 centers identified all six. 5/6 false positive results were in the same case suggesting that staining or interpretation were not random. 14/16 laboratories showed 100% specificity (one laboratory had 93% specificity due to staining failure on one slide and one lab demonstrated 45% specificity due to weak or absent staining in most cases. Re-review of returned hMSH2 slides shoed lack of

Intra or inter-hospital reliability data	Describe
	internal positive control staining in at least 2 of the 6 hMSH2-negative cases from 8 of 16 centers. The other 8 centers had 100% sensitivity and 93-100% specificity on re-review. The slides that lacked internal positive control staining wre largely accounted for by two cases suggesting the possibility of fixation or processing variation. Variation of staining quality and interpretation was much greater for hMLH1 than for hMSH2. individual centers reported 0 to 100% sensitivity and 40 to 100% specificity. Re-review of the returned slides resulted in sensitivities of 0-90%. 12 centers experienced difficulty with lack of internal positive control or high background. Overall, four laboratories performed relatively well with both hMLH1 and hMSH2 staining protocols. The key element common to these and distinguishing them from the rest was a heated antigen retrieval step. Steam treatment in the presence of EDTA provided the best results although steam and citrate buffer also provided acceptable results.

	Study Quality	Yes	No	Unc
1	Was the description of how MSI or IHC and other genetic techniques described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
2	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
3	Was MSI, IHC, other genetic testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
4	Was there a clear description of which mismatch repair mutations were being tested for?			
5	Were quality control methods described for the molecular and genetic tests?			
6	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
7	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			
8	Was microdissection performed?			
9	Did the study specify whether the biological tissues were from patients known to have HNPCC clinically?			
10	Did the study include a control group in which biological material was obtained from patients known not to have HNPCC clinically			
11	**Overall rating (A B C)**			

Clinical Validity

Study: (Author, year, UI)

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?		
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?		
Was a minimum of hMLH1 and hMSH2 sequencing performed?		

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**				**Predictors analyzed (check all that apply)**		**How were deleterious, missense/variants mutations defined (ND if not described)**
				Y	N	?	Am 1		
			≥5 MSI markers used?				Am R		
			MSI-H defined by ≥ 2 markers?				Beth 1		
			Microdissection?				Beth R		
			Gene screening?				MSI-H		
			Deletion analysis?				MSI-L		
			Conversion analysis?				IHC		
							Age <50		
							Suggestive family history		
							Specify		
							Other		
							Specify		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		

If yes, which clinical criteria (check all that apply)?	
Am 1 +	
Am R +	
Beth 1 +	
Beth R +	
Age <50	
Suggestive family history (specify)	
Other (specify)	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population (i.e. from table above)? "ND" if not a restricted population *(e.g. Patients with CRC who had a suggestive family history)*	Index tests	Check ***ONE***	Index test	Number with MMR+	Number with MMR-
	Am 1 +		+	(A)	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			
2	Inclusion criteria clear?			
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			
7	Were withdrawals from the study explained?			
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
12	Was there a clear description of which mismatch repair mutations were being tested for?			
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			
14	Were quality control methods described for the molecular and genetic tests?			
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Benefits and Harms

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes / No / Uncl		

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	**2,3**	**5**			**8c**	**1,6a,6b,7,10**
Family member/other high risk individual	**2,3**	**5**	**9**	**8a**	**8b,8c**	**1,6b,9,10,11**
Public health or policy	**2,3**	**5**	**9**	**8a**	**8b,8c**	**1,6,a,6b,7,10**

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias						A (strong)	B (moderate)	C (weak)

(RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")								
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			

Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Appendix C. Evidence Tables

Study: Aaltonen 1998

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
509 consecutive patients with CRC from 9 hospitals in Finland between May 1994 and April 1996 ie does not overlap with Salovaara mean age 67	MSI wad done in all patients (Battery of MSI was not common to all), founder mutation 1 in MLH1 was looked for in all patients; MLH1 and MLH2 sequencing was done in MSI positive and founder mutations negative patients	Founder mutation 1 in MLH1 and other mutations in MLH1 and MLH1 (NB Salovaara tested founder mutation 2 also)	≥5 MSI markers used? N MSI-H defined by ≥ 2 markers? N Microdissection? ? Gene screening? Y Deletion analysis? ? Conversion analysis? ? Genetic testing varied between first 198 and subsequent 311 pts. MSI testing differed between pts, all however received the BAT26 test and correlation was OK.	Am 1 Am R Beth 1 Beth R MSI-H MSI-L X IHC Age <50 Suggestive family history *Specify* Other x *MSI*	Literature and previous analyses on the founder mutation and comparison wit with non-cancer controls

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	Proportion with a mismatch repair mutation (describe which MMR gene). *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	Proportion of overall population positive for each predictor *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	Correlation of predictors to one another (e.g. IHC versus MSI) *Report what study found such as correlation coefficients or other measures of correlation*	Study conclusion *(What did the authors conclude about the testing strategy or other major findings).*	Implications to family /other findings or comments from authors relevant to any key question	Quality grade (see checklist below) and add specific comments about study quality/concerns here
509 mean age ? males ?	10 MMR/509 patients with CRC 9 MLH1 and 1 MSH2 NOT COUNTING THE NON-PATHOGENIC MUTATIONS	4/509 (0.8%) Am I	Other miniSATs + - BAT + 58 6 26 - 5 440	Large scale molecular screening for HNPCC can be done by 2-stage procedure: screening for founders mutations, then MSI, then sequencing for those with + MSI To select who to screen for MSI, use Age <50, family Hx of EC or CRC or multiple tumor in same pt.	no	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam	Amsterdam 1	X	Combined 4/4

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
criteria) what proportion had an MMR gene mutation?	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	Combined 0/4
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		NA
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X
If yes, which clinical criteria (check all that apply)?		X
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Consecutive CRCs	Am 1 +		+	4	0
	Am R +		-	6	499
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	Combined Am1&R	X			

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Consecutive CRCs	Am 1 +		+	10	53
	Am R +		-	0	446
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI, Other Sats	X			

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Consecutive CRCs	Am 1 +		+	10	54
	Am R +		-	0	443
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI, BAT26	X			

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	X		
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Aarnio, 1997 RefID 2366 Multicenter, Finland

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Focused on same patients as Aarnio 1995 Ref ID 2666 but reported different outcomes. Focused on 51 families with HNPCC affected by gastric cancer. Of 92 families registered in HNPC registry, a mutated MLH1 gene segregated in 45 and a mutated MSH2 in 2 while four met Amsterdam I criteria. In these 51 families, there were 570 family members affected by cancer of whom 62 (10.9%) had gastric cancer and formed the basis for the study.	Suspected HNPCC by Amsterdam I, some families with known mutation. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: X No: Uncl:	History of gastric cancer and member of an HNPCC family.	Examined 24 gastric cancer specimens for histology and H. pylori staining. Performed IHC for P53 expression and MSI on 20 cases. Also assessed survival in a total of 45 patients.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
45 patients with gastric cancer (24 men, 21 women) mean age 56 (range 31-85). Metachronous other cancer occurred in 23 (51%). Total number of tumors 90. There was variation in the occurrence of gastric cancer in families with different MLH1 or MSH2 genes but difference was not statistically significant. 19/24 were intestinal type; 6 (32%) were poorly differentiated, four others (17%) classified as diffuse or mucinous. 7/15 samples (47%)with suitable tissue were MSI-H H. pylori positive in 3/15. Overall 5-year survival 15%; 48% in patients in whom radical surgery was performed.	ND	Gastric cancer was an HNPCC-associated tumor on average in 11% of affected family members. Predominant tumor type was intestinal. Existence of this tumor in patients younger than 60 suggests HNPCC in the background. Relative rarity in HNPCC families makes cost-effectiveness of endoscopic screening questionable.	C Significance of survival data is unclear since there was no information on staging or how patients were selected for treatment. Incomplete verification of tissues samples, and tissue samples were assessed only in a subset of patients. Unclear how many patients of eligible population agreed to participate. Unclear how survival/death was ascertained.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3 x	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	X No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			
Analysis						A (strong)	B (moderate)	X

Domain/question	***Place an "X" in one***					***Overall rating***		
								C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	No	X ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	X Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	X Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	X Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	X No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	X Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Adams, J 2003, RefID 653 Multicenter UK

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Secondary analysis of data recorded for a study on polyp prevention (a database) on chemopreventive effects of aspirin. Study had included 600 patients with HNPCC; of these 154 were available for analysis and full data were available on 134. Of these 134, 49 had a personal history of CRC. Socioeconomic status assessed using the Town-Deprivation Score (TDS) of the current enumeration district of residence. This score is based upon relative material deprivation based upon census data.	Amsterdam II criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Inclusion criteria included age >25 and, in those with a previous diagnosis of CRC, at least five years since resection.	Primary outcome was relationship between TDS and age of resection of first colorectal cancer.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
There was a statistically non-significant correlation between the TDS and the age of first resection indicating that individuals in less deprived areas tended to have their colorectal cancer resected at an earlier age than those living in more deprived areas.	Most dropouts from missing data due to wrong postal codes	More affluent individuals from HNPCC families tended to undergo surgical resection for CRC earlier than less affluent individuals. This relationship bordered on statistical significance and probably represents socio-economic variations in access to treatment.	C Poorly defined populations, interventions, documentation of cancers, no correlation with ages of family members who may have resided in affluent areas. Reasons for resection not stated.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	X Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	X NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	X No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	X No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	X C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	X	ND					

Domain/question	***Place an "X" in one***					***Overall rating***		
		No						
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	X Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	X Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Aktan-Collan 2000 Ref ID 1798, same patients as Atkan-Collan 2000-2001, Ref ID 866, 1419 and 1835 but studied different outcomes

Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
Counseling offered to members of 36 families in which a genetic mutation had been identified. Questionnaires filled out before, counseling and then 1 month and 1 year afterward. Of 446, 90% consented to participate, 381 (85%) returned a baseline questionnaire. Education/counseling attended by 347 of whom 333 (96%; 75% of total population) opted for predictive testing. Of these, 271 filled out all three questionnaires. These form the basis of the study group.	Mutation identified in families Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	As per study description	Surveys

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
334/381 accepted genetic testing of whom 30% were mutation-positive. Acceptors differ from decliners in more often having a spouse or partner, being employed and having higher education. On multivariate analysis, the only significant factor for accepting genetic testing was employment status. At 1-month, there was no difference in satisfaction in decision to undergo testing in mutation positive and negative subjects. At one-year, subjects who had not inherited a mutation were slightly more satisfied. Most common reasons cited against undergoing testing were: being old already and having no children (23%), would not trust result (15%), timing found unsuitable because of pregnancy 1 (8%), no reason given (54%)	ND	The possibility of early detection and treatment of colorectal tumors makes predictive testing for HNPCC beneficial. The test was welcomed by the majority of individuals at risk for HNPCC, and few reported having regretted the decision to make it. Thus, we suggest that all members of families with HNPCC should be actively informed about the predictive test by their physician.	B

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						X A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A	X	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	B (moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Atkan-Collan, 2000 RefID 1835 Multicenter Finland, Same patients as Atkan-Collan 2001, Ref ID 866 and 1419 but different outcomes

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
446 family members from 36 HNPCC families with known mutations at 50% risk offered counseling and genetic testing. Families contacted by letter. Along with counseling and testing, all sent questionnaires before counseling, 1 month, and 1 year after testing. Of 446 eligible, 401 (90%) agreed to participate and 381 (85%) returned a baseline questionnaire. Educational counseling attended by 347, of whom 333 (96%, 75% of whole group) opted for predictive genetic testing. Study focused on 271 subjects (61% of whole group) who had been tested and returned all questionnaires.	Families known to have a genetic mutation. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	See study description.	Surveys

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Mean age 43 (range 19-77, SD 13) At one month 88% indicated counseling was easily comprehended. 10% wanted minor changes, most commonly asking for more written material. Those with a university education suggested changes more than those without At one year, pretest counseling considered very useful by 49%, fairly useful by 40% and slightly useful by 10%. Women and those with a lower level of education were significantly more likely to consider counseling useful. At post-test follow-ups, 89 and 95%, respectively, considered a single post-test session sufficient. 53% indicated that they might have used professional psychological support along with counseling if it had been provided. The support might have been used by women with children more than by others. Mutation positive subjects more often reported the greatest need for support soon after hearing the results.	As per study description`	A protocol that includes one-comprehensive pre-test counseling session and a test disclosure session, supplemented with option of professional psychological support, seems to be sufficient for both the educational and supportive needs of counselees. Only a minority expressed a need for post-test follow-up sessions.	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5 x	9 x	8a x	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	**Place an "X" in one**					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						X A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A	X	C

Domain/question	**Place an "X" in one**					***Overall rating***		
						(strong)	B (moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Aktan-Collan 2001, Ref ID 1419, Multicenter Finland. Same patients as Aktan-Collan 2001 Ref ID 866 but studied different outcomes

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Counseling offered to members of 36 families in which a genetic mutation had been identified. Questionnaires filled out before, counseling and then 1 month and 1 year afterward. Of 446, 90% consented to participate, 381 (85%) returned a baseline questionnaire. Education/counseling attended by 347 of whom 333 (96%; 75% of total population) opted for predictive testing. Of these, 271 filled out all three questionnaires. These form the basis of the study group.	Mutation in participating families Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Unclr:	See study description and Aktan-Collan 2001	Surveys

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Data analyzed according to mutation positive or negative. Fear of cancer, fear of dying soon and satisfaction were life were all modestly (but significantly) different between those who turned out to be mutation positive and negative even after adjusting for age. Mutation positive subjects who had just received their results had significantly higher scores than mutation-negative subjects. After 1 year, the fear of cancer had decreased from baseline in both groups. Mutation-positive subjects were slightly, although significantly, more afraid of cancer at every measurement.	As per study description	Counseling and testing relieve fear of cancer; no harmful emotional impact was detectable at 1-year follow-up. These findings should be studied after a longer interval.	B

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						X A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A	X	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	B (moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Aktan-Collan 2001, Ref ID 866 Multicenter Finland

Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
Described insurance behavior of 271 patients who had undergone predictive genetic testing for HNPCC. 446 family members from 36 HNPCC families with known mutations at 50% risk offered counseling and genetic testing. Families contacted by letter. Along with counseling and testing, all sent questionnaires before counseling, 1 month, and 1 year after testing. Of 446 eligible, 401 (90%) agreed to participate and 381 (85%) returned a baseline questionnaire. Educational counseling attended by 347, of whom 333 (96%, 75% of whole group) opted for predictive genetic testing. Study focused on 271 subjects (61% of whole group) who had been tested and returned all questionnaires.	Mutation identified in family. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	See study description.	Surveys

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Mutation-positive subjects reported that they already had a life insurance before entering study more often than mutation-negative subjects (36 versus 28%) but results were not statistically significant. The difference in possessing a health insurance plan before genetic counseling between mutation-positive and negative individuals was significant (21 versus 11%). 3% indicated that they had purchased a life insurance policy and 2% correspondingly a health insurance policy before they were tested.	See study description	Mutation-positive subjects did not differ from others in purchase of life or health insurance policies. However, the mutation-positive individuals reported that they possessed health insurance policies before entering the study more often than their counterparts.	B Incomplete reporting of data on denials, extent to which insurers requested information about genetic testing. Validity of surveys unclear.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						X A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A	X	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	B (moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Arrigoni, A 2005, Ref ID 276 Multicenter, Italy

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Individuals form families fulfilling Amsterdam I criteria advised to take place in a surveillance program as follows (all tests repeated every two years) Colonoscopy starting at age 25 GYN exam, and transvaginal US starting at age 30 Urinary ultrasonography and urine analysis starting at age 30 Endoscopy starting at age 30 Thyroid ultrasonography starting at age 35 Breast mammography starting at age 35	Amsterdam criteria I Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	As per study description	Surveillance procedures as outlined in study description

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
22 families identified with a total of 331 at-risk living relatives of whom 40 had already developed CRC. 199 accepted surveillance while 132 refused. Patients who accepted or refused did not differ significantly for by sex or age. Six individuals accepting surveillance were found to have CRC (3%). Overall incidence of CRC was 7/199 in patients undergoing surveillance and 5/132 in those not (p=NS). However, CRC detected in those undergoing surveillance was significantly less advanced. Seven at-risk individuals were found to have extracolonic cancer during surveillance. Extracolonic cancers were detected in six of 132 who refused.	As per outcomes.	Our study confirms the importance of collecting a careful family history for each patient with CRC. Data on a relatively short followup seem to support the efficacy of repeat colonoscopies in reducing CRC rate in at-risk members of HNPCC. Studies with longer followup are needed.	C Data do not support their conclusions. Possibility of lead-time bias in earlier stage detection. Data not analyzed using survival analysis.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3 x	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	X No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	X No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			
Analysis						A	X	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	B (moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	Yes	X No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Arver B 2004 Ref ID 433 Multicenter Sweden

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
1000 families underwent "oncogenetic counseling" between 1995 and 1995. In those where an autosomal dominant breast/ovarian or colon cancer gene mutation was suspected, genetic screening of BrcA1 and BRCA2 or MLH1 and MSH2 offered. A total of 295 BRCA1 and BRCA2 and 57 MLH1 and MSH2 offered. 114 healthy men and women from 73 of the 80 families with an identified mutation opted for genetic testing. Study sample consisted of 90 healthy women.	Women at risk for a genetic syndrome as defined in study description. HNPCC defined genetically. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Healthy females belonging to a family with a known mutation in BRCA1, BRCA2, MLH1 and MSH2 desiring genetic testing, age>18 and Swedish speaking. Men were excluded except if they had a personal history of cancer.	Surveys conducted before testing and then 1 week, 2, 6 and 12 months after results were given. Hospital anxiety and depression scale Swedish SF-36

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Al but one woman agreed to genetic testing. The one exception worried about consequences of insurance and employment in the future. Mean age in 87 women was 38.1 in mutation carriers and 41 in non-mutation carriers. A statistically significant decrease in anxiety mean scores over time was observed in total sample irrespective of tested gene or carrier status. Anxiety scores were similar to a normative Swedish population. Women tested for breast cancer predisposing genes showed an average lower levels of depression measured over time than women tested for colon cancer genes but difference was not statistically significant. QOL subscores did not change significantly during observation. Women tested for colon cancer genes showed increased vitality scores over time. However, carriers of colon cancer genes had a decrease in vitality scores two to six months after the result of disclosure, followed by increased levels while levels in non-carriers were more stable. The difference between carriers and non-carriers over time was statistically significant. No differences were found between study group and Swedish normative data on any of the five QOL parameters. 83% thought decision to undergo genetic counseling was “easy”. All but one woman would have redone the testing again.	1 because of worries about insurance and employment, 1 non Swedish-speaking, 1 did not receive surveys	Predictive testing for hereditary breast/ovarian or colon cancer gene mutations will not confer impaired mental health in motivated individuals who receive thorough pre- and post-test information and support and for whom preventive surveillance is offered.	B Did not describe details of surveillance and relationship with survey results. Mixed population of HNPCC and BRC.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	X Yes	No						
Were data collection tools shown or are they known to be reliable?	X Yes	No						
Withdrawals and Dropouts						X A	B (moderate)	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)		
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			
Analysis						X A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	X Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?			
Benatti, 2001 RefID 1411 Italy, multicenter			
Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
HNPCC families from the data of a population-based CRC registry were identified in 1984. All incident CRC cases diagnosed in the period 1984-1997 were collected and then stratified according to the presence of 6 clinical features. When a patient had 2 or more of these features, an extended genealogic pedigree was traced and then analyzed for the presence of diagnostic criteria for HNPCC. Once identified, families were considered eligible for the study if they met the inclusion criteria. 29 families met the inclusion criteria: 10 carried MMR gene mutations (group A), 10 were characterized by MSI phenotype but not by MMR gene mutation (group B), and 9 did not show mutations or MSI (group C). Time of followup was calculated from the date of HNPCC diagnosis in the index case to 12/31/1999. Mean duration was 8.2, 6.7, and 9.4 years for group A, B, and C, respectively.	1) fulfillment of AM-II for HNPCC diagnosis, (2) MSI testing in at least 1 case of CRC or endometrial cancer in the family, or (3) availability of hMLH1 and hMSH2 mutation analysis results. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: x No: Uncl:	(1) fulfillment of AM-II for HNPCC diagnosis, (2) MSI testing in at least 1 case of CRC or endometrial cancer in the family and (3) availability of hMLH1 and hMSH2 mutation analysis results.	Observation only

<table>
<tr><th>Outcomes</th><th>Dropouts, explanations, other comments</th><th>Study Conclusions</th><th>Quality score* (comments)</th></tr>
<tr>
<td>
Baseline clinical features of the 3 groups examined:
<table>
<tr><th></th><th>Group A: MMR+</th><th>Group B: MSI&MMR-</th><th>Group C: MSS&MMR-</th><th>P value</th></tr>
<tr><td>No. of family</td><td>10</td><td>10</td><td>9</td><td></td></tr>
<tr><td>Subjects (M/F)</td><td>361 (188/173)</td><td>241 (129/112)</td><td>355 (169/186)</td><td>ns</td></tr>
<tr><td>Tumor spectrum
- Colon (% of total)
- Extracolonic HNPCC-related sites</td><td>66 (58.9)

29 (25.9)</td><td>38 (78.0)

6 (12.0)</td><td>47 (66.2)

5 (7.0)</td><td><0.005</td></tr>
<tr><td>Mean age at diagnosis (years)
- Colon
- HNPCC-related sites
- Extra-HNPCC sites</td><td>
47.6
48.4
52.5</td><td>
49.0
61.0
53.2</td><td>
63.4
65.6
57.6</td><td><0.05
<0.05
ns</td></tr>
<tr><td>MSI-H tumors</td><td>25/25 (100%)</td><td>23/25 (92%)</td><td>2/26 (7.7%)</td><td></td></tr>
</table>
I. Cancer occurrence in the follow-up of the 3 groups:
In the follow-up, among HNPCC-related neoplasms, endometrial cancer was more frequent for gastric carcinoma in groups A and B, whereas in group C the stomach was the only extracolonic organ involved. Patients developing CRC in the follow-up were significantly younger (p=0.001) in groups A and B than in group C.

II. CRC-specific Survival:
The log-rank test revealed a significantly better prognosis for patients from families with a germline MMR gene mutation or MSI (p=0.0001). Furthermore, the presence of MMR gene mutations or MSI, together with stage, appeared as an independent prognostic factor by Cox regression multivariate analysis.
</td>
<td>
Retrospective cohort analyses.
No dropouts described.

Due to significant differences in many factors between the 3 groups at baseline, followup results from univariate analyses (such as analyses for cancer occurrence and log-rank analyses) are not useful.

Only results from Cox regression multivariate analyses (variables in the model: groups, sex, age ≤50 vs. >50, Stage, and site) might have some implications for the benefit-and-harm question.

Our interpretation of the Cox survival analyses results:
After controlling for sex, age, cancer stage, and site of CRC tumor, hazard ratio of dying is significantly higher for Group C (MSS and MMR negative) patients (HR=2.51 95%CI 1.32-4.79), when compared to the Group A (presence of MMR gene mutations) patients. There was no statistical difference in the hazard ratio of dying between group A and group B patients after controlling for sex, age, cancer stage, and site of CRC tumors.
</td>
<td>
Authors' conclusions:
"Our results support the hypothesis that the biomolecular heterogeneity of HNPCC is reflected in heterogeneous clinical features and confirm the value of MSI analysis as a surrogate marker for MMR gene mutations. The clinical similarities between group A and group B patients indicate the MSI testing could be useful to better define clinical HNPCC families, identifying groups with different risks of carrying genetic defects."
</td>
<td>C</td>
</tr>
</table>

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B x (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely x	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	X NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C x (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes x	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No x	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes x	No						
Blinding						A (strong)	B (moderate)	C x (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND x	NA				
Data Collection methods						A x (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes x	No						
Were data collection tools shown or are they known to be reliable?	Yes x	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND x	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e.	Yes	No	Can't					

Domain/question	***Place an "X" in one***					***Overall rating***		
intention to treat) rather than the actual intervention received?			tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Bermejo 2005 UI 16344057 Sweden Multicenter

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
The families of the Swedish Family-Cancer Database with at least 4 generations (N=566,877) were classified according to Amsterdam or Bethesda criteria. Survival methods were used to assess the risk of cancer in the classified families, the prognosis of cancer patients, and the risk of subsequent malignancies after CRCs and after CRCs/endometrial carcinomas.	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: x ; Uncl:	Families with at least 4 generations were included. If a founder parent of the family was missing or if they had married several times, the family was excluded. Overlap between families was possible, eg, individuals in the 4th generation could belong to 4 different families. The ages of the parents were unlimited, but the maximum age in the second generation was 70 years.	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
N=566,877 families 21 fulfilled Am I; 42 fulfilled Am II; 5095 fulfilled Beth (0.9%) 1. By age 75 yr, the cumulative risk of CRC in Am I families was 57.1% (95% CI, 46-68.8%); the cumulative risk of CRC in Am II families was 41.2% (95%CI, 33.3-50.1%) 2. The cumulative risk of CRC in Beth families was 41.7% (95%CI, 39.4-44.1%) in men, and 23.3% (95%CI, 22.2-24.4%) in women. 3. The cumulative risk of endometrial cancer in Am II by age 75 yr was 45.4% (95%CI, 34.1-58.5%), and it was 8.7% (95%CI, 8-9.5%) in Beth. 4. For ovarian cancer, the cumulative risk by age 75 yr in Beth was 4.7% (95%CI, 4.2-5.3%); for gastric cancer, it was 2.3% (95%CI, 2-2.7%). 5. Families that fulfilled the Beth criteria showed increase risk of cancer in the colorectum, endometrium, small bowel, ovary, stomach, bile ducts, renal pelvis, and ureter; members of Beth criteria families were at decreased risks of lung and cervical cancers. 6. The prognosis of cancer in the ureter, renal pelvis, stomach, ovary, and colorectum, but not in the endometrium, was better in Beth criteria than in nonclassified families.		"Most malignancies in the classified families reflect typical features of HNPCC. The data presented should help to define surveillance strategies for members of families that fulfill the criteria for HNPCC testing."	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Bertario 1999, Ref ID 2106 Multicenter, Italy

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
2340 CRC patients consisting of 3 groups (144 HNPCC, 161 FAP, 2035 sporadic). Comparative survival estimated. FAP=familial adenomatous polyposis	Amsterdam criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	As per study description	Cumulative probability of five-year survival. For HNPCC and FAP patients, the follow-up procedures and adjuvant-treatment protocols were the same as for patients with sporadic colorectal carcinoma. Taking into consideration the increased risk of extracolonic manifestations, hereditary-colorectal cancer patients were also subjected to periodical instrumental examinations tailored to the different spectrum of the disease.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
No substantial survival advantage for patients with HNPCC and FAP versus sporadic group after adjustment for age, gender, stage and tumor location.	ND	No substantial survival advantage for HNPCC and FAP compared with sporadic group after adjustment for age, gender, stage, and tumor location. HR for HNPCC was 1.01 (95% CI 0.72-1.39) compared with sporadic.	B Short followup. No details on reliability of survival data. Study may have been underpowered.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			
Analysis						A	B	X

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	(moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	X No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	X Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	X Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Berth 2002 Ref ID 985 Germany single center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Survey sent to 2076 persons of the general population and 36 patients at high risk for HNPCC or FAP. FAP=familial adenomatous polyposis	ND Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	As per study description	Survey regarding attitudes about genetic testing.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
"overall, both the general population and at-risk persons hold a favorable attitude toward genetic testing.	ND	Attitudes of persons at risk for HNPCC and FAP similar to general population.	C Validity of survey instruments unclear, no details provided about response rates, differences among responders and non-responders, definition of at-risk individuals or any other details about at-risk individuals.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	X B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	X Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Bleiker, EM 2005, Ref ID 268 Multicenter, Netherlands

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
178 patients at high risk for CRC (27 Amsterdam I criteria 43 familial CRC occurrence). All had received counseling from a geneticist and were advised to have periodic colonoscopy.	As per study description. Selected patients who fulfilled Amsterdam were tested for mutations. 21% had been treated for CRC and/or endometrial cancer in the past.	As per study description	Objective and self-reported compliance

Did all patients have a personal history of an HNPCC-related cancer? Check one		
Yes	No	Uncl
	X	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
149/178 returned a completed questionnaire. Respondents significantly older than nonrespondents. Average f/u 5.8 years 61% of study sample fulfilled Amsterdam I Objective compliance data available for 132/149. Of these 95 (72%) were found to be compliant with screening advise. Of 37 (28%) noncompliant, 20 had postponed screening for at least 1 year, 13 were screened only once and 4 never underwent screening. Compliers were significantly younger than noncompliers and there was a nonsignificant trend to increased level of education, mutation status and type of colon screening. No difference between perceived colon cancer risk. Noncompliers significantly more likely to perceive barriers to screening. Noncompliers rated screening as being more embarrassing. On multivariate analysis only the perceived number of barriers to screening was associated significantly with noncompliance (OR 1.2, 95% CI 1.1-1.3). Of self-reported data, noncompliance was associated with embarrassment with screening procedure and sigmoidoscopy rather than colonoscopy. Compliers were significantly more likely to have remembered receiving a reminder letter.	ND	Although few high-risk individuals abstain from screening entirely, approximately 1 in 4 deviates significantly from the recommended frequency of screening. Increased compliance may be achieved by reducing the discomfort and embarrassment associated with the procedure and the use of reminder letters.	B No details on whether noncompliers had poor access/economic barriers to screening. Survey instruments novel and have not been validated.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						X A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	X 80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						X A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			
Analysis						X	B	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						A (strong)	(moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	X Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	X Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						X A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	X No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Boks, DE 2002, Ref ID 1059 Multicenter, Netherlands

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
66 patients with endometrial cancer from 46 families in an HNPCC registry.	Had an HNPCC mutation OR met revised Amsterdam criteria 38/50 patients included in final analysis had a mutation (19 MLH1, 16 MSH2 and 3 MSH6)	Cancer diagnosis verified by medical record or pathology report.	Survival compared with an age and stage-matched control group (2 controls per case) of patients with endometrial cancer from a population registry who lacked other malignancies.

Did all patients have a personal history of an HNPCC-related cancer? Check one		
Yes	No	Uncl
X		

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
No significant difference in distribution of tumor histologic types between cases and control. Survival rates for cases and controls were not significantly different. Cumulative 5-years survival 88 versus 82% for HNPCC versus sporadic. No difference in survival was observed between patients in the study group with a mutation identified and those without an mutation. No significant difference in survival for age or stage matching.	16 patients excluded from survival analysis because stage of cancer could not be verified or because a matched control	The outcomes in survival for endometrial cancer in the general population and women from families with HNPCC do not differ significantly.	C Treatment not described.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						X A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						X A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	X Yes	No						
Were data collection tools shown or are they known to be reliable?	X Yes	No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						X A (strong)	B (moderate)	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	X No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	X Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	X Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	X No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	X NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Bradshaw 2003, Ref ID 692 Single center UK

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
A two year cohort study described results of colonoscopy surveillance in a total of asymptomatic patients with a family history of CRC at a young age but who do not fulfill the Amsterdam criteria and those who fulfill the Amsterdam criteria.	Amsterdam I Criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	Asymptomatic individuals attending an appointment for CRC risk assessment with a genetic counselor between 2000 and 2002. Risk definitions: Low risk - Anyone not fulfilling medium or high risk criteria. Moderate risk – with family history of CRC but not fulfill Amsterdam criteria High risk - with a family history fulfilling the Amsterdam criteria 1 Fifty three individuals with a high risk family history and 123 individuals assessed at moderate risk were eligible for and had colonoscopic evaluation. Excluded patients with FAP.	Individuals with a family history fulfilling the Amsterdam criteria 1 (high risk, table 1) were recommended to undergo colonoscopy every two years from the age of 30 years. Moderate risk cases were offered colonoscopy at the age of 35 years, or five years before the youngest age of diagnosis of CRC in the family if this resulted in colonoscopy at a younger age and, if this colonoscopy was normal, a second at the age of 55 years.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
No cases of CRC were detected. 4 patients (8%) in high-risk group had adenomas. In moderate group, overall five individuals had an adenoma (2%). Proportion of patients younger than 50 found to have an adenoma was significantly greater in the high risk group than those in the moderate risk group (p=0.05).	448 attended genetic service for risk assessment. 53 with a high risk family history and 123 at moderate risk had a colonoscopy. Colonoscopy was complete to the cecum in 92%. See Figure 1 in original paper for detailed accounting of dropouts.	These findings indicate that the prevalence of significant neoplasia in groups defined by family history is low, particularly in younger age groups. These prospective data call into question the value of colonoscopy before the age of 50 in moderate risk individuals.	C Low completion rate of colonoscopy suggests examinations were not performed optimally (possible variation in endoscopists' experience). Analyses were post-hoc. Study likely to be underpowered. Short followup. No comment as to whether endoscopists were aware of the family history.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	X 1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	X 1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	X 80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						X A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 0-100	60-79	<60	ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	B (moderate)	X C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	Yes	X No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	X No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Brain, K, 2003, Ref ID 895 Single center UK

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Survey obtained from 16 regional genetic centers involved in predictive testing for HNPCC. Purpose was to gain evidence regarding core elements of the pre-test counseling protocol for HNPCC.	ND Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Center performs genetic testing for HNPCC.	Questionnaire involving core features of protocol for predictive testing.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Average number of pre-test counseling sessions was 2 (range 1-3) with an average of 4 weeks between sessions. Minimum number of sessions was 1 (range 1-2). Counselors included clinical geneticists and genetic nurse and gastroenterologists. Some included home visits, psychiatric consultation, explicit circumstances in which a client would be excluded or postponed from testing. Four centers reported that they were considered shortening the counseling protocol. Centers described a total of 144 topics as being core topics of prêt-test counseling.	16/20 centers participated. One declined because of few patients counseled. No other details provided.	"The present findings suggest that although some UK centers are favorable towards shortening pretest counseling for HNPCC, there is some uncertainty regarding the key elements of genetic counseling. There is currently little empirical evidence regarding which components of pretest counseling are effective in promoting good psychological outcomes. Controlled trials are needed."	B Very little detail provide about characteristics of centers/patient populations educational level etc. that might have bearing on the need for specific counseling protocols.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	**2,3**	**5**			**8c**	**1,6a,6b,7,10**
Family member/other high risk individual	**2,3**	**5**	**9**	**X** **8a**	**8b,8c**	**1,6b,9,10,11**
Public health or policy	**2,3**	**5**	**9**	**X** **8a**	**8b,8c**	**1,6,a,6b,7,10**

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	**Place an "X" in one**					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	X NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	X No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						X A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			

Domain/question	**Place an "X" in one**					***Overall rating***		
Analysis						A (strong)	X B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	No	X ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	X Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	X Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						X A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	X No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Brain, K 2005, Ref ID 74 Single center UK

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Randomized controlled trial involving 26 high-risk individuals for HNPCC who were randomly assigned to extended or shortened genetic counseling.	Mutation Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Unaffected individuals from families with a known HNPCC mutation.	Extended (two sessions of education and reflection held one month apart) or shortened (a single educational session) of genetic counseling. Questionnaires administered prior to and immediately after counseling (two "in house" measures and "validated measures" of systemic decision-making, satisfaction and psychological distress Also qualitative interviews.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
At baseline, counseling preferences were significantly more likely to be for information than for support (Z=-298, p=0.003). Participants were very positive in their evaluations of genetic counseling, reporting high levels of satisfaction with the information and support received in both groups (p=0.13). There were high levels of decision satisfaction regardless of study group (p=0.40). Levels of psychological distress were not significantly different (p=0.98). The effect of counseling protocol on knowledge was not significant. Analysis of counseling sessions indicated that counselor's reflective questions occurred equally in both protocols.	51 family members of proband invited, 34 agreed to participate, consent form returned by 31 of whom four subsequently declined and one withdrew for "practical" reasons.	This exploratory study indicates that shortening genetic counseling may be an appropriate means of supporting decisions already made by individuals about HNPCC testing. However, participants would benefit from preparatory information to help them reflect on issues not previously considered, which can then be explored more fully as part of a tailored counseling approach.	C No details regarding baseline familiarity, education level and other potentially relevant characteristics across study groups to assure randomization was successful.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	X 8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	X 8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	X <60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	X C (weak)
Is the method of random allocation stated?	Yes	X No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	X No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	X <60	ND	NA			
Analysis						A (strong)	X B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	X No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	X <60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?			
Brodersen, N 2004, Ref 385 Multicenter UK			
Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
Cross sectional study to examine anticipated reactions to genetic testing for HNPCC in 437 asymptomatic people with a family history of colorectal cancer	Amsterdam 1 criteria/mutations Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Registered with St. Mark's family cancer clinic as being at increased risk for CRC because of a family history. All receiving regular colonoscopy screening. No FAP in family. No personal history of cancer.	Questionnaires (non-validated) regarding anticipated emotional response and intended actions upon receiving test results.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
A number of significant differences between men and women in anticipated emotional response. More women anticipated feeling fairly or extremely worried if they did not have the test (36 versus 23 percent, p =0.009) or if the test was positive (64 versus 40 percent, p =0.008). More women anticipated that they would feel fairly or extremely regretful if the test showed that they were at high risk (30 versus 20 percent, p =0.03). More women thought that they would feel fairly or extremely angry if the test showed that they were at high risk (33 versus 13$, p <0.001). There was only one significant difference between men and women in anticipated reaction following genetic testing: if at low risk more women then men would try to adopt a healthier lifestyle (p=0.007. When asked if they would have more screening if found to be at high risk, 416/432 (96%) said that they probably (23%) or definitely (74%) would.	743 questionnaires sent out, 516 returned (71%), 79 who had cancer excluded.	The emotional effects of receiving a test result are unlikely to be much more severe than the effects of choosing not to have a genetic test, and in some cases not having a genetic test is likely to result in a poorer emotional adjustment. Women anticipate more extreme emotional reactions than men.	C Access, educational levels, familiarity with CRC screening or HNPCC not described.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	X 9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	X 9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A	X	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	B (moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	X 60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Brown, S 1998, Ref ID 2147 Single center, UK

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
157 with a history of CRC who developed a second primary in the HNPCC spectrum (CRC, stomach, urinary, ovary, endometrial) compared with 444 with a single colorectal cancer.	Amsterdam I criteria and modified criteria. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: X; No: ; Uncl:	History of CRC Second primary in HNPCC spectrum Second CRC between 1992-1995 Comparison group: history of CRC but no evidence of second primary cancer in HNPCC spectrum, colitis, or FAP.	Interview/family history Compared observed risk of CRC in 1st degree relatives with expected incidence of CRC for the population adjusted for age and sex.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
For multiple primary group, 24 probands (16%) had one 1st-degree relative with CRC, five (4%) had two, and five (4%) had three or more first degree relatives. The frequency of familial cancer was similar to that of the comparison group. In the study group, 10 (8%) fulfilled the Amsterdam criteria and six additional were considered to be likely to have HNPCC. This compared with only three families (0.7%) from the comparison group who were considered likely to have HNPCC. RR of CRC among 1st degree relatives was 3.2 ($p<0.00001$). RR of extracolonic cancers: In single primary group, both HNPCC associated and non-HNPCC associated extracolonic cancers were seen at the expected frequency. In the multiple primary group, extracolonic non-HNPCC cancers were also seen at the expected frequency. However, HNPCC associated extracolonic cancers were seen at over twice the frequency of the general population.	Control patients: 595 approached, 4 excluded because of IBD, 1 because of FAP, 4 because they were adopted. 56 had died and had not traceable next of kin while 34 patients and 15 next of kin refused to give a history, leaving 478 patients. Of these 34 had documented multiple primaries and were excluded leaving 444. 166 met multiple primary cancer criteria of whom 157 were approached and 128 provided a complete family history.	This study highlights the importance of taking a family history in patients with multiple primary cancers and indicates the risk of malignancy in their relatives.	C No information given as to whether patients had undergone screening. No adjustment for length of followup. Small sample size of patients with HNPCC. Validity of tumor verification and family history unclear.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	X 8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	X 8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	X No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	B (moderate)	X C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	X ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	X 60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Study: Calistri, D 2000

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Multicenter, Italy 45 unrelated patients with CRC either: fulfilling Amsterdam criteria, from families meeting 2/3 Amsterdam criteria, CRC < 50 but no family history, at least one-first degree relative with CRC, or multiple neoplasms	Tissue samples from cancer analyzed for MSI. DNA from peripheral blood samples analyzed for hMSH2 and hMLH2	hMSH2 and hMLH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? Y Deletion analysis? ? Conversion analysis? ?	Am 1 Am R Beth 1 Beth R MSI-H X MSI-L X IHC Age <50 Suggestive family history X *Specify* See inclusion criteria Other *Specify*	Authors noted that some mutations had been described previously but did not comment as to whether they were known to be deleterious or whether the two additional mutations they detected were considered to be deleterious.

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
45 enrolled, no dropouts 25/45 male this is a convenience sample; dropouts do not apply, mean age 51.5	8 mutations noted 4 hMLH1 and 4 hMSH2	16/45 tumors MSI-H 4/45 tumors MSI-L 25/45 MSS	ND	As the most likely mutation carriers, HNPCC subjects [i.e. Amsterdam criteria] might be directly analyzed for gene mutations while in non-HNPCC subjects, MSI should be used to select for MMR.	ND	C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	7/13
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	11/13 MSI-H
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1	X	ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Amsterdam 1 criteria **versus**	Am 1 +	X	+	(A) 7	(B) 6

other family history as above	Am R +		-	(C) 1	(D) 31
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
>2/3 Amsterdam criteria OR "partially documented" Amsterdam criteria **versus** other family history as above	Am 1 +		+	(A) 7	(B) 11
	Am R +		-	(C) 1	(D) 26
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Amsterdam criteria	Am 1 +		+	(A) 6	(B) 5
	Am R +		-	(C) 0	(D) 2
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				

	IHC (no staining)	
	Other (specify)**	

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
"Family history"	Am 1 +		+	(A) 1	(B) 1
	Am R +		-	(C) 0	(D) 15
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
"Juvenile cancer"	Am 1 +		+	(A) 0	(B) 0
	Am R +		-	(C) 0	(D) 7
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

Note, did not extract for "multiple tumors" since only one patient.

