

EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome

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EXECUTIVE SUMMARY

An original evidence review examined screening and diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC) and the subsequent outcomes in a population of newly diagnosed cases of colorectal cancer (CRC). This supplementary evidence review focuses on five issues of further interest to the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG), as summarized below.

1. *Clarifying how to define the clinical disorder—Lynch syndrome.* In this supplementary review, Lynch syndrome refers to individuals with a predisposition to CRC and certain other malignancies as a result of a germline mismatch repair (MMR) gene mutation—including those with an existing cancer and those who have not yet developed cancer. This definition allows planned analyses of clinical validity and utility to be more straightforward. Several recent editorials and publications recommend that the ambiguous term HNPCC be abandoned and that this clarified definition of Lynch syndrome should be used instead.
2. *Removing family history from consideration as a preliminary test.* A previous evidence review showed that screening performance of both the Amsterdam and the Bethesda criteria to identify individuals with Lynch syndrome were highly heterogeneous, possibly due to differences among the populations tested. In a general population, Amsterdam criteria are associated with relatively low sensitivity (28–45%), but high specificity (99%), whereas Bethesda criteria are associated with higher sensitivity (73–91%), but at the cost of lower specificity (82–77%). Neither provides the necessary high sensitivity/specificity in a reliable and consistent manner. There are also gaps in knowledge relating to the time required to

collect family history, the consistency with which it is collected, and the accuracy of the information. These shortcomings have led us to remove family history from consideration as a preliminary test in individuals newly diagnosed with CRC. However, family history may still be an important component of CRC risk assessment in the general population.

3. *Documenting the clinical validity of DNA-based preliminary tests.* Because of rapid advances in knowledge and technology regarding molecular testing and Lynch syndrome, we generally limited this review to publications from 2003 and later. Although not formally studied, this is a likely reason why several of our estimates differ from those provided in an earlier evidence report. There was “Adequate” (a formal EWG term) evidence showing the sensitivity of microsatellite instability (MSI) testing to be about 89% (for mutations in the MMR genes *MLH1* and *MSH2*), with a lower sensitivity of about 77% for *MSH6* mutations. Sensitivity was higher when three or more mononucleotide markers were included in the panel. Specificity was estimated to be 90.2%, with an adequate level of evidence. There was also good evidence showing the sensitivity of immunohistochemical (IHC) testing to be 83%, regardless of the underlying MMR gene involved. Specificity was more variable with a central estimate of 88.8%, and an adequate level of evidence. Inadequate evidence was available to determine the distribution of mutations in the MMR genes, but the limited data suggest 32% will be in *MLH1*, 38% in *MSH2*, 14% in *MSH6*, and 15% in *PMS2*. Adequate evidence was available to estimate sensitivity (69%) and specificity (point estimate of 100%) for identifying Lynch syndrome using a specific mutation in the *BRAF* gene among those with absent IHC staining for *MLH1*. An alternative to *BRAF* mutation testing might be direct testing of *MLH1* methylation status, but this was not evaluated.
4. *Benefits and harms to probands and relatives with Lynch syndrome.* Between 2 and 12 first-degree relatives of probands (newly diagnosed CRC cases with Lynch syndrome, or index cases) can be contacted, based on resources and methodology. There was adequate evidence to document uptake of counseling among these first-degree relatives who were contacted (52%) and subsequently targeted for MMR gene mutation testing (95%). Adequate evidence was found showing the risk of CRC by age 70 to be approximately 45% for men and 35% for women among relatives with Lynch syndrome. This is lower than earlier estimates, because of the more severe family histories included in earlier studies. Among relatives with Lynch syndrome, risks for endometrial cancer by 70 years of age are variable and range from as low as 31% to as high as 64%. Some of the higher estimates,

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however, may be subject to family history bias. The U.S. Multisociety Task Force on Colorectal Cancer recommends colonoscopy every 1 or 2 years for first-degree relatives of individuals diagnosed with Lynch syndrome, and uptake among this group is about 80%. The most serious adverse events associated with colonoscopy in the general population are bleeding (1.1/1000 individuals), perforation (3.3/1000), and death (0.08/1000). Adequate evidence on the effectiveness of routine colonoscopy in relatives with Lynch syndrome is available from a controlled trial in Finland and from an observational cohort study in the Netherlands. Evidence, overall, was rated as Level IIb. By using an intention to treat analysis, the Finnish study reported that CRC incidence was reduced by 62%, with no deaths among those undergoing surveillance, compared with nine in the control group. Other less direct studies suggest efficacy of periodic colonoscopy. Adequate evidence exists that 63% of women will adhere to endometrial cancer surveillance. Inadequate data are available to document that transvaginal ultrasound and endometrial biopsy can reduce the incidence of endometrial cancer. Hysterectomy and bilateral salpingo-oophorectomy are effective in reducing the risk for endometrial cancer, but uptake is low (19%) and it has not been the standard of care to recommend this procedure.

5. *Economic modeling of programmatic costs and costs per Lynch syndrome detected using four different testing strategies.* Data from this supplementary review, along with other published information, were used to perform a cost-consequences analysis. Rather than looking at health outcomes, this type of analysis focuses on the direct consequences of testing. In this analysis, the costs per Lynch syndrome case detected were determined for four strategies that represent a wide range of possible testing/diagnostic scenarios. The list of strategies is not intended to be exhaustive but to provide examples. It is assumed that the initial test in each of the four strategies described below would have 67% uptake so that detection rates for Lynch syndrome can be compared between strategies. Subsequent actions are modeled at rates found in the literature (e.g., uptake of counseling and testing among their relatives). Total program costs (preliminary testing, counseling, diagnostic testing, contacting relatives and targeted testing) are computed assuming a cohort of 150,000 newly diagnosed CRC cases with a 3% prevalence of Lynch syndrome.

- a. *Strategy 1.* Individuals with newly diagnosed CRC (probands) would have MMR gene sequencing/deletion testing for *MLH1*, *MSH2*, and *MSH6*. This strategy will have the highest sensitivity for Lynch syndrome (about 85% or 2537 of the 3000 cases), and cost about \$111,000 (90% CI, \$83,000–\$148,000) per proband with Lynch syndrome detected. If relatives of the proband are included in the analysis as well, the cost per adult with Lynch detected is reduced to about \$72,000 (90% CI, \$49,000–\$101,000). Costs are reduced when relatives are included because they require only counseling and targeted testing for the family mutation. Total program costs for this strategy are about \$281 million.
- b. *Strategy 2.* All probands would have quality MSI testing; those with high instability would have sequencing/deletion testing for the three MMR genes. This strategy will have a lower overall detection rate

(73% or 2198 cases) because the MSI is not high in all individuals with Lynch syndrome. The cost per proband with Lynch syndrome detected is \$47,000 (90% CI, \$33,000–\$64,000). When relatives are included, the cost per case detected is reduced to \$31,000 (90% CI, \$20,000–\$44,000). Total program costs are about \$104 million.

- c. *Strategy 3.* All probands would have quality IHC testing; those with negative staining would have sequencing/deletion testing for the some of the three MMR genes. This strategy will have a slightly lower overall detection rate (70% or 2105 cases), compared with Strategy 2, because IHC testing seems to be slightly less reliable when identifying probands with Lynch syndrome. The cost per Lynch syndrome proband detected is \$21,000 (90% CI, \$14,000–\$29,000). When relatives are included, the cost per case detected is reduced to \$14,000 (90% CI, \$9,000–\$20,000). The lower costs are because IHC testing provides information about which MMR gene(s) are likely to contain the mutation, thereby reducing testing costs for the probands. Total program costs are about \$46 million.
- d. *Strategy 4.* Strategy 3 is modified so that probands with an IHC *MLH1* negative stain are all tested for the specific *BRAF* mutation. If that mutation is not found, the individual continues on for MMR gene(s) testing. If the mutation is found, no further testing (sequencing) is required as the chance of having Lynch syndrome is very low. The overall detection rate remains at about 70% (or 2097 cases), as the sensitivity of *BRAF* mutation testing is close to 100%. The cost per Lynch syndrome proband detected is \$19,000 (90% CI, \$13,000–\$26,000). When relatives are included, the cost per case detected is reduced to \$13,000 (90% CI, \$8,000–\$18,000). Total program costs are about \$41 million.

IMPORTANT GAPS IN KNOWLEDGE

- What is the clinical validity of sequentially applied screening tests (e.g., MSI, then IHC testing)?
- Is methylation testing useful as part of preliminary testing for Lynch syndrome?
- Additional information needs to be collected regarding the methods of identifying, approaching, educating, counseling, and testing relatives of probands with Lynch syndrome.
- Although randomized trials are unlikely, observational studies could provide additional information on whether systematic surveillance is effective in reducing Lynch syndrome–related morbidity and mortality for both CRC and other related cancers.
- Should the clinical care of CRC patients with Lynch syndrome be altered?
- Among females with Lynch syndrome, is endometrial cancer surveillance effective?
- A comprehensive cost-effectiveness analysis (CEA) should be performed as a way to help inform policymakers about which strategy(s) might be recommended.

AIMS OF THIS SUPPLEMENTARY EVIDENCE REVIEW

The aims of this supplementary evidence review are to reconsider aspects of the evidence regarding: (1) a clarified DNA-based definition of the clinical disorder; (2) performance characteristics of preliminary, laboratory-based tests, taking into

account the technical laboratory issues and new markers; (3) an updated and expanded exploration of implications for other family members (psychosocial, as well as other benefits and harms); (4) the usefulness of considering family history as a preliminary test; and (5) an economic model for detecting Lynch syndrome that includes both probands and their first-degree relatives.

BACKGROUND AND JUSTIFICATION

Recently, an evidence report was released¹ regarding the use of gene-based tests in the diagnosis and treatment of HNPCC. Its purpose was to inform the EGAPP Working Group (EWG) in making recommendations. Because these tests are rapidly evolving and the evidence report highlighted important gaps in knowledge, the EWG requested a supplementary report to provide updated information and an expanded evidence base upon which to build its recommendations.² The updated information relies on the original evidence report as its basis, but adds to and extends those findings. This supplemental evidence was targeted at five specific areas after discussions with a Technical Evaluation Panel consisting of EWG members, Centers for Disease Control and Prevention (CDC) consultants and staff, and experts in the field of identifying Lynch syndrome (H. Hampel) and laboratory testing for Lynch syndrome (S. Thibodeau). These five areas are summarized below and the first four are reviewed in detail in the remainder of this document.

Clarifying how to define the clinical disorder—Lynch syndrome

Defining Lynch syndrome (sometimes referred to as HNPCC) clinically, using family and personal history of cancer, is problematic. The original Amsterdam criteria were designed to identify a suitable group for further study of an inherited form of CRC; not to define a specific clinical disorder. Subsequent modifications to these criteria (e.g., Bethesda criteria) were interpreted as a more sensitive test for identifying inherited forms of CRC. However, such a group is heterogeneous, with some cancers caused by MMR gene mutations, others by inactivated MMR genes, and many others of unknown etiology. This has led to confusion in the literature. As a result, specifications for the primary, evidence-based review were difficult to shape, and it was determined that interpretation of data from that review would require supplementary information. The initial review helped the EWG realize that the clinical scenario needed to be refined. Consequently, the present document replaces a family history–based definition with a molecular definition that has recently been promulgated in the research community—individuals with an identifiable MMR gene mutation are defined as having Lynch syndrome, whether or not an existing CRC or other cancer is present.