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			X
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction for Analytic Validity

Study: (Casey, 2005, RefID 258)

Inclusion criteria (all must be yes)

	Yes	No
Did study evaluate biological material from patients with CRC considered to be at risk for HNPCC?	x	
Did the study report ANY of the following? (check which one below)		
1) Proportion MSI-H with NIH markers versus other markers		
2) Sensitivity or specificity of MSI-H using NIH markers compared with a reference standard that the study claims is better		
3) Sensitivity or specificity of IHC compared with an immunohistochemical standard that study claims is better		
4) Sensitivity or specificity of a genetic technique compared with a reference standard (or combination of standards)	x	
5) Reliability of MSI/IHC/genetic method across laboratories or within a laboratory		
Are data (proportions or 2 X 2 tables) extractable or reliability data extractable?	x	

*NIH markers are BAT-25, BAT-26 D2S123, DS346 and D17S250

Exclusion criteria (exclude if yes)

	Yes	No
Did the study include the index test in the reference standard?		x

Describe source of biological materials (and whether patients were known to have an HNPCC phenotype)	Summarize how materials were processed and analyzed
Genomic DNA was isolated from lymphoblastoid cell lines from each participant using a blood kit (Qiagen Inc, Valencia, Calif). The majority of cases (85 of 89) were selected because they also had prior evidence of a defect in mismatch repair due to having either a tumor with high microsatellite instability or loss of expression of a mismatch repair protein by immunohistochemistry.	Goal: Conversion analysis vs. conventional analysis and DNA sequencing (MLH1, MSH2, and MSH6). Cases were selected for study based on a number of clinical characteristics and the presence of defective mismatch repair either by microsatellite instability testing or immunohistochemistry, or both. Cases included 64 CRC patients from HNPCC Amsterdam 1 Criteria Families, 8 HNPCC-like cases (at least 2 first- or second-degree relatives with colorectal cancer or 1 relative with endometrial cancer and at least 1 other relative with colorectal cancer who did not meet Amsterdam 1 criteria), and 17 colorectal cancer cases diagnosed prior to age 50 years. All patients received both conversion analysis and conventional analysis and DNA sequencing for mutation. The identity of the participant samples, family history of cases, and data on MSI or IHC were blinded until the end of study. DNA sequencing: PCR technique. DNA sequencing was performed on all cases for mutations in *MLH1* and *MSH2*. *MSH6* sequencing was performed only on those cases (n=23) that were negative for deleterious mutations in *MLH1* or *MSH2*. Mutations were confirmed by repeating the PCR amplification reaction and sequencing. Conversion analysis: Hybrid cell lines were generated following conversion technology protocols by fusing lymphoblastoid cells from participants with E2 mouse cells. The E2 cell line is an immortal mouse embryonic cell line. The markers used for genotyping were derived from the linkage mapping set (version 2.5, MD10; Applied Biosystems). Polymerase chain reaction amplification of the DNA markers was performed with Taq and reaction buffer (Invitrogen, Carlsbad, Calif). The PCR products were fractionated by capillary electrophoresis using an ABI 3100 automated DNA sequencer. Total RNA was isolated using the RNeasy Kit (Qiagen) and cDNA generated using Super Script II reverse transcriptase (Invitrogen). Both positive and negative reverse transcriptase cDNA reactions were performed from each RNA source. Conversion analysis combines the separation of alleles into hybrids along with analysis of cDNA sequence changes and effects on mRNA expression. Changes in mRNA transcript size or levels of mRNA expression of *MSH2* and *MLH1* genes were determined by reverse transcriptase PCR from hybrids containing chromosomes 2 or 3.

Genetic technique with a reference standard

Overall results; results for each subgroup of patients can be segregated.

		Reference standard genetic technique: conventional analysis and DNA sequencing	
		Positive: deleterious	**Negative**
Index genetic technique: Conversion analysis	**Positive:** deleterious	**35**	**18**
	Negative	**28**	**8** (5 no mutation and 3 insufficient data reported as unclassified variant)

*Mutations were considered pathogenic (or deleterious) if the change met any of the following criteria. a frameshift mutation that would be predicted to result in a truncated protein; nonsense mutations; missense mutations if additional mRNA expression data revealed aberrant splicing or exon skipping; splice site mutations if additional data revealed aberrant splicing; large genomic deletions that removed at least 1 exon; or duplication of exons. Four in-frame deletions and missense mutations of unknown clinical significance were not classified as deleterious because additional data are needed.

Intra or inter-hospital reliability data	**Describe**
ND	

	Study Quality	Yes	No	Unc
1	Was the description of how MSI or IHC and other genetic techniques described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
2	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
3	Was MSI, IHC, other genetic testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
4	Was there a clear description of which mismatch repair mutations were being tested for?	x		
5	Were quality control methods described for the molecular and genetic tests?	x		
6	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
7	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	x		
8	Was microdissection performed?			x
9	Did the study specify whether the biological tissues were from patients known to have HNPCC clinically?	x		
10	Did the study include a control group in which biological material was obtained from patients known not to have HNPCC clinically		x	
11	**Overall rating (A B C)**	A/B (Blinded analyses)		

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Cats 1995; Ref ID 2753, Multicenter, Netherlands

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
30 patients at risk for HNPCC randomly assigned to calcium carbonate (1.5g) or placebo three times daily for 12 weeks. Primary endpoint was epithelial cell proliferation in colon and rectum.	Unclear Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: ; Uncl: X	1st-degre relatives of patients with HNPCC participating in regular screening program. At 50% lifetime risk of developing CRC	Calcium supplementation or placebo.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Cytolytic activity decreased with calcium but difference was not statistically significant. No difference in sigmoid colon or rectum.	53 invited 30 participated, reasons for not participating not described.	Oral calcium was shown to cause only a minor nonstatistically significant reduction in epithelial cell proliferation in the rectum compared with placebo and have no effect in the sigmoid and descending colon. These results cast doubt on the value of calcium supplementation in the prevention of CRC, especially in individuals already consuming an adequate amount of dietary calcium.	B Small sample size. Could not fully adjust for baseline calcium consumption.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	X C (weak)
Is the method of random allocation stated?	Yes	X No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	X No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	X No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	X No						
Blinding						X A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	X Yes	No	ND	NA				
Data Collection methods						X A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	X Yes	No						
Were data collection tools shown or are they known to be reliable?	X Yes	No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs	80-100	X	<60	ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
by groups, record the lowest).		60-79						
Analysis						X A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	X Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	X No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						X A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	X No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Cederquist, 2001 Ref ID 1536 Multicenter, Northern Sweden

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Family histories were obtained on persons with colon and endometrial cancer. The cancer risks in their first degree relatives were analyzed; they were estimated in relation to MSI status and age at diagnosis in proband. Regional Cancer Register identified 89 cases; in 10 cases, unable to localize the patient, to verify the diagnosis or to obtain approval to contact the family. Thus, 36 colon-endometrial cancer cases and 43 colon-colon cases were included in this study.	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: X No: Uncl:	First degree families of CRC and endometrial cases (N=649) Probands- Colon-endometrial cancer cases (n=36) and colon-colon cases (n=43)	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
In ≤ 50-year-old cohorts with MSI positive status, standardized incidence ratio (SIR) for colon cancer 21.13 (95% CI 11.82-34.85). In ≤ 50-year-old with MSI negative status, significant increase in SIR for stomach and uterus cancer. In > 50-year-old with MSI positive status, significant increase in SIR for rectal and uterus cancer. In > 50-year-old with MSI negative status, no increased risk at all was observed. In total, 649 FDR were analyzed (328 males and 321 females) with a total of 17,088 person years. Among first degree relatives, 111 cancers were observed with an expected number of cancers of 65.86, yielding a SIR of 1.69 (95% CI, 1.39-2.03); colon, rectal and uterus cancer exhibited a significantly increased risk. In the ≤ 50-year-old cohort, the overall risk was 2.67 (95% CI 2.08-3.38) compared with the >50-year-old cohort where no increased risk was observed (SIR 1.04, 95% CI 0.74-1.40). In the ≤ 50-year-old cohort, significant increased risk were found for most other HNPCC-associated tumors such as stomach, colon, rectal, uterus and ovary. Colon cancer had a pronounced increased risk with a SIR of 12.57 (95% CI 7.96-18.86).		"A first degree relative to a person with a primary cancer of the colorectum or the colon/endometrium have a significantly increased risk of having a colorectal or other HNPCC-associated cancers if the proband is diagnosed with one of the cancers before age 50."	B

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely X	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100 X	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell X					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No X	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No X						
Were data collection tools shown or are they known to be reliable?	Yes	No X						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100 X	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No X					
Is there a statistically significant difference between groups?	Yes	No	ND					

Domain/question	***Place an "X" in one***					***Overall rating***		
	X							
Are the statistical methods appropriate?	Yes X	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Christensen, M 2002

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Multicenter, Denmark 42 patients with colorectal cancer selected based upon clinical and family history meeting either Amsterdam I criteria (n=11) or a suggestive family history.	MSH2 and MLH1 genes sequenced in 31 patients. MSI obtained in 35 patients IHC performed in 40 patients. Compared sensitivity/specificity of these tests against sequencing as the reference standard.	hMLH1 and hMSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? Y Deletion analysis? ? Conversion analysis? ?	Am 1 Am R Beth 1 Beth R MSI-H X MSI-L IHC X Age <50 Suggestive family history *Specify* Other *Specify*	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
42 enrolled, all analyzed as above, sex not described, age not described	MMR detected in 13/42 patients [Note that only 31 pts got gene sequencing!] 8 hMSH2 5 hMLH1 (one patient had both)	11/42 Amsterdam I 11/25 tested had MSI-H 9/29 tested had lack of staining on IHC	There was concordance between sequencing and the combined MS/IHC approach for 94% (16/17) tumors. Concordance among the three methods was found for 74% (17/23) tumors	IHC should be used in combination with MSI analysis to prescreen suspected HNPCC patients for selection for sequencing.	ND	C Authors did not explain how patients were selected for sequencing or index tests. Had incomplete verification in cohort.

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	4/11
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	8/11
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		4/11
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients who fulfilled Amsterdam I or were otherwise considered at increased risk for HNPCC	Am 1 +		+	(A) 11	(B) 6
	Am R +		-	(C) 1	(D) 16
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients who fulfilled Amsterdam I or were otherwise considered at increased risk for HNPCC	Am 1 +		+	(A) Only one patient has MSI-L so not extracted	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*	X			
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients who fulfilled Amsterdam I or were otherwise considered at increased risk for HNPCC	Am 1 +		+	(A) 9	(B) 3
	Am R +		-	(C) 4	(D) 15
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

Grade	**Explanation for Quality Scoring**
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?		X	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants		X	
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?		X	
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Claes, E 2004, Ref ID337 Single center, Belgium

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Describe motivation, recall of cancer risks and illness representations of 40 individuals who had a predictive test for HNPCC.	Mutation Did all patients have a personal history of an HNPCC-related cancer? Check one Yes:	No: X	Uncl:	Self-referred unaffected persons who had a predictive test for HNPCC.	Surveys pretest and one-month post-test

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Motivation for predictive testing: Most common motivations cited were early detection of CRC (95%), early detection of endometrial cancer (93%), knowledge of children's risk (70%), reduction of uncertainty (38%). **Recall and perception of risk:** 36/40 correctly recalled the 50% risk of being a mutation carrier **Awareness of cancer risk:** The majority provided an "accurate" estimation of the lifetime risk of developing CRC. Distress (STAI-Trait scale): Differences between carriers and non-carriers regarding mean pre- and post-test IES scores for colorectal cancer and State-anxiety were not statistically significant. Although there was a decrease of distress over time in the total group, the decrease was only significant for State-anxiety and avoidance for colorectal cancer. The difference between pre- and post-test scores in the subgroup of carriers was not statistically significant while the decrease in the group of non-carriers was statistically significant for State-anxiety (Wilcoxon signed ranks test, $P < 0.01$) **Early detection behavior:** 26/40 had at least one colonoscopy before predictive testing. All carriers had the intention of having colonoscopies on a regular basis. All female carriers had intention of having yearly examinations of the endometrium. The majority of noncarriers either did not have the intention to have a colonoscopy or only when indicated.	40/48 included 1 patient who had difficulty concentrating 1 patient did not fill in survey 2 patients who were >70 4 did not fill out survey at 1 month	"Our results in the first applicants of predictive genetic testing for HNPCC who came forward on their own initiative within our clinic-based genetic testing program illustrate that predictive genetic testing did not induce major short-term psychological problems and/or reluctance to adopt health-related behavior in the future when it is offered in the context of a multidisciplinary approach."	C Small sample, highly-selected (since self-referred). Followup short. Some data collected by telephone and others by written instrument; unclear if mode of administering affected the results.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	X 80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	X C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	X No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Claes, E 2005 Ref ID 185. **Note** same patients as Claes, E 2004, Ref ID337 Single center, Belgium

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Evaluated illness representations, distress, and health-related behavior one-year after disclosure of predictive genetic tests results for HNPCC in 36 carriers and 36 noncarriers.	Mutations Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	Predictive testing offered to unaffected persons from families in which a DNA MMR mutation had been identified. Excluded age >70	Surveys before and one-year after predictive testing.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
General distress: Mean scores within normal ranges or lower than population means. Carriers and noncarriers did not differ significantly before testing but carriers had significantly higher scores than noncarriers on cancer-specific distress scores for CRC and endometrial cancer. **Impact of genetic testing result:** about ½ of carriers (19/36) and less than 20% of noncarriers (6/36) reported at least one disadvantage of knowing the predictive test result. The main reported disadvantages for carriers were "burden of regular medical examinations" (22%) and the psychological burden (20%). The main disadvantage for noncarriers was that they experienced difficulties due to their favorable test results toward carrier relatives (e.g. survivor guilt).	One excluded because of difficulty concentrating others because age >70. 72/79 participated.	"Our study did not reveal major psychological problems, measured by psychometric measurements, as a consequence of predictive testing when offered in the context of a multidisciplinary counseling context. However, interview data indicated some individually different problems specifically related to the predictive test (e.g. worry, difficulties in relation to other relatives burden of regular follow-up). The presented data are promising regarding the impact on health-related behavior."	B Highly-selected (since self-referred). Some data collected by telephone and others by written instrument and not clear if mode of administering affected the results.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
All but one carrier and all but 2 noncarriers reported at least one advantage. The two advantages most cited by carriers were "instrumental advantage" (89%) and certainty/reduction of uncertainty (33%). For noncarriers, the two most cited advantages were "reassurance" (50%) and the fact that the children were not at risk anymore (39%). **Health-related behavior within the year after the predictive test:** 11 carriers (31%) never had a colonoscopy prior to genetic testing. Within the year after disclosure, 27 carriers had a colonoscopy. The three who did not were <25. Thus, compliance with recommendation was 100%. 22 noncarriers (61%) had at last one colonoscopy prior to genetic testing. None had a colonoscopy within the year after testing.			

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	X 80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			
Analysis						A	B	X

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	(moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	X No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Codori, A 1999, Ref ID 2053 Single center, USA

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Evaluated uptake of genetic testing for HNPCC among 1st-degree relatives of CRC by comparing 77 test acceptors and 181 decliners. 505 1st-degree relatives of CRC from 118 kindreds invited to participate. Final sample included 77 acceptors and 181 decliners (see intervention for definitions).	Amsterdam criteria I or mutation in family. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	>18, no personal history of CRC, at least one 1st-degree relative with CRC. Flow: 505 invited; 233 interested 264 not interested and 8 undeliverable. Of the 233 Interested, 104 completed phase I (8/104 withdrew, 19/104 entered phase 2 awaiting blood draw and **77**/104 entered phase II and had blood draw). Of the 264 not interested 247 were called for interview (the remaining have not been interviewed yet); **181**/147 completed the interview.	Invitation to undergo genetic counseling Acceptors = accepted counseling and testing Decliners = declined counseling and testing Decliners had telephone interview, acceptors filled out a questionnaire

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Significant predictors of acceptance on multivariate analysis were: perceived ability to cope with a positive gene test result, risk perception, frequency of thoughts about CRC (not at all or rarely versus sometimes or more) and CRC screening history. Coping: ≥90% confident OR 4.0 (95% CI 1.53-10.3, p=0.005) CRC screening: never had a colonoscopy (OR 0.45, 95% CI 0.21-0.93, p=0.030) Cancer thoughts: Rarely/never versus sometimes, often, a lot: OR 0.44 95% CI 0.17-1.13, p =0.088)	ND	"Our study showed that people who believe they will get CRC, think about getting it, believe that they can cope with the "bad news," and already engage in cancer prevention behaviors are more likely to be tested."	B Validity of survey instruments and telephone interviews were not established.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	X <60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						X A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	X Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	X No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	X <60	ND	NA			
Analysis						A (strong)	X B	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
							(moderate)	
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	X <60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Collins, V 2005, Ref ID 140 Multicenter, Australia

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
134 invited to participate, 114 returned the baseline questionnaire, 98/114 returned a follow-up questionnaire Evaluate screening and preventive behaviors following predictive testing for HNPCC. Annual colonoscopy beginning at age 25 or 5 years earlier than youngest affected family members recommended in those at risk for HNPCC. Those not carrying the mutation are advised to follow population screening guidelines. Endometrial screening "can be considered" from age 30-35 or even 25 generally with annual transvaginal ultrasound and endometrial sampling in premenopausal women and annual transvaginal ultrasound and CA 125 testing for postmenopausal women. Prophylactic oophorectomy and TAH also considered for known mutation carriers ages 30 to 35 or when childbearing is complete.	Mutation. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	Individuals undergoing predictive genetic testing at 1 of 5 familial cancer clinics in Australia between1998-2002. Individuals were eligible who had never had CRC or any of the cancers associated with HNPCC. They were excluded if they had limited literacy in English.	Surveys

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Of 114 participants, 32 identified as MMR carriers and 82 as noncarriers. At baseline, 3/12 <25 and 74/101 ≥25 reported a colonoscopy. On multivariate analysis, perceived risk of developing bowel carcinoma was the only variable significantly associated with having a colonoscopy (OR 103, 95% CI 1.00-1.05, p=.03) At 12-month follow-up, 71% of carriers reported having a colonoscopy in the 12 months since receiving their genetic results. Among noncarriers, 8 (12%) also reported a colonoscopy. A significantly higher proportion of those who reported a colonoscopy were mutation carriers or women. This group also had higher levels of perceived risk. However, only carriers status was statistically significant in a multivariate model (OR 20, 95% CI 5.8-68, p<.0001). At baseline 19/63 women (30%) reported ever having a transvaginal ultrasound and 4/58 (7%) an endometrial sampling. At 12 months, 47% (8 of 17)of carriers reported having a transvaginal ultrasound and 53% (9 of 17) endometrial sampling. 39 noncarriers reported either a transvaginal ultrasound (10%) or endometrial sampling (5%). At baseline, no one reported having a prophylactic colectomy; at 12 months, one indicated intention to do so.	ND	The majority of individuals reported appropriate screening behaviors after predictive testing for HNPCC. The small group of noncarriers who had screening after genetic testing might benefit from additional counseling.	B 12 month data subject to Hawthorne Effect. Self-report without attempt at verification of procedures. Issues of access to screening procedures not addressed.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) **Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence**

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						X A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	X 80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						X A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			
Analysis						X A	B (moderate)	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)		
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Study: Colombino 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**				**Predictors analyzed (check all that apply)**		**How were deleterious, missense/variants mutations defined (ND if not described)**
Multicenter, Italy 362 patients proven to be of Sardinian origin with histologically proven CRC. All are consecutive, unselected CRC patients	Family history assessed. Patients classified as sporadic if no CRC in 1st and 2nd-degree relatives, low familial recurrence if one additional family member, familial if at least three family members. HNPCC diagnosed based on Amsterdam criteria. Patients with familiar CRC underwent gene sequencing Sporadic were screened for mutations identified in "familial"	hMLH1 and hMSH2		Y	N	?	Am 1	X	Compared mutations to 103 unrelated normal individuals There were 8 different mutations and half of them were missense. No separate data given, unfortunately
			≥5 MSI markers used?				Am R		
			MSI-H defined by ≥ 2 markers?				Beth 1		
			Microdissection?				Beth R		
			Gene screening?	X			MSI-H		
			Deletion analysis?			X	MSI-L		
			Conversion analysis?			X	IHC		
							Age <50	X	
							Suggestive family history	X	
							Specify As in inclusion criteria		
							Other		
							Specify		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
179/362 men=49% Median age at dx 60 (27-87)	Of 48 patients tested, 14 had a germline MMR: 12 on MSH2, 2 in MLH1	103/362 "familial" 55/162 two cases 48/362 had at least three family members 13/362 fulfilled Amsterdam	ND	Strongly reaffirms several previous predictors (earlier diagnosis age, presence in family of three or more CRC) for occurrence of MLH1 and MSH2 mutations	ND	C Incomplete verification and reporting.

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	10/13
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with one additional family member with CRC vs sporadic	Am 1 +		+	(A) 16	(B) 87
	Am R +		-	(C) No data (5)	(D) No data (254)
	Beth 1 +			In parentheses, because the "sporadic" were screened only for the mutations found in the "familial"	
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Among 103 with >=2 CRC in family				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Two cases in family	Am 1 +		+	(A) 2	(B) 53
	Am R +		-	(C) No data	(D) No data
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
3 cases in family	Am 1 +		+	(A) 14	(B) 35
	Am R +		-	(C) No data	(D) No data
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
	Am 1 +	X	+	(A) 10	(B) 3
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Age <= 45	Am 1 +		+	(A) 6	(B) 55
	Am R +		-	(C) No data	(D) No data
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			X
7	Were withdrawals from the study explained?			X
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			X
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			X
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Curia 1999

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
30 unrelated HNPCC patients from the files of the University of Florence; 17 has Amsterdam criteria 26 have CRC and 15 are Am1 among them	SSCP and sequencing were done in all patients; IHC were done in 24 patients; MSI were done in 22 patients We caution that the MSI and IHC data are completely convenience-based	HMLh1 and hMSH2	See table below	See table below	Based on the literature, predicted alteration and contrast with controls

	Y	N	?
≥5 MSI markers used?		X*	
MSI-H defined by ≥ 2 markers?	X		
Microdissection?	X		
Gene screening?	X		
Deletion analysis?		X	
Conversion analysis?		X	

They screened with 3 and if none was unstable up to a total of 7.

Am 1	
Am R	
Beth 1	
Beth R	
MSI-H	
MSI-L	
IHC	
Age <50	
Suggestive family history	
Specify	
Other	
Specify	

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
30 unrelated patients, but only 26 have CRC	7 hMLH1 and 5 MSH2 (3 pathogenic, 4 uncertain, 5 polymorphisms) in 30 unrelated patients Pathogenic: 3 (2mlh1 , 1 msh2)	15/26 eligible were AM1 Unclear if 2 or 1 they refer to "Am criteria" 23/26 were <50 y 12/26 were <40y	Notably, the lack of hMLH1 or hMSH2 immunostaining and microsatellite instability were observed in tumors from patients in whom a pathogenic mutation could not be identified by SSCP screening of the coding sequence.	Constitutional alterations in hMLH1 and hMSH2 transcript expression may represent genetic markers for HNPCC carrier status also in cases in which mutational analysis did not detect a definite pathogenic variant.		B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	1/15 Am1 Eligible has pathogenic mutation
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		12/15 were MSI-H (Am1)
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		7/15 had IHC (Am1)
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		x
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Unrelated patients with HNPCC	Am 1 +	X	+	1	16
	Am R +		-	2	11
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Unrelated patients with HNPCC with available specimens	Am 1 +		+	1	13
	Am R +		-	0	10
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				
	MSI only				

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Unrelated patients with HNPCC with available specimens	Am 1 +		+	1	18
	Am R +		-	0	3
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI unstable	X			

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			X
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			x
7	Were withdrawals from the study explained?	x		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	x		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	x		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			x
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			x
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			x

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: De Abajo 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Referrals to the clinic for familial cancer at the San Carlos University Hospital in Madrid. All index cases were CRC	All tested for MLH1 and MSH2 and the ones who were negative were tested for MSH6 too.	MLH1 MSH2 MSH6 (among negative for the above)	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? Y Gene screening? Y Deletion analysis? N Conversion analysis? N The NCI/Bethesda marker ser was used	Am 1 X Am R X Beth 1 X Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other *Specify*	Predicted alteration; comparison with 100 controls; literature and databases

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
132 nd on age and sex dropouts NA (only 19 not MSI-typed)	24 mlh1 17 msh2 3 msh6	56/132 AM1 67/132 AM2	NA	MSH6 disease causing mutations are very infrequent in Spanish CRC referral cases	ND	B

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		AM1 → 32/56 (MLH1 and MSH2) and 1/56 MSH6 AM2 → 36/67 (MLH1 and MSH2) and 3/56 MSH6
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		Am1 → 42/55 analyzed were MSI-H Am2 → 44/62 analyzed were MSI-H
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Referrals to the clinic for familial cancer at the San Carlos University Hospital in Madrid. All index cases were CRC MSH6 is included	Am 1 +	X	+	33	23
	Am R +		-	11	65
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Referrals to the clinic for familial cancer at the San Carlos University Hospital in Madrid. All index cases were CRC MSH6 is included	Am 1 +		+	39	28
	Am R +	X	-	5	60
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Referrals to the clinic for familial cancer at the San Carlos University Hospital in Madrid. All index cases were CRC MSH6 is included	Am 1 +		+	44	60
	Am R +		-	0	28
	Beth 1 +	X			
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Among AM2 patients MSH6 is included	Am 1 +	X	+	33	23
	Am R +		-	6	5
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Among Beth patients MSH6 is included	Am 1 +		+	39	28
	Am R +	X	-	5	37
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Among Beth patients MSH6 is included	Am 1 +	X	+	33	23
	Am R +		-	11	37
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
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Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			X
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?	X		
7	Were withdrawals from the study explained?			X
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?		X	
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?	X		
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			X
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		x	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Debniak 2000

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
168 consecutive patients with CRC in whom FAP was excluded. GROUP B: 25were SHNPCC based on age <=40, familial HNPCC-related Ca or synchronous or Metachronous Ca 143 were the rest, apparently sporadic: GROUP A: 43/143 Patients apparently sporadic , ie a) late-onset (>40) no family history of HNPCC-related tumors and no syn-or metachornous cancer presumably sporadic Poland Center	IHC performed in all patients MSI examined in all Sequencing performed in all from group B and those from group A that showed abnormal IHC or MSI	MLH1, MSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? ? Deletion analysis? ? Conversion analysis? ?	Am 1 Am R Beth 1 Beth R MSI-H X MSI-L IHC X Age <50 Suggestive family history X *Specify* Absence of sporadic criteria Other *Specify*	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
168 but only 68 analyzed, age ND, dropouts ND	6 mutations detected of the 68 included 4 MLH1 2 MSH2	Group A: normal protein expression in 42/43 1 patient probably negative staining MSI present in 4/43 tumors Group B: Normal protein expression 18/25 tumors Staining definitively negative MLH1 3 cases, MSH2 in two while in 2 patients, MLH1 was probably absent MSI was present in 9/25 cancers	Among 9 patients with MSI 2 were "probably abnormal" for protein staining.	Performing pedigree analysis/clinical data (for exlusion of late-onset sporadic CRC) in conjunction with IHC was lowest cost but missed 1/6 mutations. Additional preselection by IHC and MSI was required to detect all mutations.	ND	C Not clear how "sporadic" subgroups was chosen

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	1/3
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		2/3
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		2/3
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	X
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify) Absence of criteria that suggested sporadic disease above: Also performed testing on a sample ? random of low-risk population	x	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Selected patients considered to Have sporadic colorectal cancer Because of absence of clinical Features suggestive of HNPCC PLUS a group of patients considered To be at high risk for HNPCC	Am 1 +		+	(A) 5	(B) 20
	Am R +		-	(C) 0	(D) 43
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	Suggestive family History as above	X			

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with clinical history Suggestive of Lynch syndrome	Am 1 +		+	(A) 5	(B) 4
	Am R +		-	(C) 1	(D) 15
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with clinical history Suggestive of Lynch syndrome	Am 1 +		+	(A) 1	(B) 1
	Am R +		-	(C) 5	(D) 18
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with clinical history Suggestive of Lynch syndrome	Am 1 +		+	(A) 5	(B) 2
	Am R +		-	(C) 1	(D) 17
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	IHC OR MSI	X			

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients without a clinical history Suggestive of the Lynch syndrome	Am 1 +		+	(A) 0	(B) 1
	Am R +		-	(C) 0	(D) 42
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients without a clinical history Suggestive of the Lynch syndrome	Am 1 +		+	(A) 0	(B) 5
	Am R +		-	(C) 0	(D) 38
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	IHC OR MSI	X			

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			X
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?	X		
7	Were withdrawals from the study explained?		X	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants		X	
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?			X
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Dieumegard 2000

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Multicenter, France 34 patients with CRC who represented one of three groups: 1) Amsterdam I criteria 2) Incomplete Amsterdam (missing at least one criteria but strong family history) 3) Age <50 and absence of HNPCC-related cancer sin family	All patients tested for MSI. Patients with MSI-H and nine MSS cases underwent germline testing for MMR. IHC was performed in all but 4 patients.	hMLH1 and hMSH2	See table below	See table below	Reported results as "non-ambiguous:(frame-shift or splice site) and missense"

	Y	N	?
≥5 MSI markers used? (20 markers)	X		
MSI-H defined by ≥ 2 markers?	X		
Microdissection?			X
Gene screening?	X		
Deletion analysis?			X
Conversion analysis?			X

Am 1	X
Am R	
Beth 1	
Beth R	
MSI-H	X
MSI-L	
IHC	X
Age <50	
Suggestive family history	X
Specify See inclusion criteria	
Other	
Specify	

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
34: 10 group 1, 7 group 2 and 17 group 3, sex not described Age at cancer diagnosis group 1 26-55 (range), group 2 37-56, group 3 17-50	6 patients with MMR in group 1, 3 in group 2 and none in group 3. In total, 9 MMRs detected 6 inMLH1 and 3 in MSH2	15/34 MSI-H Lack of protein expression observed in 10 tumors (of 30 who had complete testing)	Lack of protein expression in 10 tumors all being MSI-H. All sporadic MSS tested had normal staining.	Simple tests such as MS study combined with hMSH2 and hMLH1 protein immunostaining performed on tumor tissues may provide valuable information to distinguish between familial and probably hereditary, and sporadic CRC cases	ND	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	6/10
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	9/10
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1	X	4/8 (not all tested)
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +		
Beth 1 +		
Beth R +		
Age <50	X	
Suggestive family history (specify) Amsterdam I except missing 1 criteria	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients fulfilling Am 1	Am 1 +		+	(A) 6	(B) 3
	Am R +		-	(C) 0	(D) 1
	Beth 1 +				
	Beth R +				
	MSI-H*+L	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients fulfilling Am 1	Am 1 +		+	(A) 2	(B) 2
	Am R +		-	(C) 2	(D) 2
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients fulfilling Am 1 minus 1 criteria	Am 1 +		+	(A) 3	(B) 3
	Am R +		-	(C) 0	(D) 1
	Beth 1 +				
	Beth R +				
	MSI-H*+L	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients fulfilling Am 1 minus 1 criteria	Am 1 +		+	(A) 2	(B) 3
	Am R +		-	(C) 1	(D) 0
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients age <50 and no family History of HNPCC cancers	Am 1 +		+	(A) 0	(B) 0
	Am R +		-	(C) 0	(D) 7
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients age <50 and no family history of HNPCC cancers	Am 1 +		+	(A) 0	(B) 0
	Am R +		-	(C) 0	(D) 7
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

Grade	Explanation for Quality Scoring
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Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
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6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?	X		
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?	X		
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
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MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Dove-Edwin, 2002 UK, The Netherlands Multicenter

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
UK family clinic: 184 women from HNPCC (67) or HNPCC-like families (117) had annual or biennial endometrial carcinoma surveillance by ultrasound. Amsterdam Criteria had not yet been established so modification included no cancer cases under 50 years, or cases of endometrial carcinoma The Netherlands registry: 108 women from 38 HNPCC families had transvaginal ultrasound scan annual or biennial. 25 families were AC positive, 3 ACII positive, 10 families suggestive of HNPCC and HNPCC mutations present	Amsterdam Criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	See study description	Annual or biennial endometrial transvaginal ultrasound scan, if unavailable transabdominal pelvic scans recommended Netherlands registry: Women were advised to have a transvaginal ultrasound scan annually or biennially from age 30-35 years.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
522 scans carried out with ration of 0.63 for years scanned/years of risk. No cases of endometrial carcinoma detected by ultrasound but one case each of endometrial carcinoma from each group. Both women had hMLH1 mutation	292 women offered screening – data on 269 (171 from HNPCC families, 98 from HNPCC-like families). Limited data on dropouts – 8 died from unrelated causes	"Endometrial carcinoma surveillance in hereditary colorectal carcinoma may not offer obvious clinical benefits."	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Durno C 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Multicenter, Canada Patients with CRC at ≤ age 24 (selected from a total of 1382 patients in a cancer registry.	Tumors analyzed for MSI, IHC and blood for MMR	hMLH1 and hMSH2 and PMS2 (select patient not described who)	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? Y Gene screening? Y Deletion analysis? ? Conversion analysis? ?	Am 1 X Am R X Beth 1 Beth R MSI-H X MSI-L IHC X Age <50 Suggestive family history X *Specify* Age ≤ 24 Other *Specify*	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=16, all analyzed, 6/16 = 38% male	MMR 6 of 12 tested; 2 with MLH1, 3 with MSH2 and 1 with PMS2	8/16 fulfilled revised Amsterdam criteria MSI-H 11/16 IHC abnormal in 2 of 8 tested	ND	Patients with early onset CRC often have an inherited predisposition to the disease. Tumors with MSI-H and germline MMR mutations are sufficiently common in this population that they should be considered even though family histories may not satisfy the stringent Amsterdam criteria for HNPCC.	7/16 patients developed a second cancer during followup (mean 1.8 years). Majority (75 %), were in the GI tract.	C Partial verification: IHC/MSI/MMR were tested only in a subset of patients and authors did not explain why. Extent of gene testing seemed to vary but authors did not explain why.

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	3/5 Amsterdam 1 (not all patients were tested) 3/6 Amsterdam 2 (not all patients were tested)
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	4/6 (not all tested) 4/6 Am2 (ie the Am1) no other Am R were tested
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1	X	1/5 Amsterdam I 1/5 Am2 (ie the Am1) no other Am R were tested
	Amsterdam R	X	
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
CRC ≤ age 24	Am 1 +		+	(A) 5	(B) 3
	Am R +		-	(C) 0	(D) 1
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
CRC ≤ age 24	Am 1 +		+	(A) 3	(B) 1
	Am R +		-	(C) 1	(D) 3
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

Grade	**Explanation for Quality Scoring**
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.

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Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?		X	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants		X	
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?		X	
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

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Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Farrington, SM 1998

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**				**Predictors analyzed (check all that apply)**		**How were deleterious, missense/variants mutations defined (ND if not described)**
50 unrelated patients with CRC at age <30 identified retrospectively from cancer registrations since 1970 compared with 26 age matched volunteers without cancer. Edinburgh, Scotland, Single center	Detailed family history obtained from cases, paraffin-embedded archival tumor material obtained along with matched normal tissue from 42 patients. Tumor and normal tissue analyzed for MSI. Genomic sequencing done on all patients and controls using peripheral blood.	hMSH2 and hMLH1		Y	N	?	Am 1	X	"[mutations] not considered pathogenic unless either there was a nonconservative amino acid change or the variant arose at a conserved sequence around a splice site"
			≥5 MSI markers used?	X			Am R		
			MSI-H defined by ≥ 2 markers?	X			Beth 1		
			Microdissection? "Only areas with >90% tumor tissue used"	X			Beth R		
			Gene screening? "genomic sequencing and IVSP analysis"	X			MSI-H		
			Deletion analysis?	X			MSI-L		
			Conversion analysis?			X	IHC		
							Age <50		
							Suggestive family history	X	
							Specify 4 categories: 0= no family hx; 1= relative with non-CRC; 2 = relative with CRC but not Amsterdam,; 3 =Amsterdam		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g., of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
50 patients enrolled of whom 42 samples could be assessed for MSI	14 mutations identified from 50 patients (7 hMLH1, 7 hMSH2)	MSI-H 19/50	As below	IVSP complemented genomic sequencing by detection of mutations not identified by genomic analysis….An appreciable proportion of young colon cancer probands carry a germ-line mutation in a DNA mismatch-repair gene	Sensitivity of genomic sequencing 80% and IVSP 64%; IVSP and genomic sequencing complementary.	**B** Population selected from a registry thus would have selected for survivors. If survival influenced by mutation status, assessment of prevalence of mutations may be biased. However, authors found no such association .

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	3/6
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	5/5 (one did not have tissue for MSI)
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify) CRC <30	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC <30	Am 1 +		+	(A) 12	(B) 7
	Am R +		-	(C) 2	(D) 19
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC < 30	Am 1 +		+	(A) 4	(B) 10
	Am R +		-	(C) 3	(D) 8
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Family history 1 vs family history 0 as noted above				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC < 30	Am 1 +		+	(A) 7	(B) 3
	Am R +		-	(C) 3	(D) 8
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Family history 2 vs family history 0 as noted above				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC < 30	Am 1 +		+	(A) 3	(B) 3
	Am R +		-	(C) 3	(D) 8
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Family history 3 vs family history 0 as noted above (i.e. Amsterdam 1 positive)				

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			X
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?	X		
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Fujita, 1996 (RefID 2533) UI 8895676 Japan Single center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
3356 patients with CRC underwent surgery and follow up between 1968 and 1993. They were classified into 4 categories: Amsterdam 14 Japanese A 31 Japanese B 100 Sporadic 1604 Kaplan-Meier was performed	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: x; No: ; Uncl:	Subjects who died within 30 days of surgery were excluded from calculation	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
5-year survival rate: Amsterdam 92.3% Japanese A 81.2% Japanese B 66.5% Sporadic 60% The first 2 groups were better than the latter 2 groups (P<0.05).	Not all patient with CRCs were accounted for?	"Prognosis of HNPCC is better than that of sporadic CRC, and Japanese A criteria can be used to select putative HNPCC patients from among those with sporadic CRC."	B-

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely X	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND X	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
done?								
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Graff 2005; Ref ID 125 Single center Australia

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Investigated experiences of 12 individuals with HNPCC in a semi structured telephone interview. All patients had been asked to inform family members.	Pathogenic mutation causing HNPCC Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: X; No: ; Uncl:	Pathogenic mutation, English speaking, excluded those with inconclusive test results. All had received genetic counseling. All had an HNPCC-related cancer.	Semi-structured telephone interview.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
12 subjects (4 men and 3 women) Respondents older than nonrespondents (55 versus 49), had more cancer diagnoses per individual and were more likely to be female (no statistical comparisons made). All had told some (mainly immediate family) at-risk family members about predictive testing. Family reactions ranged form interest to disinterest. Men expressed a need for guidance and support in communicating to relatives more than women. Letters and booklets were thought to enhance the quality of information but further aids were unlikely to increase the number of relatives made aware of predictive testing by the probands.	19 invited, 2 declined and five failed to respond	Respondents informed their immediate family about the availability of genetic testing but more distant relatives were not directly informed. Men expressed a need for guidance or support in communicating to relatives more than women. Letters and booklets were thought to enhance the quality of information.	C Very small sample size. No information about experience of probands (e.g., with screening or cancer treatment), which might have influenced their attitude. No validation of what relatives were actually told.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						X A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	X NA			
Analysis						A (strong)	X B	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
							(moderate)	
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	No	X ND					
Are the statistical methods appropriate?	Yes	No	X ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Green S 1995, Ref ID 2706 Single center UK.

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Data on all screening colonoscopies performed in 61 HNPCC family members from start of a screening program in 1990-1994 analyzed.	Amsterdam I criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	Member of family fulfilling Amsterdam I criteria and undergoing a colonoscopy.	Analysis of colonoscopy results.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
2 patients (3 %) had a malignant neoplasm (one Dukes B and the other dukes C) at ages 66 and 41, respectively. Another patient presented with an obstructing sigmoid carcinoma. Seven patients had adenomas (mean age 45.3, range 26-63).	ND	These findings support the hypothesis that adenomas do not occur in large numbers in HNPCC families but, because of the high malignant conversion rate, biennial colonoscopy with removal of polyps is recommended.	C Retrospective, uncontrolled, small sample size, unclear if data collection methods were valid.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	X 1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	X 1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			
Analysis						A (strong)	B (moderate)	X C

Domain/question	***Place an "X" in one***					***Overall rating***		
								(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	X No	ND					
Are the statistical methods appropriate?	Yes	No	X ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	X Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	X NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Gritz E 2005, Ref ID 227 Single center US. Note, same patients as Vernon, SW: participation in colorectal cancer screening: a review. J Natl Cancer Inst 1997; 89:1406.

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
155 affected and unaffected persons with HNCC completed psychological tests before genetic testing and at 2 weeks, 6 and 12 months afterward. Among 89 affected participants, 33 were HNPCC predisposing mutation carriers. Among unaffected participants, 19 were HNPCC mutation carriers and 47 were noncarriers who received definitive negative test results.	Amsterdam or suggestive family history followed by mutation testing. (Not clear if original or revised Amsterdam). Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	≥18 Probands: fulfilled Amsterdam or suggestive family history: Family of mutation positive probands at 25 to 50% risk of carrying a mutation. "Affected" included any index patients or relatives with a prior diagnosis of any cancer excluding non-melanoma skin cancer; unaffected included relatives with no personal history of cancer.	Short form of the Social support Questionnaire (SSQ). Center for Epidemiologic Studies Depression scale (CES-D) State anxiety subscale of the State-Trait Anxiety inventory (STAI-S). Ferrans and Powers Quality of Life Index (QQLI) Cancer worries scale Perceived risk of colorectal cancer

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Affected participants: Nonwhites had significantly higher mean scores on the CES-D, STAI-S and RIES over time on all measures compared with whites; Those with lower educational levels had significantly higher scores than those who had higher educational levels. Carriers had significantly higher mean RIES scores at 2 weeks and 6 months postdisclosure compared with affected participants and uninformative results. Scores decreased significantly form 2 weeks to 12 months postdisclosure except for those with uninformative results, which did not changed. **Unaffected participants:** Race or ethnicity were significantly associated with mean CES-D and STAI-S scores over time with nonwhites having significantly higher means scores compared with whites and those with lower educational levels having significantly higher scores compared with those who had higher educational levels. Nonwhites also had significantly higher mean RIES scores compared with whites. Carriers had significantly higher mean RIES scores compared with noncarriers at the 2 week and 6 month postdisclosure time points. Post hoc comparisons showed a significant reduction in mean RIES scores for both unaffected carriers and noncarriers form 2 weeks to 6 months post disclosure. **Effect of baseline distress on postdisclosure outcomes:** Cluster analysis identified two well-defined subgroups for affected and unaffected participants using the mean baseline scores on the CES-D, STAIS-S, QLI, and SSQ scales: a high, and low-distress cluster. The proportion comprising the high-and low-distress groups was the same among affected and unaffected participants (30.3%). The high-distress groups had significantly higher scores (regardless of mutation status) at two weeks, 6 and 12 months postdisclosure. Cancer worries and perceived risk scores were not affected by distress, and changes in these scores were primarily related to mutation status.	126 affected participated (79 families) but 15 (12%) did not receive their results. 178 unaffected relatives of mutation-positive index patients invited; 68 (38%) refused and 24 (13%) completed the questionnaire only. Of 86 unaffected who completed the questionnaire and gave blood samples for testing, 4 (5%) did not receive their results. Individuals who declined their genetic test results did not differ from those who received results on demographic or distress variables.	Although HNPCC genetic testing does not result in long-term adverse psychological outcomes, unaffected mutation carriers may experience increased distress during the immediate postdisclosure time period. Furthermore, those with higher levels of baseline mood disturbance lower quality of life, and lower social support may be at risk for both short and long-term increased distress.	A Would have benefited if form more detail regarding effect of actual screening behaviors and issues related to access and psychological distress.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5	X	X	8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	X 9	X 8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	X 9	X 8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						X A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						X A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	X No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						X A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	X Yes	No						
Were data collection tools shown or are they known to be reliable?	X Yes	No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						X A	B (moderate)	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)		
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	X 60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Hadley, DW 2003, Ref ID 946 Multicenter, US (one author from Ireland but patients US). Same patients as Hadley Ref ID 657)

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Elucidate factors affecting decisions regarding genetic testing in individuals form families with newly identified HNPCC. 165 adult men and women from 15 families identified with HNPCC mutations invited to participate; 104 agreed to participate.	Mutations Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	Members of families with newly identified HNPCC with any of the following features: HNPCC-associated cancer demonstrating MSI or a family history suggestive of HNPCC and 1st-degree relatives at 50% risk of inheriting the mutation. Had to agree to fill out questionnaires, undergo counseling and participate in telephone interviews 6 and 12 months later.	Individuals contacted by telephone, filled out baseline questionnaire, received genetic education, contacted by telephone 6 and 12 months later.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
104/165 (63%) eligible patients (including index cases and 1st degree relatives agreed to participate. This included 54 probands (of whom 87% agreed to participate) and 111 family members of whom 51% agreed to participate. **Awareness of genetic testing**: those at higher household income levels were more aware of genetic testing for cancer (p=0.001) and colon cancer (p=0.009) than those at lower household income levels. There was no significant association between participants' awareness and age, sex, personal cancer history or number of 1s-degree relatives. **Perceived risk of being a carrier**: Participant's feelings about their chances of getting colon cancer were significantly associated with their beliefs about the likelihood that they carry a mutation (p<0.001) **Intentions toward genetic testing:** The intention to pursue genetic testing was found to have a positive association with participants beliefs that cancer may be explained by family heredity (p=0.006). Concern about the psychosocial effect of genetic testing on the family demonstrated a negative association with their intention to pursue testing (p=0.001). Participant's concerns about their ability to handle the emotional aspects of genetic test results demonstrated a negative association with their intentions to pursue testing (p<0.001). No association between age, sex or cancer status in regard to their intentions toward genetic testing. **Reasons for pursuing genetic tests:** 50% of respondents believe that the most important	ND	Genetic counseling and testing offers the potential to focus cancer screening resources in individuals truly at increased risk, thereby reducing mortality and morbidity. Fears of discrimination and concerns about psychological and psychosocial issues may present barriers to the use of current cancer prevention strategies, including genetic counseling and testing.	B Surveys used had not been validated. Selection bias is likely to have influenced the results since almost one-half of family members declined to participate. Authors did not explore differences in those who declined from those who agreed. Access to resources not described. Perceptions about screening procedures, ability to prevent cancer not fully explored. Conclusions are not fully supported by data presented since mortality and morbidity were not a focus of the study.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
reason for undergoing genetic testing was to learn about their children's risk. Second most important reason (17%) was to guide cancer screening; third (13%) as to confirm their belief that they carry a mutation. With respect to the importance of genetic testing for reproductive decision making, a statistically significant difference was detected between those younger than the median age of 43 or older (p=0.002). **Reasons for not pursuing testing**: Worry about losing health insurance (39%), concerns about how it might affect the family (27%), concerns about handling the results emotionally (10%). A statistically significant difference was detected between those younger than the median age of 43 compared with those older with respect to concerns about handling the emotional aspects of genetic testing (p=0.006). **Testing decisions:** 44/54 (81%) of eligible probands eventually chose to undergo genetic testing for HNPCC. 56/111 eligible first-degree relatives chose to pursue genetic testing (intention-to-treat analysis). Of those agreeing to participate in the study, 44/47 (94%) probands agreed to undergo genetic testing and 56/57 (98%) of family members agreed.			

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5		X	8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a X	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a X	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	X <60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	X <60	ND	NA			
Analysis						A (strong)	X B	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
							(moderate)	
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	X <60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Hadley, DW 2004, Ref ID 657 **(same patients as Hadley Ref ID 946)** Multicenter, United States

Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
Assess impact of genetic counseling and mutation testing on endoscopic screening procedures and adherence to recommended endoscopic screening guidelines in 56 at-risk individuals from families known to carry an HNPCC mutation.	Deleterious mutation Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	≥18, no personal history of cancer, 50% risk of carrying their families previously identified deleterious HNPCC mutation who chose to undergo genetic testing and who completed questionnaires at baseline, 6 and 12 months. Excluded those simultaneously participating in clinical trials involving colonoscopy	Genetic counseling and surveys as described in Hadley 2003.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
30/56 patients had at least one colonoscopy before genetic counseling and testing. 12/56 had at least one colonoscopy within 12 months after testing. Mutation-positive individuals were more likely to undergo colonoscopy. Among mutation-negative individuals, use of colonoscopy and flexible sigmoidoscopy decreased significantly between pre and post testing. Among mutation-positive individuals, a nonsignificant increase in use was noted. On logistic regression, colonoscopy was significantly more likely in mutation-positive individuals (OR 61, 95% CI 5.8-652, p=0.006), and increasing age (OR 1.1, 95% CI 1.008-1.2, p=.03). Employment, income, sex or colonoscopy status before testing did not predict use. 11/56 were classified as nonadherent with colonoscopy recommendations 12 months after testing . Mutation positive (OR 7.5, 95% CI 1.3-42.2, p=0.02) and unemployed individuals (OR 8.6, 95% CI 1.3-56.6, p=0.025) were significantly less likely to adhere to guidelines for endoscopic screening. Age, sex, income, or pre-testing colonoscopy status were not associated with adherence.	ND.	Genetic counseling and testing for HNPCC significantly influences the use of colonic endoscopy and adherence to recommendations for colon cancer screening.	B Small sample size. Not clear if authors fully adjusted for eligibility for followup procedures in the year following counseling. Compliance was assessed by self-report rather than by verifying whether patients had procedures. No exploration about access or other barriers to colonoscopy.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	X 5	X 9	8a	8b,8c	X 1,6b,9,10,11
Public health or policy	2,3	X 5	X 9	8a	8b,8c	X 1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			
Analysis						A (strong)	X B	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
							(moderate)	
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Halbert, CH 2004, Ref ID 405 Multicenter, United States

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Evaluate whether genetic testing for HNPCC mutations and receipt of positive test results have an effect on the use of colonoscopy. Identify factors associated with adherence to identify potential barriers to patient compliance. 222 eligible, 98 in final sample.	Mutations. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	25% risk of having HNPCC identified in family. Excluded individuals with colon cancer or a history of colectomy.	All eligible invited by mail to participate; those who agreed were interviewed by telephone. They were then invited to participate in a information/education session. Subjects requesting genetic testing were notified by letter when mutation results became available. Following disclosure, they were contacted by telephone to reassess screening behaviors at 1, 6 and 12 months.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
134/222 participated but after exclusions, 98 were available for analysis. Men and subjects who were younger were significantly more likely to be lost to follow-up or not participate. Colonoscopy use before genetic counseling was not significantly different between test result groups. During the 12 months following genetic testing, 73% (16/22) of mutation carriers and 16% (8/49) noncarriers and 22% (6/27) decliners reported having a colonoscopy (p<.001). Mutation carriers were significantly more likely than noncarriers and decliners to have a colonoscopy. No clinical or sociodemographic factors were significantly associated with colonoscopy use during the 12 months following-genetic counseling.	Excluded 25/14 due to a prior history of colectomy or colon cancer and 2/134 who developed colon cancer during the study, and 9/134 who received genetic test results during follow-up.	Genetic testing may motivate increased colonoscopic screening among HNPCC mutation carriers. Increased efforts may be needed to assess patients' family histories of colon cancer and provide appropriate referrals for genetic counseling and testing to target colonoscopic screening to high-risk individuals.	B All colonoscopy data based upon self-report. High dropout rate. Issues related to access and perceptions of colonoscopy not assessed fully.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	X 5	X 9	8a	8b,8c	X 1,6b,9,10,11
Public health or policy	2,3	X 5	X 9	8a	8b,8c	X 1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	**Place an "X" in one**					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	X <60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	X Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	X <60	ND	NA			
Analysis						A (strong)	X B	C (weak)

Domain/question	**Place an "X" in one**					***Overall rating***		
							(moderate)	
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	X No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	X <60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Hampel 2005, Ref ID 29

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
70 HNPCC families including 88 probands and 373 mutation-positive family members evaluated for risk of CRC and endometrial cancer.	Mutations and clinical criteria, mostly Amsterdam I or II Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Excluded if mutation unknown diagnosis or data incomplete	Cohort 1, 45 HNPCC families in which a mutation for MLH1 had been detected. Probands had presented with CRC. Cohort 2, family members from 25 HNPCC families in whom a mutation for MLH1 or MSH2 had been detected. The probands had been diagnosed with CRC.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Mean age of diagnosis of CRC 55.1 (95% CI 52.6-57.6)) for men and 60.3 (95% CI 58.0-62.6) for women; approximately 10-15 years older than previous estimates of age at onset for CRC among HNPCC patients. Lifetime risk of CRC was 68.7% for men and 52.2% for women. Mean age of onset for endometrial cancer in women was 58.6 (95% CI 56.9-60.3). Lifetime risk of endometrial cancer was approximately 54% Mean age of ovarian cancer was 54.1 (95% CI 53.5-54.7). Women had a 13.5% lifetime risk for ovarian cancer. Among probands, average age at diagnosis of CRC in men was 44.8 (95% CI 41.6-48.0) and for women was 47.0 (95% CI 43.0-51.0) Among probands, average age of diagnosis of endometrial cancer was 53.1 (95% CI 50.5-55.7)	ND	A markedly later age of onset of CRC at 61 year than previously reported (approximately 44 years) is suggested, resulting mainly from a more rigorous method of analysis in which all gene-positive individuals (both affected and unaffected with cancer) are considered. Lifetime cancer risk may be lower for CRC and endometrial cancer than presently assumed. If confirmed, these data suggest a need to alter counseling practices, and to consider HNPCC in older individuals than before.	B Description of followup, surveillance procedures and dropouts incomplete making it unclear whether the relatively advanced age of diagnosis of cancer in family members may have been due to detection of precursor lesions.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	**2,3**	**5**			**X** **8c**	**1,6a,6b,7,10**
Family member/other high risk individual	**2,3**	**5**	**9**	**8a**	**X** **8b,8c**	**1,6b,9,10,11**
Public health or policy	**2,3**	**5**	**9**	**8a**	**X** **8b,8c**	**1,6,a,6b,7,10**

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	X B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	No	X ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	X NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Jarvinen, H 2000, Ref ID 5 Multicenter, Finland. Same patients (and includes) Jarvinen HJ. Gastroenterology 1995; 108:1405 Ref ID 2746

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Incidence of CRC and survival compared in 2 cohorts of at-risk members of 22 families with HNPCC. Colonic screening at 3-year intervals arranged for 133 subjects while 119 had no screening.	Amsterdam I criteria or other suggestive family history (some mutation positive). Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	Asymptomatic member of an HNPCC family at 50% risk of. Other inclusion/exclusion criteria as per Jarvinen 1995; 108:1405 as follows:	Initial colonoscopy or double contrast barium enema and sigmoidoscopy and then repeated examinations at 3-year intervals. The proportion of colonoscopy was 56% on the second round and 83% on the third round and 100% on the fourth round.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Cancer morbidity data, survival and causes of death checked at the Finnish Cancer Registry and the Population Registry of Finland. Significantly more patients developed CRC in control group (19 (16%) versus 8 (6%), p=0.014; RR CRC 0.377 (95% CI 0.171-0.829). Among mutation-positive subjects, the frequency of CRC was 18% (8 of 44) in the study group and 41% (19 of 46) in the control group , p=0.02; RR 0.440 (95% CI 0.215-0.900). Mortality was greater in study group (9 of 119 (8%) versus 0/133 in study group (p<0.001).Overall there we re 10 deaths (8%) in the study group compared with 26 deaths (22%) in the control group during a 15-year period (p<0.001). Relative risk of deaths for the screened group was 0.344 (95% CI 0.173-0.683) overlap and 0.348 (95% CI 0.122-0.999) for mutation positive subjects.	252 asymptomatic individuals belonging to 22 HNPCC families and being at 50% risk. Of those invited to participate, 78 eligible declined and 40 could not be traced; these patients constituted the control group.	Colonoscopic screening at 3-eyar intervals more than halves the risk of CRC, prevents CRC deaths, and decreases overall mortality by about 65% in HNPCC families.	B Nonrandomized design; not clear if those who declined screening has same baseline risk. However, the treatment effect was very large. Validity of cancer/mortality database not clear.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	**2,3**	**5**			**8c**	**X 1,6a,6b,7,10**
Family member/other high risk individual	**2,3**	**5**	**9**	**8a**	**8b,8c**	**X 1,6b,9,10,11**
Public health or policy	**2,3**	**5**	**9**	**8a**	**8b,8c**	**X 1,6,a,6b,7,10**

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						X A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	X Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	X 80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	X B (moderate)	C (weak)
Is the method of random allocation stated?	X Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	X No						
Was the method of random allocation reported as concealed?	Yes	X No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						X A (strong)	B (moderate)	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	X 80-100	60-79	<60	ND	NA			
Analysis						X A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	X 80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	X No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Study: Katballe Gut 2002

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Consecutive Danish patients with CRC in 4 counties (11/1995 to 10/1998) Inclusion criteria: Amsterdam criteria I or II or on extended pedigree; Amsterdam criteria with HNPCC related cancers; Amsterdam criteria except youngest was 50-55yrs; pts ≤40 with at least one CRC among family members; Proband and one first degree relative with CRC before 55 yrs	Prospective population study; if a family met criteria for suspected HNPCC, screening for hMSH2 or hMLH1 were done and tumor were analyzed for MSI; HNPCC defined as Amsterdam I or II; or suspected HNPCC based on history plus pathogenic hMLH1 or hMSH2 mutation.	MLH1 and MSH2	See table below	See table below	Based on previous reports by them and other authors; in a case based on the fact that splice mutations are usually pathogenic.

	Y	N	?
≥5 MSI markers used?	X		
MSI-H defined by ≥ 2 markers?	X (>=2 bands, but OK)		
Microdissection?	X		
Gene screening?	X		
Deletion analysis?			X
Conversion analysis?			X

Am 1	
Am R	
Beth 1	
Beth R	
MSI-H	
MSI-L	
IHC	
Age <50	X
Suggestive family history	X
Specify	
Other	

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
1514 with 1° CRC (median age 71 yrs) 1328 eligible, 1200 in study, excluded pts who died shortly after dx, psychological problems, ethical reasons, did not complete questionnaires	10 pts were found with MMR among the 41 HNPCC who were sequenced → or among the 1200 CRC who were included.	11/1200 (0.9%, 0.5-1.6%) fulfilled Amsterdam I 18/1200 (1.5%, 95% CI 0.8-2.25%) fulfilled Amsterdam II	The sensitivity of MSI is only 61% in terms of detecting families that satisfied the Amsterdam criteria I or II .	"The sensitivity of MSI is only 61% in terms of detecting families that satisfied the Amsterdam criteria I or II → MSI is not as appropriate to identify the families with suspected HNPCC." "The majority of mutation carriers present with a strong family Hx of HNPCC-related Cancers."	No	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	5/11 Am1 people; (4/10 independent, ie without familial clustering) 8/18 Am2 (including AM1) people; (6/16 independent)
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	Am1 →7/10 analyzable had MSI-H Am1 →0/10 analyzable had MSI-L Am2 →10/16 analyzable has MSI-H Am2 →1/16 analyzable has MSI-L
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +	X	
Beth 1 +		
Beth R +		
Age <50	X	
Suggestive family history (specify)	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
All 45 Patients with CRC and S-HNPCC as defined above (actually 41, because 4 were not analyzed)	Am 1 +	X	+	5	6
	Am R +		-	5	25
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
All 45 Patients with CRC and S-HNPCC as defined above (actually 41, because 4 were not analyzed)	Am 1 +		+	8	10
	Am R +	X	-	2	21
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
MSI-H+MSI-L vs MSI-S among all people analyzable out of the 45	Am 1 +		+	10	5
	Am R +		-	0	20
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*	X			
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
MSI-H vs MSI-S among all people analyzable out of the 45	Am 1 +		+	10	3
	Am R +		-	0	20
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MSI-H or L**	**Number with MSI-S**
	Am 1 +		+	11	7
	Am R +	X	-	4	18
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	X		
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?	X		
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?	X		
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Keller, 2002 Ref ID 984 Germany Single center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Longitudinal study, data reported come from self-completed questionnaires pre- and post-counseling (4-6 weeks after counseling) 94 individuals received counseling between 7/2000 and 8/2001 73/82 completed a post-counseling questionnaire; 8 of those did not have a precounseling questionnaire; Final sample size: 65, 31 patients and 34 unaffected individuals	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	>18 year olds, patients with HNPCC-related cancer or unaffected individuals from families at risk for HNPCC; referred by physician or self-referred	Standardized protocol of interdisciplinary team counseling by geneticists, pathologists, molecular biologists, surgeons, and psycho-oncologists; comprised of genetic, clinical, and psychosocial counseling

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Distress and worries related to HNPCC declined after counseling. An increase was found in personal belief in control of cancer risk. A trend was observed toward greater anticipated ability to cope with a positive gene test after counseling. Changes after counseling were generally more pronounced for persons at risk, as compared to patients with cancer. 1/3 of the sample reported enhanced communication specific to hereditary disease within the family after counseling. A substantial minority, however, experienced increased worry and physical symptoms after counseling.		"Counselees demonstrated less stress and perceived cancer threat as well as enhanced beliefs regarding personal control over cancer, suggesting an overall beneficial impact of comprehensive counseling. Further research is needed to identify those individuals most at risk for increased fear and worry related to HNPCC so that they may be most appropriately counseled."	B

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence

(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence

(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e.	Yes	No	Can't					

Domain/question	***Place an "X" in one***					***Overall rating***		
intention to treat) rather than the actual intervention received?			tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?			
Keller, 2004 RefID 585 Germany Single center			
Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
Of 140 eligible patients and their families, 36 families participated in the information session, 25 participants were patients, and 11 were spouses or relatives of patients who had died or were too ill to attend. Participation rate 26%. Questionnaire completed by spouses or relatives were not included in the analysis. Of the 104 nonparticipating patients addressed by mail, 61 responded (59%), 12 died , 48 returned a completed questionnaire. Sample here consisted of 73 patients: 25 participants and 48 nonparticipants	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: ; Uncl:	>18 year olds, had surgery for CRC-associated or HNPCC-associated cancer between 1994 and 1998, met Amsterdam or Bethesda or FH suggestive of HNPCC FAP were excluded	Eligible patients received a letter announcing the discovery of HNPCC mutations and the availability of free genetic counseling and testing. Patients and their families were invited to partcipate in comprehensive information session on HNPCC. Participation in an information session was followed by answering a questionnaire.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
"Among participants, 60% reported being very worry because of familial cancer; among non-participants 35% reported being very worry because of familial cancer ($P<0.05$). 28% of participants reported impairment of daily life because of these worries compared to 19% of nonparticipants (difference not significant). Similarly, perceived distress of spouse and family from patient's point of view was higher in participants than in nonparticipants (difference not significant). Regardless of whether patients participated in the information session, most were in favor of genetic testing. Twice as many nonparticipants (25%) as participants (13%) were undecided about whether to accept genetic testing. All participants definitely expressed the wish to know whether a mutation is present in the family, compared to 81% of nonparticipants."	No actual genetic testing was done.	"Expressed intention and attitude toward genetic testing do not reliably predict actual uptake of counseling or testing. Thorough interdisciplinary counseling should be provided to every patient with clinical criteria suggestive of HNPCC. The considerable distress related to the hereditary disorder should be adequately addressed, as should be communication issues."	C

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Kruhoffer, 2005, RefID 129 Country: Denmark and Finland Center: 15

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Colorectal cancer tissue from a total of 151 patients was collected from 15 different clinics in Denmark and Finland. A sample set consisting of 101 stages II and III cancers (34 MSI-H; 67 MSI-L and MSS) were used. MSI classification data were correlated to the overall survival of the patients for stage II (in 36 patients) and III (in 65 patients) tumors separately.	Among the 101 stages II and III cancer patients, 19 had sporadic MSI tumors, 15 had hereditary MSI tumors, and 67 had MSS tumors. MSI tumors with HNPCC origin were classified by a maximum likelihood MSI classifier with a "leave-one-out" cross validation scheme basically as described (Dyrskjot et al, 2003). All HNPCC cases included in this study carry MLH1 or MSH2 mutations identified by sequencing.	A sample set consisting of 101 stages II and III cancers (34 MSI-H; 67 MSI-L and MSS) was included in the analyses for the relation between microsatellite status, cancer stage, and patient survival.	65 patients with Stage III tumors receiving adjuvant chemotherapy

Did all patients have a personal history of an HNPCC-related cancer? Check one		
Yes	No	Uncl
	x	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Kaplan-Meier survival analyses were performed for stage II and III tumors separately. Note that MSS in this study includes MSI-L. The overall survival was highly significantly related to the classification in 36 Stage II patients, as 10 out of 11 patients that died within 5 years belonged to the MSS group (p=0.0014). Among 65 patients with Stage III tumors receiving adjuvant chemotherapy, 16 were classified as MSI tumors and 49 as MSS tumors. As 6 MSI and 30 MSS patients died within 5 years of follow-up, there was no significant difference in overall survival between these group (p=0.55)	No "dropouts" due to retrospective design.	"Our findings are in accordance with other recent publications, the MSI classification (or MSI-H) clearly proved to be a strong predictor of survival in stage II disease, and there is a relation between MSI classification and a good prognosis in stage II patients."	C Retrospective design and likely to have selection bias

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak) x
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely x					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND x	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
done?								
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction for Analytic Validity

Study: (Author, year, UI) Kurzawki, G, 2002, Ref ID 1253

Inclusion criteria (all must be yes)

	Yes	No
Did study evaluate biological material from patients considered to be at risk for HNPCC?	X	
Did the study report ANY of the following? (check which one below)		
5) Proportion MSI-H with NIH markers versus other markers		
6) Sensitivity or specificity of MSI-H using NIH markers compared with a reference standard that the study claims is better		
7) Sensitivity or specificity of IHC compared with an immunohistochemical standard that study claims is better		
8) Sensitivity or specificity of a genetic technique compared with a reference standard (or combination of standards)	X	
Are data (proportions or 2 X 2 tables) extractable?	X	

Exclusion criteria (exclude if yes)

	Yes	No
Did the study include the index test in the reference standard?		X

Describe source of biological materials (and whether patients were known to have an HNPCC phenotype)	Summarize how materials were processed and analyzed
46 unrelated patients with CRC: nine families fulfilled Amsterdam II and in 37 there was a strong suspicion of HNPCC. In 19, MLH1 or MSH2 mutations or polymorphisms had been identified by DNA sequencing	Genomic DNA analyzed using DHPLC and sequencing

MSI Proportion (add additional 2 X 2 tables where relevant)

Proportion MSI-H using NIH markers (≥2 markers)	Proportion MSI-H using other markers

MSI with a reference standard

		MSI-H using another reference standard	Describe
		Positive	Negative
MSI-H using NIH markers	MSI-H (≥2 markers)		
	MSI-S or MSI-L		

IHC with a reference standard

		IHC using another immunohistochemical reference standard	Describe
		Positive	Negative
IHC	Positive		
Describe	Negative		

Genetic technique with a reference standard

		Reference standard genetic technique	Describe Sequencing
		Positive	**Negative**
Index genetic technique	**Positive**	**42**	**NR**
Describe: **DHPLC**	**Negative**	**42**	

	Study Quality	Yes	No	Unc
1	Was the description of how MSI or IHC and other genetic techniques described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
2	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			X
3	Was MSI, IHC, other genetic testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
4	Was there a clear description of which mismatch repair mutations were being tested for?	X		
5	Were quality control methods described for the molecular and genetic tests?	X		
6	Did the authors attempt to address the reproducibility of results (reliability of tests)?			X
7	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	
8	Was microdissection performed?			
9	Did the study specify whether the biological tissues were from patients known to have HNPCC clinically?	X		
10	Did the study include a control group in which biological material was obtained from patients known not to have HNPCC clinically		X	
11	**Overall rating (A B C)**	C		

Study: Lamberti 1999

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
I do not know from which population these index pts come from (!) 160 index pts who were **GR1** Am1 (57); **GR2** (Loose criteria with relatives with extracolonic HNPCC malignancies →) Am2 NOT Am1 (12); **GR3** CRC aged <50 AND with relatives with nonCRC HNPCC related cancer (n=45); **GR4** Apparently sporadic CRC <50y OR sporadic CRC with synchronous or metachronous CRC tumor (no age limit; n=46) German Study,	Blood sample ant tumor tissues from all index pts (when possible); All Gr1 and Gr2 pts were screened for mutations irrespectively of MSI status; Gr3 and GR4 screened only if MSI was positive	hMLH1 and hMSH2	≥5 MSI markers used? Y: X MSI-H defined by ≥ 2 markers? Y: X (By >40% of loci being unstable) Microdissection? ?: X Gene screening? Y: X Deletion analysis? N: X Conversion analysis? N: X D2S123, D2S136, D3S1298, D3S1611, D5S346, D6S470, D18S35, D18S37, BAT25, HBAP1	Am 1: X Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other	With references to other papers; other are not.

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
Overall N=160 index pts; Am1 N=57; Am2 N=69 (57 + 12); % male unknown; dropouts not described [[57 Am I mean age at dx of CRC = 44 (11.8); 12 non-Am I mean age at dx of CRC = 37 (10.8)]]	17 with MMR/69 patients with CRC and Am2; 11 MLH1; 6 MSH2	[Of the enrolled population, **which is not described.**] 57/160 are Am1 and 69/160 essentially AM2	ND	A positive MSI is an efficient indicator of a germline mutation in the mismatch repair genes MSH2 and MLH1, both in patients meeting the clinical criteria for HNPCC and pts with sporadic CRC. It is a matter of debate whether the age cutoff should be used at all for the MSI test. Pts with CRC <50 y should be examined anyhow, even if F Hx is negative.	No	C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	Am1: 15/57 Am2 (INCLUDING AM1): 17/69
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	MSI-H: Am1: 35/49 MSI-H Am2: 39/57 [Not all tumors assessed for MSI]
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X (But, again, the population is not really defined)
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Among people with Am2,	Am 1 +	X	+	15	42
	Am R +		-	2	10
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Contrast BETWEEN MSI-H vs MSI-S & MSI-?	Am 1 +		+	15	24
	Am R +		-	2	16
AMONG PEOPLE with AMI2	Beth 1 +				
In total 52 that could be assessed	Beth R +				
This is the only contrast you can extract with certainty;	MSI-H*	X			
You cannot compare against MSI-S only, because of the way the data are reported.	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			X
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			X
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			X
7	Were withdrawals from the study explained?		X	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants		X	
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Lee, 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	Design and analytic strategy *(brief description of strategy used for testing patients with CRC)*	Which MMR mutations were sought?	Characteristics of laboratory testing (see definitions below)	Predictors analyzed (check all that apply)	How were deleterious, missense/variants mutations defined (ND if not described)
Inclusion: CRC with Am I or Am II family history; or CRC ≤ 40; or CRC with 1st degree relative with CRC or HNPCC-like cancer (cancer of endometrium, small bowel, biliary tract, pancreas, stomach, ureter, ovary, renal pelvis or glioblast); or proband ≥ 2 primary cancers classical of HNPCC (CRC or HNPCC-like cancer) Exclusion: familial adenomatous polyposis Singapore Single center	Blood sample for genotyping and paraffin-embedded colon cancer tissue blocks for MSI testing	MLH1 and MSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? Y Gene screening? ? Deletion analysis? N Conversion analysis? N	Am 1 Am R x Beth 1 Beth R MSI-H x MSI-L IHC Age <50 x Suggestive family history x *Specify* Other *Specify*	Deleterious mutation: truncated protein (nonsense or frameshift mutations) or if previously reported in HNPCC kindreds

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=46 Median 39 yr (18-82) 30 (65%) men	7/46 WITH DELETERIOUS MUTATIONS	5/46 Am2 23/46 >=2 first degree relatives 5 multiple tumors 13 apparently sporadic aged <40	ND	Singapore patients may have the same key characteristics for HNPCC as those described widely in the western families.	ND	B But 96% are CRC

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		3/5 AM2
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	MMR+	MMR-
Am2	3	2
3 1st degree family members CRC	2	7
2 1st or 2nd degree family members CRC	1	13
CRC with Multiple cancers (not adenomas)	1	4
Sporadic aged <40	0	13

	MMR+	MMR-
MSI-H	4	7
MSI-L	0	2
MSI-S	1	21

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
	Am 1 +		+	(A)	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			X
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?	X		
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Lerman, 1996 RefID 2617, US Single Center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
24 consecutive index colon cancer patients (who did not fulfill the Amsterdam criteria) identified 61 first degree relatives for the study (index patient consent rate = 83%). Of these first degree relatives, 44 (72%) completed the interview, 2 (2%) declined, 16 (26%) could not be reached. This was a pilot study in preparation for a prospective study of genetic testing for HNPCC-associated mutations.	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: x ; Uncl:	Person who had a prior diagnosis of cancer were excluded.	15-minute telephone interview

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
"Anticipated reactions to positive and negative results of a genetic test: Over ½ of respondents indicated that they would become depressed or very anxious if they tested positive. Most subjects reported that they would react by changing their health habits, including diet and screening behaviors. In response to negative test result, about 1/3 of respondents anticipated they would feel guilty and ½ would still worry because the test might be wrong. About ½ of respondents expected that they would decrease their use of screening tests and make fewer attempts to reduce dietary fat if they tested negative."	Pilot study, no actual genetic tests were done.	"These preliminary results underscore the importance of educating patients about the potential risks, benefits, and limitations of genetic testing, with particular emphasis on the possibility of adverse psychological effects and implications for health insurance. The potential for false reassurance following a negative test result should be addressed by emphasizing the residual risks of cancer among non-carriers of predisposing mutations."	C

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e.	Yes	No	Can't					

Domain/question	***Place an "X" in one***					***Overall rating***		
intention to treat) rather than the actual intervention received?			tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Liljegren 2004, Ref ID 541 SAME PATIENTS AS Lindgren G. Gut 2002; 50:228-234. Single center Sweden

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Evaluated psychological aspects of colorectal cancer screening in 240 individuals at high risk for CRC.	Patients categorized into: 1) mutation positive; 2) suggestive family history but not fulfill Amsterdam I; 3) two close relatives affected by CRC.	Subjects from Cancer Family Clinic at Karolinska Hospital form 1990-1999 who fell into three categories described.	Colonoscopies were performed every 2-3 years in patients enrolled over a 10-year period between 1990 and 1999. In 1999 all eligible patients were asked to fill out a questionnaire about experiences with colonoscopy and to complete the Hospital Anxiety and Depression Scale (HAD), Medical Outcomes Study Short-Form 36 (SF-36).

Did all patients have a personal history of an HNPCC-related cancer? Check one		
Yes	No	Uncl
	X	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Perceived benefit of colonoscopy was rated an 8/10 on a visual analogue scale where 0 = no benefit and 10 =great benefit. Risk perception: Among 28 mutation-positive subjects 17 (61%) reported their personal risk as being 40% or less and 36% perceived a 1 to 20% lifetime risk of CRC. In risk groups 2 and 3 (129 and 83 individuals), approximately 50% either under-or over-estimated their personal lifetime risk of colorectal. Cancer. HAD scores we within the normative data range. SF-36 scores were significantly lower on mental health (p=.013) and vitality (p=0.16) compared with the reference population. Women and significantly lower scores on vitality (p=0.48) while men had lower scores on mental health (p=0.47). 88% of subjects gave a correct answer to the questions "Why have you been offered regular colonoscopies".	304 included of whom 23 later proven to be mutation negative and were excluded. 16 excluded because address could not be found. 265 sent questionnaire of 91% returned usable data.	A majority of the study sample understood why they were under surveillance and regular colonoscopies were well-tolerated. The wide range of risk perception as well as low-risk perception in mutation positive subjects is acceptable as long as these individuals adhere to surveillance programs and do not demonstrate increased levels of anxiety or depression.	C Data do not fully support authors' conclusions. Small sample size of mutation-positive patients. No control group of patients who declined to undergo surveillance so there may have been selection bias for patients with relatively favorable psychological profiles. Unclear if any of these patients had a personal history of cancer. Results not adjusted for potential covariates such as age, personal history of cancer, educational level.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	X 9	X 8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	X 9	X 8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	X 60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	X B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	X Yes	No						
Were data collection tools shown or are they known to be reliable?	X Yes	No						
Withdrawals and Dropouts						A (strong)	X B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	X 60-79	<60	ND	NA			
Analysis						A	B	C

Domain/question	***Place an "X" in one***					***Overall rating***		
						(strong)	(moderate)	(weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	X 60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Lindgren, G 2002, Ref ID 1300 SAME PATIENTS AS Lilegren A 2004, Ref ID 541 Single Center, Sweden

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Evaluate outcomes in 304 patients at increased risk for CRC including HNPCC who were undergoing surveillance colonoscopy.	Patients categorized into: 1) mutation positive; 2) suggestive family history but not fulfill Amsterdam I; 3) two close relatives affected by CRC.	Subjects from Cancer Family Clinic at Karolinska Hospital form 1990-1999 who fell into three categories described.	Screening colonoscopy approximately every two years except those with an adenoma in whom it was repeated every year. Adenoma prevalence for normal population estimated from three autopsy studies.

Did all patients have a personal history of an HNPCC-related cancer? Check one		
Yes	No	Uncl
	X	

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
304 subjects underwent 765 colonoscopies 10 colorectal cancers found in 9 individuals before recruitment into the surveillance program. 4 had CRC at first colonoscopy and two developed Metachronous cancers during surveillance. RR of adenoma before age 54 compared with general population 2.6 (p=0.001) RR for tested gene carriers in HNPCC families 4.5.	ND	There was clear overrepresentation of adenomas in all three family types compared with the reference population. In HNPCC, we found earlier onset of adenomas and faster progression to cancer.	C Non-uniform protocol, endoscopists were unblinded and thus may have been more vigilant for adenomas in these high risk patients. It is likely that clustering effects were occurring among patients that were not accounted for in the analysis. The control group was based upon autopsy studies rather than adenoma detection rates based upon colonoscopy.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	X 1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	X 1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	B (moderate)	X C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	Yes	X No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Study: Liu 2004 Ref ID 445

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
28 independent Chinese families: 15 fulfill Am1 criteria; 13 not Am1 but fulfill Japanese Clinical Diagnosis Criteria for HNPCC.	DHPLC analysis was performed	HMSH2 and hMLH1	≥5 MSI markers used? N MSI-H defined by ≥ 2 markers? N Microdissection? N Gene screening? Y Deletion analysis? N Conversion analysis? N	Am 1 x Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other *Specify*	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
28 probands from 28 families are described onwards. They all meet the Japanese HNPCC criteria; they are assumed to be with CRC – not clearly stated though. Overall, 128 pts among all family members has CRC. Among these 128: 45.9 yrs mean age onset of CRC, 55% male	7 patients with MMR/28 patients with CRC; 2 with hMSH2 and 5 with hMLH1	15/28 probands from AM1 families	ND	"Three novel germline mutations were found, the germline G204X nonsense mutation in the 3rd exon of hMSH2 is the first MMR gene mutation found in Chinese Mongolian people."		C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	5/15 7/28 Japanese HNPCC criteria
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		x
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Japanese Criteria for HNPCC; Sample seems to be a convenience Sample; unknown selection.	Am 1 +	x	+	5	10
	Am R +		-	2	11
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			x
2	Inclusion criteria clear?		x	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		x	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?			x
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			x
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?		x	
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			x
14	Were quality control methods described for the molecular and genetic tests?		x	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			x

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Luce, 1995

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	Design and analytic strategy *(brief description of strategy used for testing patients with CRC)*	Which MMR mutations were sought?	Characteristics of laboratory testing (see definitions below)	Predictors analyzed (check all that apply)	How were deleterious, missense/variants mutations defined (ND if not described)
Consecutive cases of CRC referred to a tertiary hospital. Patients reporting 2 or more cases of colorectal or endometrial cancer in first-degree relatives or only 1 affected first-degree relative younger than 50 years old were interviewed, and detailed pedigrees were obtained. Country: US Center: Single	Genetic testing was done in all enrolled patients with CRC	hMLH1 & hMSH2	≥5 MSI markers used? (Y / N / ?) MSI-H defined by ≥ 2 markers? (Y / N / ?) Microdissection? (Y / N / ?) Gene screening? (Y / N / ?) Deletion analysis? (Y / N / ?) Conversion analysis? (Y / N / ?) None of these testing was done	Am 1: x Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other *Specify*	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=19 cases/families Mean age=40.3 %male=ND Dropouts=0	6 cases/families with MMR / 19 cases/families with CRC 4 MLH1 2 MSH2	19 cases/families with CRC, 63% positive AM	ND		ND	B Small sample size

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	6/12
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		x
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
See definition above; Essentially they are not Am1 Selected Familial CRCs (proband and 1 or 2 1st degree)	Am 1 +	x	+	(A) 6	(B) 6
	Am R +		-	(C) 0	(D) 7
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	x		
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	x		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).	N/A		
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?	N/A		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			x
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	N/A		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?	N/A		
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?	N/A		
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	x		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Lynch, HT 1996, Ref ID 2661 Single center USA.

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Evaluated reaction of 51 members of Navaho Indian CRC patients who received MLH1 genetic testing underwent genetic counseling.	Mutation Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	As per study description.	Family reservation visited three times with interceding genetic testing and counseling.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Many family members believed the family's fate was influenced by factors such as fear, a taboo, or a curse. Four family members denied their heightened cancer genetic risk. Several who had not undergone gene testing wanted their children testing.	ND	DNA-based genetic counseling requires compassion and empathy coupled with intensive pre-education regarding potential penalties and advantages that might emanate from this knowledge. Special care must be given to patients' culture.	C No formal instrument used to assess subjects' reactions. Important covariates such as educational level and personal history of cancer not described.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	X 8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	X 8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	X Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	X NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	B (moderate)	X C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	Yes	No	X ND					
Are the statistical methods appropriate?	Yes	No	X ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	X Can't tell					
Intervention Integrity						A (strong)	X B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Lynch 1997 (RefID 2355) US; also see Lynch 1996 for similar information Single Center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
High-risk members from 4 extended HNPCC families that were selected from Creighton's HNPCC resource, which comprised about 100 HNPCC extended kindreds Emotional responses to receiving genetic test results in 130 individuals from 4 extended families with HNPCC. 36% had positive test 64% had negative test	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Of the 89 members did not enroll in the study, 67% did not respond to the invitation, 22% had their counseling postponed, and 10% refused, citing fear of knowing, fear of insurance discrimination, surviving guilt and disinterest.	Genetic counseling

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
1. When mutation positive, 20% exhibited sadness, particularly because of concern of the potential risk of transmission of the deleterious mutation to their children. Others were concerned about their personal cancer destiny, particularly the possibility of an unfavorable prognosis that could lead to their early death and may put their children in jeopardy of not having a biological parent to care for them. 2. Following a positive test result, 67% were considering prophylactic colectomy. 3. Following a positive test result, 87% were considering prophylactic total abdominal hysterectomy and bilateral salpingo-oophorectomy. 4. 18/130 were concerned with sharing information with their insurance company.		"DNA testing should be restricted to well-verified candidate families and accompanied by genetic counseling…patients exhibited a wide range of emotional responses when told of their genetic testing status…Whether… the test results, has a long-term effect on behavior, quality of life, or on cancer morbidity and mortality remains to be seen…."	C

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) **Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence**

Domain/question	**Place an "X" in one**					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	**Place an "X" in one**					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Meiser 2004 Australia Multicenter

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Psychological impact of predictive genetic testing in 114 individuals (32 carriers and 82 non-carriers) were assessed after disclosure of carrier status.	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes / No / Uncl	Only individuals in whose families a known HNPCC mutation had already been identified, who chose to receive a predictive genetic testing result and had never had CRC or any of HNPCC-related cancers were included in the study.	Self-answered questionnaires pre-test, post-test at 2 wk, 4 mo and 12 mo after carrier status disclosure

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
1. Compared to baseline, carriers showed a significant increase in mean scores for intrusive and avoidant thoughts about CRC and a significant decrease in mean depression scores 2 wk and 4 mo post notification of results. 2. Carriers had significantly higher intrusive and avoidant thoughts 2 wk and 12 mo post-notification compared to non-carriers. 3. Compared to baseline, non-carriers showed a significant decrease in mean scores for intrusive and avoidant thoughts, and mean depression scores at all follow up time points.		Predictive genetic testing for HNPCC leads to psychological benefits amongst non-carriers, and no adverse psychological outcomes were observed amongst carriers.	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

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(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Mesters 2005 Netherlands Single

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
30 persons from the database of the Netherlands Foundation for the Detection of Hereditary Tumors (the article did not explicitly state who these subjects were but 37% had confirmed carrier status) were interviewed at home.	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: Uncl: X		

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
1. Disclosure was stimulated if people felt morally obliged to do so or when they anticipated regret if something happened because it is preventable. Presence of external cues (eg, professionals) appeared important for disclosure as well. 2. Disrupted and tense family relations were reasons not to disclose, as well as young age of the message recipients and negative experience at their first attempt to disclose. 3. Disclosure was restricted to the nuclear family. A personal approach was preferred. 4. Participant disclosed only the presence of the hereditary defect and the possibility of testing. It was mostly considered the recipients' responsibility and own choice to obtain further information. 5. Limitations of this study include that findings might be affected by recall bias since the interviews focused on prior experiences about HNPCC disclosure.		See paper concerning practice implications.	C

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

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(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Moslein, 1996

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X*	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

*although not all study patients had CRC but data on those patients with CRC are extractable

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Patients having sporadic, familial colorectal carcinoma, and HNPCC meeting the Amsterdam criteria Convenience samples Country: Germany Center: multi	The DNA sequence of hMSH2 and hMLH1 was evaluated in all patients	HMSH2 and hMLH1	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? N Deletion analysis? ? Conversion analysis? ?	Am 1 x Am R Beth 1 Beth R MSI-H X MSI-L IHC Age <50 Suggestive family history x *"Familial CRC"* Other	Based on predicted transcript alteration

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=36 Mean age=51 %Male=ND Dropouts=0	13 patients with MMR/ 36 patients with CRC 7 MLH1 4 MSH2; 1 MLH1&MSH2	NA	5/9 patients who met Amsterdam criteria (HNPCC) had positive MSI 8/12 patients who had familial CRC had positive MSI	"In this study, MSI was present in 100% (10/10) tumors (whether from familial or HNPCC cases) in which mutations in either hMLH1 or HMSH2 were subsequently found. These results suggest that MSI may be a very sensitive method for distinguishing a subset of HNPCC colon cancers (i.e., those resulting from defective mismatch repair) from sporadic colon cancers."	ND	C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	6/14
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	x	5/9 positive MSI (≥ 2 markers showing instability) Note: 5 (14-9) patients who met Amsterdam criteria did not have tissue available for MSI testing
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	x	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	x	
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (*Familial CRC*)	x	
Other (MSI+)	x	

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC who met Amsterdam criteria (n=14) Note: 5 patients did not have tissue for MSI testing, so that they are not in the 2x2 table	Am 1 +		+	(A) 5	(B) 0
	Am R +		-	(C) 0	(D) 4
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with sporadic CRC (n=7) Note: 5 patients did not have tissue for MSI testing, so that they are not in the 2x2 table	Am 1 +		+	(A) 1	(B) 6
	Am R +		-	(C) 0	(D) 0
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with familial CRC (n=15)	Am 1 +		+	(A) 4	(B) 4
	Am R +		-	(C) 0	(D) 4
Note: 3 patients did not have tissue for MSI testing, so that they are not in the 2x2 table	Beth 1 +				
	Beth R +				
	MSI-H*	x			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	"MSI"				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?		x	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?		x	
14	Were quality control methods described for the molecular and genetic tests?		x	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			x

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction for Analytic Validity

Study: (Author, year, UI) Muller 2001, Ref ID 731

Inclusion criteria (all must be yes)

	Yes	No
Did study evaluate biological material from patients considered to be at risk for HNPCC?	X	
Did the study report ANY of the following? (check which one below)	X	
9) Proportion MSI-H with NIH markers versus other markers		
10) Sensitivity or specificity of MSI-H using NIH markers compared with a reference standard that the study claims is better		
11) Sensitivity or specificity of IHC compared with an immunohistochemical standard that study claims is better		
12) Sensitivity or specificity of a genetic technique compared with a reference standard (or combination of standards)		
5) Reliability of MSI/IHC/genetic method across laboratories or within a laboratory	X	
Are data (proportions or 2 X 2 tables) extractable?	X	

*NIH markers are BAT-25, BAT-26 D2S123, DS346 and D17S250

Exclusion criteria (exclude if yes)

	Yes	No
Did the study include the index test in the reference standard?		X

Describe source of biological materials (and whether patients were known to have an HNPCC phenotype)	Summarize how materials were processed and analyzed
20 colorectal cancers from different institutions including those known to be MSI-H or have DNA mismatch repair mutations of hMLH1 (n=6) or hMSH2 (n=11) or negative controls (n=3)	A set of two unstained slides from each case were sent to participating medical centers with capability of performing immunoperoxidase assays for hMLH1 and hMSH2

MSI Proportion (add additional 2 X 2 tables where relevant)

Proportion MSI-H using NIH markers (≥2 markers)	Proportion MSI-H using other markers

MSI with a reference standard

		MSI-H using another reference standard	Describe
		Positive	Negative
MSI-H using NIH markers	MSI-H (≥2 markers)		
	MSI-S or MSI-L		

IHC with a reference standard

		IHC using another immunohistochemical reference standard	Describe
		Positive	Negative
IHC	Positive		
Describe	Negative		

Genetic technique with a reference standard

		Reference standard genetic technique	Describe
		Positive	Negative
Index genetic technique	Positive		
Describe	Negative		

Intra or inter-hospital reliability data	Describe
	Of 18 participating centers 2 were excluded: one because slides were damaged in transit and the other because of insufficient staining. Sensitivity for detecting loss of hMSH2 2 expression ranged from 84 to 100%; 10 centers identified all six. 5/6 false positive results were in the same case suggesting that staining or interpretation were not random. 14/16 laboratories showed 100% specificity (one laboratory had 93% specificity due to staining failure on one slide and one lab demonstrated 45% specificity due to weak or absent staining in most cases. Re-review of returned hMSH2 slides shoed lack of internal positive control staining in at least 2 of the 6 hMSH2-negative cases from 8 of 16 centers. The other 8 centers had 100% sensitivity and 93-100% specificity on re-review. The slides that lacked internal positive control staining were largely accounted for by two cases suggesting the possibility of fixation or processing variation. Variation of staining quality and interpretation was much greater for hMLH1 than for hMSH2. individual centers reported 0 to 100% sensitivity and 40 to 100% specificity. Re-review of the returned slides resulted in sensitivities of 0-90%. 12 centers experienced difficulty with lack of internal positive control or high background. Overall, four laboratories performed relatively well with both hMLH1 and hMSH2 staining protocols. The key element common to these and distinguishing them from the rest was a heated antigen retrieval step. Steam treatment in the presence of EDTA provided the best results although steam and citrate buffer also provided acceptable results.