Removing family history from consideration as a preliminary test

On the basis of the original evidence report, the EWG Technical Evaluation Panel members decided against using the family history as the initial screening test (e.g., Bethesda criteria) in this population. This was based on the difficulty and the costs of obtaining reliable family history, and the overall poor sensitivity and specificity.

Documenting the clinical validity of DNA-based preliminary tests

Significant new information is available in the literature, and we also reviewed whether any information in the original evi-

dence review had become outdated. For example, older reports containing results for MMR gene testing used less sensitive technologies (e.g., denaturing gradient gel electrophoresis or single strand conformation polymorphism analyses) and did not check for large deletions. In addition, older tests for MSI relied on only a few repeated sequences, compared with more recent expanded testing panels that utilize three or more mononucleotide repeats. Given the rapid advances in both knowledge and technology, we also restricted literature searches to more recent dates and stratified results by technology to determine the impact of newer test panels. For example, we stratified the analysis of MSI testing by factors known to improve sensitivity (e.g., number of mononucleotide repeats).

Benefits and harms to probands and relatives with Lynch syndrome

The original evidence report (www.ahrq.gov/downloads/pub/evidence/pdf/hnpcc/hnpcc.pdf) addressed anxiety and psychosocial issues, but did not address the medical harms related to screening and diagnosis in probands and relatives (e.g., additional colonoscopies) or the benefits in probands and relatives (e.g., avoidable CRC and endometrial cancer). In addition, neither uptake rates for subsequent screening nor effectiveness of screening tests were addressed. This type of information is necessary when balancing the benefits and harms of testing and diagnosis, and also to inform decision analysis/economic models. Data from the original evidence report were reanalyzed to determine whether more data from the breast cancer literature could be included to inform the review.

Economic modeling of programmatic costs and costs per Lynch syndrome detected using four different testing strategies

A more comprehensive set of models using updated information was requested in order that the EWG might create broad recommendations. For example, the model in the original evidence review did not consider the benefits that IHC testing provided in directing which MMR gene to sequence, nor did it include relatives of probands. Although it was not possible to perform a comprehensive CEA, it was possible to conduct a cost-consequences analysis to help inform recommendations.

METHODS

The EWG has explicit methods for both identifying published and gray data, and for ranking the quality of data sources (1 being the highest quality and 4 being the lowest) and quality of evidence (convincing being the highest, adequate and inadequate being the lowest).² Criteria for both the quality of data sources and quality of evidence differ for analytic validity, clinical validity, and clinical utility. Specific details regarding the identification of data are contained within each section. In general, data identification was based on explicit search strategies for each question of interest, with occasional use of gray data, referral to the original evidence report,² and in some instances, existing structured reviews. Although the analytic framework used for the original evidence review remains relevant, the present review does not address all aspects of the overarching question. For example, we did not systematically collect additional data regarding the analytic validity of preliminary DNA-based tests. An interpretation of the data are also provided for each question of interest that includes an assessment of quality of data and quality of evidence and identification of possible biases and gaps in knowledge.

RESULTS

Clarifying the definition of the disorder

In this supplementary review, we will use the terminology and definitions as proposed by Jass³ and Lindor et al.⁴ Lynch syndrome will refer to an individual with a germline MMR gene mutation who has a predisposition to CRC and to certain other malignancies, or is diagnosed with one of these cancers. The term HNPCC will generally not be used except in direct reference to a publication. The following sections provide the rationale for this definition.

The term HNPCC is problematic and has multiple definitions (e.g., defined by family history, clinical, and/or pathologic features). In a 2005 editorial, Terdiman⁵ provides an example of how the terms Lynch syndrome and HNPCC have multiple and overlapping definitions which can cause confusion.

HNPCC, also called Lynch syndrome after Henry T. Lynch, MD, a pioneer in the field, is an autosomal dominant hereditary cancer syndrome which accounts for upwards of 3% of all CRC, and is associated with an increased risk of endometrial, ovarian, and other extracolonic cancers . . . The syndrome originally was defined in clinical terms by the stringent Amsterdam criteria, although over time, more relaxed clinical definitions have been suggested, culminating in the recently published revised Bethesda guidelines. Many cases of clinically defined Lynch syndrome are caused by a germline mutation in one of a set of genes responsible for DNA mismatch repair.

A solution to the confusion of terms is best summarized by Jass.³

The term HNPCC is a poor descriptor of the syndrome described by Lynch. Over the last decade, the term has been applied to heterogeneous groups of families meeting limited clinical criteria, for example, the Amsterdam criteria. It is now apparent that not all Amsterdam criteria-positive families have the Lynch syndrome. The term HNPCC has also been applied to clinical scenarios in which CRCs with DNA microsatellite instability are diagnosed but in which there is no vertical transmission of an altered DNA mismatch repair gene. A term that has multiple, mutually incompatible meanings is highly problematic, particularly when it may influence the management of an individual family. The Lynch syndrome is best understood as a hereditary predisposition to malignancy that is explained by a germline mutation in a DNA MMR gene. The diagnosis does not depend in an absolute sense on any particular family pedigree structure or age of onset of malignancy.

These definitions were extended by Lindor et al.⁴ who suggest using the term Familial CRC Type X to describe individuals in families satisfying stringent family history criteria (e.g., Amsterdam) who have no evidence of a MMR gene mutation.

The use of HNPCC as a label needs to be refined or made obsolete. The term HNPCC encompasses considerable heterogeneity and has come to mean different entities to different people. We prefer the term “Lynch syndrome” or “HNPCC Lynch syndrome” to specify those individuals or families with germline mutations in the DNA MMR

genes . . . It may be reasonable to introduce a term for families similar to our group B families, who have a clustering of CRC but in whose tumors no DNA MMR gene defect is evident. We suggest the term “familial colorectal cancer type X.” This term does not define these groups as having hereditary CRC (which usually implies single-gene etiology), and it acknowledges our lack of understanding of the etiology (thus the “X”) . . . Regardless of what term is eventually adopted, it is essential that the term HNPCC not be used without clearly defining it, to acknowledge that families with Lynch syndrome (hereditary DNA MMR deficiency) and those with familial colorectal cancer type X are not equivalent entities.