	Study Quality	Yes	No	Unc
1	Was the description of how MSI or IHC and other genetic techniques described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
2	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
3	Was MSI, IHC, other genetic testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?	X		
4	Was there a clear description of which mismatch repair mutations were being tested for?	X		
5	Were quality control methods described for the molecular and genetic tests?		X	
6	Did the authors attempt to address the reproducibility of results (reliability of tests)?	X		
7	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	X		
8	Was microdissection performed?			X
9	Did the study specify whether the biological tissues were from patients known to have HNPCC clinically?	X		
10	Did the study include a control group in which biological material was obtained from patients known not to have HNPCC clinically	X		
11	**Overall rating (A B C)**	**B**		

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Murakami 2004 Japan Single

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
47 participants who had genetic testings.42 completed 1-month psychologic follow up	6/27 probands had positive genetic test 21/27 probands received uninformative test result 5/15 relatives received positive test 10/15 received negative test Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: X Uncl:	Probands or unaffected relatives whose family members had been identified as carrying the hMSH2/hMLH1 mutation before or during this psychologic study procedure, and age ≥ 20 yrs.	Structured Clinical Interview based on DSMIIIR or DSMIV

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
3/27 probands had minor depression at 1 month (1 had minor depression at baseline). 2/42 had posttraumatic stress symptoms at 1 month (both had positive test results). 5/42 had feelings of guilt. The only predictor of psychologic distress found was the presence of a major or minor depression (OR 19.41; 95%CI, 1.42-264.95).	See Figure 1 in original paper.	Disclosure of genetic test results for HNPCC may not cause significant psychologic distress in Japanese probands or relatives, but healthcare providers should not neglect to assess these individuals for psychologic responses, such as minor depression or posttraumatic stress symptoms.	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Nakahara, 1997

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Patients who met the Japanese registry's clinical diagnostic criteria for HNPCC proposed at the 34th Annual Meeting of the JSCCR Country: Japan Center: Single	MSI was performed in all 32 patients with (multiple) colonic and/or extracolonic cancers. PCR-SSCP analysis of hMSK2 and hMLH1 mutations was performed in DNA samples from 11 MSI phenotype cases (of the 18 MSI+ cases).	hMSK2 and hMLH1	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? N Deletion analysis? ? Conversion analysis? ? Not the NCI marker set.	Am 1 x Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other x *MSI phenotype, defined as* "MSI+ in cancer lesions at most of the microsatellite loci examined."	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=32 Mean age=59.3 %Male=41 Exclusions=66%, MSI- (n=14) or non-MSI phenotype cases (n=7) For the 11 patients with CRC and MSI phenotype: Mean Age: 45 Male: 45%	7 patients with MMR / 11 patients with CRC and MSI phenotype 1 MLH1, 2 MSH2; 4 MLH1&MSH2	32 patients with CRC, 22% positive AM, 56% positive MSI	5 (28%) of the 18 MSI+ patients fulfilled the Amsterdam criteria 2 (14%) of the 14 MSI- patients fulfilled the Amsterdam criteria	"The 'Japanese criteria' have the advantage of being able to detect more HNPCC kindreds from borderline HNPCC kindreds, compared to the Amsterdam criteria. We propose that a microsatellite assay is necessary to detect more "true-HNPCC kindreds" from borderline HNPCC kindreds, and molecular findings of MSI phenotype should be added to the criteria for HNPCC."	ND	C Two of the 11 patients analyzed were from the same family. Small sample size. Highly selected sample.

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	5/7 MSI high
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	x	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (Japanese registry's clinical diagnostic criteria for HNPCC)	x	

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC who fulfilled the Japanese registry's clinical diagnostic criteria for HNPCC and had MSI phenotype (MSI+ in cancer lesions at most of the microsatellite loci examined) NOTE THAT WE DO NOT KNOW HOW MANY OF THE MSI-L&S WERE AM1 SO WE CANNOT ESTIMATE THE SAME TABLE ASSUMING THAT ALL MSI-S&L ARE NEGATIVE FOR MUTATIONS	Am 1 +	x	+	(A) 4	(B) 1
	Am R +		-	(C) 3	(D) 3
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
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Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		x	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?	x		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants		x	
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	x		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		x	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
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MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Norum 2000 Norway Single Center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
11 health insurers in Norway were mailed a questionnaire to evaluate two hypothetical individuals' requests for insurance; one has HNPCC mutation, the other has BRCA1/BRCA2	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes / No / Uncl		

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
9/11 insurers responded. 2 companies reported results on personal indemnity insurance. There were no restrictions for individuals with a genetic risk of breast CA when seeking life insurance or disability pension. One company raised premium on the one with genetic risk of CRC. Another company offered standard or higher rate of premium on personal indemnity insurance in the person with genetic risk of CRC.		In Norway: "According to the survey, individuals with hereditary risk for breast or CRC may experience insurance discrimination simply based on family history of cancer. Genetic testing results are generally not included in determination of health insurance. Therefore, based on the present state, Norwegians should have no need for hesitation in undergoing genetic testing out of fear of insurance discrimination."	C

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Park, 1999, 2002, & 2007

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
123 pts (123 families) with Suspected-HNPCC which were not Am1; from 8/58 centers (7 countries) of ICG-HNPCC. *Criteria:* **Cat1:** (vertical CRC transmission OR 2 CRC siblings in family) AND (multiple CR tumors including adenoma OR >=1 CRC before 50y OR extracolonic cancer endometrium/urinary tract/small intestine/stomach/hepatobiliary /ovary) **Cat2 (Sporadic):** 1 CRC patient AND (onset<40y OR endometrial, Urinary tract, or small intestine cancer in patient or a sibling <50y OR 2 siblings with other integral colonic cancers, one aged <50y) They compare them against 154 Am1 families which have received genetic testing	All 123 S-HNPCC received genetic testing. All 154 excluded Am1 have received genetic testing (Discussion) All detected mutations were verified by sequencing	HMLH1 (n=123) HMSH2 (n=122) HPMS1 (n=27) HPMS2 (n=24)	Y / N / ? ≥5 MSI markers used? — X MSI-H defined by ≥ 2 markers? — X Microdissection? — X Gene screening? — X Deletion analysis? — X Conversion analysis? — X	Am 1 Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history — X *Specify* Other *Specify*	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=123 S-HNPCC; ND on %M; dropouts not pertinent	24 with MMR/123 with CRC; 16 with hMLH1 / 123 ; 8 with hMSH2 /122	N=123 S-HNPCC-NOT-AM1 and n=154 AM1 pts	NA	Criteria I detected a mutation rate of 28% which is sufficiently high to recommend genetic testing in such families; useful in clinical setting; should be offered genetic testing. Criteria II have been ineffective as a means of identifying individuals with mutations; should not be offered genetic testing.	NO	C In the definitions of Criteria II, authors added the <40y age factor post hoc; 50/56 crit2 pts are because of the age factor -only 3/50 had mutations (see Discussion).

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	19/67 for Cat1; 5/56 for Cat2 → 24/123 S-HNPCC 77/154 for Am1
	Amsterdam R		
	Other (specify)	X	
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (***see the description of the population***)	X	
Other (Discussion: 50 centers didn't test because the PIs are clinicians; testing is expensive; as of that paper, it was unclear whether non Am1 pts need testing)	X	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Defined above. Am1 vs Cat1 & Cat2 S-HNPCC	Am 1 +	X	+	77	77
	Am R +		-	24	99
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?		X	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			X
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			X
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			X
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?			X
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			X
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Peel, 2000

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Referral HNPCC case families (Amsterdam criteria) These were from the ICG HNPCC group database (but they give MSI-HL vs MSI which is not covered in the other papers)	MSI were performed in 10 families; MSH2 and MLH1 were done in 11 families	MSH2 and MLH1	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? ? Microdissection? Y Gene screening? Y Deletion analysis? ? Conversion analysis? ?	Am 1 X Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other *Specify*	2 MLH1 Were truncating and expected to be pathogenic; 1 MLH1 was a nonsense mutation; 2 MSH2 were predicted to cause missense changes and likely to disrupt MSH2 function

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
11 families	5 families/11 families have MMR 3 MSH2 and 2 MLH1; 2 mutations were previously identified; 3 were novel mutations	5/556 had Am1 (.9% ; 0.3 to 2.1%)	NO comments	"The prevalence of HNPCC in the general population is most likely closer to 1% rather than 5% Previously reported characteristics of HNPCC like proximal tumor location may not always hold true in a population-based sample."	No	C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	5/11
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	4/9 MSI H or Low
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X?	
Am R +	X?	
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
	Am 1 +		+	3	1
	Am R +		-	0	5
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*	X			
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only	X			

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
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Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			X
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?		X	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?		X	
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?		X	
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	X		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Perrin, 2001, RefID 1361 Country: France Center: single

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
A retrospective series of 225 patients diagnosed with sporadic CRC. 208 tumors were tested for IHC, and correlations with survival were performed by log-rank test and Cox model.	ND Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: ; Uncl: x	A retrospective series of 225 patients diagnosed between 1990 and 1998 with sporadic colorectal cancers underwent surgery. There were 121 men and 104 women. 46% were >65 years old.	ND

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
27 of 208 (13%) of the tumors tested showed abnormal MSH2 and/or MLH1 expression. For the whole population, there was no association of MSH2 and/or MLH1 status (by IHC) with either overall or disease-free survival. 56/225 tumors were located in the proximal colon. 15/56 (31%) tumors were negative for either protein or both. Negativity of MLH1, and "MSH2 and/or MLH1" were correlated with a localization in the proximal colon ($p=0.02$ and $p<0.01$, respectively). Negativity of MSH2 was close to being significantly associated to this localization ($p=0.07$); the absence of significance may be due to a low number of cases (n=10). For the subpopulation of proximal tumors, MSH2 and/or MLH1 negativity was associated with a longer disease-free survival.	17/225 (7.5%), no IHC testing	Abnormal IHC measurement of MLH1 and /or MSH2 protein were frequently tumors of proximal colon; in this subpopulation or proximal tumors, MSH2 and/or MLH1 negativity was associated with a longer disease-free survival.	C Retrospective series of sporadic CRC cases No information on treatments.

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate) x	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely x	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND x	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Peterson 2003 (RefID 833) US Single Center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
39 members of 5 families, representing 30 separate households were interviewed by telephone. Each family included a proband with HNPCC mutation who had genetic counseling and testing through clinical research protocol, each family included at least 5 members estimated to be at 50% risk of carrying a mutation (regardless if they have had actual genetic testing)	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: x Uncl:		

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
1. The identification of a cancer-predisposing gene mutation was new information that confirmed their beliefs, and families were not shocked or surprised by the news. This information was not viewed as a medical crisis. 2. No one reported that the news of the mutation was intentionally withheld from any family members. They willingly shared this information with other relatives, including those who did not participate in the study. 3. Compared with mutation carriers, those who tested negative for a mutation and those who did not test perceived those topics as less personally relevant and were less involved in discussing the mutation and the need for genetic testing. Spouse's reactions were similar. 4. Probands assumed that each adult who was told about the mutation would be responsible for telling his or her children; however, probands did not follow up with these adults to find out if news about the mutation had been actually conveyed to those children. Participants (other than probands) viewed themselves as primarily responsible for notifying their nuclear family and encouraging them to seek counseling or testing.		"Family members who were persuaded to seek those services by the proband were more likely to have counseling and testing and were more likely to seek those services sooner. Genetic counseling should attempt to identify the existing communication norms within families and ways that family members can take an active role in encouraging others to learn about their cancer risk and options for testing. Interventions may also need to target beyond the immediate family and to unaffected family members who may be central to the communication process (eg, spouses of mutation carriers)."	C

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Pinol, 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Newly diagnosed CRC; Spain, multi-center Excluded FAP, IBD, patient or family refusal to participate	MSI and IHC performed in all patients with CRC; testing centralized in 2 centers; pts with MSI and/or IHC of MSH2 or MLH1 underwent germline genetic testing for MSH2 and MLH1 by probe amplification and sequencing	MSH2 and MLH1	See table below	See table below	Predicted transcript alteration; literature and databases

	Y	N	?
≥5 MSI markers used?		X*	
MSI-H defined by ≥ 2 markers?			
Microdissection?		X	
Gene screening?		X	
Deletion analysis?	X		
Conversion analysis?			X

Pre screen with BAT26 and if non-conclusive the remaining battery of the 5 marker set

Am 1	
Am R	
Beth 1	X
Beth R	X
MSI-H	
MSI-L	
IHC	X
Age <50	X
Suggestive family history	X
Specify	
Other	
Specify	

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
1222/1872 eligible	11 among the 91 tumor samples that were genotyped (MSI OR IHC abnormalities) [To clarify: 91 were MSI OR IHC abnormal; 73 were abnormal in MSI AND IHC; 10 ONLY in MSI and 8 ONLY in IHC]	24% Beth revised 18% Beth original 1.8% (22)Am2	See tables for various data	MSI testing and IHC testing are equivalent in terms of cost-effectiveness to screening for the MMR.	Unclear	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		4/22 = 18% (but 4 MSI-S and IHC(+) were not genetically tested) AMONG THE GENETICALLY TESTED 4/18 = 22%
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X, on MSI or IHC results
If yes, which clinical criteria (check all that apply)?	[redacted]	
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
	Am 1 +		+	(A)	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	X		
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).	X		
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)		X	
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?		X	
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?	X		
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Pistorius, 2000

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
106 pts with Fam Hx or early Hx of Cancer were referred 72 patients met Bethesda, but not all of them CRC. Patients from Germany and Czech Republic (multicenter?)	MSI was performed; sequencing was done in patients with MSI-H IHC only in those with mutations (negative stain in all)	hMLH1 and hMSH2 and hmsh6	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? Y Deletion analysis? ? Conversion analysis? ? The NCI marker set was used	Am 1 X Am R x Beth 1 x Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other *Specify*	Based on predicted alteration, literature

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
72 index patients fulfilled Bethesda; Not all 72 CRC 19 Am2 and they are all CRC	15 patients with MMR in 38 MSI-H patients, 6 MLH1 8 MSH2 1 MSH6 Among 19 Am2, 17 were MSI-H and 12/17 had mutations	72/106=68% had Beth Among Beth: 50/72 Beth1-3 19/72 Am1		Family history and MSI-H are both strong indicators of germline mutations in MSH2, MLH1 and MSH6 genes. Identification of mutation status allows clear-cut decisions on whether or not inclusion in surveillance programs is indicated.	Of the 15 families with mutations, 60 patients and family members were analyzed, 26 were affected and 8 were asymptomatic. They were included in the HNPCC surveillance program, where as 26 noncarriers were excluded from this program. But 3 of 26 noncarriers presented with colon CA at age 35, testicular CA at age 30, and 2 colon polyps at age 58, respectively.	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	12/17 (Amsterdam and MSI-H)
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		38/72
	Amsterdam R		
	Bethesda	x	
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +	x	
Beth R +	X	
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC who fulfilled Bethesda and had MSI-H	Am 1 +		+	12	5
	Am R +	x	-	3	18
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
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C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		x	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			x
7	Were withdrawals from the study explained?		x	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	x		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	x		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			x
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			x

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Plaschke 2004, Ref ID 382, Multicenter Germany

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Clinical and molecular data on families with an established mutation in MSH6 were compared with families with MLH1 and MSH2 mutations	Fulfillment of Amsterdam 1 or 2 plus Bethesda plus absence of MSH6 expression by protein staining plus normal expression of MLH1 and MSH2 by protein staining Patients selected from a registry based upon Amsterdam I and II criteria without age restriction and Bethesda guidelines A total of 706 families had been registered. Available tumors were analyzed for MLH1 and MSH 2 protein expression and selected patients in whom these proteins were expressed underwent additional testing for MSH6 expression. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: No: Uncl: X	1) Patients had developed tumors that were either low or high MSI 2) MLH1 and MSH2 were expressed in the tumor cells Tumor samples from selected patients were studied for MSH6 expression with IHC and by sequencing.	Observation only (not clear if patients underwent any screening)

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Among 396 enrolled members of families with pathogenic MSH6 mutations, 115/29%) were affected by malignant disease. This rate was lower than for families with MLH1 or MSH2 mutations (37.5%). Colorectal cancer was statistically less frequent among all tumors (42.4 versus 66 percent). Such a difference was not observed for any other HNPCC tumors. Median age of onset of colorectal cancer in putative mutation carriers was 10- years higher than for MLH1 and MSH2 (54 versus 44 percent). Median age of onset of any tumor was 8 years higher for MSH6 (51 versus 43). Cumulative risk by age to develop CRC or any tumor was statistically lower for MSH6 mutation carriers compared with MLH1 and MSH2.	ND	"Later age of disease onset and lower incidence of colorectal cancer may contribute to lower proportion of identified MSH6 mutations in families suspected of HNPCC. However, in approximately half of these families, at least one patient developed colorectal or endometrial cancer in the fourth decade of life. Therefore, a surveillance program as stringent as that for families with MLH1 or MSH2 mutations is recommended."	**C** Potential for selection bias. Extent to which a mutation was confirmed genetically in MLH1 and MSH2 families unclear. Any interventions, screening was not described. How tumors were verified/ascertained was not described.

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	X C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	X Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	X No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	B (moderate)	X C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	B (moderate)	X C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
Analysis						A (strong)	B (moderate)	X C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	X Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	X Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	X NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	X ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	X Yes	No	Can't tell					

Study: Ponz de Leon 1999

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
1899 colorectal malignancies and 1831 patients registered of whom a family history could be obtained in 1721 Cancer registry in Modena, Italy and surrounding communities	Patients and families classified into five categories to a more or less likely genetic basis: 1) HNPCC (Amsterdam 1) 2) suspected HNPCC (2 generations with CRC or other HNPCC tumor plus at least one before 50 plus 50% or more of siblings affected by cancer), 3) juvenile cases 4) A specific cancer aggregation; 5) Sporadic cases. MSI and mutation analysis performed in 18 families with HNPCC and 18 with suspected HNPCC	hMLH1 and hMSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? Y Gene screening? Y Deletion analysis? ? Conversion analysis? ?	Am 1 X Suggestive family history X MSI-H X *Specify* Family history categories 1-5 as in column 2	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
18 in HNPCC and 18 suspected	3 mutations detected of 36 patients analyzed 2 hMSH2 1 MLH1	Of 1721 in whom a family history could be obtained, 47 individuals fulfilled Am-1 and 84 suspected HNPCC	ND	Incidence of mutations using a population-based approach lower than previously reported and limited to families with full-blown HNPCC.	ND	C

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	3/18
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	11/18
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify) "Suspected HNPCC as above"	X	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Amsterdam criteria 1 positive	Am 1 +		+	(A) DATA CANNOT BE EXRACTED	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			X
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			X
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Porteous, 2003, RefID 736, UK, Single

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
A total of 160 patients who were approached for the Colorectal Cancer Genetic Susceptibility (COGS) study between February 1999 and July 2000, were eligible to participate in the patient feedback study. Of the 160 questionnaires given to patients, 111 (69%) were returned completed. Only one of the 111 patients who completed the questionnaire had declined to participate in the COGS study.	Newly diagnosed CRC patients under the age of 55 years are offered genetic testing for 3 mutations in MMR genes (i.e. MLH1, MSH2, MSH6) Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: x; No: ; Uncl:	Patients who were approached for the Colorectal Cancer Genetic Susceptibility (COGS) study between February 1999 and July 2000, were eligible to participate in the patient feedback study.	N/A

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
"Of the 111 participants, 61 (55%) were male and 50 (45%) were female ranging in age from 31 to 55 years (mean=48.6, s.d.=5.5). The risk of HNPCC for their relatives was low for 60% of participants (n=65), moderate for 39% (n=42) and high for 2% (n=2). The majority were married or living with a partner (n=90, 83%), and had been educated up to age 16 years only (n=56, 60%). In all, 81% (n=89) had at least one child. After receiving the information sheet about the COGS study, but prior to discussing participation with a member of staff, 91% (n=101) reported that they had decided to take the genetic test, one individual (1%) had decided not to take the test and nine (8%) were undecided. Most participants (n=97, 87%) reported some degree of difficulty coming to terms with the diagnosis of cancer, with 15% (n=17) overall reporting that it had been very difficult. Similarly, the majority of participants (n=89, 80%) indicated that it had been difficult to cope physically with their illness and treatment, with eight participants (7%) overall reporting that it		"The majority of participants found it highly acceptable to have information about HNPCC brought to their attention at a time when they were coping with a new diagnosis of colorectal cancer, despite a lack of prior awareness that the disease could run in families. The vast majority had decided to accept the offer of a genetic test and reported high levels of subjective understanding concerning genetic testing. The results suggest that although receiving information about HNPCC did not cause most participants undue worry, a minority of participants rated this worry at the top end of the scale. This was despite the fact that most had been informed that their relatives were at low risk of HNPCC. Likewise, these participants tended to have rated worry about their cancer and its treatment at the top end of the scale."	B Results from this survey can only apply to those newly diagnosed CRC patients who decided to accept mutation testing.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
had been very difficult. A total of 45% of participants (n=48) rated their current worry about their cancer and its treatment at or above the midpoint of 4 on a 1 (not at all) to 7 (all the time) scale. In total, 19% of participants (n=21) rated their current level of worry caused by the genetics information at or above the midpoint of 4 on a 1 (not at all) to 7 (all the time) scale. Of these 21 participants, 80% (n=16) had rated their current worry about their cancer and its treatment at or above the midpoint, 57% (n=12) and 43% (n=9) had been informed that their relatives were at low or moderate risk of HNPCC, respectively."			

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3 x	5 x			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Raedle 2001

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Single center, Germany 125 consecutive, unrelated patients with CRC	Family history obtained from all, MSI performed on all MLH1 promoter methylation analysis performed on all MSI Genomic sequencing performed in all patients with MSI	MLH1 and MSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? Y Gene screening? N Deletion analysis? ? Conversion analysis? ?	Am 1 X Am R X Beth 1 X Beth R MSI-H MSI-L IHC Age <50 Suggestive family history X *Specify* Analyzed each component of Bethesda 1 Other *Specify*	Reported as "pathogenic" based upon previous reports but details not described.

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
125 no dropouts 56 women 69 men Mean age 52 +/- 14.4 (range 22-95)	11 had pathogenic mutation in MLH1 (4) MSH2 (8) or both (1) NB. Doesn't add up to 11, because they double count the common.	58/125 fulfilled at least one of seven Bethesda criteria (31 met one, 12 met 2, 8 met 3 and 7 met 4). Criteria B4 mostly commonly satisfied (i.e. CRC or endometrial cancer diagnosed age <45) MSI 22/125	ND	"Bethesda guideline useful for selecting patients for MSI. MLH1 and MSH2 should be recommended in all patients with CRC and MSI who fulfill at least one Bethesda criteria. MLH1 promoter methylation may accompany rather than initiate carcinogenesis in patients with CRC who have mismatch repair gene defects." Authors present diagram suggesting that patients with CRC who fulfill AM II should undergo genetic testing for MLH1 and MSH2. Those who do not should be asked Bethesda. If positive, MSI testing should be done and genetic testing done if MSI positive.	ND	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	ND [CANNOT ANSWER THIS QUESTION, BECAUSE WE HAVE MMR AMONG MSI-H]
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	Based on the proportions and the CIs reported in the paper: Sens of Am1 is 6/22 Sens of Am-R is 10/22 Spec of Am1 is 97/103 Spec of Am-R is 93/103
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +	X	+	(A) 6	(B) 0
	Am R +		-	(C) 5	(D) 11
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 8	(B) 2
	Am R +	X	-	(C) 3	(D) 9
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 11	(B) 6
	Am R +		-	(C) 0	(D) 5
	Beth 1 +	X			
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 9	(B) 6
	Am R +		-	(C) 2	(D) 5
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	BG, items 1-3				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 2	(B) 2
	Am R +		-	(C) 10	(D) 8
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	BG, item #2				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 8	(B) 1
	Am R +		-	(C) 4	(D) 9
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	BG, Item #3:				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 8	(B) 3
	Am R +		-	(C) 4	(D) 7
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Individuals with CRC or endometrial Cancer <45				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 1	(B) 0
	Am R +		-	(C) 11	(D) 10
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Individuals with right-sided CRC with an undifferentiated pattern (solid, Cribriform, defined as poorly differentiated or undifferentiated Carcinoma composed of irregular, solid sheets of Large eosinophilic cells and containing small gland-like Spaces).				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 3	(B) 0
	Am R +		-	(C) 9	(D) 10
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Individuals with signet-ring cell type CRC diagnosed At age <45				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Nonselected patients with CRC who were MSI-H	Am 1 +		+	(A) 0	(B) 0
	Am R +		-	(C) 12	(D) 10
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Individuals with adenomas Dx <40				

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	X		
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			X
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Renkonen-Sinisalo, 2000, RefID 1701 Finland, Single

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
The patients came from 57 different families (49 families with a known mutation, 8 families that met the Amsterdam criteria). There were 137 CRC patients. They were divided into 2 groups, depending on how CRC had been diagnosed. 35 cases of CRC in 33 patients had been diagnosed via the surveillance program. Of these, 13 patients in whom CRC had been detected during very first surveillance examination (age range, 28-59 years). Among the others, 20 patients had been aware of their inherited susceptibility to cancer and had undergone thorough colonic examination at least once. 115 carcinomas had been found in 104 patients merely because of symptoms. These patients had undergone colonic examination and mostly not been aware of their susceptibility to cancer nor had they taken part in any surveillance program.	Known mutation or Amsterdam criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: x No: Uncl:	All new CRC cases occurring in known HNPCC families in Finland from 1983 to the end of 1997.	Surveillance program involving use of colonoscopy or double-contrast barium enema and signoidoscopy

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
No significant difference in age or gender distribution between the 2 groups of patients. The stage distribution of the 34 tumors in the surveillance group was significantly more favorable then that of the 114 tumors in the nonsurveillance group. In the nonsurveillance group, CRC was considered disseminated or nonoperable in 19 cases (17%). Synchronous CRC occurred in 2 patients in the surveillance group and in 10 in the nonsurveillance group. During this 15-year period, metachronous CRC was observed in 2 and 11 patients in the surveillance and nonsurveillance group, respectively. Five of 33 patients (15%) in the surveillance group had died, compare to 40 of 104 patents (38%) in the nonsurveillance group. CRC resulted in 2 deaths in the surveillance group and 33 deaths in the nonsurveillance group. The cumulative CRC-specific survival was 93% in the surveillance group, significantly higher than the 68% in the nonsurveillance group ($p<0.02$) 10 years after surgery. Similar results were shown for patients' overall survival.	Retrospective analysis; some data was missing	"Colonoscopic surveillance is beneficial in at-risk members of the HNPCC families. Even if CRC cannot be prevented by polypectomies, tumors found during surveillance usually are detected at least so early that expectation of survival is excellent and significantly better than if there had been no surveillance."	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10 x
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11 x
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?			
Rijcken, 2003 The Netherlands Single center			
Study description (N enrolled)	**How was HNPCC defined?**	**Inclusion/exclusion criteria**	**Intervention(s)**
Females from HNPCC families identified by physicians and national registry. Annual screening performed and results treated accordingly. Women encouraged to report symptoms Women included in the program had a known MMR gene mutation and/or belonged to a family fulfilling the Amsterdam criteria II 41 enrolled and followed for 5 year (median, 5 months to 11 years) At enrollment: 85% premenopausal Genetic status known for following: 8 MLH1 2 MSH2 1 MSH6	MMR gene mutation or Amsterdam criteria II Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	MMR gene mutation or Amsterdam criteria II, 30-35 years	Annual screening consisting of gynecological exam, transvaginal ultrasound (TVU), serum levels of CA125 (normal ≤35 kU/L). Endometrial sampling as indicated

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
179 TVU performed over 197 patient years, Endometrial sampling for 11 from 17 TVU results, 4 from clinical symptoms 14/17 endometrial samplings showed no severe pathology, 3 premalignant lesions detected resulting in 1 hysterectomy for premenopausal woman and 2 hysteroscopy and curettage procedures with normal histology for 1 pre- and 1 postmenopausal women. Another postmenopausal woman reporting symptoms was diagnosed with interval endometrial cancer outside regular screening program resulting in hysterectomy and bilateral salpingo-oophorectomy 115 serum CA125 levels ranged from 1–24 kU/L with median of 7 kU/L No ovarian cancers detected by screening or outside of annual screening	None	"No asymptomatic malignant lesions detected but asymptomatic premalignant lesions detected and treated appropriately. Annual gynecologic screening with TVA as triage for endometrial sampling remains justified for women motivated for it but also that recognition and reporting of clinical symptoms by the women is of utmost importance."	B Data gather retrospectively

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	**2,3**	**5**			**8c**	**1,6a,6b,7,10**
Family member/other high risk individual	**2,3 x**	**5**	**9**	**8a**	**8b,8c**	**1,6b,9,10,11 x**
Public health or policy	**2,3**	**5**	**9**	**8a**	**8b,8c**	**1,6,a,6b,7,10**

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Rossi, 2002

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	Design and analytic strategy *(brief description of strategy used for testing patients with CRC)*	Which MMR mutations were sought?	Characteristics of laboratory testing (see definitions below)	Predictors analyzed (check all that apply)	How were deleterious, missense/variants mutations defined (ND if not described)
25 different Brazilian families with suspected hereditary CRC were consecutively enrolled from 1/1995 to 7/1999 Country: Brazil Center: Single • Am2 criteria, or • A relative (degree?) with CRC or HNPCC-related cancer, or • Proband aged <50y with or without affected relatives, or Multiple neoplasms in a proband	All patients underwent gene sequencing. Note: MSI was not considered as a standard preliminary test in this study	HMSH2 and hMLH1	≥5 MSI markers used? (Y / N / ?) MSI-H defined by ≥ 2 markers? (Y / N / ?) Microdissection? (Y / N / ?) Gene screening? (Y / N / ?) — N: x Deletion analysis? (Y / N / ?) — N: x Conversion analysis? (Y / N / ?) — N: x No MSI	Am 1: x Am R: x Beth 1: Beth R: MSI-H: MSI-L: IHC: Age <50: X Suggestive family history: X *Familial CRC without fulfilling Amsterdam criteria*: Other:	ND

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=25 Mean age=46.16 Dropout=0 Probands 60% males	10 patients with MMR / 25 patients with CRC 8 MLH1 2 MSH2	25 patients with CRC, 20% positive AM-I, 4% AM-II, 28% familial CRC w/o fulfilling AM, 44% probands <50 years	ND	"In addition to the Amsterdam criteria I and II, the physician in Brazil should also consider HNPCC when a patient is young and has CRC or when a patient has CRC and a family history of HNPCC, even if it is nontypical, with specific attention being given to gastric tumors."	ND	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	1/4 (4 have Am1)
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		2/5 (5 have Am2= 4with Am1 and 1 with Am2)
	Amsterdam R	x	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	x	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	x	
Am R +	x	
Beth 1 +		
Beth R +		
Age <50	x	
Suggestive family history (*Familial CRC without fulfilling Amsterdam criteria*)	x	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC who had suspected hereditary CRC (fulfilled AM-1 or AM-II, Familial CRC without fulfilling Amsterdam criteria, probands presented at an early age (<50years) or a proband presented with multiple CRCs)	Am 1 +		+	(A) 2	(B) 3
	Am R +	X	-	(C) 8	(D) 12
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Of the 5 pts with Am2, 4 had Am1! So among those with AM2 NB very small table	Am 1 +	X	+	(A) 1	(B) 3
	Am R +		-	(C) 1	(D) 0
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		x	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			N/a
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?			N/a
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			x
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			N/a
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?		x	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	x		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Salovaara, 2000

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
535 consecutive patients with CRC from 9 hospitals in Finland mean age 67	MSI wad done in all patients, founder mutations 1 & 2 in MLH1 were looked for in all patients; MLH1 and MLH2 sequencing was done in MSI positive and founder mutations negative patients	Founder mutations 1 and 2 in MLH1 and other mutations in MLH1 and MLH1	≥5 MSI markers used? N MSI-H defined by ≥ 2 markers? N Microdissection? ? Gene screening? N Deletion analysis? ? Conversion analysis? ?	Am 1 x Am R x Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other x *MSI*	Literature and previous analyses on the founder mutations, and comparison with non-cancer controls

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
535 mean age 67	18 MMR/535 patients with CRC 17 MLH1 and 1 MSH2	3/535 (0.6%) Am I 2/535 (0.4%) Am R		Large scale molecular screening for HNPCC can be done by a 2-stage procedure: screening for founders mutations, then MSI, then sequencing for those with + MSI. The authors also proposed that a strategy that screened for MSI based on age<50, familial disease or multiple tumor, would identify most tumors.		B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	Combined 5/535
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	X	Combined 4/5
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		
If yes, which clinical criteria (check all that apply)?		X
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Consecutive CRCs	Am 1 +		+	4	1
	Am R +		-	14	516
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	Combined Am1&R	X			

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Consecutive CRCs	Am 1 +		+	18	48
	Am R +		-	0	469
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI	X			

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	X		
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			X
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Samowitz, 2001

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Population based sample from the Keiser Permanente Medical care program of Northern California or a 8 county area in Utah. Eligible were incident CRC cases aged between 30 and 79 who were mentally competent	1917 people were contacted, but complete questionnaire data and MSI screening in 1066; Then the 130/171 MSI-H who could be sequenced/had good quality DNA in paraffin blocks → MMR mutations	MLH1 & MSH2	See table below 3 measures of instability 1: BAT 26; 2: TGFbetaRII 126-128; 3: 10 tetranucleotide repair markers used previously (ref); Measures 1 and 3 are reported to be are highly correlated with the 5 2nucleotide marker set. ** with >=one measure of instability.	See table below	Based on the predicted change in the gene and on the HNPCC mutations database.

	Y	N	?
≥5 MSI markers used?	X		
MSI-H defined by ≥ 2 markers?		X**	
Microdissection?		X	
Gene screening?		X	
Deletion analysis?	X only for Finnish mutation		
Conversion analysis?		X	

Am 1	
Am R	
Beth 1	
Beth R	
MSI-H	
MSI-L	
IHC	
Age <50	
Suggestive family history	X
CRC in Fam	
Other	X
Endom Ca in Fam *Age <55*	

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
1917 people were contacted, but complete questionnaire data and MSI screening in 1066; Then the 130/171 MSI-H who could be sequenced/had good quality DNA in paraffin blocks → MMR mutations	Among the 130 MSHI-H 7 MMR mutations (5 ML1; 3 MSH2 – one person had both mutations)	Of 130 MSI-H: 18% (23/129)with CRC in family 5% (6/129) with endometrial ca in family 16% (21/130) aged<55y	ND	The clinical criteria notwithstanding, MSI testing is important on its own. Ought to be done in all CRC. Incorporation of MSI testing in clinical practice might lessen the need for clinical criteria. The estimated prevalence of HNPCC in the CRC population (0.86%) might be an underestimate.	ND	B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify) MSI-H	X	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Among 130 CRC with MSI-H	Am 1 +		+	4	17
	Am R +		-	3	107
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Age <55y				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Among 130 CRC with MSI-H	Am 1 +		+	4	19
	Am R +		-	3	103
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**	X			
	Familial CRC				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	NA		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).		X	
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	NA		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
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MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Schmeler, K, 2006, US, Multicenter

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
315 women with HNPCC identified. Those who had undergone prophylactic TAH (14) compared with those who had undergone prophylactic TAH/BSO (47) compared with age-matched, mutation positive women who had not undergone either procedure (n=210 for endometrial cancer and n=223 for ovarian cancer)	Documented MLH1, MSH2 or MSH6 germline mutation. Did all patients have a personal history of an HNPCC-related cancer Check one: Yes: ; No: ; Uncl: X	Women with genetic variants of unknown functional significance excluded.	TAH versus TAHBSO versus observation

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score
0/61 who had undergone a hysterectomy developed endometrial cancer compared with 69/210 controls ($p<0.001$) 0/47 women who had undergone oophorectomy developed ovarian cancer compared with 12/223 controls ($p=0.09$) Surgical complications in 1/61 women who underwent prophylactic surgery (ureteral injury, ureterovaginal and ureteroeneteric and then rectovaginal fistula))	Patients selected from a registry, and thus presumably all had a family history of HNPCC. ? selection bias for groups with increased penetrance? Did not describe how patients were assigned to surgery or no surgery ? selection bias. Did not describe what type of screening was performed on women who did not undergo the procedures. Other risk factors for gynecologic malignancy (eg, BMI) not assessed. Effects on survival or death not assessed. Quality of life not assessed	Findings support consideration of prophylactic TAH/BSO in women with Lynch syndrome after the age of 35, or once childbearing has been completed. (Median age of diagnosis of endometrial cancer was 46, ovarian cancer 42).	B

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Scott, 2001 RefID 1607 Australia, single

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Index patients from 95 families with HNPCC. Each pedigree was classified as either Amsterdam-criteria positive or Bethesda-criteria positive. Genetic analysis was performed on a fresh blood specimen from the youngest living affected proband in each family. Disease verification in affected individuals was based on either examination of pathology reports or death certificates.	Amsterdam-criteria positive or Bethesda-criteria positive Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: x; No: ; Uncl:	ND	ND

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Of the 95 families, 12 harbored a genetic change in the hMSH2 gene, 22 harbored a change in the hMLH1gene, and 61 were found not to harbor any change in either gene. When mutation status based on the Amsterdam criteria was compared with that based on the Bethesda criteria, differences between the two groups were observed. As expected, 60% (20/33) of Amsterdam-criteria families were mutation positive, compared with 20% of Bethesda-criteria families (12/32). There appeared to be no relationship, between the sites of mutations, that could be used to establish a genotype-phenotype correlation, for either hMLH1 or hMSH2. Within each of the two groups, a comparison between the age at onset of CRC, the spectrum of extracolonic disease, and how this related to the mutation-negative group was made. Cancer Occurrence The overall percentage of tumors observed—in the total population, in the mutation-negative group, and in the hMSH2 mutation–positive and hMLH1 mutation– positive groups—is shown in table 3 of the paper. Briefly, the 9.86% of the total population had CRC and 1.37% had endometrial or ovarian carcinoma. Other types of malignancy were less prevalent.	ND	"In conclusion, use of the Bethesda criteria for the selection of families for gene analysis results in a reduced probability of mutation detection. Nevertheless, families or patients that can be identified on the basis of these criteria should be offered genetic testing for genes associated with HNPCC, since this will result in an increased rate of identification of gene-mutation carriers. To improve the probability of mutation detection if the Bethesda criteria are adopted, we would suggest that mutation analysis be performed in conjunction with DNA microsatellite testing and, possibly, immunohistochemical staining for DNA-mismatch-repair proteins. Subdivision of the mutation-positive and mutation-negative groups makes it possible to tease out subtle differences between the various populations, such as similar ages at onset of disease in the mutation-negative and *hMLH1* mutation–positive groups and disease-spectrum	B Not sure how representable of the sample population. Familial clustering was considered in analyses

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
The relative standardized incidence rates (SIRs), including 95% confidence intervals (95% CIs), for all cancers identified in each group are shown in table 4 of the paper. Among all patients the SIR for CRC was 159.5 (139 – 182.9). This was of the same order among MLH1 and MSH2 carriers (196.8 [143-270.7] and 134.2 [99.1-181.8], respectively), as well as in the mutation negative part of the population (158.6 [132.8 – 189.4]). Data on other malignancies were more sparse, but the estimated SIRs were of similar order across all subgroups (see Table 4 of the paper). II. CRC The age distributions of the hMSH2 mutation–positive group and the hMLH1 mutation–positive group were essentially identical, but there was an about 5-year shift in the distribution seen in the mutation-negative group. The average age at diagnosis in hMSH2 mutation-positive families was 45.77 years, and that in hMLH1 mutation-positive families was 47.16 years. When the authors adjusted for familial clustering there was no statistical difference between the two groups. The average age at diagnosis in the mutation-negative group was 52.7 years. The average age at diagnosis was significantly different from that in the hMSH2 mutation-positive group but was not statistically different from that in the hMLH1 mutation-positive group. III. Extracolonic cancers: The frequencies of the following cancer types were found not to differ between the hMSH2 mutation–positive group, the hMLH1 mutation–positive group, and mutation-negative group: lymphoproliferative disease, renal/renal tract cancers, endometrial/ovarian cancers, and stomach cancer. The authors of the paper note that all these malignancies were overrepresented in all three groups, compared with the expected frequency in the general population.		differences within each mutation-positive group. Better knowledge of the disease spectrum associated with mutation status will aid in the management of these families. Better classification of the mutationnegative group will aid in identification of additional genes associated with this disorder.”	

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely x	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND x	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND x	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					

Domain/question	***Place an "X" in one***					***Overall rating***		
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Shia, 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
A group of 112 colorectal adenocarcinomas (n=83) or adenomas (n=29) obtained from 110 patients treated at the cancer center was assembled. These cases had a family history that fulfilled one of the following criteria: 1) Amsterdam criteria I or II, 2) a set of relaxed AC that we referred to as "HNPCC-like", and 3) Bethesda criteria Country: US Center: single	All patients started with MSI testing, followed by mutation analysis	MLH1 and MSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? N Microdissection? Gene screening? Y Deletion analysis? Conversion analysis? *MSI markers were scored for the appearance of novel locus-specific bands in the tumor but not in the DNA from normal tissue. A tumor was scored "MSI present" when the proportion of successfully typed loci exhibiting instability was ≥0.3.	Am 1 Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other *Specify*	"Mutations were determined to be disease-causing based on sequencing results, segregation analysis, and published data and mutation databases."