This is not to say that all experts in the field are consistently using this terminology at this point in time. In a 2007 article by Lynch et al.,⁶ the terms HNPCC and Lynch syndrome are still, apparently, used interchangeably.

Before molecular genetic diagnosis came of age in the 1990s, a comprehensive family history was the only basis on which familial risk of CRC could be estimated. In the case of HNPCC, also known as Lynch syndrome, the historic perspective offered by Warthin in 1895 has not changed appreciably.

Performance of DNA-based tests in the context of a molecularly defined disorder

What is the clinical sensitivity of MSI testing to identify individuals with Lynch syndrome?

The optimal study design for this purpose would be population based, enrolling a large group of individuals consecutively diagnosed with CRC. Initially, MMR gene mutation testing, accounting for as many major mutations as possible, would be performed on all of these cases. MSI testing would then be performed on samples from all cases with a mutation. Testing (both for MSI and MMR mutations) would utilize the technology currently in use. We restricted the search to articles published in 2003 and later, to help ensure that retrieved studies utilized current testing technologies. By that time, testing laboratories often would have (1) incorporated the basic National Cancer Institute (NCI) panel⁷ for MSI testing; (2) included additional mononucleotide markers to improve performance^{8,9}; (3) routinely tested for mutations in *MSH6* and, possibly, *PMS2*; and (4) routinely tested for large deletions in MMR genes using multiplex ligation-dependent probe amplification. We searched PubMed from 2003 through June 2007, using the MeSH terms “(Colorectal Neoplasms or Hereditary Nonpolyposis) and (MSI or microsatellite instability),” restricted to humans and the English language. Overall, 212 articles were identified. Two of us (G.E.P. and S.M.) reviewed the 212 abstracts and agreed that 28 full articles should be reviewed for appropriateness. Of these 28 articles, 11 met the following inclusion criteria.^{10–20} (1) MMR gene mutations were identified without knowledge of MSI status (in at least an identifiable subset of the data); (2) MSI testing was attempted on all patients with Lynch syndrome; (3) the MSI testing methodology was described in sufficient detail to rate test quality; and (4) the MMR gene with the mutation was identifiable.

The analysis was restricted to individuals with CRC (a few excluded studies included only patients with endometrial or breast cancer). In some studies, a few individuals had multiple CRC tumors tested. We chose the earliest sample with complete test results (i.e., MSI and IHC), to best simulate what might

happen as part of routine evaluation in the future. In some studies, there were a few instances of multiple family members being tested. We chose to use the family member with the youngest age of onset for a CRC who had complete test results. Assessment of MSI test quality was defined before reviewing the articles and consisted of four questions: (1) did the authors discuss whether microdissection was performed, and whether it was manual or via laser; (2) how many mononucleotide markers were included in the panel; (3) were both tumor and normal tissue used in determining MSI status; and (4) was a minimum proportion of tumor cells required (e.g., 30% or higher).

Of the 11 studies examined,^{10–20} only one was close to being population based, but it was restricted to CRC diagnosed under age 55.¹⁰ Because of the relatively low prevalence of Lynch syndrome (about 2–4%), comprehensive MMR gene testing among all CRC patients in the general population would be expensive. It is for this reason that the 11 studies used various definitions of high-risk populations, such as the Amsterdam or the Bethesda criteria, some other family history–based definition, or early age of onset for the proband (e.g., <55 years of age). If a study included some patients in whom MMR gene mutations were sought because of a positive MSI test result, that study was used only if an identifiable subset of the study population was identified with nonbiased criteria (e.g., family history).¹⁹ The studies were performed in North America,¹³ Europe,^{10–12,15–18,20} Asia,¹⁴ and Australia.¹⁹ The smallest included five individuals/families¹⁴ with Lynch syndrome; the largest included 26 patients¹⁰ (average 15); MSI results were available for a total of 150 Lynch syndrome patients.

Table 1 shows the estimated clinical sensitivities for MSI testing (positive defined as MSI-high, negative as MSI-low or MSI-stable) to identify MMR mutations. Eleven studies reported MSI results for 81 Lynch syndrome patients with mutations in *MLH1*. Combining the study results using a random effects model, the sensitivity of MSI testing was 85% (95% CI, 75–92%). The bolded rows indicate summary information for a particular MMR gene. Non-bolded clinical sensitivity estimates (last column) indicate results stratified by MSI test quality. The same studies identified 59 Lynch syndrome patients with a *MSH2* mutation and found the sensitivity of MSI testing to be 85% (95% CI, 73–93%). Five studies identified 67 patients with *MSH6* mutations and found the sensitivity of MSI testing to be 69% (95% CI, 46–85%). When assessing test quality, we found that all 11 studies reported using both tumor and normal tissue to assign MSI status. None of the studies explicitly reported laser microdissection, which has been reported to be the optimal method for sample preparation.²¹ In addition, none of the studies reported a minimum proportion of tumor cells. Roughly half of the studies, however, relied solely on the 1998 NCI recommended panel⁷ that includes only two mononucleotide markers (labeled as a 2 under the “MSI test quality” column), whereas the remaining studies utilized three or more mononucleotide markers (labeled as a 3+ under the MSI test quality column). When the results are stratified by this quality measure (last column in Table 1), the clinical sensitivities of studies using three or more mononucleotide markers are consistently higher (91% vs. 80% for *MLH1*, 87% vs. 84% for *MSH2*, and 77% vs. 55% for *MSH6*). This provides evidence supporting several methodological studies^{8,9,21,22} and a more recent NCI report²³ suggested that additional mononucleotide markers (up to five) be included in an MSI panel for clinical testing. Only one study reported a Lynch syndrome patient with a *PMS2* mutation¹⁹ and found an MSI-stable result, but only 4 of the 10 MSI test markers provided interpretable results.