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
110 patients derived from 84 kindreds., 48/110 males, 50.5 (range 23-78) years old Total number of tumors assessed was 112, including 83 primary colorectal adenocarcinomas and 29 colorectal adenomas	49 patients with MMR / 92 patients with CRC analyzed. 13 MLH1, 31 MSH2, 5 MSH6 36 patients (from 32 families) with disease-causing MMR / 92 patients with CRC analyzed. 9 MLH1, 23 MSH2, 4 MSH6 13 patients with variants of uncertain clinical significant MMR / 92 patients with CRC analyzed. 4 MLH1, 8 MSH2, 1 MSH6	83 colorectal carcinomas, 51% AM I or II, 39% HNPCC-like patients, and 11% Beth 29 colorectal adenomas, 45% AM I or II, 55% HNPCC-like patients	Abnormal IHC was detected in 31 of 41 tumors that exhibited MSI, whereas all 63 tumors that did not exhibit MSI showed presence of expression of all three proteins.	Even though the authors found that IHC could not replace MSI testing, the authors recommended the use of IHC as a first line screening method to identify patients for mutation analysis because of the simplicity and ready availability.	ND	C Family clustering not accounted for; primarily tumor analyses; dropouts were not described; a “convenience sample” study

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1	x	31/48 tumors MSI present
	Amsterdam R	x	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		4/43 tumors MSI present
	Amsterdam R		
	Other (HNPCC-like)	x	
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1	x	29/55 tumors
	Amsterdam R	x	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		4/44 tumors
	Amsterdam R		
	Other (HNPCC-like)	x	

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	x	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	x	
Am R +	x	
Beth 1 +	x	
Beth R +		
Age <50		
Suggestive family history ("HNPCC-like", defined as 3 or more colorectal cancers among the first- and second-degree relatives)	x	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Colorectal adenomas (n=17) and carcinomas (n=64), fulfilled one of the inclusion criteria: 1) Amsterdam criteria I or II, 2) a set of relaxed AC that we referred to as "HNPCC-like", and 3) Bethesda criteria	Am 1 +		+	(A) 29	(B) 9
	Am R +		-	(C) 4	(D) 39
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	x			
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Colorectal adenomas (n=12) and carcinomas (n=61), fulfilled one of the inclusion criteria: 1) Amsterdam criteria I or II, 2) a set of relaxed AC that we referred to as "HNPCC-like", and 3) Bethesda criteria	Am 1 +		+	(A) 30	(B) 1
	Am R +		-	(C) 7	(D) 35
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (MSI present)**	x			

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			
2	Inclusion criteria clear?			
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			
7	Were withdrawals from the study explained?			
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
12	Was there a clear description of which mismatch repair mutations were being tested for?			
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			
14	Were quality control methods described for the molecular and genetic tests?			
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: (Southey, 2005, RefID 3071)

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Men and women from Victorian Colorectal Cancer Family Study who were younger than age 45 years when diagnosed with a histologically confirmed, first primary adenocarcinoma of the colon or rectum from July 1, 1992 to September 30, 1996. A random selection of 222 patients were asked to participate. Country: Australia Single center	Patients answered a risk factor questionnaire, received IHC and MSI screening. Germline MMR mutation testing was conducted for all patients with one or more of the following characteristics: a family history that fulfilled the Amsterdam Criteria for hereditary nonpolyposis colorectal cancer (HNPCC); having a tumor that was high MSI, low MSI, or that lacked expression of at least one MMR protein; and presence in a random sample of 23 patients selected from those who had tumors that were MS stable and did not lack expression of any MMR protein.	*hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2*	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? Y Gene screening? Y Deletion analysis? Y* Conversion analysis? ? * Only in 10 people with IHC abnormality and no MMR mutation detected	Am 1 Am R x Beth 1 Beth R MSI-H x MSI-L IHC x Age <50 Suggestive family history *Specify* Other *Specify*	Variants were defined to be deleterious if they could be predicted to produce (or were known to produce) a shortened or truncated protein product, or were missense mutations that have been reported previously to be deleterious.

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=131, ND, ND Attrition due to death (12.6%), refusal (doctor, 10.4%; patient, 12.6%), or because the patient moved and was not located (5.4%) resulted in 131 consenting to participate (59.0% of those eligible). The median time between diagnosis and interview was 9 months. Invasive tumor samples of primary colorectal adenocarcinoma were obtained from	18 patients with deleterious MMR / 105 patients with CRC 9 hMLH1 4 hMSH2 4 hMSH6 1 hPMS2	105 patients with CRC, 11% AM II, 17% MSI-H, 15%MSI-L, 19% IHC abnormal	ND	"Because we did not conduct germline MMR testing on all patients who had tumors that were MS stable and IHC normal, we could not calculate sensitivity and specificity directly. The formula used to estimate the sensitivity and specificity were in Appendix." See Table 2 in original paper for detailed results. Loss of MMR protein Expression: Sensitivity (95%CI) 1.00 (0.82-1.00); Specificity (95%CI) 0.91 (0.83-0.96) High or low MSI: Sensitivity 0.94 (0.73 –1.00); Specificity 0.80 (0.71-0.88) Any family history: Sensitivity 0.67 (0.41-0.87); Specificity 0.63 (0.52-0.73)	ND	B

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
hospitals and private pathology laboratories for 118 patients (90%). An assessment of MSI was not successful for 13 (12%) tumor samples because of technical reasons related to tumor DNA quality, which left 105 tumors tested.				Conclusion: "Tumor IHC analysis of four MMR proteins and MSI testing provide a highly sensitive strategy for identifying MMR gene mutation–carrying, early-onset colorectal cancer patients, half of whom would have been missed using Amsterdam Criteria alone. Tumor-based approaches for triaging early-onset colorectal cancer patients for MMR gene mutation testing, irrespective of family history, appear to be an efficient screening strategy for HNPCC."		

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		9/12 [NOT FOR THE PREVALENCE QUESTION, THESE ARE ALL AGED <45]
	Amsterdam R	x	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		7/9 MSI-H; 2/9 MSI-L [NOT FOR THE PREVALENCE QUESTION, THESE ARE ALL AGED <45]
	Amsterdam R	x	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		9/9 [NOT FOR THE PREVALENCE QUESTION, THESE ARE ALL AGED <45]
	Amsterdam R	x	
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		x
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC (assuming all MSI-S and IHC(-) are MMR(-)) WILL NOT USE THIS IN THE SUMMARY TABLES (AM2 WHO ARE <45y)	Am 1 +		+	(A) 9	(B) 3
	Am R + (<45y)	x	-	(C) 9	(D) 84
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC who got tested for MMR (All Am2, signal in MSI or IHC and 23 randomly selected out of the sporadics) All <45y	Am 1 +		+	(A) 13	(B) 5
	Am R +		-	(C) 5	(D) 36
	Beth 1 +				
	Beth R +				
	MSI-H*	x			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC (All Am2, signal in MSI or IHC and 23 randomly selected out of the sporadics) all <45y *Note the 18 patients with MSI-H were not included in this analysis	Am 1 +		+	(A) 4	(B) 12
	Am R +		-	(C) 1	(D) 24
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*	x			
	Age <50				
	IHC (no staining)				
	Other (specify)**				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients with CRC *"of the 79 patients with tumors that expressed all proteins, no mutations were detected in the 33 who were tested"	Am 1 +		+	(A) 18	(B) 8
	Am R +		-	(C) 0	(D) 33/79*
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	x			
	Other (specify)**				

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	X		
2	Inclusion criteria clear?	X		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			X
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?	X		
7	Were withdrawals from the study explained?	X		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			X
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Stormorken 2002

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?		X
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	Design and analytic strategy *(brief description of strategy used for testing patients with CRC)*	Which MMR mutations were sought?	Characteristics of laboratory testing (see definitions below)				Predictors analyzed (check all that apply)		How were deleterious, missense/variants mutations defined (ND if not described)
Index patients from the first 56 families registered at the Norwegian Radium hospital	The patients with the mutations have been described in the Wijnen logistic regression model.	Mlh1 (1) Msh2 (4) Msh6 (2)		Y	N	?	Am 1	X	ND
			≥5 MSI markers used?		NA		Am R	X	
			MSI-H defined by ≥ 2 markers?		NA		Beth 1		
			Microdissection?		NA		Beth R		
			Gene screening?			X	MSI-H		
			Deletion analysis?			X	MSI-L		
			Conversion analysis?			X	IHC		
							Age <50		
							Suggestive family history		
							Specify		
							Other		
							Specify		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
56 index cases not only with CRC, but we focus on 20 AM2 and 12 AM1 who had CRC	3/12 AM1 7/20 AM2	12/56 AM1 20/56 AM2	NA	Should use IHC as a screening		C Unclear description of molecular methods and characterization of pathogenic mutations

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		3/12 AM1 7/20 AM2
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		3/12 AM1 4/20 AM2
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

[NOT APLPICABLE FOR THE REST OF THE EXTRACTION FORM BECAUSE WE ARE NOT SURE THAT ALL PARTICIPANTS HAD CRC]

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
	Am 1 +		+	(A)	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
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Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		X	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).	X		
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)	X		
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?	X		
7	Were withdrawals from the study explained?			X
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	X		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).		X	

Genetic and molecular testing methods

	Examples of tests
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MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Syngal, 2000 [see also Wahlberg 2002]

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Families were identified by self or health care provider referral and were enrolled on the basis of multiple cases of CRC, early age of CRC diagnosis, or the familial association of CRC with other HNPCC associated tumors	Each pedigree were classified into Amsterdam, Amsterdam Mod, Amsterdam II; each proband had genetic analysis, results entered into a 2X2 analysis with respect to clinical classification	MSH2 or MLH1	≥5 MSI markers used? — Y MSI-H defined by ≥ 2 markers? — Microdissection? — Gene screening? — N Deletion analysis? — ? Conversion analysis? — ?	Am 1 — x Am R — x Beth 1 — x Beth R — MSI-H — MSI-L — IHC — Age <50 — Suggestive family history — *Specify* Am Mod — x *Specify*	Based on predicted major change in the protein or on others' results from the literature.

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
70 families were enrolled, representing 297 CRCs and 364 other cancer diagnoses	18 MMR/70 families	28/70 (40%) Am I 39/70 (56%) Am M 34/70 (49%) Am II 56/70 (80%) Beth		The most sensitive clinical criteria for identifying pathogenic MSH2 and MLH1 were Bethesda Guidelines.		B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	11/28 Am1 14/34 AmII 13/39 Modified Am (this is like Am1, but small families so that 2 1st degree related CRC, 2 generations, <55y OR 2 1st degree CRC and 3rd with early onset of HNPCC related) 17/56 Beth 14/56 Beth 1to3
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		See Wahlberg
	Amsterdam II	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		See Wahlberg
	Amsterdam R		
	Am Mod	X	

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Families were identified by self or health care provider referral and were enrolled on the basis of multiple cases of CRC, early age of CRC diagnosis, or the familial association of CRC with other HNPCC associated tumors	Am 1 +	X	+	11	17
	Am R +		-	7	35
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Families were identified by self or health care provider referral and were enrolled on the basis of multiple cases of CRC, early age of CRC diagnosis, or the familial association of CRC with other HNPCC associated tumors	Am 1 +		+	14	20
	Am II	X	-	4	32
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Families were identified by self or health care provider referral and were enrolled on the basis of multiple cases of CRC, early age of CRC diagnosis, or the familial association of CRC with other HNPCC associated tumors	Am Mod	X	+	13	26
	Am R +		-	5	26
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Families were identified by self or health care provider referral and were enrolled on the basis of multiple cases of CRC, early age of CRC diagnosis, or the familial association of CRC with other HNPCC associated tumors	Am 1 +		+	17	39
	Am R +		-	1	13
	Beth 1 +	x			
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	x		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			x
7	Were withdrawals from the study explained?			x
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants		x	
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?		x	
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?		x	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	x		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Terdiman, 2001

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
Eligible families had to have 2 or more first-degree relatives with CRC at any age, an individual with CRC diagnosed before 50 years of age, or a single individual with synchronous or metachronous CRCs. Probands were selected based on convenience and age at cancer diagnosis. When multiple family members were available for molecular testing, the individual with cancer diagnosed at the youngest age was selected as proband. Country: US Center: Single	Paraffin-embedded tumor samples were obtained from all probands for MSI analysis and MSH2/MLH1 immunostaining → Subjects found to have tumors demonstrating MSI-H (n=47) were invited for germline genetic testing of MSH2 and MLH1 → Gene testing was carried out in 32 of the 47 eligible families. Eight probands refused testing for fear of insurance discrimination. In 7 instances, the proband was deceased (n=4) or could not be recontacted (n=3)	MSH2 and MLH1	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? Y Microdissection? ? Gene screening? Y Deletion analysis? N Conversion analysis? N MSI-H was if 3 or more of the 7 marker loci exhibited band size shifts or if 2 or more loci exhibited hand size shifts when 1 of the involved loci was either BAT26or BAT40	Am 1 X Am R Beth 1 X Beth R MSI-H X MSI-L X IHC X Age <50 Suggestive family history *Specify* Other *Specify*	Predicted alteration , previous studies, (literature)

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g., 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
N=114 Mean age=ND %Male=ND Dropouts=5 (4%), no tumor tissue or medical records	16 patients with deleterious MMR / 32 patients with CRC 7 MLH1 9 MSH2 5 patients with MMR of variants of uncertain significance / 32 patients with CRC 4 MLH1 1 MSH2	109 patients with CRC, 43% MSI-H, 3% MSI-L, 23% positive AM, 70% positive Beth	0/40 MSI-L or MSS tumors had positive IHC 45/56 MSI-H tumors had positive IHC; 6/38 MSI-H tumor had ambiguous IHC 21/44 (48%) MSI-H patients met Amsterdam criteria 42/44 (95%) MSI-H patients met Bethesda criteria 3/61 (48%) MSI-L or MSS patients met Amsterdam criteria 30/61 (49%) MSI-L or MSS patients met Bethesda criteria	"In clinical practice today, detection of germline MSH2 or MLH1 mutations is best approached by a 2-step method in which the tumor of high-risk patients are tested either for MSI-H or for the absence of MSH2 or MLH1 protein staining."	ND	B Since study started from MSI testing and gene sequencing only performed in patients with MSI-H, the MMR prevalence questions could not be answered.

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		21/24 (AM2)
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?	[redacted]	
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (CRC and MSI-H)	X	

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+ (deleterious and variants of uncertain significance)**	**Number with MMR-**
Patients with CRC who had MSI-H	Am 1 +		+	(A) 16	(B) 29
	Am R +		-	(C) 1	(D) 4
*6 ambiguous IHC were excluded from this analysis	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)	X			
	Other (specify)**				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+ (deleterious and variants of uncertain significance)**	**Number with MMR-**
Patients with CRC who had MSI-H	Am 1 +		+	(A) 15	(B) 4
	Am R +	X	-	(C) 6	(D) 7
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)	x		
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		x	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			x
7	Were withdrawals from the study explained?	x		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			x
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	x		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?		x	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		x	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	X for IHC		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
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Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Van Dalen 2003, RefID 851 Country: US Center: Multicenter

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
To document the patterns of colorectal surgery performed for cancer-bearing patients who are part of an Amsterdam criteria-positive family, and to compare rates of metachronous cancers that followed each type of index operation. 39 HNPCC families with 93 affected patients. The number of patients per family ranged from 1 to 13. There were 127 CRC. Median age at diagnosis of index cancer was 47 (range, 25-81) years. A total of 16 patients (17%) had metachronous cancers, whereas it was 14 (4%) had synchronous cancer.	Amsterdam criteria defined by Vasen et al in 1991 Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: x; No: ; Uncl:	Family kindreds fulfilling the Amsterdam criteria for HNPCC were identified from the David G. Jagelman Inherited Colorectal Cancer registries. All patient for whom surgical and pathology records were available were eligible to be included in the study. The type of surgery and the outcome of subsequent follow-up were abstracted.	Patients were divided into 2 groups: those treated at the Cleveland Clinic Foundation (CCF) and those treated elsewhere. 33 patients had their initial operation at CCR, and 60 had the surgery elsewhere. Primary surgery performed including colectomy and proctosigmoidectomy. 16 (48%) of the 33 patients who had surgery at CCF had a total colectomy vs. 7 (12%) of the 60 patients who had surgery elsewhere ($p<0.001$) The median follow-up for patients who underwent surgery at CCR was 13 (range 4-49) years, whereas it was 14 (range 1-42) years for those who had surgery elsewhere.

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Incidence of 2nd surgeries for metachronous cancer: only 1 patient who had surgery at CCF had a second operation, this after an initial right colectomy. Thus, 1 (6%) of 17 patients had a segmental resection. 15 (28%) of 53 patients who had surgery elsewhere needed a second resection ($p=0.094$).		"There is potentially a high risk of metachronous colorectal cancer if an index cancer in an HNPCC patient defined according to Amsterdam criteria is treated by partial colectomy. However, this risk can be lowered, either by performing a total colectomy at the time of index surgery or possibly by effective postoperative surveillance."	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3 x	5			8c	1,6a,6b,7,10 x
Family member/other high risk individual	2,3	5	9	8a	8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			

Domain/question	***Place an "X" in one***					***Overall rating***		
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Vasen, 1995, UI7577010 The Netherlands National registry

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
5 year followup (1-20 yr) of surveillance program from registry of families with CRC including colonoscopy or sigmoidoscopy with barium enema every 2-3 years. 50 families with 238 CRC (control pts), 388 (79%) first-degree relative screened of 493 could be traced from 597 high-risk relatives	Amsterdam criteria Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: X No: Uncl:	See study description	Surveillance/screening

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
CRCs in index group were at an advanced histopathological stage compared to screened group; 33 (8.5%) subjects with ≥1 adenomas and 11 (2.8%) CRCs; 5 of 11 CRC patients had negative screening exam 11mo – 3.6 yr previously; in addition to 33 patients with adenoma there were adenomas in 26 patients with CRC. Mortality lower in screened versus control group, 9% vs 45%; 5 year survival higher in screened versus control group, 87% vs 63% 1 patient with severe complication due to screening exam (cardiac arrest with good response to resuscitation); 1 patient had inappropriate exam (sigmoidoscopy rather than colonoscopy)	80% compliance	The screened group had high detection rate CRC, better 5 year survival rate, earlier histopathological stage than the control group. Periodic examination of HNPCC families allow cancer detection at earlier stage than patients not under surveillance. Recommend screening interval of 1-2 years because of aggressive nature of polyps associated with HNPCC	B

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate) **X**	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely **X**	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
		X						
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")	**NA**					A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated	Yes	No						
If the method of random allocation is stated, is it appropriate	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak) **X**
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell **X**					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding	**NA**					A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong) **X**	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes **X**	No						
Were data collection tools shown or are they known to be reliable?	Yes **X**	No						
Withdrawals and Dropouts						A (strong) **X**	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			

Domain/question	***Place an "X" in one***					***Overall rating***		
	X							
Analysis						A (strong)	B (moderate)	C (weak) **X**
Is there a sample size calculation or power calculation	Yes	Partially	No **X**					
Is there a statistically significant difference between groups?	Yes	No	ND **X**					
Are the statistical methods appropriate?	Yes	No	ND **X**					
Indicate the unit of allocation	Community	Organization/group	Provider	Client **X**	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client **X**	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA **X**					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong) **X**	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA **X**			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes **X**	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell **X**					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Vasen, HF 2001, Ref ID 1364 Multicenter, Netherlands/Norway

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Survival analysis performed in 138 families with HNPCC.	Amsterdam I or II or high suspicion +/- mutation testing. Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: ; Uncl: X	Family with HNPCC included in Netherlands HNPCC registry or mutation carriers from a Norwegian registry.	Cumulative cancer rates evaluated in three groups: 1) "Putative" carriers (those relatives affected with CRC or endometrial cancer if a mutation had not been excluded 2) Obligate carriers because of their position in the pedigree in relation to relatives with a mutation 3) Those carrying a mutation

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Pathogenic mutations in 71 Dutch and 8 Norwegian families (34 MLH1 and 40 MSH2 and 5 MSH6). Mean age at diagnosis of CRC higher in carriers of MSH6 compared with MLH1 or MSH2 (50 versus 43 and 44, respectively), statistical significance not reported. Lifetime risk of developing cancer at any age was significantly higher in MSH2 than MLH1 ($p<0.01$). Risk of CRC higher in MSH2 than MLH1 ($p=0.13$). Overall, risk of CRC in male mutation carriers was higher than risk of CRC in female mutation carriers but difference was statistically significant only for MSH2 mutation carriers ($p<0.01$). The risk of developing endometrial cancer was higher in MSH2 mutation carriers than MLH1 ($p=.057$) MSH2 carriers had significantly higher risk of developing cancer of the urinary tract by age 70 (12%, $p<0.05$).	ND	Pending large prospective studies, the extension of the current surveillance program in MSH2 mutation carriers with the inclusion of the urinary tract should be considered.	C No description as to whether the difference may have been observed by clustering of urinary tract cancer in only a subset of families. No description of whether all families received the same degree of surveillance; could incident cancers have been missed in the clinical and pathological databases due to slow growth etc.?

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			X 8c	1,6a,6b,7,10
Family member/other high risk individual	2,3	5	9	8a	X 8b,8c	1,6b,9,10,11
Public health or policy	2,3	5	9	8a	X 8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	X B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	X Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	X ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	X C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	X Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	X NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	X Yes	No						
Blinding						A (strong)	X B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	X ND	NA				
Data Collection methods						A (strong)	X B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	X No						
Were data collection tools shown or are they known to be reliable?	Yes	X No						
Withdrawals and Dropouts						A (strong)	B (moderate)	X C (weak)

Domain/question	***Place an "X" in one***					***Overall rating***		
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	X ND	NA			
Analysis						A (strong)	X B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	X No					
Is there a statistically significant difference between groups?	X Yes	No	ND					
Are the statistical methods appropriate?	X Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	X Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	X Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	X NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	X Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	X C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	X ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	X Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	X Can't tell					

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?
Wagner, 2005, #16341806 The Netherlands Single center

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
Long-term surveillance & counseling to relatives of HNPCC cases. Mutation carriers referred to local specialist for follow-up and surveillance consisting of colonoscopy every 1-2 years from age 20-25 years and on. Female carriers offered gynecological screening by US and CA125-measurements of blood from age 30-35 years and on. Additional screening for stomach, duodenum or urinary tract as advised.	MMR genetic testing Did all patients have a personal history of an HNPCC-related cancer? Check one Yes: ; No: X ; Uncl:	First- and second-degree relatives of patients with HNPCC-related tumor	Surveillance by screening (colonoscopy, gynecologcial screening, or screening of stomach, duodenum or urinary tract), psychological support & counseling program

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)
Long-term satisfaction with screening, counseling procedure Compliance Worry and consequences on daily activities, work, insurance	21/115 (18%) mutation carriers died or moved away 70 (74%) responded to questionnaires 28 (40%) were already diagnosed with HNPCC-related tumor at time of testing 5 has had no screening –burdensome of procedure (1), lack of time (1), plan to go (3)	Colonoscopy satisfaction of health gene carriers: 88% (37) healthy mutation carrier had colonoscopy screening 1-2 years colonoscopy unpleasant 57% fearful 32% painful 51% shameful 16% Faith of colonoscopy effectiveness 90% Worry about complications of colonoscopy 14% Preferred another screening method 71% Long-term satisfaction with counseling: 84% approved of how they were informed of HNPCC genetic testing 97% approved information given during counseling 4/10 had life, disability or mortgage insurance problems, none had health insurance or getting job	B

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	2,3	5			8c	1,6a,6b,7,10
Family member/other high risk individual	2,3 x	5 x	9 x	8a x	8b,8c	1,6b,9,10,11 x
Public health or policy	2,3	5	9	8a	8b,8c	1,6,a,6b,7,10

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias (RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")						A (strong)	B (moderate)	C (weak)
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					

Domain/question	***Place an "X" in one***					***Overall rating***		
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			
Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					

Study: Wahlberg 2002 also see Syngal 2000 for additional details

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**				**Predictors analyzed (check all that apply)**		**How were deleterious, missense/variants mutations defined (ND if not described)**
Families were identified by self or health care provider referral and were enrolled on the basis of multiple cases of CRC, early age of CRC diagnosis, or the familial association of CRC with other HNPCC associated tumors	48 families with tumors available for MSI analysis, 24 of them also had tumor material of sufficient quality for IHC analysis, gene sequencing done previously in Syngal 2000; they were also resequenced using new sequencers	MSH2 or MLH1		Y	N	?	Am 1	X	By predicting major Alteration in the protein structure and based on the literature
			≥5 MSI markers used?	X (NCI)			Am Mod	X	
			MSI-H defined by ≥ 2 markers?	X			Beth 1	X	
			Microdissection?	X			Beth R		
			Gene screening?		X		MSI-H		
			Deletion analysis?			X	MSI-L		
			Conversion analysis?			X	IHC		
							Age <50		
							Suggestive family history		
							Specify		
							Other		
							Specify		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
48 families with available tumor tissue for MSI analysis were included (original study by Syngal 2000 enrolled 70 families)	See Syngal 2000			A combination of the Bethesda criteria for HNPCC and an MSI-H phenotype defined the smallest number of cases having all of the germ-line MSH2 and MLH1 mutations that could be detected by DNA sequencing.		B like Syngal, same study

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		See Syngal 2000
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		MSI H and MSI-L 15/19 Am1 and 3/19 18/28 Modif Amsterdam like Syngal and 4/28 24/36 Beth1 and 4/36 20/28 Beth 1 to 3 and 4/28
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		5/19 Am1 7/28 Md Am1 like Syngal 8/28 Beth 1 to 3 9/36 Beth
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		X
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify)		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MSI-H (NCI)**	**Number with MSI-L or S (NCI)**
48 families with CRCs with available tumor tissue for MSI analysis	Am 1 +	X	+	15	4
	Am R +		-	13	16
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MSI-H (NCI)**	**Number with MSI-L or S (NCI)**
48 families with CRCs with available tumor tissue for MSI analysis	Am 1 +		+	24	12
	Am R +		-	4	8
	Beth 1 +	X			
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			
2	Inclusion criteria clear?			
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			
7	Were withdrawals from the study explained?			
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
12	Was there a clear description of which mismatch repair mutations were being tested for?			
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			
14	Were quality control methods described for the molecular and genetic tests?			
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Wang 1999 & 1997

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
75 unrelated families from France, selected from genetic consultations, multicenter 22 Amsterdam 33 not Amsterdam 12 sporadic CRC 8 CRC+extra-colonic tumor	Combined DNA and RNA-based screening for hMLH1, hMSH2, hPMS1, hPMS2, and hMSH6	hMLH1, hMSH2	≥5 MSI markers used? — N MSI-H defined by ≥ 2 markers? — N Microdissection? — Gene screening? — Y Deletion analysis? — Y Conversion analysis? —	Am 1 — x Am R — Beth 1 — Beth R — MSI-H — MSI-L — IHC — Age <50 — x Suggestive family history — *Specify* Other — *Specify*	They defined them by predicting protein structure loss and based on the literature Missense of unknown pathogenicity were excluded from the analysis

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
See above	26 deleterious mutations were found; 19 MLH1 and 7 MSH2 in 55 unrelated families			Data confirmed that hPMS1, hPMS2, and HMSH6 germline mutations are rare in familial aggregation of CRCs.		B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	14/22
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	x	
If yes, which clinical criteria (check all that apply)?	[redacted]	
Am 1 +	x	
Am R +		
Beth 1 +		
Beth R +		
Age <50	x	
Suggestive family history (specify)	x	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND"** **if not a restricted population** *(e.g. Patients with CRC* *who had a suggestive family* *history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Unrelated kindreds with HNPCC, some fulfilled Amsterdam, some did not	Am 1 +	x	+	14	8
	Am R +		-	8	25
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
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Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?		x	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	x		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			x
7	Were withdrawals from the study explained?			x
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	x		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?		x	
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			x
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			x

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Wolf, 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
81 unrelated patients from Austria who met at least the Beth R guidelines Unclear if all are CRCs,	Pts who met Beth R, Beth 1, Am Mod, or Am II were preselected; DNA sequencing was done in all, and MSI was done in available tumor samples	MLH1 and MSH2	≥5 MSI markers used? Y MSI-H defined by ≥ 2 markers? ? Microdissection? ? Gene screening? N Deletion analysis? ? Conversion analysis? ?	Am 1 Am R X Beth 1 X Beth R X MSI-H MSI-L IHC Age <50 Suggestive family history *Specify* Other X Am Mod (2004)	Predicted alteration; literature; contrast with healthy controls

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsatellite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	Proportion with a mismatch repair mutation (describe which MMR gene). *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	Proportion of overall population positive for each predictor *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	Correlation of predictors to one another (e.g. IHC versus MSI) *Report what study found such as correlation coefficients or other measures of correlation*	Study conclusion *(What did the authors conclude about the testing strategy or other major findings).*	Implications to family /other findings or comments from authors relevant to any key question	Quality grade (see checklist below) and add specific comments about study quality/concerns here
81 pts; 48% M	19 pts with MMR/81 pts with CRC 12 MLH1 7 MSH2	Not applicable	Detection of mutation was correlated with Am R and Am Mod (2004) (*P*=0.011 and *P*=0.038)	MSI enhanced specificity in screening; recommend the use of Am Mod (2004) to select for patients for primary sequence analysis if MSI is not possible.		B

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		13/35
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		16/24
	Amsterdam R	X	
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +	X	
Beth 1 +	X	
Beth R +	X	
Age <50		
Suggestive family history (specify)		
Other (specify) Am Mod (2004)	X	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients who fulfilled Beth R	Am 1 +		+	13	22
	Am R +	X	-	6	40
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients who fulfilled Beth R	Am 1 +		+	19	53
	Am R +		-	0	9
	Beth 1 +	X			
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patients who fulfilled Beth R	Am 1 +		+	16	36
	Am R +		-	3	26
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	** Am Mod (2004)	X			
	MSI only				

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
Patient who fulfilled Beth R	Am 1 +		+	13	9
	Am R +		-	0	33
	Beth 1 +				
	Beth R +				
	MSI-H*	X			
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?	x		
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?		x	
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?	x		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants	X		
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	x		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			x
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	x		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Yuan 1998 [included data from Han 1996]

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**				**Predictors analyzed (check all that apply)**		**How were deleterious, missense/variants mutations defined (ND if not described)**
31 families (from Korean Hereditary CRC registry) with suspected HNPCC and 45 patients with sporadic early-onset CRC	One CRC patient from each family had DNA testing except for one family (hyperplastic polyp); sequencing done in patients with altered mobility on SSCP bands only	hMLH1 and hMSH2		Y	N	?	Am 1		Predicted change in the transcripts; literature (at least in 1 case)
			≥5 MSI markers used?		x		Am R		
			MSI-H defined by ≥ 2 markers?		x		Beth 1		
			Microdissection?				Beth R		
			Gene screening?	x			MSI-H		
			Deletion analysis?				MSI-L		
			Conversion analysis?				IHC		
							Age <50		
							Suggestive family history	X	
							Suspect HNPCC		
							Other		
							Specify		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
31 suspected HNPCC; 45 sporadic CRC Dx <40y (table1 range) suspected HNPCC 50y 65%male Sporadic CRC diagnosed <40y 39y 49% male	7/31 in suspected HNPCC (5 hMLH1, 2 hMSH2); 1/45 in sporadic CRC (hMSH2)			The Korean definition of suspected HNPCC is useful in the diagnosis of HNPCC and for identifying those families suspected of having HNPCC.		C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		ND
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify) Korean criteria HNPCC		
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with Abnormal PCR-SSCP who had hMLH1 or hMSH2 pathogenic mutations**	**Number with Normal PCR-SSCP (no further DNA sequencing)**
	Am 1 +		+	7	24
	Am R +		-	1	44
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Korean criteria	X			
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
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Item	Criteria	Yes	No	Un clr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		X	
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	X		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).	NA		
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			X
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		X	
7	Were withdrawals from the study explained?	NA		
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?		X	
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	X		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			X
12	Was there a clear description of which mismatch repair mutations were being tested for?	X		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	X		
14	Were quality control methods described for the molecular and genetic tests?		X	
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?		X	
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			X

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Study: Yuan 2004

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	x	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	x	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	x	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
14 families that fulfilled the Chinese HNPCC criteria; 3 of them fulfilled Amsterdam	DHPLC was performed, DNA sequencing was performed if DHPLC were abnormal	HMLH1 and hMSH2	See table below	See table below	

	Y	N	?
≥5 MSI markers used?		x	
MSI-H defined by ≥ 2 markers?		x	
Microdissection?		x	
Gene screening?	x		
Deletion analysis?	x		
Conversion analysis?			x

Am 1	
Am R	
Beth 1	
Beth R	
MSI-H	
MSI-L	
IHC	
Age <50	
Suggestive family history	
Chinese criteria	
Other	x
Specify	

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**
14 probands	12 abnormal DHPLC; all 12 had point mutations in either hMLH1 or hMSH2 3 pathogenic mutations identified (2 mhl1 and 1 msh2)	3 were Am1		Detection of large genomic deletions should be involved in the routine screening of HNPCC families.		C

	How was Lynch Syndrome defined (check all that apply)?		Specify numerator and denominator and any comments (ND if not described)
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	x	1/3 were Am1 all other were Chinese criteria
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		NA
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		NA
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	x	
If yes, which clinical criteria (check all that apply)?		
Am 1 +		
Am R +		
Beth 1 +		
Beth R +		
Age <50		
Suggestive family history (specify) Chinese HNPCC		
Other (specify) Chinese HNPCC criteria		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with Abnormal DHPLC that had pathogenic mutation by sequence analysis**	**Number with Abnormal DHPLC that had no pathogenic mutation by sequence analysis**
Patients who fulfilled Chinese HNPCC criteria	Am 1 +	X		2	1
	Am R +			2	9
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Study: Zhu, 2005

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?	X	
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?	X	
Was a minimum of hMLH1 and hMSH2 sequencing performed?	X	

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**	**Predictors analyzed (check all that apply)**	**How were deleterious, missense/variants mutations defined (ND if not described)**
1. 45 unrelated patients with familial CRC, 21 fulfilled Amsterdam 2. 20 patients with CRC <50 yr 3. 13 patients with CRC > 50 yr China	Screen for large deletions and duplications in MSH2 and MLH1 by multiplex ligation-dependent probe amplification (MLPA_	MSH2 and MLH1	≥5 MSI markers used? NA MSI-H defined by ≥ 2 markers? NA Microdissection? NA Gene screening? N: X Deletion analysis? Y: X Conversion analysis? ?: X	Am 1: X Am R Beth 1 Beth R MSI-H MSI-L IHC Age <50: X Suggestive family history *Specify* Other *Specify*	Unclear

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male	**Proportion with a mismatch**	**Proportion of overall population**	**Correlation of predictors to one**	**Study conclusion** *(What did the authors*	**Implications to family /other findings**	**Quality grade (see checklist below)**

dropouts, reasons for dropouts	**repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	*conclude about the testing strategy or other major findings).*	**or comments from authors relevant to any key question**	**and add specific comments about study quality/concerns here**
See above	Genomic aberration was detected in 4/21 patients with Amsterdam	Genomic aberration was detected in 4/21 patients with Amsterdam; 3/24 patients without Amsterdam; 2/20 patients with CRC < 50 yr		Genomic aberrations, large-fragment deletions and duplications, in MSH2 and MLH1 play a role in the pathogenesis of Chinese CRC patients with a family history.		B

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1	X	4/21 has genomic aberration
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		
	Amsterdam R		
	Family history		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?	X	
If yes, which clinical criteria (check all that apply)?		
Am 1 +	X	
Am R +		
Beth 1 +		
Beth R +		
Age <50	X	
Suggestive family history (specify)	X	
Other (specify)		

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population **(i.e. from table above)? "ND" if not a restricted population** *(e.g. Patients with CRC who had a suggestive family history)*	**Index tests**	**Check** *ONE*	**Index test**	**Number with MMR+**	**Number with MMR-**
45 unrelated patients with familial CRC, 21 fulfilled Amsterdam	Am 1 +	X	+	4	17
	Am R +		-	3	21
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				
	MSI only				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)		x	
2	Inclusion criteria clear?		X	
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?	x		
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			x
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			x
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?		x	
7	Were withdrawals from the study explained?		x	
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			X
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?	x		
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?	x		
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			x
12	Was there a clear description of which mismatch repair mutations were being tested for?	x		
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?	x		
14	Were quality control methods described for the molecular and genetic tests?			x
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			x
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).	x		

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Appendix D. List of Excluded Studies

Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, et al. Clues to the pathogenesis of familial colorectal cancer.[comment]. Science 1993 May 7;260(5109):812-6. **Linkage analysis**

Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la CA, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999 Apr 12;81(2):214-8. **Not population of interest**

Abdel-Rahman WM, Ollikainen M, Kariola R, Jarvinen HJ, Mecklin JP, Nystrom-Lahti M, et al. Comprehensive characterization of HNPCC-related colorectal cancers reveals striking molecular features in families with no germline mismatch repair gene mutations. Oncogene 2005 Feb 24;24(9):1542-51. **Didn't report any of 5 options**

Ainsworth PJ, Koscinski D, Fraser BP, Stuart JA. Family cancer histories predictive of a high risk of hereditary non-polyposis colorectal cancer associate significantly with a genomic rearrangement in hMSH2 or hMLH1. Clin Genet 2004 Sep;66(3):183-8. **Not population of interest**

Akrami SM, Dunlop MG, Farrington SM, Frayling IM, MacDonald F, Harvey JF, et al. Screening for exonic copy number mutations at MSH2 and MLH1 by MAPH. Fam Cancer 2005;4(2):145-9. **Didn't report any of 5 options**

Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 2001;158(2):527-35. G**enetic testing not compared with an index test**

Andrew SE, Whiteside D, Buzin C, Greenberg C, Spriggs E. An intronic polymorphism of the hMLH1 gene contributes toward incomplete genetic testing for HNPCC. Genet Test 2002;6(4):319-22. **Didn't report any of 5 options**

Anonymous. American Gastroenterological Association medical position statement: hereditary colorectal cancer and genetic testing. Gastroenterology 2001 Jul;121(1):195-7. **Reference**

Anonymous. MSH6 mutations and risk of uterine cancer. Cancer Biol Ther 2004 Jun;3(6):491. **News article, not study**

Anonymous. Practice parameters for the identification and testing of patients at risk for dominantly inherited colorectal cancer. Dis Colon Rectum 2001 Oct;44(10):1403. **Guideline**

Anonymous. Statement of the American Society of Clinical Oncology: genetic testing for cancer susceptibility, Adopted on February 20, 1996. J Clin Oncol 1996;14(5):1730-6. **Not relevant**

Apessos A, Mihalatos M, Danielidis I, Kallimanis G, Agnantis NJ, Triantafillidis JK, et al. hMSH2 is the most commonly mutated MMR gene in a cohort of Greek HNPCC patients. Br J Cancer 2005 Jan 31;92(2):396-404. **Not population of interest**

Arnold CN, Goel A, Compton C, Marcus V, Niedzwiecki D, Dowell JM, et al. Evaluation of microsatellite instability, hMLH1 expression and hMLH1 promoter hypermethylation in defining the MSI phenotype of colorectal cancer. Cancer Biol Ther 2004 Jan;3(1):73-8. **Not population of interest**

Ashktorab H, Smoot DT, Carethers JM, Rahmanian M, Kittles R, Vosganian G, et al. High incidence of microsatellite instability in colorectal cancer from African Americans. Clin Cancer Res 2003 Mar;9(3):1112-7. **Genetic testing not compared with index test**

Ashktorab H, Smoot DT, Farzanmehr H, Fidelia-Lambert M, Momen B, Hylind L, et al. Clinicopathological features and microsatellite instability (MSI) in colorectal cancers from African Americans. Int J Cancer 2005 Oct 10;116(6):914-9. **Genetic testing not compared with an index test; hMLH1 and hMSH2 sequencing not performed**

Bacher JW, Flanagan LA, Smalley RL, Nassif NA, Burgart LJ, Halberg RB, et al. Development of a fluorescent multiplex assay for detection of MSI-High tumors. Dis Markers 2004;20(4-5):237-50. **Biological material not evaluated**

Bai YQ, Akiyama Y, Nagasaki H, Lu SL, Arai T, Morisaki T, et al. Predominant germ-line mutation of the hMSH2 gene in Japanese hereditary non-polyposis colorectal cancer kindreds. Int J Cancer 1999 Aug 12;82(4):512-5. **Not population of interest**

Bandipalliam P. Syndrome of early onset colon cancers, hematologic malignancies & features of neurofibromatosis in HNPCC families with homozygous mismatch repair gene mutations. Familial Cancer 2005;4(4):323-33. **Case series**

Banno K, Susumu N, Hirao T, Yanokura M, Hirasawa A, Aoki D, et al. Identification of germline MSH2 gene mutations in endometrial cancer not fulfilling the new clinical criteria for hereditary nonpolyposis colorectal cancer. Cancer Genet Cytogenet 2003 Oct 1;146(1):58-65. **Not population of interest**

Banno K, Susumu N, Hirao T, Yanokura M, Hirasawa A, Aoki D, et al. Two Japanese kindreds occurring endometrial cancer meeting new clinical criteria for hereditary non-polyposis colorectal cancer (HNPCC), Amsterdam Criteria II. J Obstet Gynaecol Res 2004 Aug;30(4):287-92. **Not population of interest**

Banno K, Susumu N, Yanokura M, Hirao T, Iwata T, Hirasawa A, et al. Association of HNPCC and endometrial cancers. Int J Clin Oncol 2004 Aug;9(4):262-9. **Not population of interest**

Bapat BV, Madlensky L, Temple LK, Hiruki T, Redston M, Baron DL, et al. Family history characteristics, tumor microsatellite instability and germline MSH2 and MLH1 mutations in hereditary colorectal cancer. Hum Genet 1999 Feb;104(2):167-76. **Not population of interest**

Bartosova Z, Fridrichova I, Bujalkova M, Wolf B, Ilencikova D, Krizan P, et al. Novel MLH1 and MSH2 germline mutations in the first HNPCC families identified in Slovakia. Hum Mutat 2003 Apr;21(4):449. **Not population of interest**

Batra S, Valdimarsdottir H, McGovern M, Itzkowitz S, Brown K. Awareness of genetic testing for colorectal cancer predisposition among specialists in gastroenterology. Am J Gastroenterol 2002 Mar;97(3):729-33. **Not population of interest**

Baudhuin LM, Burgart LJ, Leontovich O, Thibodeau SN. Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for lynch syndrome. Fam Cancer 2005;4(3):255-65. **Not primary study**

Baudhuin LM, Ferber MJ, Winters JL, Steenblock KJ, Swanson RL, French AJ, et al. Characterization of hMLH1 and hMSH2 gene dosage alterations in Lynch syndrome patients. Gastroenterology 2005 Sep;129(3):846-54. **Didn't report any of 5 options**

Baudhuin LM, Mai M, French AJ, Kruckeberg KE, Swanson RL, Winters JL, et al. Analysis of hMLH1 and hMSH2 gene dosage alterations in hereditary nonpolyposis colorectal cancer patients by novel methods. J Mol Diagn 2005 May;7(2):226-35. **Didn't report any of 5 options**

Baudi F, Fersini G, Lavecchia A, Terracciano R, Leone F, Quaresima B, et al. A novel missense germline mutation in exon 2 of the hMSH2 gene in a HNPCC family from Southern Italy. Cancer Lett 2005 Jun 8;223(2):285-91. **Not population of interest**

Beck NE, Tomlinson IP, Homfray T, Frayling I, Hodgson SV, Harocopos C, et al. Use of SSCP analysis to identify germline mutations in HNPCC families fulfilling the Amsterdam criteria. Hum Genet 1997 Feb;99(2):219-24. **Didn't report any of 5 options**

Beck NE, Tomlinson IP, Homfray T, Hodgson SV, Harocopos CJ, Bodmer WF. Genetic testing is important in families with a history suggestive of hereditary non-polyposis colorectal cancer even if the Amsterdam criteria are not fulfilled. Br J Surg 1997 Feb;84(2):233-7. **Not population of interest**

Beck NE, Tomlinson IP, Homfray TF, Frayling IM, Hodgson SV, Bodmer WF. Frequency of germline hereditary non-polyposis colorectal cancer gene mutations in patients with multiple or early onset colorectal adenomas. Gut 1997 Aug;41(2):235-8. **Did not meet inclusion criteria for population**

Benatti P, Roncucci L, Ganazzi D, Percesepe A, Di Gregorio C, Pedroni M, et al. Clinical and biologic heterogeneity of hereditary nonpolyposis colorectal cancer. Int J Cancer 2001 Sep 20;95(5):323-8. **Duplicate publication**

Benatti P, Sassatelli R, Roncucci L, Pedroni M, Fante R, Di Gregorio C, et al. Tumour spectrum in hereditary non-polyposis colorectal cancer (HNPCC) and in families with "suspected HNPCC". A population-based study in northern Italy. Colorectal Cancer Study Group. Int J Cancer 1993 May 28;54(3):371-7. **Genetic testing not compared with an index test; hMLH1 and hMSH2 sequencing not performed**

Berends MJ, Hollema H, Wu Y, van der ST, Mensink RG, ten Hoor KA, et al. MLH1 and MSH2 protein expression as a pre-screening marker in hereditary and non-hereditary endometrial hyperplasia and cancer. Int J Cancer 2001 May 1;92(3):398-403. **Not population of interest**

Berends MJ, Wu Y, Sijmons RH, Mensink RG, van der ST, Hordijk-Hos JM, et al. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. Am J Hum Genet 2002 Jan;70(1):26-37. **Not population of interest**

Berends MJ, Wu Y, Sijmons RH, van der ST, Ek WB, Ligtenberg MJ, et al. Toward new strategies to select young endometrial cancer patients for mismatch repair gene mutation analysis. J Clin Oncol 2003 Dec 1;21(23):4364-70. **Not population of interest**

Bisgaard ML, Jager AC, Myrhoj T, Bernstein I, Nielsen FC. Hereditary non-polyposis colorectal cancer (HNPCC): phenotype-genotype correlation between patients with and without identified mutation. Hum Mutat 2002 Jul;20(1):20-7. **Not population of interest**

Bocker T, Diermann J, Friedl W, Gebert J, Holinski-Feder E, Karner-Hanusch J, et al. Microsatellite instability analysis: a multicenter study for reliability and quality control. Cancer Res 1997 Nov 1;57(21):4739-43. **Genetic test not compared to index test**

Boland CR, Fishel R. Lynch syndrome: form, function, proteins, and basketball. Gastroenterology 2005;129(2):751-5. **Not relevant**

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998 Nov 15;58(22):5248-57. **Review**

Broaddus RR, Lynch PM, Lu KH, Luthra R, Michelson SJ. Unusual tumors associated with the hereditary nonpolyposis colorectal cancer syndrome. Mod Pathol 2004 Aug;17(8):981-9. **Case report**

Brown GJ, St John DJ, Macrae FA, Aittomaki K. Cancer risk in young women at risk of hereditary nonpolyposis colorectal cancer: implications for gynecologic surveillance. Gynecol Oncol 2001;80(3):346-9. **Not relevant**

Buerstedde JM, Alday P, Torhorst J, Weber W, Muller H, Scott R. Detection of new mutations in six out of 10 Swiss HNPCC families by genomic sequencing of the hMSH2 and hMLH1 genes. J Med Genet 1995 Nov;32(11):909-12. Not population of interest

Burgart LJ. Testing for defective DNA mismatch repair in colorectal carcinoma: a practical guide. Arch Pathol Lab Med 2005 Nov;129(11):1385-9. **Not relevant**

Burke W, Petersen G, Lynch P, Botkin J, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. Jama 1997;277(11):915-9. **Not primary study**

Cai Q, Sun MH, Lu HF, Zhang TM, Mo SJ, Xu Y, et al. Clinicopathological and molecular genetic analysis of 4 typical Chinese HNPCC families. World J Gastroenterol 2001 Dec;7(6):805-10. **Case series**

Cai SJ, Xu Y, Cai GX, Lian P, Guan ZQ, Mo SJ, et al. Clinical characteristics and diagnosis of patients with hereditary nonpolyposis colorectal cancer. World J Gastroenterol 2003 Feb;9(2):284-7. **Not relevant**

Caldes T, Godino J, de la HM, Garcia C, I, Perez SP, Eng C, et al. Prevalence of germline mutations of MLH1 and MSH2 in hereditary nonpolyposis colorectal cancer families from Spain. Int J Cancer 2002 Apr 10;98(5):774-9. **Not population of interest**

Cameron BH, Fitzgerald GW, Cox J. Hereditary site-specific colon cancer in a Canadian kindred. CMAJ 1989 Jan 1;140(1):41-5. **Case series, no relevant data**

Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer.[see comment]. Gastroenterology 2004 Feb;126(2):394-401. **Not relevant**

Chai SM, Zeps N, Shearwood AM, Grieu F, Charles A, Harvey J, et al. Screening for defective DNA mismatch repair in stage II and III colorectal cancer patients. Clin Gastroenterol Hepatol 2004 Nov;2(11):1017-25. **hMLH1 and hMSH2 sequencing not performed**

Chan TL, Yuen ST, Chung LP, Ho JW, Kwan KY, Chan AS, et al. Frequent microsatellite instability and mismatch repair gene mutations in young Chinese patients with colorectal cancer. J Natl Cancer Inst 1999 Jul 21;91(14):1221-6. **No data**

Chapusot C, Martin L, Mungra N, Rageot D, Bouvier AM, Bonithon KC, et al. Sporadic colorectal cancers with defective mismatch repair display a number of specific morphological characteristics: relationship between the expression of hMLH1 and hMSH2 proteins and clinicopathological features of 273 adenocarcinomas. Histopathology 2003 Jul;43(1):40-7. **Genetic testing not compared with an index test; hMLH1 and hMSH2 sequencing not performed**

Chapusot C, Martin L, Puig PL, Ponnelle T, Cheynel N, Bouvier AM, et al. What is the best way to assess microsatellite instability status in colorectal cancer? Study on a population base of 462 colorectal cancers. Am J Surg Pathol 2004 Dec;28(12):1553-9. **Genetic test not compared to index test**

Chaves P, Cruz C, Lage P, Claro I, Cravo M, Leitao CN, et al. Immunohistochemical detection of mismatch repair gene proteins as a useful tool for the identification of colorectal carcinoma with the mutator phenotype. J Pathol 2000 Aug;191(4):355-60. **Genetic testing not compared with index test**

Chen S, Watson P, Parmigiani G. Accuracy of MSI testing in predicting germline mutations of MSH2 and MLH1: a case study in Bayesian meta-analysis of diagnostic tests without a gold standard. Biostatistics 2005 Jul;6(3):450-64. **Meta-analysis**

Chung DC, Rustgi AK. The hereditary nonpolyposis colorectal cancer syndrome: genetics and clinical implications. Ann Intern Med 2003;138(7):560-70. **Not primary study**

Church J, Simmang C. Practice parameters for the treatment of patients with dominantly inherited colorectal cancer (familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer). Dis Colon Rectum 2003 Aug;46(8):1001-12. **Guideline**

Church J. Hereditary colon cancers can be tiny: a cautionary case report of the results of colonoscopic surveillance. Am J Gastroenterol 1998 Nov;93(11):2289-90. **Case report**

Claro I, Cravo M, Gloria L, Gaspar C, Albuquerque C, Lage P, et al. Colonic cancer in a 34-yr-old woman: should it prompt microsatellite instability studies and mismatch repair gene testing? Am J Gastroenterol 1998 Oct;93(10):1991-2. **Case report**

Coggins RP, Cawkwell L, Bell SM, Crockford GP, Quirke P, Finan PJ, et al. Association between family history and mismatch repair in colorectal cancer. Gut 2005 May;54(5):636-42. **Did not meet inclusion criteria**

Cravo M, Lage P, Albuquerque C, Chaves P, Claro I, Gomes T, et al. BAT-26 identifies sporadic colorectal cancers with mutator phenotype: a correlative study with clinico-pathological features and mutations in mismatch repair genes. J Pathol 1999 Jul;188(3):252-7. **Data not extractable**

Crijnen TE, Janssen-Heijnen ML, Gelderblom H, Morreau J, Nooij MA, Kenter GG, et al. Survival of patients with ovarian cancer due to a mismatch repair defect. Familial Cancer 2005;4(4):301-5. **Not relevant**

De Felice C, Parrini S, Chitano G, Gentile M, Dipaola L, Latini G. Fordyce granules and hereditary non-polyposis colorectal cancer syndrome. Gut 2005 Sep;54(9):1279-82. **Not population of interest**

de Jong AE, van Puijenbroek M, Hendriks Y, Tops C, Wijnen J, Ausems MG, et al. Microsatellite instability, immunohistochemistry, and additional PMS2 staining in suspected hereditary nonpolyposis colorectal cancer. Clin Cancer Res 2004 Feb 1;10(3):972-80. **hMLH1 and hMSH2 sequencing not performed**

de la CA. Testing tumors for microsatellite instability. Eur J Hum Genet 1999 May;7(4):407-8. **Review**

Dietmaier W, Hofstadter F. Detection of microsatellite instability by real time PCR and hybridization probe melting point analysis. Lab Invest 2001 Oct;81(10):1453-6. **Not relevant**

Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997 Nov 1;57(21):4749-56. **Incomplete data**

Domingo E, Laiho P, Ollikainen M, Pinto M, Wang L, French AJ, et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet 2004 Sep;41(9):664-8. **hMLH1 and hMSH2 sequencing not performed**

Domingo E, Niessen RC, Oliveira C, Alhopuro P, Moutinho C, Espin E, et al. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. Oncogene 2005 Jun 2;24(24):3995-8. **Not relevant, select patients**

Douglas JA, Gruber SB, Meister KA, Bonner J, Watson P, Krush AJ, et al. History and molecular genetics of Lynch syndrome in family G: a century later. Jama 2005;294(17):2195-202. **Case report**

Dovrat S, Figer A, Fidder HH, Neophytou P, Fireman Z, Geva R, et al. Mutational analysis of hMsh6 in Israeli HNPCC and HNPCC-like families. Familial Cancer 2005;4(4):291-4. **Genetic testing not compared with index test**

Drobinskaya I, Gabbert HE, Moeslein G, Mueller W. A new method for optimizing multiplex DNA microsatellite analysis in low quality archival specimens. Anticancer Res 2005 Sep;25(5):3251-8. **Genetic test not compared to index test**

Dunlop MG, Farrington SM, Carothers AD, Wyllie AH, Sharp L, Burn J, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997;6(1):105-10. **Not relevant**

Dunlop MG, Farrington SM, Nicholl I, Aaltonen L, Petersen G, Porteous M, et al. Population carrier frequency of hMSH2 and hMLH1 mutations. Br J Cancer 2000 Dec;83(12):1643-5. **Not population of interest**

Eisen GM, Weinberg DS. Narrative review: screening for colorectal cancer in patients with a first-degree relative with colonic neoplasia. Ann Intern Med 2005;143(3):190-8. **Review**

Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 2000;355(9217):1745-50. **Not population of interest**

Engel C, Forberg J, Holinski-Feder E, Pagenstecher C, Plaschke J, Kloor M, et al. Novel strategy for optimal sequential application of clinical criteria, immunohistochemistry and microsatellite analysis in the diagnosis of hereditary nonpolyposis colorectal cancer. Int J Cancer 2006 Jan 1;118(1):115-22. **Not outcomes of interest**

Ensenauer RE, Michels VV, Reinke SS. Genetic testing: practical, ethical, and counseling considerations. Mayo Clin Proc 2005 Jan;80(1):63-73. **Not relevant**

Ericson K, Nilbert M, Bladstrom A, Anderson H, Olsson H, Planck M. Familial risk of tumors associated with hereditary non-polyposis colorectal cancer: a Swedish population-based study. Scand J Gastroenterol 2004 Dec;39(12):1259-65. **Not relevant**

Evans DG, Walsh S, Jeacock J, Robinson C, Hadfield L, Davies DR, et al. Incidence of hereditary non-polyposis colorectal cancer in a population-based study of 1137 consecutive cases of colorectal cancer. Br J Surg 1997 Sep;84(9):1281-5. **Genetic testing not compared with index test**

Fallik D, Borrini F, Boige V, Viguier J, Jacob S, Miquel C, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Res 2003 Sep 15;63(18):5738-44. **Not population of interest**

Faragher IG, Whitehead RH. Rapid diagnostic test for hereditary nonpolyposis colon cancer kindred using polymerase chain reaction. Dis Colon Rectum 1998 Jul;41(7):938-40. **Not relevant**

Fidalgo P, Almeida MR, West S, Gaspar C, Maia L, Wijnen J, et al. Detection of mutations in mismatch repair genes in Portuguese families with hereditary non-polyposis colorectal cancer (HNPCC) by a multi-method approach. Eur J Hum Genet 2000 Jan;8(1):49-53. **Unknown if patients had CRC**

Frazier ML, Sinicrope FA, Amos CI, Cleary KR, Lynch PM, Levin B, et al. Loci for efficient detection of microsatellite instability in hereditary non-polyposis colorectal cancer. Oncol Rep 1999 May;6(3):497-505. **Didn't report any of 5 options**

Froggatt NJ, Brassett C, Koch DJ, Evans DG, Hodgson SV, Ponder BA, et al. Mutation screening of MSH2 and MLH1 mRNA in hereditary non-polyposis colon cancer syndrome. J Med Genet 1996 Sep;33(9):726-30. **Data not extractable**

Furlan D, Tibiletti MG, Taborelli M, Albarello L, Cornaggia M, Capella C. The value of microsatellite instability in the detection of HNPCC families and of sporadic colorectal cancers with special biological features: an investigation on a series of 100 consecutive cases. Ann Oncol 1998 Aug;9(8):901-6. **No genetic testing**

Furukawa T, Konishi F, Shitoh K, Kojima M, Nagai H, Tsukamoto T. Evaluation of screening strategy for detecting hereditary nonpolyposis colorectal carcinoma. Cancer 2002 Feb 15;94(4):911-20. **Data not extractable**

Gaglia P, Atkin WS, Whitelaw S, Talbot IC, Williams CB, Northover JM, et al. Variables associated with the risk of colorectal adenomas in asymptomatic patients with a family history of colorectal cancer. Gut 1995 Mar;36(3):385-90. **Not population of interest**

Geirdal AO, Reichelt JG, Dahl AA, Heimdal K, Maehle L, Stormorken A, et al. Psychological distress in women at risk of hereditary breast/ovarian or HNPCC cancers in the absence of demonstrated mutations. Fam Cancer 2005;4(2):121-6. **Results of FBOC and HNPCC combined in analyses**

Genuardi M, Anti M, Capozzi E, Leonardi F, Fornasarig M, Novella E, et al. MLH1 and MSH2 constitutional mutations in colorectal cancer families not meeting the standard criteria for hereditary nonpolyposis colorectal cancer. Int J Cancer 1998 Mar 16;75(6):835-9. **Data not extractable**

Giardiello FM, Brensinger JD, Petersen GM. AGA technical review on hereditary colorectal cancer and genetic testing. Gastroenterology 2001;121(1):198-213. **Review**

Gill S, Lindor NM, Burgart LJ, Smalley R, Leontovich O, French AJ, et al. Isolated loss of PMS2 expression in colorectal cancers: frequency, patient age, and familial aggregation. Clin Cancer Res 2005 Sep 15;11(18):6466-71. **Genetic testing not compared with index test, hMLH1 and hMSH2 sequencing not performed**

Giraldo A, Gomez A, Salguero G, Garcia H, Aristizabal F, Gutierrez O, et al. MLH1 and MSH2 mutations in Colombian families with hereditary nonpolyposis colorectal cancer (Lynch syndrome)--description of four novel mutations. Familial Cancer 2005;4(4):285-90. **Not population of interest**

Gologan A, Krasinskas A, Hunt J, Thull DL, Farkas L, Sepulveda AR. Performance of the revised Bethesda guidelines for identification of colorectal carcinomas with a high level of microsatellite instability. Arch Pathol Lab Med 2005 Nov;129(11):1390-7. **Genetic testing not compared with index test, hMLH1 and hMSH2 sequencing not performed**

Grover S, Stoffel EM, Bussone L, Tschoegl E, Syngal S. Physician assessment of family cancer history and referral for genetic evaluation in colorectal cancer patients. Clin Gastroenterol Hepatol 2004;2(9):813-9. **Not relevant**

Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342(2):69-77. **Not population of interest, hMLH1 and hMSH2 sequencing not performed**

Hall NR, Finan PJ, Ward B, Turner G, Bishop DT. Genetic susceptibility to colorectal cancer in patients under 45 years of age. Br J Surg 1994 Oct;81(10):1485-9. **Genetic testing not compared with index test, hMLH1 and hMSH2 sequencing not performed**

Halling KC, French AJ, McDonnell SK, Burgart LJ, Schaid DJ, Peterson BJ, et al. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. J Natl Cancer Inst 1999;91(15):1295-303. **Not relevant**

Halvarsson B, Lindblom A, Johansson L, Lagerstedt K, Nilbert M. Loss of mismatch repair protein immunostaining in colorectal adenomas from patients with hereditary nonpolyposis colorectal cancer. Mod Pathol 2005 Aug;18(8):1095-101. **Not outcomes of interest**

Halvarsson B, Lindblom A, Rambech E, Lagerstedt K, Nilbert M. Microsatellite instability analysis and/or immunostaining for the diagnosis of hereditary nonpolyposis colorectal cancer? Virchows Arch 2004 Feb;444(2):135-41. **ND on germline mutations**

Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, et al. The molecular basis of Turcot's syndrome. N Engl J Med 1995 Mar 30;332(13):839-47. **Not population of interest**

Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352(18):1851-60. **Not outcomes of interest**

Hatch SB, Lightfoot HM, Jr., Garwacki CP, Moore DT, Calvo BF, Woosley JT, et al. Microsatellite instability testing in colorectal carcinoma: choice of markers affects sensitivity of detection of mismatch repair-deficient tumors. Clin Cancer Res 2005 Mar 15;11(6):2180-7. **Genetic test not compared to index test**

Hegde M, Blazo M, Chong B, Prior T, Richards C. Assay validation for identification of hereditary nonpolyposis colon cancer-causing mutations in mismatch repair genes MLH1, MSH2, and MSH6. J Mol Diagn 2005 Oct;7(4):525-34. **Didn't report any of 5 options**

Heinimann K, Scott RJ, Buerstedde JM, Weber W, Siebold K, Attenhofer M, et al. Influence of selection criteria on mutation detection in patients with hereditary nonpolyposis colorectal cancer. Cancer 1999 Jun 15;85(12):2512-8. **Not population of interest**

Hendriks YM, Jagmohan-Changur S, van der Klift HM, Morreau H, van PM, Tops C, et al. Heterozygous Mutations in PMS2 Cause Hereditary Nonpolyposis Colorectal Carcinoma (Lynch Syndrome). Gastroenterology 2006 Feb;130(2):312-22.

Hitchins M, Williams R, Cheong K, Halani N, Lin VA, Packham D, et al. MLH1 germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. Gastroenterology 2005 Nov;129(5):1392-9. **Genetic testing not compared with index test**

Ho JW, Yuen ST, Chung LP, Kwan KY, Chan TL, Leung SY, et al. Distinct clinical features associated with microsatellite instability in colorectal cancers of young patients. Int J Cancer 2000 Jul 20;89(4):356-60. **No data**

Hodgson SV, Harocopos C, Gaglia P. Screening results in a family cancer clinic: five years experience. Anticancer Res 1993 Nov;13(6B):2581-5.

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Vasen HF, Mecklin JP, Watson P, Utsunomiya J, Bertario L, Lynch P, et al. Surveillance in hereditary nonpolyposis colorectal cancer: an international cooperative study of 165 families. The International Collaborative Group on HNPCC. Dis Colon Rectum 1993 Jan;36(1):1-4. **Not relevant**

Vasen HF, Offerhaus GJ, Hartog Jager FC, Menko FH, Nagengast FM, Griffioen G, et al. The tumour spectrum in hereditary non-polyposis colorectal cancer: a study of 24 kindreds in the Netherlands. Int J Cancer 1990 Jul 15;46(1):31-4. **No benefit and harms data**

Vasen HF, Taal BG, Griffioen G, Nagengast FM, Cats A, Menko FH, et al. Clinical heterogeneity of familial colorectal cancer and its influence on screening protocols. Gut 1994 Sep;35(9):1262-6. **Not outcomes of interest**

Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999;116(6):1453-6. **Not primary study**

Vasen HF, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology 1996;110(4):1020-7. **Not relevant**

Velayos FS, Allen BA, Conrad PG, Gum J, Jr., Kakar S, Chung DC, et al. Low rate of microsatellite instability in young patients with adenomas: reassessing the Bethesda guidelines. Am J Gastroenterol 2005 May;100(5):1143-9. **Not population of interest**

Velayos FS, Lee SH, Qiu H, Dykes S, Yiu R, Terdiman JP, et al. The mechanism of microsatellite instability is different in synchronous and metachronous colorectal cancer. J Gastrointest Surg 2005 Mar;9(3):329-35. **Genetic testing not compared with index test**

Verma L, Kane MF, Brassett C, Schmeits J, Evans DG, Kolodner RD, et al. Mononucleotide microsatellite instability and germline MSH6 mutation analysis in early onset colorectal cancer. J Med Genet 1999 Sep;36(9):678-82. **hMLH1 and hMSH2 sequencing not performed; genetic testing not compared to index test**

Vernon SW, Gritz ER, Peterson SK, Amos CI, Perz CA, Baile WF, et al. Correlates of psychologic distress in colorectal cancer patients undergoing genetic testing for hereditary colon cancer. Health Psychol 1997 Jan;16(1):73-86. **Not relevant**

Vernon SW, Gritz ER, Peterson SK, Perz CA, Marani S, Amos CI, et al. Intention to learn results of genetic testing for hereditary colon cancer. Cancer Epidemiol Biomarkers Prev 1999 Apr;8(4 Pt 2):353-60. **Not relevant**

Viel A, Genuardi M, Capozzi E, Leonardi F, Bellacosa A, Paravatou-Petsotas M, et al. Characterization of MSH2 and MLH1 mutations in Italian families with hereditary nonpolyposis colorectal cancer. Genes Chromosomes Cancer 1997 Jan;18(1):8-18. **Family level data reported, unsure all pts were CRC**

Viel A, Novella E, Genuardi M, Capozzi E, Fornasarig M, Pedroni M, et al. Lack of PMS2 gene-truncating mutations in patients with hereditary colorectal cancer. Int J Oncol 1998 Sep;13(3):565-9. **Not outcomes of interest**

Viel A, Petronzelli F, Della PL, Lucci-Cordisco E, Fornasarig M, Pucciarelli S, et al. Different molecular mechanisms underlie genomic deletions in the MLH1 Gene. Hum Mutat 2002 Nov;20(5):368-74. **Didn't report any of 5 options**

Vilkki S, Launonen V, Karhu A, Sistonen P, Vastrik I, Aaltonen LA. Screening for microsatellite instability target genes in colorectal cancers. J Med Genet 2002 Nov;39(11):785-9. **Genetic testing not compared with index test**

Wagner A, Barrows A, Wijnen JT, van der KH, Franken PF, Verkuijlen P, et al. Molecular analysis of hereditary nonpolyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene. Am J Hum Genet 2003 May;72(5):1088-100. **Not population of interest**

Wagner A, van der KH, Franken P, Wijnen J, Breukel C, Bezrookove V, et al. A 10-Mb paracentric inversion of chromosome arm 2p inactivates MSH2 and is responsible for hereditary nonpolyposis colorectal cancer in a North-American kindred. Genes Chromosomes Cancer 2002 Sep;35(1):49-57. **Didn't report any of 5 options**

Wahlberg S, Liu T, Lindblom P, Lindblom A. Various mutation screening techniques in the DNA mismatch repair genes hMSH2 and hMLH1. Genet Test 1999;3(3):259-64. **Not population of interest**

Wahlberg SS, Nystrom-Lahti M, Kane MF, Kolodner RD, Peltomaki P, Lindblom A. Low frequency of hMSH2 mutations in Swedish HNPCC families. International Journal of Cancer 1997 Feb 20;74(1):134-7. **Family level data reported, unsure all pts were CRC**

Wang L, Baudhuin LM, Boardman LA, Steenblock KJ, Petersen GM, Halling KC, et al. MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. Gastroenterology 2004 Jul;127(1):9-16. **Genetic testing not compared with index test, hMLH1 and hMSH2 sequencing not performed**

Wang Y, Friedl W, Lamberti C, Jungck M, Mathiak M, Pagenstecher C, et al. Hereditary nonpolyposis colorectal cancer: frequent occurrence of large genomic deletions in MSH2 and MLH1 genes. Int J Cancer 2003 Feb 20;103(5):636-41. **Not population of interest, data not extractable**

Wang Y, Friedl W, Lamberti C, Nothen MM, Kruse R, Propping P. A novel missense mutation in the DNA mismatch repair gene hMLH1 present among East Asians but not among Europeans. Hum Hered 1998 Mar;48(2):87-91. **Genetic testing not compared with index test**

Wang Y, Friedl W, Lamberti C, Ruelfs C, Kruse R, Propping P. Hereditary nonpolyposis colorectal cancer: causative role of a germline missense mutation in the hMLH1 gene confirmed by the independent occurrence of the same somatic mutation in tumour tissue. Hum Genet 1997 Sep;100(3-4):362-4. **Not population of interest; genetic testing not compared with index test**

Wang Y, Friedl W, Sengteller M, Jungck M, Filges I, Propping P, et al. A modified multiplex PCR assay for detection of large deletions in MSH2 and MLH1. Hum Mutat 2002 Mar;19(3):279-86. **Didn't report any of 5 options**

Ward R, Meldrum C, Williams R, Mokany E, Scott R, Turner J, et al. Impact of microsatellite testing and mismatch repair protein expression on the clinical interpretation of genetic testing in hereditary non-polyposis colorectal cancer. J Cancer Res Clin Oncol 2002 Aug;128(8):403-11. **Not population of interest**

Ward RL, Turner J, Williams R, Pekarsky B, Packham D, Velickovic M, et al. Routine testing for mismatch repair deficiency in sporadic colorectal cancer is justified. J Pathol 2005;207(4):377-84. **Genetic testing not compared with an index test; hMLH1 and hMSH2 sequencing not performed**

Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71(3):677-85. **Not relevant**

Weber TK, Chin HM, Rodriguez-Bigas M, Keitz B, Gilligan R, O'Malley L, et al. Novel hMLH1 and hMSH2 germline mutations in African Americans with colorectal cancer. Jama 1999 Jun 23;281(24):2316-20. **Genetic testing not compared with index test**

Weber TK, Conlon W, Petrelli NJ, Rodriguez-Bigas M, Keitz B, Pazik J, et al. Genomic DNA-based hMSH2 and hMLH1 mutation screening in 32 Eastern United States hereditary nonpolyposis colorectal cancer pedigrees. Cancer Res 1997 Sep 1;57(17):3798-803. Not population of interest

Weber TK, Conroy J, Keitz B, Rodriguez-Bigas M, Petrelli NJ, Stoler DL, et al. Genome-wide allelotyping indicates increased loss of heterozygosity on 9p and 14q in early age of onset colorectal cancer. Cytogenet Cell Genet 1999;86(2):142-7. **Genetic testing not compared with index test**

Wei SC, Shun CT, Tsai-Wu JJ, Wu CH, Sheu JC, Wang CY, et al. Microsatellite instability and hMLH1 and hMSH2 gene expression in Taiwanese hereditary nonpolyposis colorectal cancer. J Formos Med Assoc 2004 May;103(5):331-6. **Not population of interest**

Wei SC, Yu CY, Tsai-Wu JJ, Su YN, Sheu JC, Wu CH, et al. Low mutation rate of hMSH2 and hMLH1 in Taiwanese hereditary non-polyposis colorectal cancer. Clin Genet 2003 Sep;64(3):243-51. **Not population of interest**

Whelan AJ, Babb S, Mutch DG, Rader J, Herzog TJ, Todd C, et al. MSI in endometrial carcinoma: absence of MLH1 promoter methylation is associated with increased familial risk for cancers. Int J Cancer 2002 Jun 10;99(5):697-704. **Not population of interest**

Whitehall VL, Wynter CV, Walsh MD, Simms LA, Purdie D, Pandeya N, et al. Morphological and molecular heterogeneity within nonmicrosatellite instability-high colorectal cancer. Cancer Res 2002 Nov 1;62(21):6011-4. **Genetic testing not compared with index test, hMLH1 and hMSH2 sequencing not performed**

Whiteside D, McLeod R, Graham G, Steckley JL, Booth K, Somerville MJ, et al. A homozygous germ-line mutation in the human MSH2 gene predisposes to hematological malignancy and multiple cafe-au-lait spots. Cancer Res 2002 Jan 15;62(2):359-62. **Not population of interest**

Wijnen J, Khan PM, Vasen H, van der KH, Mulder A, Leeuwen-Cornelisse I, et al. Hereditary nonpolyposis colorectal cancer families not complying with the Amsterdam criteria show extremely low frequency of mismatch-repair-gene mutations. Am J Hum Genet 1997 Aug;61(2):329-35. **Not population of interest**

Wijnen JT, Vasen HF, Khan PM, Zwinderman AH, van der KH, Mulder A, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med 1998 Aug 20;339(8):511-8. **Family level data reported, no primary data**

Wikman FP, Katballe N, Christensen M, Laurberg S, Orntoft TF. Efficient mutation detection in mismatch repair genes using a combination of single-strand conformational polymorphism and heteroduplex analysis at a controlled temperature. Genet Test 2000;4(1):15-21. **Didn't report any of 5 options**

Winawer S, Fletcher R, Rex D, Bond J, Burt R, Ferrucci J, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. Gastroenterology 2003;124(2):544-60. **Not primary study**

Woerner SM, Gebert J, Yuan YP, Sutter C, Ridder R, Bork P, et al. Systematic identification of genes with coding microsatellites mutated in DNA mismatch repair-deficient cancer cells. Int J Cancer 2001 Jul 1;93(1):12-9. **Not outcomes of interest**

Woerner SM, Kloor M, Mueller A, Rueschoff J, Friedrichs N, Buettner R, et al. Microsatellite instability of selective target genes in HNPCC-associated colon adenomas. Oncogene 2005 Apr 7;24(15):2525-35. **Not population of interest**

Wu Y, Berends MJ, Mensink RG, Kempinga C, Sijmons RH, Der Zee AG, et al. Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. Am J Hum Genet 1999 Nov;65(5):1291-8. **Not population of interest**

Wu Y, Berends MJ, Post JG, Mensink RG, Verlind E, van der ST, et al. Germline mutations of EXO1 gene in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and atypical HNPCC forms. Gastroenterology 2001 Jun;120(7):1580-7. **Not outcomes of interest**

Wu Y, Berends MJ, Sijmons RH, Mensink RG, Verlind E, Kooi KA, et al. A role for MLH3 in hereditary nonpolyposis colorectal cancer. Nat Genet 2001 Oct;29(2):137-8. **Not population of interest**

Wullenweber HP, Sutter C, Autschbach F, Willeke F, Kienle P, Benner A, et al. Evaluation of Bethesda guidelines in relation to microsatellite instability. Dis Colon Rectum 2001;44(9):1281-9. **No data**

Xu DQ, Mattox W. Identification of a splicing enhancer in MLH1 using COMPARE, a new assay for determination of relative RNA splicing efficiencies. Human Molecular Genetics 2006 Jan 15;15(2):329-36. **Not relevant**

Yagi OK, Akiyama Y, Nomizu T, Iwama T, Endo M, Yuasa Y. Proapoptotic gene BAX is frequently mutated in hereditary nonpolyposis colorectal cancers but not in adenomas. Gastroenterology 1998 Feb;114(2):268-74. **Not outcomes of interest**

Yamamoto H, Min Y, Itoh F, Imsumran A, Horiuchi S, Yoshida M, et al. Differential involvement of the hypermethylator phenotype in hereditary and sporadic colorectal cancers with high-frequency microsatellite instability. Genes Chromosomes Cancer 2002 Mar;33(3):322-5. **Not outcomes of interest**

Yamamoto H, Sawai H, Weber TK, Rodriguez-Bigas MA, Perucho M. Somatic frameshift mutations in DNA mismatch repair and proapoptosis genes in hereditary nonpolyposis colorectal cancer. Cancer Res 1998 Mar 1;58(5):997-1003. **hMLH1 and hMSH2 sequencing not performed**

Yamashita K, Arimura Y, Kurokawa S, Itoh F, Endo T, Hirata K, et al. Microsatellite instability in patients with multiple primary cancers of the gastrointestinal tract. Gut 2000 Jun;46(6):790-4. **Not population of interest**

Young J, Simms LA, Biden KG, Wynter C, Whitehall V, Karamatic R, et al. Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: parallel pathways of tumorigenesis. Am J Pathol 2001 Dec;159(6):2107-16. **Genetic testing not compared with an index test**

Yuen ST, Chan TL, Ho JW, Chan AS, Chung LP, Lam PW, et al. Germline, somatic and epigenetic events underlying mismatch repair deficiency in colorectal and HNPCC-related cancers. Oncogene 2002 Oct 24;21(49):7585-92. **No data**

Zhao B, Wang ZJ, Xu YF, Wan YL, Li P, Huang YT. Report of 16 kindreds and one kindred with hMLH1 germline mutation. World J Gastroenterol 2002 Apr;8(2):263-6. **Not relevant**

Zhou XL, Eriksson U, Werelius B, Kressner U, Sun XF, Lindblom A. Definition of candidate low risk APC alleles in a Swedish population. Int J Cancer 2004 Jul 1;110(4):550-7. **Not population of interest, different question**

Zhou XP, Kuismanen S, Nystrom-Lahti M, Peltomaki P, Eng C. Distinct PTEN mutational spectra in hereditary non-polyposis colon cancer syndrome-related endometrial carcinomas compared to sporadic microsatellite unstable tumors. Hum Mol Genet 2002 Feb 15;11(4):445-50. **Not outcomes of interest**

Zhou XP, Loukola A, Salovaara R, Nystrom-Lahti M, Peltomaki P, de la CA, et al. PTEN mutational spectra, expression levels, and subcellular localization in microsatellite stable and unstable colorectal cancers. Am J Pathol 2002 Aug;161(2):439-47. **Not outcomes of interest**

Appendix E. Peer Reviewers

We gratefully acknowledge the following individuals who reviewed the initial draft of this Report and provided us with constructive feedback. Acknowledgments are made with the explicit statement that this does not constitute endorsement of the report by the peer reviewers.

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Appendix F. Summary Tables on Clinical Validity

Appendix F contains summary Tables (Appendices F-1 to F-29). Appendices F-1 to F-5 summarize the prevalence on mismatch repair (MMR) gene mutations, microsatellite instability or negative immunohistochemistry among patients fulfilling Amsterdam I or II criteria. Appendices F-6 to F-29 summarize the diagnostic ability of clinical and laboratory predictors to detect MMR gene mutations among different populations. Table 14 of the evidence report provides a roadmap to the different summary tables. For convenience, the information included in Table 14 of the Evidence Report is repeated after the following table of contents.

Note that usually only MLH1 and MSH2 were assessed. Whenever additional genes were assessed (MSH6 or PMS2), they are clearly noted in the Tables and footnotes.

Table of contents for Appendix F

Overview of available evidence on the sensitivity and specificity of various predictors for MMR mutation rates

Predictor	All studies	Specific CRC populations defined by increasingly selective criteria					
		Nonselected CRC probands	Revised Bethesda guidelines	Bethesda guidelines	Modified Amsterdam criteria	Amsterdam II criteria	Amsterdam I criteria
Amsterdam I criteria	F-6 (n=17)	F-7 (n=2)	ND	F-8 (n=1)	ND	F-9 (n=4)	
Amsterdam II criteria	F-10 (n=11)	F-11 (n=2)	F-12 (n=1)	F-13 (n=2)	ND		
Modified Amsterdam criteria	F-14 (n=2)	ND	ND	ND			
Bethesda guidelines	F-15 (n=4)	F-16 (n=1)	ND				
Revised Bethesda guidelines	F-17 (n=1)	F-17 (n=1)					
Young age of onset	F-18 (n=5)	F-19 (n=4)	ND	ND	ND	ND	ND
Family history	F-20 (n=9)	F-21 (n=4)	ND	ND	ND		
Multiple tumors in the same patient	F-22 (n=3)	F-22 (n=3)	ND	ND	ND	ND	ND
Age <50 years, family history, or multiple tumors in same patient	F-23 (n=3)	F-23 (n=3)	ND	ND	ND	ND	ND
Replication errors (MSI-high; MSI-high and MSI-low)	F-24 (n=16)	F-25 (n=2)	F-26 (n=1)	ND	ND	ND	F-27 (n=3)
Loss of protein expression (negative immunostaining)	F-28 (n=9)	ND	ND	ND	ND	ND	F-29 (n=2)

Table rows represent different predictors; the table columns describe the populations addressed in the summary tables. Each cell in the table refers to a corresponding Summary Table of the appendix. For example, the second cell of the second row shows that there were 17 studies that described the ability of Amsterdam I criteria to predict MMR status. The studies are described in Appendix F-6. The third cell of the second row shows that of these 17 studies, only two performed MMR testing in nonselected patients; these studies are described in Appendix F-7, and so on.
ND: No data

Appendix F-1: Prevalence of mismatch repair gene mutations among colorectal cancer probands who fulfill Amsterdam I criteria

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Genetic testing strategy C. Definition of deleterious mutations	N_{Am1}/ N_{total}	MLH1 and MSH2 mutations		Quality
			Positive/ Analyzed	Proportion [%] (95% CI)	
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases, included in the counts) A. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	56/132	32/56	57 (42, 70)	B
Syngal 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	C. Selection among referrals to a specialized center D. PCR, sequencing E. Predicted non-conservative transcription alteration; literature	28/70	11/28	39 (22, 59)	B
Wang, 1999 (1939); France Single-center	A. Sampled among referrals to a genetic consultation center B. ∅RT-PCR → IVSP; ♦PCR → HD; *in vivo* hML1 expression C. Predicted transcription alteration; literature	22/70	14/22	64 (41, 83)	B
Curia, 1999 (1959); Italy Single-center	A. Sampled from pathology registries, unclear selection criteria B. ♦RT-PCR → SSCP → Sequencing of abnormal patterns C. Predicted non-conservative transcription alteration; literature; comparison with non-cancer controls	15/30	1/15	7 (0, 32)	B
Luce, 1995 (2703); US Single-center	A. Selection among referrals to a specialized center[a] B. ♦PCR → IVTT → Sequencing of abnormal peptides C. Unclear	12/19[a]	6/12	50 (21, 79)	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Selection from a population of 1514 incident CRC B. ♦PCR → SSCP & HD →Sequencing of abnormal patterns C. Predicted non-conservative transcription alteration; literature	11/45	5/11	45 (17, 77)	B
Dieumegard, 2000 (1791); France Multi-center	A. Sample assembled with unclear selection process B. ♦PCR → SSCP → Sequencing of abnormal patterns C. Predicted non-conservative transcription alteration; literature	10/34	6/10	60 (26, 88)	B
Aaltonen, 1998 (2282); Finland Multi-center	A. Selection of all incident unrelated CRC from 9 hospitals between 05/1994 and 04/1996 B. ♦PCR → DGGE (for some samples) → Sequencing; PCR → Sequencing (for all other samples); ∅ and PCR for founder mutations (all samples) C. Literature; and comparison with healthy controls	4/509	4/4	100 (40, 100)	B

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Genetic testing strategy C. Definition of deleterious mutations	N_{Am1}/ N_{total}	MLH1 and MSH2 mutations		Quality
			Positive/ Analyzed	Proportion [%] (95% CI)	
Rossi, 2002 (1146); Brazil Single-center	A. Selected from consecutive CRC referrals B. PCR → Sequencing C. Unclear	4/25	1/4	25 (0, 81)	B
Park, 1999 (2007); 7 countries Multi-center (8 centers)	A. Study sample assembled from the ICG-HNPCC database B. ♦PCR → SSCP; and ∅ southern blotting (no details) C. Unclear[b]	154/277	77/154[b]	50 (42, 58)	C
Lamberti, 1999 (2036); Italy Single-center	A. Study sample assembled with unclear selection process B. ♦PCR → SSCP, and RT-PCR → PTA C. Literature	57/160	15/57	26 (16, 40)	C
de Leon, 1999 (2012); Italy Single-center	A. Sample selected from CRC patient registries B. ♦PCR → SSCP → Sequencing of abnormal patterns C. Unclear	18/36	3/18	17 (4, 41)	C
Liu, 2004 (445); China Single center	A. Study sample assembled with unclear selection process B. ♦PCR → DHPLC → Sequencing of abnormal patterns C. Unclear	15/28	5/15	33 (12, 62)	C
Moslein 1996 (2545); US & Germany Multi-center	A. Sample assembled from various databases; only cases stated to have had CRC are analyzed[c] B. PCR → Sequencing C. Predicted non-conservative transcription alteration; literature	14/46	6/14[c]	43 (18, 71)	C
Callistri, 2000 (1797); Italy Multi-center	A. Study sample assembled with unclear selection process B. ♦PCR → SSCP C. Unclear	13/45	7/13	54 (25, 81)	C
Colombino, 2005 (1058); Italy Single-center	A. Selection from consecutive CRC cases enrolled over 3 years[a] B. ♦PCR → DHPLC→Sequencing of abnormal patterns C. Compared mutations to 103 people without cancer	13/362[a]	10/13	77 (46, 95)	C
Stormorken, 2001 (721) Norway Single-center	A. First 56 families in the Norwegian Radium hospital registry B. Not described (was already done) (MSH6 was assessed also, included in the counts) C. Not described	12/56	3/12	25 (5, 57)	C
Debniak, 2000 (1784); Poland Single-center(?)	A. Sampled from consecutive CRC, selection process not transparent B. PCR → Sequencing C. Unclear	3/168	1/3	33 (0, 91)	C
Yuan, 2004 (303); China	A. Sampled with unclear selection process; all fulfill Chinese HNPCC criteria[d]	3/14	1/3	33 (0, 91)	C

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Genetic testing strategy C. Definition of deleterious mutations	N_{Am1}/ N_{total}	MLH1 and MSH2 mutations		Quality
			Positive/ Analyzed	Proportion [%] (95% CI)	
Single-center	B. ♦PCR → DHPLC → Sequencing of abnormal products C. Predicted non-conservative transcription alteration; literature; comparison with non-cancer controls				

Studies are ordered by quality and then by decreasing number of patients fulfilling Amsterdam I criteria. Demographic data (on age and gender distributions) were not available for probands fulfilling Amsterdam I criteria. Primary studies did not describe how many of the MMR mutations were MLH1 or MSH2 for the subset of CRC fulfilling Amsterdam I criteria.

CI: confidence interval; CRC: colorectal cancer; DHPLC: denaturating high performance liquid chromatography; HD: heteroduplex formation; IVSP: *in vitro* synthesized protein assay; IVTT: *in vitro* transcription-translation; N_{Am1}: number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction; PTA: protein truncation assay; RT-PCR: reverse transcriptase PCR; SSCP: Single-stranded conformation polymorphism

Conversion analysis was not used in any study.

♦: Gene screening as been used

∅: Analysis for large deletions has been used

[a] Unclear if all probands come from unrelated families.

[b] This study probed for hPSM1 or hPSM2 mutations in some but not all patients; none was found

[c] Although the paper states that 20 patients fulfilled Amsterdam 1 criteria, only 14 were described to have had CRC in the pertinent data table. The other had other cancers.