A high proportion of Lynch syndrome patients with mutations in the *MLH1* and *MSH2* genes can be identified via MSI-high test results. The best estimate of sensitivity with the use of two mononucleotide markers in the NCI recommended panel is 80–84%, but with the use of at least three mononucleotide markers, the sensitivity can be increased to 87–91%. Clinical sensitivity seems to be lower for *MSH6*, with the corresponding estimates of 55% and 77% depending on the number of mononucleotide markers used. The greater discrepancy between these two estimates is likely due to a known reduction in the sensitivity of dinucleotide microsatellites to *MSH6* mutations.²⁴

What are the study limitations for the clinical sensitivity for MSI testing?

All of the studies would be ranked as quality Level 2 or 3; the lower ranking because the settings are all high-risk population (e.g., families who satisfy Amsterdam criteria) rather than population based. This may lead to an overestimate of sensitivity, if MSI test results were related to penetrance. However, this does not seem to be the case. When the population-based results for those younger than 55 years (80% for *MLH1* and 82% for *MSH2*) are compared with the other four studies that were based on strong family histories regardless of age (82% for *MLH1* and 88% for *MSH2*), the results are remarkably similar. The quality of evidence is considered adequate for estimating MSI sensitivity for *MLH1*, *MSH2*, and *MSH6*. Few data are available from individuals other than non-Hispanic whites. The one study in Asians included only five patients with Lynch syndrome,¹⁴ and it found an average MSI sensitivity of 80%. No estimate of clinical sensitivity for *PMS2* was made due to the low number of reported results. In subsequent modeling, the sensitivity of MSI testing for *PMS2* will be considered equivalent to that for *MSH6*—lower than that found for *MLH1* and *MSH2*.

How can clinical sensitivity of MSI testing be improved?

It is unlikely that clinical sensitivity would ever reach 100%, even if the laboratory test were to be improved and preanalytic/postanalytic errors eliminated. There is room for improvement, however, as it is likely that none of the studies under review used the most sophisticated MSI test now possible for identifying CRC patients with Lynch syndrome. Methodological studies have shown the importance of laser microdissection,^{21,25} the proportion of tumor tissue tested, and the number of cells tested.^{25,26} Other studies have provided ways to improve testing of poor quality samples.^{26,27} If these techniques had been included in studies under review, even higher clinical sensitivities might have been obtained. Several researchers have examined the possible reasons for an MSI-low or MSI-stable in a confirmed Lynch syndrome patient.^{21,22,25,26} In the majority of instances, a methodological reason for the initial false-negative result was identified. One study found that, for certain large deletions in *MSH2*, one of the mononucleotide markers (*BAT26*) appeared MSI-stable. It was inferred that this was due to the complete absence of the target *BAT26* sequences in the tumor sample resulting in amplification of contaminated normal DNA.²⁸

What might the clinical sensitivity be for laboratories offering MSI testing in the United States and Europe?

External proficiency testing results from the United States and elsewhere indicated that current practice for MSI testing is not optimal in most laboratories. Based on this, it is likely that clinical sensitivity in routine practice for mutations in *MLH1* and *MSH2* will generally be toward the lower end of the range

Table 1 Clinical sensitivity (detection rate) of microsatellite instability (MSI) testing to identify Lynch syndrome^a

Reference	MMR gene	Total number	Total with			Study's MSI-H (%)	MSI test quality ^b	Clinical sensitivity (%)
			MSI-H	MSI-L	MSI-S			
Barnetson et al. ¹⁰	<i>MLH1</i>	10	8	2	0	80	2	
Hoedema et al. ¹³	<i>MLH1</i>	4	2	nd	2	50	2	
Lee et al. ¹⁴	<i>MLH1</i>	4	3	0	1	75	2	
Luo et al. ¹²²	<i>MLH1</i>	2	2	0	0	100	2	
Niessen et al. ¹⁵	<i>MLH1</i>	8	8	0	0	100	2	
Overbeek et al. ¹²³	<i>MLH1</i>	3	3	0	0	100	2	
Wolf et al. ¹⁶	<i>MLH1</i>	9	8	0	1 ^c	89	2	80 (54–90)
Hendriks et al. ¹¹	<i>MLH1</i>	12	11	0	1	92	3+	
Plevova et al. et al. ¹⁸	<i>MLH1</i>	18	16	1	1	89	3+	
Southey et al. ¹⁹	<i>MLH1</i>	5	5	0	0	100	3+	
Spaepen et al. ²⁰	<i>MLH1</i>	6	6	0	0	100	3+	91 (77–96)
All	<i>MLH1</i>	81	72	3	6		Any	85 (75–92)
Barnetson et al. ¹⁰	<i>MSH2</i>	11	9	2	0	82	2	
Hoedema et al. ¹³	<i>MSH2</i>	2	1	nd	1	50	2	
Lee et al. ¹⁴	<i>MSH2</i>	1	1	0	0	100	2	
Luo et al. ¹²²	<i>MSH2</i>	1	1	0	0	0	2	
Niessen et al. ¹⁵	<i>MSH2</i>	6	6	0	0	100	2	
Overbeek et al.	<i>MSH2</i>	10	9	0	1	90	2	
Wolf et al. ¹⁶	<i>MSH2</i>	8	7 ^d	0	1 ^c	87	2	84 (68–93)
Hendriks et al. ¹¹	<i>MSH2</i>	5	5	0	0	100	3+	
Plevova et al. ¹⁸	<i>MSH2</i>	4	3	0	1	75	3+	
Southey et al. ¹⁹	<i>MSH2</i>	2	2	0	0	100	3+	
Spaepen et al. ²⁰	<i>MSH2</i>	9	9	0	0	100	3+	87 (63–96)
All	<i>MSH2</i>	59	53	2	4		Any	85 (73–93)
Barnetson et al. ¹⁰	<i>MSH6</i>	5	1	2	2	20	2	
Niessen et al. ¹⁵	<i>MSH6</i>	7	4	3	0	57	2	
Overbeek et al. ¹²³	<i>MSH6</i>	5	6	0	1	83	2	55 (21–85)
Hendriks et al. ¹²	<i>MSH6</i>	21	18	3 ^e	0	86	3+	
Plaschke et al. ¹⁷	<i>MSH6</i>	27	22	4	1	81	3+	
Southey et al. ¹⁹	<i>MSH6</i>	2	0	2	0	0	3+	77 (50–92)
All	<i>MSH6</i>	67	51	14	4		Any	69 (46–85)

Hoedema et al.¹³ collapsed the MSI-L results into the MSI-S category.