[d] The Chinese HNPCC criteria ≥2 relatives with histologically proven CRC (≥2 must be first degree relatives) and one of the following: multiple colorectal tumors, one CRC diagnosed at age younger than 50 years, or development of extracolonic HNPCC-related cancer in family members.

Appendix F-2: Prevalence of MLH1 and MSH2 mutations among colorectal cancer probands who fulfill Amsterdam II criteria

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Genetic testing strategy C. Definition of deleterious mutations	N_{Am2}/ N_{total}	MLH1 and MSH2 mutations		Quality
			Positive/Analyzed	Proportion [%] (95% CI)	
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	67/132	36/67	54 (41, 66)	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Selection from a population of 1514 incident CRC B. ♦PCR → SSCP & HD → Sequencing of abnormal patterns C. Predicted transcription alteration; literature	18/45	8/18	44 (22, 69)	B
Wolf, 2005 (23) Austria Single-center	A. Retrospective cohort of referral cancer patients fulfilling the modified Bethesda criteria B. PCR → Sequencing C. Predicted transcription alteration; literature; comparison with healthy controls	35/81	13/35	37 (21, 55)	B
Syngal 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. PCR → Sequencing C. Predicted transcription alteration; literature	34/70	14/34	41 (25, 59)	B
Zhu, 2005 (138) China Single-center	A. Study sample assembled with unclear selection process B. ∅ MLPA → Sequencing of detected aberrations C. Unclear	21/78	4/21	19 (5, 42)	B
Lee, 2005 (105) Singapore Single-center	A. Sample selected from referrals to a tertiary center B. PCR → Sequencing C. Predicted transcription alteration; literature	5/46	3/5	60 (15, 95)	B
Salovaara, 2000 (1740) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 B. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene C. Literature; comparison with non-cancer controls	5/535	4/5	80 (28, 99)	B
Rossi, 2002 (1146) Brazil Single-center	A. Selected from consecutive CRC referrals B. PCR, → Sequencing C. Unclear	5/25	2/5	40 (5, 85)	B
Lamberti, 1999 (2036); Italy Single-center	A. Study sample assembled with unclear selection process B. ♦PCR → SSCP; and RT-PCR, PTA C. Literature	69/160	17/69	25 (15, 36)	C
Stormorken, 2001 (721)	A. First 56 families in the Norwegian Radium hospital registry	20/56	7/20[a]	35 (15, 59)	C

Study, year (Ref ID); Country Single-/multi-center			N_{Am2}/ N_{total}	MLH1 and MSH2 mutations		Quality
				Positive/Analyzed	Proportion [%] (95% CI)	
Norway Single-center	B.	Not described (was already done) (MSH6 was assessed also)				
	C.	Not described				

Studies are ordered by quality and then by decreasing number of patients fulfilling Amsterdam II criteria. Demographic data (on age and gender distributions) were not available for probands fulfilling Amsterdam II criteria. Primary studies did not describe how many of the MMR mutations were MLH1 or MSH2 for the subset of CRC fulfilling Amsterdam II criteria.

Conversion analysis was not used in any study.

♦: Gene screening as been used

∅: Analysis for large deletions has been used

CI: confidence interval; CRC: colorectal cancer; HD: heteroduplex formation; MLPA: Multiple ligation-dependent probe amplification; NAm2: number fulfilling Amsterdam II criteria; Ntotal: total number of studied CRC; PCR: polymerase chain reaction; PTA: protein truncation assay; RT-PCR: reverse transcriptase PCR; SSCP: Single-stranded conformation polymorphism

[a] 2/7 mutations shown are MSH6

Appendix F-3: Prevalence of microsatellite instability among colorectal cancer probands who fulfill Amsterdam I criteria

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Definition of MSI-H	N_{Am1}/ N_{total}	Tumor microsatellite instability				Micro-dissection	NCI 5 marker set	Quality
			MSI-H/ Analyzed	MSI-H [%] (95% CI)	MSI-H&L/ Analyzed	MSI-H&L [%] (95% CI)			
Wahlberg, 2002 (1158) & Syngal 2000 (1672); US Multi-center	A. Selection among referrals to specialized center B. ≥2 out of 5 dinucleotide repeats (NCI set)	28/70	15/19	79 (54, 94)	18/19	95 (74, 100)	√	√	B
Curia, 1999 (1959); Italy Single-center	A. Sampled from pathology registries, unclear selection criteria B. Screened with 3 markers and if none was unstable up to a total of 7. MSI-H defined as ≥2 markers	15/30	12/15	80 (52, 96)	ND	ND	√	X	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Selected from a population of 1514 incident CRC B. ≥2 out of 5 dinucleotide repeats	11/45	7/10	70 (34, 93)	7/10	70 (34, 93)	√	X	B
Dieumegard, 2000 (1791); France Multi-center	A. Sample assembled with unclear selection process B. ≥10% of up to 23 markers (But all were ≥30%)	10/34	9/10	90 (55, 100)	9/10	90 (55, 100)	X	X	B
Aaltonen, 1998 (2282); Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 B. ≥30% of 16 markers for tumor analyzed with fluorescence methods or ≥2 out of 7 (≈30%) markers analyzed with radioactive technique	4/509	4/4	100 (40, 100)	4/4	100 (40, 100)	X	X	B
Lamberti, 1999 (2036); Italy Single-center	A. Study sample assembled with unclear selection process B. ≥40% out of up to 10 mono- di- and tetranucleotide repeats	57/160	35/49	71 (57, 83)	37/49	76 (61, 87)	X	X	C
de Leon, 1999 (2012); Italy Single-center	A. Sample selected from CRC patient registries B. ≥2 out of ≥5 markers	18/36	11/18	61 (36, 83)	ND	ND	√	X	C
Callistri, 2000 (1797); Italy Multi-center	A. Study sample assembled with unclear selection process B. ≥2 out of 13 microsatellite markers	13/45	11/13	85 (55, 98)	11/13	85 (55, 98)	√	√	C

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Definition of MSI-H	N_{Am1}/ N_{total}	Tumor microsatellite instability				Micro-dissection	NCI 5 marker set	Quality
			MSI-H/ Analyzed	MSI-H [%] (95% CI)	MSI-H&L/ Analyzed	MSI-H&L [%] (95% CI)			
Peel, 1999 (1660); US Multi-center	A. Referral HNPCC cases, other than the 1134 CRC probands assessed for other purposes B. Unclear; at least 5 markers were used	11/11	4/9	44 (14, 79)	4/9	44 (14, 79)	√	X	C
Moslein, 1996 (2545); US & Germany Multi-center	A. Sample assembled from various databases; we present only cases stated to have CRC[a] B. ≥30% of 9 to 34 markers analyzed (unclear which exactly)	14/ 46[a]	5/9	56 (21, 86)	ND	ND	X	X	C
Debniak, 2000 (1784); Poland Single-center(?)	A. Sampled from consecutive CRC, selection not transparent B. ≥2 out of 5 dinucleotide repeats (panel I), or ≥3 out of 10 dinucleotide repeats (panel II, used if only 1 positive in panel I)	3/168	2/3	67 (9, 99)	ND	ND	X	X	C

Studies are ordered by overall quality and then by decreasing number of patients fulfilling the Amsterdam I criteria. Demographic data (on age and gender distributions) were not available for probands fulfilling Amsterdam I criteria.

CI: confidence interval; CRC: colorectal cancer; MSI-H/H&L: microsatellite instability high/combined high and low; ND: Not described; N_{Am1}: number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC; NCI: National cancer institute

X: Was not used/not stated

√: Was used

[a] Although the paper states that 20 patients fulfilled Amsterdam I criteria, only 14 were described to have a CRC in the pertinent data table. The other had other cancers. Only the 14 with CRC are analyzed.

Appendix F-4: Prevalence of microsatellite instability among colorectal cancer probands who fulfill Amsterdam II criteria

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Definition of microsatellite instability	N_{Am2}/ N_{total}	Tumor microsatellite instability				Micro-dissection	NCI 5 marker set	Quality
			MSI-H/ Analyzed	MSI-H [%] (95% CI)	MSI-H&L/ Analyzed	MSI-H&L [%] (95% CI)			
Wolf, 2005 (23) Austria Single-center	A. Retrospective cohort of referral cancer patients fulfilling the modified Bethesda criteria B. ≥30% out of up to 10 markers	35/81	16/24	67 (45, 84)	ND	ND	√	X	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Selected from a population of 1514 incident CRC B. ≥2 out of 5 dinucleotide repeats	17/45	10/16	63 (35, 85)	11/16	69 (41, 89)	√	X	B
Salovaara, 2000 (1740) Finland Multi-center	A. Selection of all new unrelated CRC patients from 9 hospitals between 03/1996 and 06/1998 B. Single marker (BAT26) analyzed in all samples	5/535	4/5	80 (28, 99)	NA	NA	X	X	B
Lamberti, 1999 (2036); Italy Single-center	A. Study sample assembled with unclear selection process B. ≥40% out of up to 10 mono- di- and tetranucleotide repeats	69/160	39/57	68 (55, 80)	41/57	72 (58, 83)	X	X	C

Studies are ordered by quality and then by diminishing number of patients fulfilling the Amsterdam II criteria. Demographic data (on age and gender distributions) were not available for probands fulfilling Amsterdam II criteria.

CI: confidence interval; CRC: colorectal cancer; MSI-H/H&L: microsatellite instability high/combined high and low; NA: Not applicable; ND: Not described; N_{Am2}: number fulfilling Amsterdam II criteria; N_{total}: total number of studied CRC; NCI: National cancer institute

X: Was not used/not stated

√: Was used

Appendix F-5: Prevalence of negative immunostaining for MLH1 or MSH2 among colorectal cancer probands who fulfill Amsterdam I criteria

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Antibodies used for immunostaining	N_{Am1}/ N_{total}	MLH1 and MSH2 immunostaining		Quality
			Negative/Analyzed	Proportion [%] (95% CI)	
Curia, 1999 (1959) Italy Single-center	A. Sampled from pathology registries, but selection process is not transparent B. Anti-MSH2: FE11 (Oncogene Research Products); anti-MLH1: clone 14 (Oncogene Research Products)	15/30	7/15	47 (21, 73)	B
Wahlberg, 2002 (1158) & Syngal 2000 (1672); [a] US Single-center	A. Selection among referrals to specialized center B. Anti-MSH2: FE11 (Oncogene Research Products); anti-MLH1: G168-728 (PharMingen)	28/70	5/14	36 (13, 65)	B
Katballe, 2002 (1310) & Christensen, 2002 (1038);[b] Denmark Single-center	A. Selection from a population of 1514 incident CRC B. Anti-MSH2: Ab-1, Ab-2 (Oncogene Research Products); anti-MLH1: G168-15 (PharMingen)	11/42	4/11	36 (11, 69)	B
Dieumegard, 2000 (1791) France Multi-center	A. Sample assembled without a transparent selection process B. Anti-MSH2: FE11 (Oncogene Research Products); anti-MLH1: Ab-1 (Oncogene Research Products)	10/34	4/8	50 (16, 84)	B
Stormorken, 2001 (721) Norway Single-center	A. First 56 families in the Norwegian Radium hospital registry B. Anti-MSH2: FE11 (Calbiochem); anti-MLH1: G168-15 (Pharmingen); anti-MSH6: Clone 44 (transduction laboratories)[c]	12/56	3/12	25 (5, 57)	C
Debniak, 2000 (1784); Poland Single-center(?)	A. Sampled from consecutive CRC, but selection process is not transparent B. Not stated	3/168	2/3	67 (9, 99)	C

Studies are ordered by overall quality and then by decreasing number of patients fulfilling Amsterdam I criteria. Demographic data (on age and gender distributions) were not available for probands fulfilling Amsterdam I criteria. None of these studies assessed any other mismatch repair genes apart from MLH1 and MSH2.

CI: confidence interval; CRC: colorectal cancer; N_{Am1}: number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC

[a] Data from the Wahlberg et al. 2002 paper; comments on sampling from the Syngal et al. 2000 paper.

[b] Data from the Christensen et al. 2002 paper; comments on sampling from the Katballe et al. 2002 paper.

[c] 2 MSH6 MMR mutations were also picked up by the anti-MSH2 IHC exam.

Appendix F-6: Ability of Amsterdam I criteria to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Aaltonen, 1998 (2282) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 B. Yes: patients without MSI were screened only for MLH1 founder mutations C. ND, ND	A. ∅All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	4/509	4	6	0	499	67 (22, 96)	100 (99, 100)	B
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. No C. ND; ND	A. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) B. 24/17 (and 3 MSH6) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	56/ 132	33	11	23	65	75 (60, 87)	74 (63, 83)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR→sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	28/70	11	7	17	35	61 (36, 83)	67 (53, 80)	B
Wang, 1999 (1939); France Single-center	A. Sampled among referrals to a genetic consultation center: Familial CRC with Am1 or up to 2 Am1 criteria missing, or aggregation of HNPCC-related tumors, or sporadic CRC aged <50 y at diagnosis B. No C. ND; ND	A. ∅RT-PCR→ IVSP; ♦ PCR→HD→sequencing; *in vivo* MLH1 protein expression B. 19/7 C. Predicted transcription alteration; literature	22/70	14	12	8	41	50 (31, 69)	84 (70, 93)	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Familial CRC selected from a population of 1514 newly diagnosed CRC: patients fulfilling Am2 (in extended families and relaxing the age criterion to <55y) and familial CRC with early age of onset	A. ♦PCR→SSCP & HD→ Sequencing of abnormal patterns B. 4/6 C. Predicted transcription alteration; literature	11/45	5	5	6	25	50 (19, 81)	81 (63, 93)	B

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
	B. No C. ND; ND									
Curia, 1999 (1959) Italy Single-center	A. Sampled from pathology registries, unclear selection criteria: HNPCC related cancers[a] B. No C. <50y, ND	A. ♦RT-PCR→ SSCP→ Sequencing of abnormal products B. 2/1 C. Predicted non-conservative transcription alteration; literature; comparison with non-cancer controls	15/30[a]	1	2	16	11	33 (0, 91)	41 (22, 61)	B
Rossi, 2002 (1146) Brazil Single-center	A. Selected from consecutive CRC referrals: Am2 or familial CRC (including HNPCC-related cancer in family), or sporadic CRC aged <50y or with multiple tumors B. No C. 46 (all sample); 60 (all sample)	A. PCR→ sequencing B. 4/6 C. Unclear	5/25	1	9	3	12	10 (0, 45)	80 (52, 96)	B
Luce, 1995 (2703); US Single-center	A. Selection among referrals to specialized center, familial CRC: In 1st degree family ≥2 CRC or endometrial cancer; or ≥1 CRC aged <50y at diagnosis [b] B. No C. ND; ND	A. ♦RT-PCR→ IVTT → sequencing of abnormal peptides; PCR → IVTT→ sequencing of abnormal peptides B. 4/2 C. Unclear	12/19[b]	6	0	6	7	100 (54, 100)	54 (25, 81)	B
Colombino, 2005 (181); Italy Single-center	A. Consecutive CRC cases enrolled over 3 years in a tertiary center [b] B. Yes: Patients without family history of cancer were screened only for the mutations identified in the familial cases C. 62; 49	A. ♦PCR→ DHPLC→ Sequencing of abnormal patterns B. 11/10 C. Compared to 103 people without cancer	13 /362[b]	10	11	3	338	48 (26, 70)	99 (97, 100)	C
Park, 1999 (2007); 7 countries Multi-center (8 centers)	A. Study sample assembled from the ICG-HNPCC database of families: Am1; and familial CRC not fulfilling the Am1 criteria B. No C. ND; ND	D. ♦ PCR → SSCP; and ∅ Southern blotting (no details) E. 101 (MLH1 and hMLH2) F. Unclear	154/ 277	77[c]	24[c]	77	99	76 (67, 84)	56 (49, 64)	C
Lamberti, 1999	A. Study sample assembled with	A. ♦ PCR → SSCP, and	69/	15	2	42	10	88	19 (10, 33)	C

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
(2036); Italy Single-center	unclear selection process; includes Am1, Am2, other familial CRC and CRC <50y at diagnosis or multiple tumors[d] B. No C. 44 for Am1, 37 for Am2; ND	RT-PCR → PTA B. 11/6 C. Literature	160[d]					(64, 99)		
de Leon, 1999 (2012) Italy Single-center	A. Selected from CRC patient registries: Am1 or familial CRC (2 generations with CRC or HNPCC-tumor, ≥1 CRC aged <50y at diagnosis and ≥50% of siblings with CRC) B. No C. ND, ND	A. ♦PCR→SSCP→ Sequencing of aberrant products B. 1/2 C. Unclear	18/36	3	0	15	18	100 (29, 100)	55 (36, 72)	C
Liu, 2004 (445); China Single center	A. Study sample assembled with unclear selection process; all fulfill the Japanese HNPCC criteria[e] B. No C. 46; 55	A. ♦PCR→DHPLC→Sequencing of abnormal products B. 5/7 C. Unclear	15/28	5	2	10	11	71 (29, 96)	52 (30, 74)	C
Moslein 1996 (2545) US & Germany Multi-center	A. Sample assembled from various databases; includes familial and sporadic cases[f] B. No C. 51, ND	A. PCR→sequencing B. 7/6 C. Predicted non-conservative transcription alteration; literature	14/46[f]	6	6	8	17	50 (21, 79)	68 (46, 85)	C
Callistri, 2000 (1797); Italy Multi-center	A. Study sample assembled with unclear selection process: Familial CRC fulfilling Am1 criteria or missing up to 2/3 Am1 criteria, or a CRC among 1st degree family or CRC diagnosis at age <50y, or multiple tumors in the same patient B. No C. ND; 56 (all sample)	A. ♦PCR→ SSCP B. 4/4 C. Unclear	13/40[g]	7	1	6	5	88 (47, 100)	45 (17, 77)	C
Yuan, 2004 (303) China Single-center	A. Sampled with unclear selection process; all fulfill Chinese HNPCC criteria[h]	A. ♦PCR→DHPLC→ Sequencing of abnormal products ∅ Multiplex PCR for large deletions	3/14	2	1	1	10	67 (9, 99)	91 (59, 100)	C

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
	B. No C. ND, ND	B. 2/1 C. Predicted non-conservative transcription alteration; literature; comparison with non-cancer controls								
Genetic testing only among patients with suggestive MSI or ICH.										
Casey, 2005 US Multi-center	A. Referrals to the Colon Cancer Family Registry B. Yes: 85/89 were pre-selected based on suggestive MSI or IHC C. Aprox 50 ; ND	A. CRC with MSI/IHC: PCR → Sequencing; (also for MSH6) Conversion analysis B. 42 in total (1 had an MSH6 mutation) C. Predicted non-conservative transcription alteration; literature; mRNA expression for missence mutations and additional data for splice site mutations	63/89	*Practically among patients with IHC or MSI*						B
				49	14	15	10	78 (66, 87)	40 (21, 61)	
Raedle, 2001 (1363) Germany Multi-center	A. Consecutive referrals to a tertiary center. B. Yes: patients without MSI were not tested for mutation C. 52, 55	A. CRC with MSI: PCR→ Sequencing B. 8/4 (1 had mutation in both genes) C. Predicted transcript alteration; literature	12/ 125	*Among patients with MSI-H tumors:*						B
				6	5	0	11	55 (23, 83)	100 (72, 100)	
Nakahara, 1997 (2354) Japan Single-center	A. Sample assembled from hospital registries[b] B. Yes: patients without MSI were not tested for mutations C. 59, 41	A. CRC with MSI: ♦PCR→ SSCP → Sequencing of abnormal patterns B. 5/6 (4 CRC had mutations in both genes) C. Unclear	≥5/32[b]	*Among patients with MSI-H tumors:*						C
				4	3	1	3	57 (18, 90)	75 (19, 99)	

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables).
Am1/2: Amsterdam I criteria/II; CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; DHPLC: denaturating high performance liquid chromatography; HD: heteroduplex formation; IVSP: *in vitro* synthesized protein assay; IVTT: *in vitro* transcription-translation; MSI(-H): microsatellite instability (high); ND: Not described; N_{Am1}:Number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction; PTA: protein truncation assay; RT-PCR: reverse transcriptase PCR; SSCP: Single-stranded conformation polymorphism
♦: Gene screening method
∅: Detection of large genomic deletions

[a] 4 were not CRC and are excluded from the calculations
[b] Not strictly clear if all CRC probands come from unrelated families.
[c] 0/27 and 0/24 had PMS1 or PMS2 mutations; Not all patients were tested for these other MMR genes.
[d] 69/160 included cases fulfilled Amsterdam II criteria and these are analyzed here. The remaining cases were 45 CRC aged <50 y at diagnosis and relatives with HNPCC related cancers and 46 CRC aged <50 y at diagnosis or CRC with multiple tumors (no age cutoff). However, these received genetic testing inconsistently and are not included in this analysis
[e] The Japanese HNPCC criteria: ≥3 CRC among 1st degree family; or ≥2 CRC among 1st degree family and one of the following: aged <50y at diagnosis, right colon involvement, synchronous or metachronous multiple CRC, or extracolonic malignancy.
[f] Interestingly, despite the fact that the study included 46 cancer patients (characterized as CRC), in an analytic description not all were described with CRC. Only cases that were described with CRC were included in this analysis.
[g] Data from 19 (out of the 45 total studied patients) CRC who missed at most 2 out of 3 Amsterdam criteria (and had available information) are analyzed here, because MMR gene mutations were tested inconsistently among other patient subgroups.
[h] The Chinese HNPCC criteria: 2 or more relatives with histologically proven CRC (at least 2 first degree relatives) and one of the following: multiple colorectal tumors, one CRC diagnosed at age younger than 50y, or development of extracolonic HNPCC-related cancer in family members.

Appendix F-7: Ability of Amsterdam I criteria to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
Aaltonen, 1998 (2282) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 B. Yes: patients without MSI were screened only for MLH1 founder mutations C. ND, ND	A. ∅All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	4/509	4	6	0	499	67 (22, 96)	100 (99, 100)	B
Colombino, 2005 (1058); Italy Single-center	A. Consecutive CRC cases enrolled over 3 years in a tertiary center[a] B. Yes: Patients without family history of cancer were screened only for the mutations identified in the familial cases C. 62; 49	A. ♦PCR→ DHPLC→ Sequencing of abnormal patterns B. 11/10 C. Compared to 103 people without cancer	13 /362[a]	10	11	3	338	48 (26, 70)	99 (97, 100)	C

Studies are ordered by quality. None of the studies in this table assessed genes other than MLH1 and hMLH2. None of the studies used conversion analysis among their genetic testing strategies.

Am1/2: Amsterdam I criteria/II; CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; DHPLC: denaturating high performance liquid chromatography; ND: Not described; N_{Am1}:Number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

♦: Gene screening method

∅: Detection of large genomic deletions

[a] Not clear if all CRC probands come from unrelated families.

Appendix F-8: Ability of Amsterdam I criteria to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands fulfilling the Bethesda guidelines

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. No C. ND; ND	A. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) B. 24/17 (and 3 MSH6) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	56/ 132	33	11	23	37	75 (60, 87)	62 (48, 74)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR→sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	56/70	11	6	17	22	65 (38, 86)	56 (40, 72)	B

The tabulated study did not assess genes other than MLH1 and MSH2. Genetic testing did not include gene screening, analysis for large genomic deletions or conversion analysis.

Am1: Amsterdam I criteria; ND: Not described; N_{Am1}:Number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

Appendix F-9: Ability of Amsterdam I criteria to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands fulfilling Amsterdam II criteria

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am1 (+)	Am1 (-)	Am1 (+)	Am1 (-)	Sensitivity [%]	Specificity [%]	
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. No C. ND; ND	A. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) B. 24/17 (and 3 MSH6) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	56/ 132	33	6	23	5	85 (69, 94)	18 (6, 37)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR→sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	34/70	11	3	17	3	79 (49, 95)	15 (3, 38)	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Familial CRC selected from a population of 1514 newly diagnosed CRC: patients fulfilling Am2 (in extended families and relaxing the age criterion to <55y) and familial CRC with early age of onset B. No C. ND; ND	A. ♦PCR→SSCP & HD→ Sequencing of abnormal patterns B. 4/6 C. Predicted transcription alteration; literature	23/45	5	3	6	9	63 (25, 92)	60 (32, 84)	B
Rossi, 2002 (1146) Brazil Single-center	A. Selected from consecutive CRC referrals: Am2 or familial CRC (including HNPCC-related cancer in family), or sporadic CRC aged <50y or with multiple tumors B. No C. ND; ND	A. PCR→ sequencing B. 4/6 C. Unclear	5/25	1	1	3	0	50 (1, 99)	0 (0, 71)	B
Lamberti, 1999 (2036); Italy Single-center	A. Study sample assembled with unclear selection process; includes Am1, Am2, other familial CRC and CRC <50y at diagnosis or multiple tumors[a] B. No C. 44 for Am1, 37 for Am2; ND	A. ♦ PCR → SSCP, and RT-PCR → PTA B. 11/6 C. Literature	69/ 160[a]	15	2	42	10	88 (64, 99)	19 (10, 33)	C

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used deletion analysis to detect large genomic deletions or conversion analysis to detect mismatch repair gene mutations. Most studies did not provide data on demographics (age and gender distribution) for the subgroup of patients fulfilling the Amsterdam II criteria.

Am1/2: Amsterdam I criteria/II; CRC: colorectal cancer; HD: heteroduplex formation; ND: Not described; N_{Am1}:Number fulfilling Amsterdam I criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction; PTA: protein truncation assay; RT-PCR: reverse transcriptase PCR; SSCP: Single-stranded conformation polymorphism

♦: Gene screening method

[a] 69/160 included cases fulfilled Amsterdam II criteria and these are analyzed here. The remaining cases were 45 CRC aged <50 y at diagnosis and relatives with HNPCC related cancers and 46 CRC aged <50 y at diagnosis or CRC with multiple tumors (no age cutoff). However, these received genetic testing inconsistently and are not included in this analysis

Appendix F-10: Ability of Amsterdam II criteria to identify MLH1 and MSH2 mutation carriers among selected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am2}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am2 (+)	Am2 (-)	Am2 (+)	Am2 (-)	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of MSI or IHC test results										
Salovaara, 2000 (1740) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 B. Yes: patients without MSI were screened only for MLH1 founder mutations C. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	5/535	4	14	1	516	22 (6, 48)	100 (99, 100)	B
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. No C. ND; ND	A. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) B. 24/17 (and 3 MSH6) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	67/ 132	39	5	28	60	89 (75, 96)	68 (57, 78)	B
Wolf, 2005 (123) Austria Single-center	A. Retrospective cohort of cancer patients fulfilling the modified Bethesda criteria B. No C. ND, 48	A. PCR → Sequencing B. 12/7 C. Predicted transcription alteration; literature; comparison with healthy controls	35/81	13	6	22	40	68 (43, 87)	65 (51, 76)	B
Zhu, 2005 (138) China Single-center	A. Study sample assembled with unclear selection process: Familial CRC and sporadic CRC B. No C. ND, ND	A. ∅ MLPA → Sequencing of detected aberrations B. 4/5 C. Unclear	21/78	4	5	17	52	44 (14, 79)	75 (64, 85)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR → Sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	34/70	14	4	20	32	78 (52, 74)	64, (49, 77)	B
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark	A. Familial CRC selected from a population of 1514 newly diagnosed CRC: patients fulfilling Amsterdam II criteria (in extended	A. ♦PCR → SSCP & HD → Sequencing of abnormal patterns	18/45	8	2	10	21	80 (44, 97)	68 (49, 83)	B

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am2}/ N_{total}	Mutation Am2 (+)	Mutation Am2 (-)	No mutation Am2 (+)	No mutation Am2 (-)	Diagnostic performance (95% confidence interval) Sensitivity [%]	Diagnostic performance (95% confidence interval) Specificity [%]	Quality
Single-center	families and relaxing the age criterion to <55y) and familial CRC with early age of onset B. No C. ND; ND	B. 4/6 C. Predicted transcription alteration; literature								
Rossi, 2002 (1146); Brazil Single-center	A. Selected from consecutive CRC referrals: Am2 or familial CRC (including HNPCC-related cancer in family), or sporadic CRC aged <50y or with multiple tumors B. No C. 46; 60	A. PCR → Sequencing B. 4/6 C. Unclear	5/25	2	8	3	12	20 (3, 56)	80 (52, 96)	B
Lee, 2005 (105); Singapore Single-center	A. Selection among referrals to tertiary center: Amsterdam criteria, familial disease, onset <40y or multiple tumors B. No C. 39 (median), 65	A. PCR → Sequencing B. 6/1 C. Predicted transcript alteration; literature	5/46[a]	3	4	2	37	43 (10, 82)	95 (83, 99)	C
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52) Spain Multi-center	A. Selection of newly diagnosed CRC from 25 centers. B. Yes: patients without MSI or with negative immunostaining were not sequenced C. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	22/ 1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 4	7	18	1197	36 (11, 69)	99 (98, 99)	B
Raedle, 2001 (1363); Germany Multi-center	A. Consecutive referrals to a tertiary center. B. Yes: patients without MSI were not tested for mutations C. 52, 55	A. CRC with MSI: PCR→ Sequencing B. 8/4 (1 had mutation in both genes) C. Predicted transcript alteration; literature	20/ 125	*Among patients with MSI-H tumors:* 8	3	2	9	73 (39, 94)	82 (48, 98)	B
Terdiman, 2001	A. Retrospective cohort of patients	A. CRC with MSI:	19/	*Among patients with MSI-H tumors:*						B

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am2}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am2 (+)	Am2 (-)	Am2 (+)	Am2 (-)	Sensitivity [%]	Specificity [%]	
US Single-center	who had ≥2 CRC in 1st degree family, or age <50 years at CRC diagnosis or multiple tumors in the same patient B. Yes: Only patients with MSI-H were tested for mutations C. ND, ND	♦PCR→ DGGE → Sequencing of abnormal products B. 11/10 C. Unclear	114	15	6	4	7	71 (47, 89)	64 (31, 89)	
Pistorius, 2000 (1590); Germany & Czech Republic Multi-center	A. Selection among referrals to tertiary center: Bethesda guidelines met B. Yes: patients without MSI were not genetically tested C. ND, ND	A. CRC with MSI: PCR → Sequencing B. 6/8 (1 MSH6 mutation also found) C. Predicted transcript alteration; literature	19/72	*Among patients with MSI-H tumors:*						B
				12	3	5	18	80 (52, 96)	78 (56, 93)	

Only Pistorious et al. assessed genes other than MLH1 and MSH2, namely the MSH6 gene.
Am2: Amsterdam II criteria; CRC: colorectal cancer; DGGE: Denaturating gradient gel electrophoresis; HD: heteroduplex formation; MLPA: Multiple ligation-dependent probe amplification; MSI(-H): microsatellite instability (high); ND: Not described; N_{Am2}:Number fulfilling Amsterdam II criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction; SSCP: Single-stranded conformation polymorphism
Conversion analysis was not used in any study.
♦: Gene screening has been used
∅: Analysis for large deletions has been used

[a] Contains two malignant tumors that are not CRC, which could not be separated (>95% are CRC)

Appendix F-11: Ability of Amsterdam II criteria to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands.

Study, year (Ref ID); Country; Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am2}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am2 (+)	Am2 (-)	Am2 (+)	Am2 (-)	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of MSI or IHC test results										
Salovaara, 2000 (1740) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 B. Yes: patients without MSI were screened only for MLH1 founder mutations C. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	5/535	4	14	1	516	22 (6, 48)	100 (99, 100)	B
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52) Spain Multi-center	A. Selection of newly diagnosed CRC from 25 centers. B. Yes: patients without MSI or with negative immunostaining were not sequenced C. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	22/ 1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 4	7	18	1197	36 (11, 69)	99 (98, 99)	B

As mentioned in the methods, especially for unselected CRC populations we accepted that patients with MSI negative tumors would be negative for mutations.
Am2: Amsterdam II criteria; CRC: colorectal cancer; MLPA: Multiple ligation-dependent probe amplification; MSI(-H): microsatellite instability (high); ND: Not described; N_{Am2}:Number fulfilling Amsterdam II criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction
Conversion analysis or gene screening were not used in these two studies.
∅: Analysis for large deletions has been used

Appendix F-12: Ability of Amsterdam II criteria to identify MLH1 and MSH2 mutation carriers among selected colorectal cancer probands fulfilling the Revised Bethesda criteria

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am2}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am2 (+)	Am2 (-)	Am2 (+)	Am2 (-)	Sensitivity [%]	Specificity [%]	
Wolf, 2005 (123) Austria Single-center	A. Retrospective cohort of cancer patients fulfilling the modified Bethesda criteria B. No C. ND, 48	A. PCR → Sequencing B. 12/7 C. Predicted transcription alteration; literature; comparison with healthy controls	35/81	13	6	22	40	68 (43, 87)	65 (51, 76)	B

Am2: Amsterdam II criteria; CRC: colorectal cancer; ND: Not described; N_{Am2}:Number fulfilling Amsterdam II criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

Conversion analysis, gene screening or deletion analysis methods were not used.

Appendix F-13: Ability of Amsterdam II criteria to identify MLH1 and MSH2 mutation carriers among selected colorectal cancer probands fulfilling the Bethesda guidelines

Study, year (Ref ID); Country Single-/multi-center	A. Study sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Am2}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am2 (+)	Am2 (-)	Am2 (+)	Am2 (-)	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of MSI test results										
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. No C. ND; ND	A. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) B. 24/17 (and 3 MSH6) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	67/ 132	39	5	28	37	89 (75, 96)	57 (44, 69)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR → Sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	34/70	14	4	20	32	78 (52, 74)	64, (49, 77)	B
Genetic testing only in patients selected after MSI										
Pistorius, 2000 (1590); Germany & Czech Republic Multi-center	A. Selection among referrals to tertiary center: Bethesda guidelines met B. Yes: patients without MSI were not genetically tested C. ND, ND	A. CRC with MSI: PCR → Sequencing B. 6/8 (1 MSH6 mutation also found) C. Predicted transcript alteration; literature	19/72	*Among patients with MSI-H tumors:* 12	3	5	18	80 (52, 96)	78 (56, 93)	B

Only Pistorious et al. assessed genes other than MLH1 and MSH2, namely the MSH6 gene.
Am2: Amsterdam II criteria; CRC: colorectal cancer; MSI(-H): microsatellite instability (high); ND: Not described; N_{Am2}:Number fulfilling Amsterdam II criteria; N_{total}: total number of studied CRC; PCR: polymerase chain reaction
Conversion analysis, deletion analysis or gene screening were not used in these two studies.

Appendix F-14: Ability of modified Amsterdam criteria to identify MLH1 and MSH2 mutation carriers among selected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sample characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing MLH1/MSH2 B. C. Definition of deleterious mutations	N_{AmMod}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Am Mod (+)	Am Mod (-)	Am Mod (+)	Am Mod (-)	Sensitivity [%]	Specificity [%]	
Wolf, 2005 (123) Austria Single-center	A. Selection from a retrospective cohort of cancer patients fulfilling the modified Bethesda criteria [a] B. No C. ND, 48	A. PCR → Sequencing B. 12/7 D. Predicted transcription alteration; literature; comparison with healthy controls	52/81	16	3	36	26	82 (60, 97)	42 (30, 55)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR → Sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	39/70	13	5	26	26	72 (47, 90)	50 (36, 64)	B

AmMod: modified Amsterdam criteria; CRC: colorectal cancer; ND: Not described; N_{AmMod}:Number fulfilling Amsterdam criteria; N_{total}: total number of studied CRC; PCR: Polymerase chain reaction.

None of the studies used deletion analysis, conversion analysis or gene screening methods to detect mutations. No other genes apart from MLH1 and MSH2 were assessed.

[a] May include some patients without CRC.

Appendix F-15: Ability of Bethesda guidelines to identify MLH1 and MSH2 mutation carriers

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Beth}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Beth (+)	Beth (-)	Beth (+)	Beth (-)	Sensitivity [%]	Specificity [%]	
Genetic Testing irrespectively of MSI/IHC results										
De Abajo 2005; Spain Single-center	A. Selection among referrals to a specialized center B. No C. ND; ND	A. PCR → DGGE → sequencing (MSH6 assessed in MLH1/MSH2 negative cases) B. 24/17 (and 3 MSH6) C. Predicted non-conservative transcription alteration; literature; comparison with healthy controls	67/ 132	44	0	60	28	100 (92, 100)	32 (22, 43)	B
Wolf, 2005 (123) Austria Single-center	A. Retrospective cohort of cancer patients fulfilling the modified Bethesda criteria B. No C. ND, 48	A. PCR → Sequencing B. 12/7 C. Predicted transcription alteration; literature; comparison with healthy controls	72/81	19	0	53	9	100 (82, 100)	15 (7, 26)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center B. No C. ND; ND	A. PCR → Sequencing B. 18 (MLH1 and MSH2) C. Predicted transcription alteration; literature	56/70	17	1	39	13	94 (73, 100)	25 (14, 39)	B
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52) Spain Multi-center	A. Selection of newly diagnosed CRC from 25 centers. B. Yes: patients without MSI or with negative immunostaining were not sequenced C. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	224/ 1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 8	3	216	995	73 (39, 94)	82 (80, 84)	B
Raedle, 2001	A. Consecutive referrals to a	A. CRC with MSI:	58/	*Among patients with MSI-H tumors:*						B

(1363) Germany Multi-center	tertiary center. B. Yes: patients without MSI were not tested for mutations C. 52, 55	PCR→ Sequencing B. 8/4 (1 had mutation in both genes) C. Predicted transcript alteration; literature	125	11	0	6	5	100 (72, 100)	45 (17, 77)	

Beth: Bethesda guidelines; CRC: colorectal cancer; IHC: immunohistochemistry; MLPA: Multiple ligation-dependent probe amplification; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: Polymerase chain reaction

∅: Deletion analysis (detection of large genomic deletions) was used

No study used gene screening methods or conversion analysis. All studies assessed only MLH1 and MSH2 genes.

Appendix F-16: Ability of Bethesda guidelines to identify MLH1 and MSH2 mutation carriers among unselected patients with colorectal cancer

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Beth}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Beth (+)	Beth (-)	Beth (+)	Beth (-)	Sensitivity [%]	Specificity [%]	
Pinol, 2005 (52) Spain Multi-center	A. Selection of newly diagnosed CRC from 25 centers. B. Yes: patients without MSI or with negative immunostaining were not sequenced C. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	224/ 1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:*						B
				8	3	216	995	73 (39, 94)	82 (80, 84)	

Beth: Bethesda guidelines; CRC: colorectal cancer; IHC: immunohistochemistry; MLPA: Multiple ligation-dependent probe amplification; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: Polymerase chain reaction

∅: Deletion analysis (detection of large genomic deletions) was used

Pinol et al. did not use gene screening methods or conversion analysis. They assessed only the MLH1 and MSH2 genes.

Appendix F-17: Ability of revised Bethesda guidelines to identify MLH1 and MSH2 mutation carriers among unselected patients with colorectal cancer

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling characteristics B. Verification bias C. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{Beth}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Beth Rev (+)	Beth Rev (-)	Beth Rev (+)	Beth Rev (-)	Sensitivity [%]	Specificity [%]	
Pinol, 2005 (52) Spain Multi-center	A. Selection of newly diagnosed CRC from 25 centers. B. Yes: patients without MSI or with negative immunostaining were not sequenced C. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	287/ 1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:*						B
				10	1	277	934	91 (59, 100)	77 (75, 79)	

Beth Rev: Revised Bethesda guidelines; CRC: colorectal cancer; IHC: immunohistochemistry; MLPA: Multiple ligation-dependent probe amplification; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: Polymerase chain reaction

∅: Deletion analysis (detection of large genomic deletions) was used

Pinol et al. did not use gene screening methods or conversion analysis. They assessed only the MLH1 and MSH2 genes.

Appendix F-18: Ability of Ability of young age at diagnosis (early disease onset) to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Definition of early onset B. Study sample characteristics C. Verification bias D. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Early onset	No early onset	Early onset	No early onset	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Salovaara, 2000 (1740) Finland Multi-center	A. <50 y B. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	535	5	13	40	477	28 (10, 53)	92 (90, 94)	B
Aaltonen, 1998 (2282) Finland Multi-center	A. <50 y B. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. ND, ND	A. ∅All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	509	4	6	12	487	67 (22, 96)	98 (96, 99)	B
Colombino, 2005 (1058); Italy Single-center	A. <45 y B. Consecutive CRC cases enrolled over 3 years in a tertiary center [b] C. Yes: Patients without family history of cancer were screened only for the mutations identified in the familial cases D. 62; 49	A. ♦PCR→ DHPLC→ Sequencing of abnormal patterns B. 11/10 C. Compared to 103 people without cancer	362[a]	6	10	5	82	38 (15, 65)	94 (87, 98)	C
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52) Spain Multi-center	A. <50 y B. Selection of newly diagnosed CRC from 25 centers. C. Yes: patients without MSI or with negative immunostaining were not sequenced D. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 3	8	55	1156	27 (6, 61)	95 (94, 97)	B

Study, year (Ref ID); Country Single-/multi-center	A. Definition of early onset B. Study sample characteristics C. Verification bias D. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Early onset	No early onset	Early onset	No early onset	Sensitivity [%]	Specificity [%]	
Samowitz 2001, (34); US Multicenter	A. <55y B. Selection among incident CRC C. Yes: patients without suggestive MSI results were not sequenced D. ND, ND	A. CRC selected by MSI: [b]PCR → Sequencing ∅ Also PCR for founder mutations in the MLH1 gene B. 5/3[c] C. Predicted transcript alteration; literature	1066	*Assuming no mutations in the absence of MSI-H tumors:*						B
				4	3	154	863	57 (18, 90)	85 (83, 87)	

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used conversion analysis to detect mismatch repair gene mutations.

Am1/2: Amsterdam I criteria/II; CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; DHPLC: denaturating high performance liquid chromatography; MLPA: Multiple ligation-dependent probe amplification; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

♦: Gene screening method

∅: Detection of large genomic deletions

[a] Not clear if all CRC probands belong to unrelated families; only data on 103 familial cases are analyzed here, since the pertinent information was lacking for sporadic cases.

[b] 130 out of 171 of people with tumors with MSI instability could be genetically tested.

[c] One patient had mutations both in the MLH1 and in the MSH2 gene

Appendix F-19: Ability of Ability of young age at diagnosis (early disease onset) to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Definition of early onset B. Study sample characteristics C. Verification bias D. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Early onset	No early onset	Early onset	No early onset	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Salovaara, 2000 (1740) Finland Multi-center	A. <50 y B. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	535	5	13	40	477	28 (10, 53)	92 (90, 94)	B
Aaltonen, 1998 (2282) Finland Multi-center	A. <50 y B. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. ND, ND	A. ∅All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	509	4	6	12	487	67 (22, 96)	98 (96, 99)	B
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52) Spain Multi-center	A. <50 y B. Selection of newly diagnosed CRC from 25 centers. C. Yes: patients without MSI or with negative immunostaining were not sequenced D. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 3	8	55	1156	27 (6, 61)	95 (94, 97)	B
Samowitz 2001, (34); US Multicenter	A. <55y B. Selection among incident CRC C. Yes: patients without suggestive MSI results were not sequenced D. ND, ND	A. CRC selected by MSI: [b]PCR → Sequencing ∅ Also PCR for founder mutations in the MLH1 gene B. 5/3[c] C. Predicted transcript alteration; literature	1066	*Assuming no mutations in the absence of MSI-H tumors:* 4	3	154	863	57 (18, 90)	85 (83, 87)	B

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used conversion analysis to detect mismatch repair gene mutations.