^aLynch syndrome defined as an individual with an identified mutation in a mismatch repair gene.

^bBased on four quality measures: (1) sample microdissection discussed, (2) three or more mononucleotide markers, (3) use of normal and tumor tissue in scoring, and (4) minimum proportion of tumor cells in preparation. Higher numbers indicate higher compliance with quality measures.

^cIn Wolf et al.¹⁶ this family was assigned both an MSI-H and MSI-S; here they are counted as MSI-S.

^dIn Wolf et al.¹⁶ one of these families was assigned both an MSI-H and MSI-S; here they are counted as MSI-H.

^eIn Hendriks et al.¹² collapsed the MSI-L results into the MSI-S category.

nd, not done.

found earlier (about 84–90%). Corresponding clinical sensitivity for *MSH6* will be even lower.

- A 2006 study reported the results from a questionnaire completed by the five clinical laboratories in the United

Kingdom offering MSI testing as part of CRC follow-up.²⁹ The survey showed wide variation in the microsatellites selected for inclusion in the panel. Little information is available regarding tissue preparation. A survey of six laboratories in the United States in 2006³⁰ identified sev-

eral key components aimed at assuring reliable MSI testing, including (1) optimizing the PCR products, (2) utilizing three or more mononucleotide markers, (3) standardizing how to determine an “equivocal” result, and (4) performing duplicate readings to reduce human errors.

- The College of American Pathologists has offered proficiency testing for MSI testing since 2003 through the Molecular Pathology Committee.³¹ Twice yearly, one unstained paraffin section is sent to participants, along with a case description. Laboratories are asked to perform MSI testing, answer accompanying technical questions about their methodology, and provide a test interpretation. Roughly 50 laboratories (mainly in North America) participate in the survey, which found important differences in test methodology, including the number and type of markers included (e.g., mononucleotide, dinucleotide) and protocols for sample preparation (microdissection and proportion of tumor cells required for reliable testing). Data suggest that at least some of the identified errors can be attributed to test methodology. Overall, there seems to be room for improvement in the technical aspects of MSI testing in clinical laboratories.

What is the clinical specificity of MSI testing when identifying Lynch syndrome?

In this review, the false-positive rate (1 – specificity) is calculated initially, and then converted into the clinical specificity. The false-positive rate can be defined as the proportion of tested individuals in the population of interest (consecutive patients with CRC) without the disorder of interest (Lynch syndrome) that has MSI-high test results. By definition, Lynch syndrome is CRC caused by a MMR gene mutation. Most MSI-high test results that are not associated with a germline MMR gene mutation are explained by a nonfunctional MMR gene (MMR gene proteins are nonfunctional because of a somatic event). The most common cause is somatic methylation of the promoter region for *MLH1*, often referred to as sporadic MSI CRC.³² Lynch syndrome and sporadic MSI CRC have morphologic features in common (lymphocytic infiltration, mucin secretion, and poor differentiation) that clearly separate them from other sporadic CRC. Lynch syndrome, however, is more often associated with a positive family history and early age of onset, whereas MSI CRCs are more common in women and occur at later ages. Thus, the “false positive” MSI-high test results indicate that the cancer is likely caused by failure of the

MMR gene, by a different mechanism (i.e., nonheritable *MLH1* promoter methylation).

What study designs are optimal to define the clinical specificity for MSI testing?

For the setting of population-based testing of newly diagnosed CRC cases, the optimal study design would be a consecutive series of individuals, all of whose tumors are tested for both MSI and MMR gene mutations (preferably for both point mutations and large deletions in *MLH1*, *MSH2*, *MSH6*, and *PMS2*). Among those individuals without an identified mutation (non-Lynch syndrome), the proportion with an MSI-high test result would be the false-positive rate from which the specificity can be computed. Studies that enroll only Amsterdam criteria-positive patients, or limit enrollees by age of onset (e.g., <40 years) would not be appropriate. However, studies that do not identify all cases of Lynch syndrome (e.g., only identify mutations among MSI-high patients) may also be acceptable, as the incidence of sporadic MSI CRC is high when compared to the incidence of Lynch syndrome.

Clinical specificity for MSI testing in population-based cohorts of CRCs

Although we would have preferred to restrict studies included in this section to the time period of 2003 and later (so that MSI testing methodologies would be similar to those used to define clinical sensitivity), the limited number of available studies did not allow for this. Six studies provided sufficient information to compute the clinical specificity of MSI testing. The consensus estimate for the clinical specificity of MSI testing is 90.2% (95% CI, 87.7–92.7%), using a random effects model. When expressed as a false-positive rate, the estimates are 9.8% (95% CI, 7.3–13.0%). These results are heterogeneous, and this is only partially explained by study design or MSI test composition (numbers and types of microsatellites tested).

In all studies, we used only MSI-high results as being positive and included both MSI-low and MSI-stable as negative. Table 2 summarizes the results of MSI testing from studies that reported on consecutive newly diagnosed CRC from a general population. One of the studies restricted enrollment to patients under age 55.¹⁰ Another reported being conducted at a referral center, but enrolled consecutive newly diagnosed cases.³³ Overall, the studies tested 3842 patients (after those with known

Table 2 Clinical specificity of microsatellite instability (MSI) testing to identify Lynch syndrome (LS)

Reference	Location	CRC patients enrolled	Average age	Incidence of LS (%)	No. controls		Clinical specificity (%)	FPR (%)
					Tested	Positive		
Barnetson et al. ¹⁰	Scotland	870 (69)	48	4.4	322	24	92.5	7.5
Pinol et al. ³⁴	Spain	1222 (62)	70	0.9	1211	73	94.0	6.0
Hampel et al. ³⁷	USA	1066 (88)	63	2.2	1043	114	89.1	10.9
Cunningham et al. ³³	USA	257 (50)	69	2.7	250	44	82.4	17.6
Salovaara et al. ³⁵	Finland	535 (90)	67	3.4	517	48	90.7	9.3
Aaltonen et al. ³⁸	Finland	509 (67)	68	2.0	499	53	89.4	10.6
All		4459			3842	356	90.2^a (87.0–92.7)	9.8^a (7.3–13.0)

^aEstimated using a random effects model. FPR, false-positive rate (100 – clinical specificity).

mutations were removed); 356 MSI-high test results were identified in that group. Formal meta-analysis shows significant heterogeneity ($Q = 40$, $P < 0.001$), because one U.S. study reported a false-positive rate of 17.6%, whereas the remaining studies averaged about 9%.

As a way of assessing heterogeneity, we examined several possible biases. Two studies^{34,35} used only *BAT26* (a mononucleotide marker) to define MSI status. Such a test is likely to have a higher specificity (lower false-positive rate) than a more complete panel. Consistent with this expectation, both of these studies have higher specificities (lower false-positive rates) than the consensus figure. There is no obvious reason why specificity was so low in one study (82.4%).³² Given that this was a referral center, some patients might have been enrolled after having already undergone MSI testing at an outlying institution. We kept this study in the analysis, as it offsets the studies biased toward high specificity and results in assigning a broad confidence interval.

Individual study quality for the six studies that estimated MSI specificity are all rated at quality Level 2 or 3. The quality of evidence is at least adequate because of the observed heterogeneity in the results. However, there are reasonable explanations (provided above) for most high and low estimates, and it might also be reasonable to assign a convincing quality of evidence.

Clinical sensitivity of IHC testing to identify individuals with Lynch syndrome

The optimal study design for determining the clinical sensitivity of IHC testing is similar to that for MSI testing. Even though less obvious improvements may have occurred in IHC testing as compared with MSI testing, we restricted publications to 2003 and later for consistency and to avoid any temporal differences. The inclusion criteria were (1) MMR gene mutations were identified without knowledge of IHC status (in at least an identifiable subset of the data); (2) IHC testing was attempted on all patients with Lynch syndrome; and (3) the MMR gene with the mutation was identifiable. We searched the English language literature from January 2003 through June 2007, using the MeSH terms “(Colorectal Neoplasms or Hereditary Nonpolyposis) and (IHC or immunohistochemical),” restricted to humans. Overall, four articles were identified. Two of us (G.E.P. and S.M.) reviewed the abstracts and agreed that all four full articles should be reviewed. Although it was determined that none of these four satisfied the inclusion criteria, we identified nine additional articles that did. These were identified through reference lists from the four retrieved articles, through inclusion in the MSI review, or through inclusion in the original evidence report. In the few instances where articles reported on multiple cancers in the same individual or for multiple members of the same family, rules similar to those described for determining MSI sensitivity were used. All nine studies used various definitions of high-risk populations, such as the Amsterdam or the Bethesda criteria, some other family history–based definition, or early age of onset for the proband (e.g., <55 years). The studies were performed in North America,¹³ Europe,^{10–12,15,17,18,20} Asia,¹⁴ and Australia.¹⁹ The smallest included three individuals/families¹⁴ with Lynch syndrome; the largest included 31 patients¹⁰ (average 16); IHC results were available for 149 Lynch syndrome patients.

Table 3 shows the estimated clinical sensitivities for IHC testing to identify MMR mutations (a correct test result is defined as a combination of IHC test results that indicate that sequencing is warranted). For example, the result is counted as correct when MLH1 protein is reported not to be present in the nucleus of tumor tissue (usually reported as negative or absent

for protein in the tumor of a patient with a known *MLH1* mutation). We performed the analysis of IHC sensitivity twice. In one analysis, we included samples in which failures occurred and considered these to be false negatives. In a second, less strict analysis, these failures were removed and one sample (reported to have reduced, but not negative expression of MLH1 protein) was reclassified as negative. Under the second set of columns labeled “Less Strict Interpretation,” Table 3 shows that seven studies reported IHC results for 58 Lynch syndrome patients with mutations in *MLH1* and found the sensitivity of IHC testing to be 83% (95% CI, 65–93%). The same seven studies identified 40 Lynch syndrome patients with an *MSH2* mutation and found the sensitivity of IHC testing to also be 83% (95% CI, 65–92%). Finally, five studies identified 33 patients with *MSH6* mutations and found the sensitivity of IHC testing again to be 83% (95% CI, 66–93%). As expected, the “strict interpretation” results are consistently lower by three to nine percentage points. One study³⁶ reported IHC test results for mutations in *PMS2* (7 of 7 were absent protein staining). These results were not included in the table.

All of the studies providing IHC sensitivity estimates are of quality level 2 or 3 because of the high-risk population studied. The quality of evidence for sensitivity for *MLH1*, *MSH2*, and *MSH6* mutations is adequate. Few data are available to estimate sensitivity for *PMS2*, but performance is likely to be similar to that found for the other MMR genes.