Am1/2: Amsterdam I criteria/II; CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; DHPLC: denaturating high performance liquid chromatography; MLPA: Multiple ligation-dependent probe amplification; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

♦: Gene screening method

∅: Detection of large genomic deletions

Appendix F-20: Ability of familial history of malignancy to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Definition of familial history of cancer B. Definition of sporadic cancer C. Comments on sample characteristics D. Verification bias E. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Familial Cancer Hx	Sporadic Cancer	Familial Cancer Hx	Sporadic Cancer	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Salovaara, 2000 (1740); Finland Multi-center	A. 1st degree relative with CRC or endometrial cancer B. All other CRC C. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 D. Yes: patients without MSI were screened only for MLH1 founder mutations E. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	535	15	3	62	455	83 (59, 96)	88 (85, 91)	B
Aaltonen, 1998 (2282); Finland Multi-center	A. 1st degree relative with CRC or endometrial cancer B. All other CRC C. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 D. Yes: patients without MSI were screened only for MLH1 founder mutations E. ND, ND	A. ∅ All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦ CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	509	9	1	71	428	90 (55, 100)	86 (82, 89)	B
Zhu, 2005 (138); China Single center	A. CRC with cancer in family B. Apparently sporadic CRC consisting of two subgroups (<50y or ≥50y at diagnosis) C. Study subgroups assembled without a clear selection process D. No E. ND, ND	A. ∅ MLPA → Sequencing of detected aberrations B. 4/5 C. Unclear	78	7	2	38	31	78 (40, 97)	45 (33, 57)	B
Wang, 1999 (1939); France Single-center	A. CRC from families fulfilling Am1 criteria, or from families with 1 or 2 Amsterdam criteria missing, or families with aggregation of HNPCCC-related tumors B. CRC with no family Hx aged <50y at diagnosis C. Sample assembled from referrals to a	A. ∅ RT-PCR→ IVSP; ♦ PCR→HD→Sequencing; *in vivo* MLH1 protein expression B. 19/7 C. Predicted transcription alteration; literature	75	26	0	37	12	100 (87, 100)	24 (13, 39)	B

Study, year (Ref ID); Country Single-/multi-center	A. Definition of familial history of cancer B. Definition of sporadic cancer C. Comments on sample characteristics D. Verification bias E. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Familial Cancer Hx	Sporadic Cancer	Familial Cancer Hx	Sporadic Cancer	Sensitivity [%]	Specificity [%]	
	genetic consultation center D. No E. ND, ND									
Yuan 1998 (2229); Korea Single-center	A. CRC fulfilling the Korean HNPCC criteria B. CRC without family Hx of colorectal cancer aged <40y at diagnosis C. Sample assembled with unclear selection process D. No E. Familial: 50, 65; sporadic: 39, 49	A. ♦ PCR→ SSCP → Sequencing of abnormal products B. 2/1 C. Predicted non-conservative transcription alteration; literature; comparison with non-cancer controls	76	7	1	24	44	88 (47, 100)	65 (52, 76)	C
Moslein 1996 (2545); US & Germany Multi-center	A. CRC fulfilling Am1; and CRC with "familial Hx of CRC" B. CRC without a family Hx of CRC C. Sample assembled with unclear selection process D. No E. 51, ND	A. PCR→sequencing B. 7/6 C. Predicted non-conservative transcription alteration; literature	46[a]	11	1	18	6	92 (62, 100)	25 (10, 47)	C
Lee, 2005 (105) Singapore Single-center	A. CRC fulfilling Amsterdam II criteria; with 3 CRC in 1st degree family; and with 2 CRC in 1st or 2nd degree family B. CRC with no familial Hx and age <40y or with multiple HNPCC-related cancers C. Sample selected from referrals to a tertiary center D. No E. 39 (median); 65	A. PCR → Sequencing B. 6/1 C. Predicted transcript alteration; literature	46[b]	6	1	21	17	86 (42, 100)	45 (29, 62)	C

Study, year (Ref ID); Country Single-/multi-center	A. Definition of familial history of cancer B. Definition of sporadic cancer C. Comments on sample characteristics D. Verification bias E. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Familial Cancer Hx	Sporadic Cancer	Familial Cancer Hx	Sporadic Cancer	Sensitivity [%]	Specificity [%]	
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52); Spain Multi-center	A. 1st degree relative with CRC of endometrial cancer B. All other CRC C. Selection of newly diagnosed CRC from 25 centers. D. Yes: patients without MSI or with negative immunostaining were not sequenced E. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:*						B
				6	5	151	1060	55 (23, 83)	88 (86, 89)	
Samowitz 2001, (34); US Multicenter	A. Family history of CRC B. All other patients C. Selection among incident CRC D. Yes: patients without suggestive MSI results were not sequenced E. ND, ND	A. CRC selected by MSI: [c]PCR → Sequencing ∅ Also PCR for founder mutations in the MLH1 gene B. 5/3[d] C. Predicted transcript alteration; literature	1066	*Assuming no mutations in the absence of MSI-H tumors:*						B
				4	3	147	870	57 (18, 90)	86 (83, 88)	

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used conversion analysis to detect mismatch repair gene mutations.

Am1: Amsterdam I criteria; CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; HD: heteroduplex formation; Hx: History; IHC: immunohistochemistry; IVSP: *in vitro* synthesized protein assay; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: polymerase chain reaction; SSCP: Single-stranded conformation polymorphism

♦: Gene screening method

∅: Detection of large genomic deletions

[a] Interestingly, only 26/46 patients were described to have had CRC in an analytic petioent-level description, and only these are analyzed

[b] Contains 2 HNPCC-related non-CRC tumors that could not be separated (>95% of tumors in these data are CRC)

[c] 130 out of 171 of people with tumors with MSI instability could be genetically tested.

[d] One patient had mutations both in the MLH1 and in the MSH2 gene

Appendix F-21: Ability of familial history of malignancy to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Definition of familial history of cancer B. Definition of sporadic cancer C. Comments on sample characteristics D. Verification bias E. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Familial Cancer Hx	Sporadic Cancer	Familial Cancer Hx	Sporadic Cancer	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Salovaara, 2000 (1740); Finland Multi-center	A. 1st degree relative with CRC or endometrial cancer B. All other CRC C. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 D. Yes: patients without MSI were screened only for MLH1 founder mutations E. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	535	15	3	62	455	83 (59, 96)	88 (85, 91)	B
Aaltonen, 1998 (2282); Finland Multi-center	A. 1st degree relative with CRC or endometrial cancer B. All other CRC C. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 D. Yes: patients without MSI were screened only for MLH1 founder mutations E. ND, ND	A. ∅ All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦ CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	509	9	1	71	428	90 (55, 100)	86 (82, 89)	B
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52); Spain Multi-center	A. 1st degree relative with CRC of endometrial cancer B. All other CRC C. Selection of newly diagnosed CRC from 25 centers. D. Yes: patients without MSI or with negative immunostaining were not sequenced E. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:*						B
				6	5	151	1060	55 (23, 83)	88 (86, 89)	
Samowitz 2001, (34);	A. Family history of CRC B. All other patients	A. CRC selected by MSI: [a]PCR → Sequencing	1066	*Assuming no mutations in the absence of MSI-H tumors:*						B

Study, year (Ref ID); Country Single-/multi-center	A. Definition of familial history of cancer B. Definition of sporadic cancer C. Comments on sample characteristics D. Verification bias E. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Familial Cancer Hx	Sporadic Cancer	Familial Cancer Hx	Sporadic Cancer	Sensitivity [%]	Specificity [%]	
US Multicenter	C. Selection among incident CRC D. Yes: patients without suggestive MSI results were not sequenced E. ND, ND	∅ Also PCR for founder mutations in the MLH1 gene B. 5/3[b] C. Predicted transcript alteration; literature		4	3	147	870	57 (18, 90)	86 (83, 88)	

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used conversion analysis to detect mismatch repair gene mutations.

Am1: Amsterdam I criteria; CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; Hx: History; IHC: immunohistochemistry; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

♦: Gene screening method

∅: Detection of large genomic deletions

[a] 130 out of 171 of people with tumors with MSI instability could be genetically tested.

[b] One patient had mutations both in the MLH1 and in the MSH2 gene

Appendix F-22: Ability of presence of multiple tumors to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Definition of multiple tumors B. Comments on sample characteristics C. Verification bias D. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Multiple tumors	No multiple tumors	Multiple tumors	No multiple tumors	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Salovaara, 2000 (1740); Finland Multi-center	A. Synchronous or metachronous endometrial cancer or CRC B. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	535	7	11	5	512	64 (31, 89)	99 (98, 100)	B
Aaltonen, 1998 (2282); Finland Multi-center	A. Synchronous or metachronous endometrial cancer or CRC B. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. ND, ND	A. ∅ All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦ CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	509	4	6	12	487	40 (12, 74)	98 (96, 99)	B
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52); Spain Multi-center	A. Synchronous or metachronous endometrial cancer or CRC B. Selection of newly diagnosed CRC from 25 centers. C. Yes: patients without MSI or with negative immunostaining were not sequenced D. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 4	7	90	1121	36 (11, 69)	93 (91, 94)	B

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used conversion analysis to detect mismatch repair gene mutations.

CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; Hx: History; IHC: immunohistochemistry; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

♦: Gene screening method

∅: Detection of large genomic deletions

Appendix F-23: Ability of presence of combined family history of colorectal cancer, young age at onset or presence of multiple tumors to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Definition of combined criteria B. Comments on sample characteristics C. Verification bias D. Mean age (y); Males (%)	A. Genetic testing B. MLH1/MSH2 C. Definition of deleterious mutations	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
				Combined criteria	Other	Combined criteria	Other	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status										
Salovaara, 2000 (1740); Finland Multi-center	A. Age <50 or CRC or endometrial cancer in 1st degree family or personal history of CRC or endometrial cancer B. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. 67, ND	A. PCR → Sequencing ∅ PCR for founder mutations in MLH1 gene B. 17/1 C. Literature; comparison with non-cancer controls	535	17	1	100	417	94 (73, 100)	81 (77, 84)	B
Aaltonen, 1998 (2282); Finland Multi-center	A. Age <50 or CRC or endometrial cancer in 1st degree family or personal history of CRC or endometrial cancer B. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 C. Yes: patients without MSI were screened only for MLH1 founder mutations D. ND, ND	A. ∅ All CRC: PCR for founder mutations 1 & 2 in MLH1 ♦ CRC with MSI: (some) PCR → DGGE → Sequencing (Remaining) PCR → Sequencing B. 9/1 C. Literature; comparison with healthy controls	509	10	0	112	387	100 (69, 100)	78 (74, 81)	B
Genetic testing only in patients selected after MSI and/or ICH										
Pinol, 2005 (52); Spain Multi-center	A. Age <50 or CRC or endometrial cancer in 1st degree family or personal history of CRC or endometrial cancer B. Selection of newly diagnosed CRC from 25 centers. C. Yes: patients without MSI or with negative immunostaining were not sequenced D. 70, 60	A. CRC selected by MSI/IHC: PCR → Sequencing ∅ also MLPA B. 4/7 C. Predicted transcript alteration; literature and databases	1222	*Assuming no mutations in the absence of MSI-H tumors or tumors with negative immunostaining:* 8	3	306	905	73 (39, 94)	75 (72, 77)	B

Studies are ordered by quality and then by decreasing number of patients available for the calculation of sensitivity and specificity (2 by 2 tables). None of the studies used conversion analysis to detect mismatch repair gene mutations.

CRC: colorectal cancer; DGGE: Denaturing gradient gel electrophoresis; Hx: History; IHC: immunohistochemistry; MSI: microsatellite instability; ND: Not described; N_{total}: total number of studied CRC; PCR: polymerase chain reaction

♦: Gene screening method

∅: Detection of large genomic deletions

Appendix F-24: Ability of microsatellite instability to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sample characteristics B. Verification bias C. Mean age (y); Males (%) D. Definition of MSI	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
			MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Barnetson, 2006 (NA) Scotland Multi-center	A. Selection of all incident CRC aged <55 y at diagnosis between 02/1999 and 07/2003 B. No C. 48; 53 D. ≥2 out of ≥5 markers (MSI-H)	870[a]	20	10	24	298	67 (47, 83)	93 (89, 95)	√	√	A
	≥1 out of ≥5 markers (MSI-H&L)		28	2	50	272	93 (78, 99)	84 (80, 88)			
Salovaara, 2000 (1740) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 03/1996 and 06/1998 B. Yes: patients without MSI were screened only for MLH1 founder mutations C. 67, ND D. MSI based on BAT26 only	535	18	0	48	469	100 (81, 100)	91 (88, 93)	X	X	B
Aaltonen, 1998 (2282) Finland Multi-center	A. Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996 B. Yes: patients without MSI were screened only for MLH1 founder mutations C. ND, ND D. ≥30% of 16 markers for tumor analyzed with fluorescence methods or ≥2 out of 7 (≈30%) markers analyzed with radioactive technique (MSI-H)	509	10	0	53	446	100 (69, 100)	89 (86, 92)	X	X	B

Study, year (Ref ID); Country Single-/multi-center		Comments on sample characteristics	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
	A. B. C. D.	Verification bias Mean age (y); Males (%) Definition of MSI		MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Southey, 2005 (66); Australia Single-center	A.	Randomly selected from a prospective cohort of all CRC with age of onset <45 y; only patients who received genetic testing are presented [b]	105	13[b]	5[b]	4	36	72 (47, 90)	90 (76, 97)	√	√	B
	B.	No										
	C.	<45y; ND										
	D.	>5 out of 10 markers (MSI-H)										
		>1 out of 10 markers (MSIH&L)		17 [b]	1[b]	16	60	94 (73, 100)	79 (68, 87)			
Wolf, 2005 (123); Austria Single-center	A.	Selection from a retrospective cohort of cancer patients fulfilling the modified Bethesda criteria	81	13	0	9	33	100 (75, 100)	79 (63, 90)	√	X	B
	B.	No										
	C.	ND, 48										
	D.	≥30% out of up to 10 markers (MSI-H)										
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A.	Selection among referrals to specialized center[c]	70[c]	14	0	14	20	100 (77, 100)	59 (41, 75)	√	√	B
	B.	No										
	C.	ND; ND										
	D.	≥2 out of ≥5 markers (MSI-H)										
Farrington, 1998 (2205); Scotland Single-center	A.	Retrospective cohort of CRC aged <30 y at diagnosis who were still alive (since 1970)	50	12	2	7	19	86 (57, 98)	73 (52, 88)	√	√	B
	B.	No										
	C.	<30y, ND										
	D.	≥2 out of 7 markers (MSI-H)										

Study, year (Ref ID); Country Single-/multi-center			N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
				MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A.	Familial CRC selected from a population of 1514 newly diagnosed CRC: patients fulfilling Amsterdam II criteria (in extended families and relaxing the age criterion to <55y) and familial CRC with early age of onset	45	10	0	3	20	100 (69, 100)	87 (66, 97)	√	X	B
	B.	No										
	C.	ND; ND										
	D.	≥2 out of ≥5 markers (MSI-H)										
		≥1 out of ≥5 markers (MSI-H&L)		10	0	5	20	100 (69, 100)	80 (59, 93)			
Dieumegard, 2000 (1791); France Multi-center	A.	Sample assembled with unclear selection process: we present analyses only among familial CRC who were at most 1 criterion short of fulfilling Am1 (n=17); study also included 17 apparently sporadic CRC aged <50y at diagnosis.	34[d]	9	0	6	9	100 (66, 100)	60 (32, 84)	X	X	B
	B.	Yes: not all sporadic CRC underwent MMR mutation testing (these are not included in this analysis)										
	C.	Oldest at diagnosis was 56, ND										
	D.	≥10% of up to 23 markers (But all were ≥30%) (MSI-H)										
		≥10% of up to 23 markers (MSI-H&L)		9	0	6	9	100 (66, 100)	60 (32, 84)			
Curia, 1999 (1959); Italy Single-center	A.	Sampled from pathology registries, unclear selection criteria: HNPCC related cancers[e]	30[e]	1	0	15	3	100 (0, 100)	17 (4, 41)	√	X	B
	B.	No										
	C.	<50y, ND										
	D.	≥2 markers out of 3 markers or up to a total of 7 markers (MSI-H)										

Study, year (Ref ID); Country Single-/multi-center		Comments on sample characteristics	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
	A. B. C. D.	Comments on sample characteristics Verification bias Mean age (y); Males (%) Definition of MSI		MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Debniak,, 2000 (1784); Poland Single-center(?)	A. B. C. D.	Sampled from consecutive CRC, selection process not transparent. All patient with available data are included. Yes: Only 43/143 apparently sporadic CRC were tested, but it is unclear how they were selected ND, ND ≥2 out of ≥5 markers or ≥3 out of ≥10 markers (MSI-H)	168	5	1	8	54	83 (36, 100)	87 (76, 94)	√	√	C
Lee, 2005 (105); Singapore Single-center	A. B. C. D.	Selection among referrals to tertiary center: Amsterdam criteria, familial disease, onset <40y or multiple tumors No 39 (median); 65 ≥2 out of ≥5 markers (MSI-H)	46[f]	4	1	7	21	80 (28, 99)	75 (55, 89)	√	√	C
		≥1 out of ≥5 markers (MSI-H&L)		4	1	9	21	80 (28, 99)	70 (51, 85)			
Moslein 1996 (2545) US & Germany Multi-center	A. B. C. D.	Sample assembled from various databases; we present analyses only among familial cases (39/46), and only those described to have CRC. Study also included 7 sporadic CRC.[g] No 51, ND ≥30% of 9 to 34 markers analyzed (unclear which exactly) (MSI-H)	46[g]	5	4	4	8	56 (21, 86)	67 (35, 90)	X	X	C

Study, year (Ref ID); Country Single-/multi-center		Comments on sample characteristics	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
	A. B. C. D.	Verification bias; Mean age (y); Males (%); Definition of MSI		MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Callistri, 2000 (1797); Italy Multi-center	A.	Study sample assembled with unclear selection process: Familial CRC fulfilling Am1 criteria or missing up to 2/3 Am1 criteria, or a CRC among 1st degree family or CRC diagnosis at age <50y, or multiple tumors in the same patient	40[h]	7	0	5	4	100 (59, 100)	44 (14, 79)	√	√	C
	B.	No										
	C.	ND; 56 (all sample)										
	D.	≥2 out of ≥5 markers (MSI-H)										
Durno, 2005 (195); Canada Multi-center	A.	Retrospective cohort of CRC aged <24 y at diagnosis who were still alive (since 1960)	16	5	0	3	1	100 (48, 100)	25 (0, 81)	√	√	C
	B.	No										
	C.	<24y, 38										
	D.	≥2 out of ≥5 markers, or (MSI-H) ≥40% out of up to 10 markers (MSI-H)										
Peel, 1999 (1660); US Multi-center	A.	Referral HNPCC cases, other than the 1134 CRC probands who were also included but were not assessed with laboratory tests	11	3	0	1	5	100 (29, 100)	83 (36, 100)	√	X	C
	B.	No										
	C.	ND; ND										
	D.	Unclear; at least 5 markers were used (MSI-H?)										

Note that studies that assessed genetic mutations only in patients with MSI cannot be used to construct 2 by 2 tables for the ability of MSI testing to detect MMR mutations. Thus such studies are not included in this table. Not included in the table are data from Lamberti 1999, where only the contrast of MSI-H versus combined MSI-L and MSI-S was extractable.

CRC: colorectal cancer; MSI(-H/-L): microsatellite instability (-high/-low); MSS: microsatellite-stable (no instability); NA: Not applicable; ND: Not described; N_{total}: total number of studied CRC

[a] Overall, 359 patients had available tumor tissue of good quality. Study assessed for mutations in the MSH6 gene also.

[b] This study assessed MSH6 and hPSM2 also. The mutation counts include pathogenic mutations in these genes also.

[c] Data from the Wahlberg et al. publication

[d] Only 7/17 apparently sporadic CRC were genetically tested (unclear how they were selected).

[e] Four were not CRC and were excluded from the calculations; numbers among patients with available tumors; only pathogenic mutations are shown.

[f] Included are 2 malignant tumors other than CRC that could not be separated.
[g] Interestingly, despite the fact that all were characterized as CRC, in an analytic description not all were described with CRC. Only familial cases that were described with CRC are included.
[h] Data out of 20 CRC (of 45 total studied patients) who missed at most 2 out of 3 Amsterdam I criteria are analyzed here, because MMR gene mutations were tested inconsistently among the remaining patients.

Appendix F-25: Ability of microsatellite instability to identify MLH1 and MSH2 mutation carriers among unselected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center		Comments on sample characteristics	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
	A.			MSI-H	MSS	MSI-H	MSS	Sensitivity [%]	Specificity [%]			
	B.	Verification bias										
	C.	Mean age (y); Males (%)										
	D.	Definition of MSI										
Salovaara, 2000 (1740) Finland Multi-center	A.	Selection of all new unrelated CRC from 9 hospitals between 0.3/1996 and 06/1998	535	18	0	48	469	100 (81, 100)	91 (88, 93)	X	X	B
	B.	Yes: patients without MSI were screened only for MLH1 founder mutations										
	C.	67, ND										
	D.	MSI based on BAT26 only										
Aaltonen, 1998 (2282) Finland Multi-center	A.	Selection of all new unrelated CRC from 9 hospitals between 05/1994 and 04/1996	509	10	0	53	446	100 (69, 100)	89 (86, 92)	X	X	B
	B.	Yes: patients without MSI were screened only for MLH1 founder mutations										
	C.	ND, ND										
	D.	≥30% of 16 markers for tumor analyzed with fluorescence methods or ≥2 out of 7 (≈30%) markers analyzed with radioactive technique (MSI-H)										

Note that studies that assessed genetic mutations only in patients with MSI cannot be used to construct 2 by 2 tables for the ability of MSI testing to detect MMR mutations. Thus such studies are not included in this table.

CRC: colorectal cancer; MSI(-H): microsatellite instability (-high); MSS: microsatellite-stable (no instability); ND: Not described; N_{total}: total number of studied CRC

Appendix F-26: Ability of microsatellite instability to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands fulfilling the revised Bethesda criteria

Study, year (Ref ID); Country; Single-/multi-center			N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
				MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Wolf, 2005 (123); Austria; Single-center	A.	Selection from a retrospective cohort of cancer patients fulfilling the modified Bethesda criteria	81	13	0	9	33	100 (75, 100)	79 (63, 90)	√	X	B
	B.	No										
	C.	ND, 48										
	D.	≥30% out of up to 10 markers (MSI-H)										

Note that studies that assessed genetic mutations only in patients with MSI cannot be used to construct 2 by 2 tables for the ability of MSI testing to detect MMR mutations. Thus such studies are not included in this table.

CRC: colorectal cancer; MSI(-H/-L): microsatellite instability (-high/-low); MSS: microsatellite-stable (no instability); NA: Not applicable; ND: Not described; N_{total}: total number of studied CRC

Appendix F-27: Ability of microsatellite instability to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands fulfilling the Amsterdam I criteria.

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sample characteristics B. Verification bias C. Definition of MSI	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Micro-dissection	NCI 5 marker set	Quality
			MSI	MSS	MSI	MSS	Sensitivity [%]	Specificity [%]			
Moslein 1996 (2545) US & Germany Multi-center	A. Sample assembled from various databases; we present analyses only among familial cases (39/46), and only those described to have CRC. Study also included 7 sporadic CRC. B. No C. ≥30% of 9 to 34 markers analyzed (unclear which exactly) (MSI-H)	14/46	5	0	0	4	100 (48, 100)	100 (40, 100)	X	X	C
Dieumegard, 2000 (1791); France Multi-center	A. Sample assembled with unclear selection process: we present analyses only among familial CRC who were at most 1 criterion short of fulfilling Am1 (n=17); study also included 17 apparently sporadic CRC aged <50y at diagnosis. B. Yes: not all sporadic CRC underwent MMR mutation testing (these are not included in this analysis) C. ≥10% of up to 23 markers (But all were ≥30%) (MSI-H)	10/34	6	0	3	1	100 (54, 100)	25 (0, 81)	X	X	B
	≥10% of up to 23 markers (MSI-H&L)		6	0	3	1	100 (54, 100)	25 (0, 81)			
Peel, 1999 (1660); US Multi-center	A. Referral HNPCC cases, other than the 1134 CRC probands who were also included but were not assessed with laboratory tests B. No C. Unclear; at least 5 markers were used (MSI-H?)	11/11	3	0	1	5	100 (29, 100)	83 (36, 100)	√	X	C

Demographics (data on age and gender distributions) were not available among people with fulfilling the Amsterdam I criteria. Note that studies that assessed genetic mutations only in patients with MSI cannot be used to construct 2 by 2 tables for the ability of MSI testing to detect MMR mutations. Thus such studies are not included in this table.
CRC: colorectal cancer; MSI(-H/-L): microsatellite instability (-high/-low); MSS: microasatellite-stable (no instability); NA: Not applicable; ND: Not described; N_{Am1}: total number of people fulfilling Amsterdam I criteria in the study population; N_{total}: total number of studied CRC

Appendix F-28: Ability of immunohistochemistry to identify MLH1 and MSH2 mutation carriers among selected colorectal cancer probands

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Verification bias C. Mean age (y); Males (%) D. Antibodies used	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
			No staining	Staining	No staining	Staining	Sensitivity [%]	Specificity [%]	
Genetic testing irrespectively of tumor microsatellite instability status									
Barnetson, 2006 (NA) Scotland Multicenter	A. Selection of all incident CRC aged <55 y ate diagnosis between 02/1999 and 07/2003 B. No C. 48; 53 D. Anti-MSH2: (Oncogene Research Products); anti-MLH1: (PharMingen); anti-MSH6: (Transduction laboratories);	870[a]	24	3	39	285	89 (71, 98)	88 (84, 91)	A
Southey, 2005 (66) Australia Single-center	A. Randomly selected from a prospective cohort of all CRC with age of onset <45y; only patients who received genetic testing are presented [b] B. No C. <45y; ND D. Anti-MSH2: FE-11 (Oncogene Research Products); anti-MLH1: G168-728 (PharMingen); anti-MSH6: clone 44 (BD transduction laboratories); anti-PMS2: clone A16-4 (PharMingen)	105[b]	18[b]	0[b]	8[b]	33[b]	100 (81, 100)	80 (65, 91)	B
Syngal, 2000 (1672) & Wahlberg, 2002 (1158); US Single-center	A. Selection among referrals to specialized center [c] B. No C. ND; ND D. Anti-MSH2: FE11 (Oncogene Research Products); anti-MLH1: G168-728 (PharMingen)	70[c]	6	5	3	22	55 (23, 83)	88 (69, 97)	B

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Verification bias C. Mean age (y); Males (%) D. Antibodies used	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
			No staining	Staining	No staining	Staining	Sensitivity [%]	Specificity [%]	
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Selected from a population of 1514 incident CRC B. No C. ND; ND D. Anti-MSH2: Ab-1, Ab-2 (Oncogene Research Products); anti-MLH1: G168-15 (PharMingen)	42[d]	9	4	3	15	69 (39, 91)	83 (59, 96)	B
Curia, 1999 (1959) Italy Single-center	A. Sampled from pathology registries, unclear selection criteria: HNPCC related cancers[e] B. No C. <50y, ND D. Anti-MSH2: FE11 (Oncogene Research Products); anti-MLH1: clone 14 (Oncogene Research Products)	30[e]	1	0	13	10	100 (0, 100)	57 (34, 77)	B
Dieumegard, 2000 (1791) France Multicenter	A. Sample assembled with unclear selection process: familial CRC missing at most 1 Am1 criterion and sporadic CRC aged <50y at diagnosis B. Yes: only 7 sporadic CRC underwent genetic testing C. Oldest age at diagnosis 56, ND D. Anti-MSH2: FE-11 (Oncogene Research Products); anti-MLH1: Ab-1 (Oncogene Research Products)	34	4	3	5	9	57 (18, 90)	64 (35, 87)	B

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Verification bias C. Mean age (y); Males (%) D. Antibodies used	N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
			No staining	Staining	No staining	Staining	Sensitivity [%]	Specificity [%]	
Debniak,, 2000 (1784); Poland Single-center(?)	E. Sampled from consecutive CRC, selection process not transparent. All patient with available data are included. F. Yes: Only 43/143 apparently sporadic CRC were tested, but it is unclear how they were selected G. ND, ND H. Unclear	168	2	9	0	56	18 (2, 51)	100 (94, 100)	C
Durno, 2005 (195) Canada Multi-center	E. Retrospective cohort of CRC aged<24 y at diagnosis who were still alive (since 1970) F. No G. <24y; 38 H. Anti-MSH2: FE-11 (Oncogene Research Products); anti-MLH1: G168-728 (PharMingen	16	3	1	1	3	75 (19, 99)	75 (19, 99)	C
Genetic testing among patients with MSI-H tumors									
Terdiman, 2001 (1572) US Single-center	A. Retrospective cohort of CRC probands with ≥2 CRC in first degree family, age <50y at diagnosis or multiple tumors in the same patient B. Yes: only patients with MSI-H were assessed C. ND, ND D. Unclear	114	16	1	29	4	94 (71, 100)	13 (4, 30)	B

No (nuclear immuno-) staining is the abnormal response. The number of patients in the 2 by 2 tables is the number of patients with available data and is typically smaller than the total number of patients included in the study.

CRC: colorectal cancer; ND: Not described; N_{total}: total number of studied CRC

[a] Overall 359 patients had available tumor tissue of good quality.

[b] This study assessed PMS2 and MSH6 also. The mutation and IHC testing include pathogenic mutations in these genes also.

[c] Data from the publication by Wahlberg et al.

[d] Data from the publication by Christensen et al.

[e] 4 were not CRC and are excluded from the calculations; Only 24 specimens had available IHC data.

Appendix F-29: Ability of immunohistochemistry to identify MLH1 and MSH2 mutation carriers among colorectal cancer probands fulfilling Amsterdam I criteria

Study, year (Ref ID); Country Single-/multi-center	A. Comments on sampling B. Verification bias C. Mean age (y); Males (%) D. Antibodies used	N_{Am1}/ N_{total}	Mutation		No mutation		Diagnostic performance (95% confidence interval)		Quality
			No staining	Staining	No staining	Staining	Sensitivity [%]	Specificity [%]	
Katballe, 2002 (1310) & Christensen, 2002 (1038); Denmark Single-center	A. Selected from a population of 1514 incident CRC B. No C. ND; ND D. Anti-MSH2: Ab-1, Ab-2 (Oncogene Research Products); anti-MLH1: G168-15 (PharMingen)	11/42[a]	2	2	0	5	50 (7, 93)	100 (48, 100)	B
Dieumegard, 2000 (1791) France Multicenter	A. Sample assembled with unclear selection process: familial CRC missing at most 1 Am1 criterion and sporadic CRC aged <50y at diagnosis B. Yes: only 7 sporadic CRC underwent genetic testing C. Oldest age at diagnosis 56, ND D. Anti-MSH2: FE-11 (Oncogene Research Products); anti-MLH1: Ab-1 (Oncogene Research Products)	10/34	2	2	2	2	50 (7, 93)	50 (7, 93)	B

No (nuclear immuno-) staining is the abnormal response. The number of patients in the 2 by 2 tables is the number of patients with available data and is typically smaller than the total number of patients included in the study.

CRC: colorectal cancer; ; N_{Am1}: number fulfilling Amsterdam I criteria; ND: Not described; N_{total}: total number of studied CRC

[a] Data from the publication by Christensen et al.

Appendix G: One-Way Sensitivity Analyses for the Decision Trees

The three variables with the most important influence for each strategy are depicted in the corresponding figures.

Number of tests

Figure G-1 shows the one-way sensitivity analyses on the expected number of MMR genetic tests with each treatment strategy. Among the three variables that exerted the most influence on the expected number of MMR genetic tests were the diagnostic characteristics (mainly the specificities) of the clinical criteria, IHC or MSI, and the prevalence of MMR mutations. For strategies using serial screening with clinical criteria and MSI or IHC, the diagnostic characteristics of both tests (mainly their specificities) were among the three more influential variables.

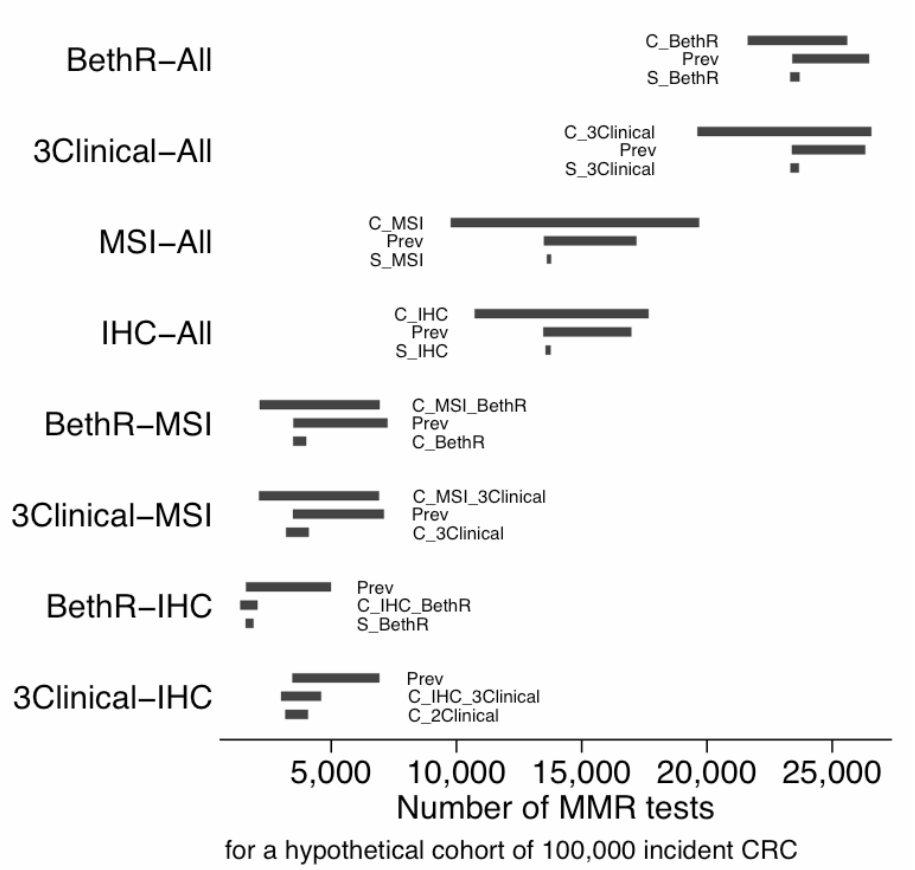

Figure G-1. One-way sensitivity analysis for the number of MMR genetic tests expected from each strategy among a hypothetical population of 100,000 incident CRC. Shown are the effects of one-way sensitivity analyses on the number of MMR tests performed in each strategy. The horizontal bars illustrate the different values that the outcome takes when a variable is varied from its lower to its higher plausible value in the sensitivity analyses. Only the three variables that had the greatest influence on each strategy are depicted (variable names are explained in Table 15 and in the text). Since all other variables had an even smaller influence, they are not depicted at all. The first strategy (MMR-All) is not shown. The first strategy assumes that all patients receive MMR testing; thus the number of expected MMR tests is always 100% of the population.

Figure G-2 shows the variation in the expected number of MSI and IHC tests for the strategies that are affected by the sensitivity analyses. The remaining strategies either do not use MSI or IHC at all (MMR-All, BethR-All, 3Clinical-All) or use one test in all patients and the other test in none (MSI-All and IHC-All).

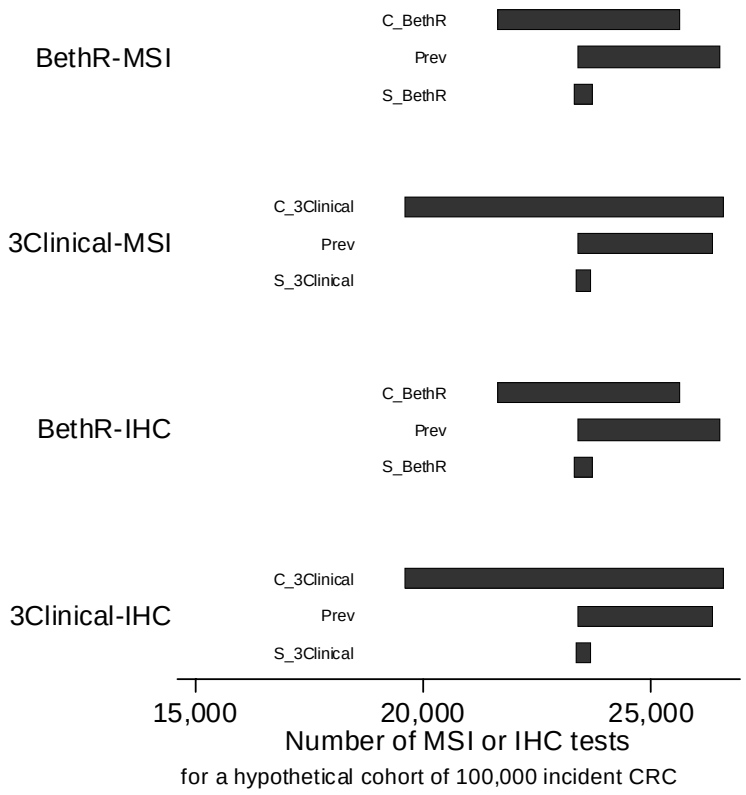

Figure G-2. One-way sensitivity analysis for the number of MSI or IHC genetic tests expected from each strategy among a hypothetical population of 100,000 incident cases of CRC. The bars for the first two strategies (BethR-MSI and 3Clinical-MSI) refer to the expected number of MSI tests. The expected number of IHC tests is zero since IHC is not used in these strategies. Similarly, the bars for the last two strategies (BethR-IHC and 3Clinical-IHC) refer to the expected number of IHC tests. The expected number of MSI tests is zero since MSI is not used in the latter strategies. Layout similar to Figure 27.

Number of people with positive test

The most important influence on the number of people with positive or true positive MMR was prevalence of MMR mutation carriers in the population. Diagnostic characteristics of the various tests also have a role. **Figure G-3** and **Figure G-4** illustrate the pertinent one-way sensitivity analyses.

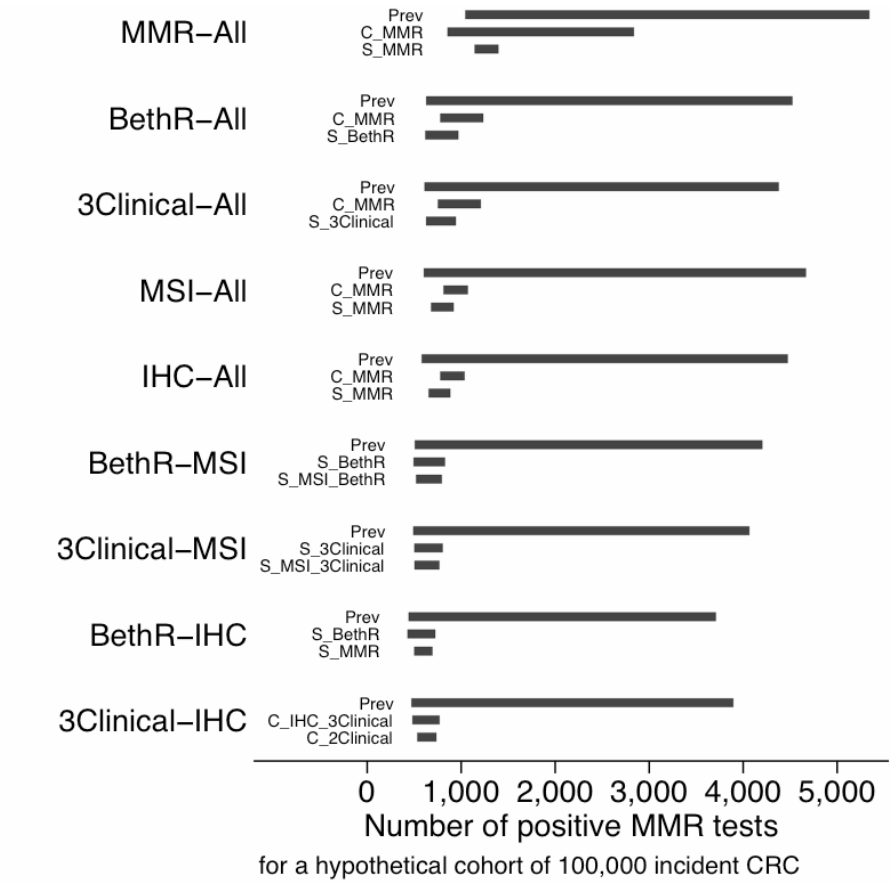

Figure G-3. One-way sensitivity analysis for the number of positive MMR tests expected from each strategy among a hypothetical population of 100,000 incident cases of CRC. The layout is similar to Figure 27.

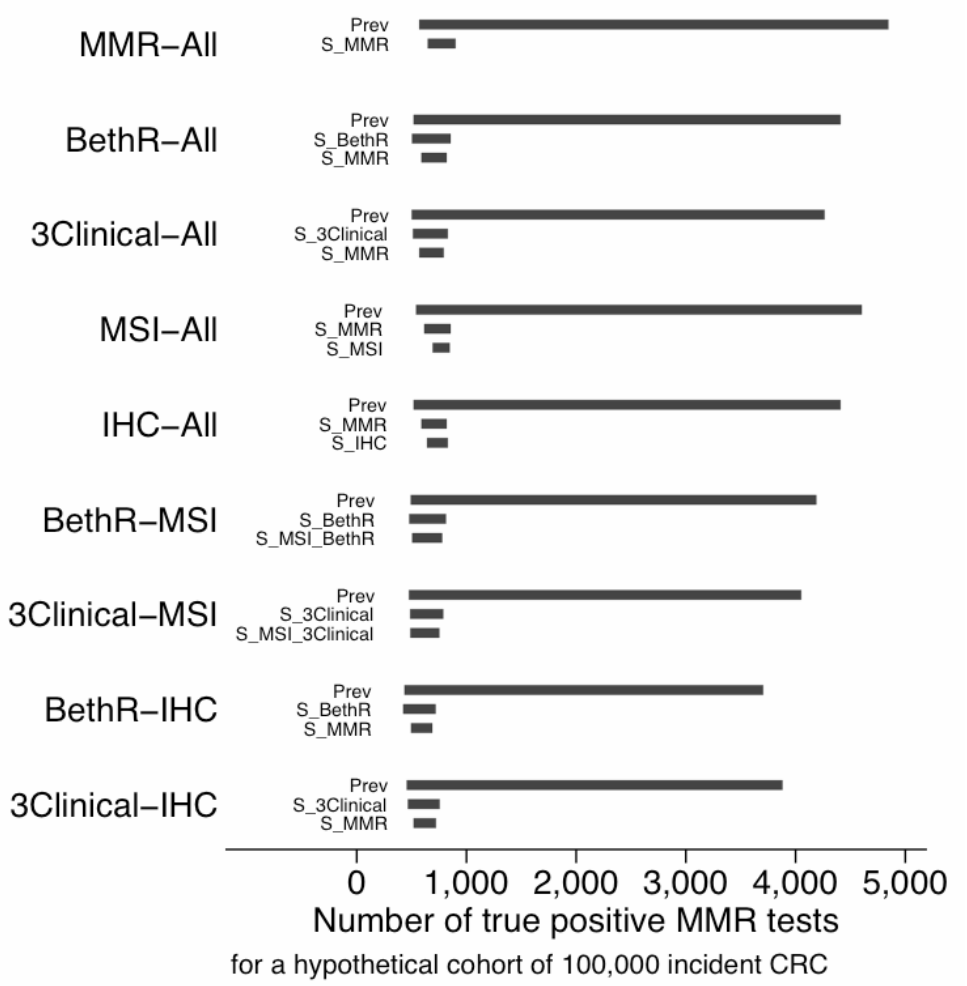

Figure G-4. One-way sensitivity analysis for the number of true positive MMR tests expected from each strategy among a hypothetical population of 100,000 incident cases of CRC. The first strategy (MMR-All) is affected only by two variables in the sensitivity analyses. The layout is similar to Figure 27.