Clinical specificity of IHC testing for Lynch syndrome in population-based cohorts of CRCs

Table 4 shows the consensus estimate for the clinical specificity of IHC testing, based on three studies. The overall estimate of specificity is 88.8% (95% CI, 67.6–94.8%), using a random effects model. When expressed as a false-positive rate, the estimate is 11.2% (95% CI, 5.2–22.4%). Analysis shows the results to be heterogeneous ($Q = 49$, $P < 0.001$), only part of which might be explained by study design or IHC test composition. All three studies are graded Level 2 or 3. Given that the heterogeneity between studies cannot be fully explained, the level of evidence is considered adequate.

Distribution of MMR gene mutations among Lynch syndrome patients in the general population

Few, if any, studies have performed comprehensive identification of all Lynch syndrome patients from a general population of newly diagnosed CRC patients. However, several of the studies included for the analysis of clinical specificity identified a high proportion of Lynch syndrome patients and an analysis of their data can be instructive, even though it may not be definitive. Table 5 shows the number of mutations identified in each of the four major MMR genes from the six studies previously examined (Tables 2 and 4). Only one study attempted to identify mutations in all four genes.³⁷ Since that study was published, further *PMS2* testing has identified three additional deleterious *PMS2* mutations (Hampel, unpublished study). Another study sequenced three genes (omitting *PMS2*) and the remaining four studies sequenced only *MLH1* and *MSH2*. The two studies from Finland^{35,38} report a very different ratio between *MLH1* and *MSH2* mutations identified (26:3), compared with the remaining studies (28:39). This is a result of a founder effect, which will not be representative of rates in other populations. The four analyzed studies are from Scotland,¹⁰ Spain,³⁴ and the United States.^{33,37} We referenced the ratio of mutations in *MLH1*, *MSH6*, and *PMS2* to the most common MMR gene to have mutations—*MSH2*. After weighting by the number of observa-

Table 3 Clinical sensitivity (detection rate) of immunohistochemical (IHC) testing to identify Lynch syndrome^a

Reference	Gene	“Strict” interpretation ^a			“Less strict” interpretation ^b		
		Number correct	IHC tested	Clinical sensitivity (%)	Number correct	IHC tested	Clinical sensitivity (%)
Barnetson et al. ¹⁰	<i>MLH1</i>	9	10	90	9	9	100
Hendriks et al. ¹¹	<i>MLH1</i>	15	19	79	15	19	79
Hoedema et al. ¹³	<i>MLH1</i>	1	1	100	1	1	100
Luo et al. ¹²²	<i>MLH1</i>	0	2	0	0	2	0
Niessen et al. ¹⁵	<i>MLH1</i>	6	8	75	6	7	86
Plevova et al. ¹⁸	<i>MLH1</i>	11	13	85	12	13	92
Southey et al. ¹⁹	<i>MLH1</i>	5	5	100	5	5	100
All	<i>MLH1</i>	47	58	78 (65–88)	48	56	83 (65–93)
Barnetson et al. et al. ¹⁰	<i>MSH2</i>	9	12	75	9	11	82
Hendriks et al. ¹¹	<i>MSH2</i>	10	10	100	10	10	100
Hoedema et al. ¹³	<i>MSH2</i>	4	4	100	4	4	100
Luo et al. ¹²²	<i>MSH2</i>	1	1	100	1	1	100
Niessen et al. ¹⁵	<i>MSH2</i>	8	8	100	8	8	100
Plevova et al. ¹⁸	<i>MSH2</i>	2	4	50	2	4	50
Southey et al. et al. ¹⁹	<i>MSH2</i>	2	2	100	2	2	100
All	<i>MSH2</i>	36	41	80 (62–90)	36	40	83 (65–92)
Barnetson et al. ¹⁰	<i>MSH6</i>	3	6	50	3	4	75
Hendriks et al. ¹¹	<i>MSH6</i>	3	4	75	3	4	75
Niessen et al. ¹⁵	<i>MSH6</i>	6	8	75	6	7	86
Plaschke et al. ¹⁷	<i>MSH6</i>	14	17	82	14	16	88
Southey et al. ¹⁹	<i>MSH6</i>	2	2	100	2	2	100
All	<i>MSH6</i>	28	37	74 (57–86)	28	33	83 (66–93)
All MMR mutations		111	136	77^c (69–84)	112	129	83^c (75–89)

^aFor “strict” interpretation, assay failures and one result identified as “present but reduced” (Plevova et al.¹⁸) are considered incorrect.

^bFor “less strict” interpretation, assay failures are not counted and one result identified as “present but reduced” is considered correct.

^cEstimated using a random effects model.

Table 4 Clinical specificity of immunohistochemical (IHC) testing among colorectal cancer (CRC) patients without Lynch syndrome

Reference	No. controls		Clinical specificity (%)	FPR (%)
	Tested	Positive ^a		
Barnetson et al. ¹⁰	359	39	88	12.1
Pinol et al. ³⁴	1211	73	94	6.0
Cunningham et al. ³³	250	51	80	20.4
All	1783	163	88.8^b (67.6–94.8)	11.2^b (5.2–22.4)

^aIt was not possible to determine whether some of the individuals might have absent staining for two or more MMR genes.

^bEstimated using a random effects model.

tions, the overall proportions were 32% *MLH1*, 39% *MSH2*, 14% *MSH6*, and 15% *PMS2*. Although based on small numbers from only one or two studies, it is interesting that slightly over one quarter of the MMR gene mutations identified occur on the *MSH6* and *PMS2* genes. In the future, it is likely that even more

markers for detecting Lynch syndrome will be identified as more comprehensive DNA analyses become possible, and this missing information could be expressed by having the four MMR gene proportions add up to <100%. Because of the lack of information regarding which gene(s) might have future mu-

Table 5 Estimating the proportion of Lynch syndrome attributable to each of the four major mismatch repair genes

Study	Mismatch repair gene				Total
	<i>MLH1</i>	<i>MSH2</i>	<i>MSH6</i>	<i>PMS2</i>	
Barnetson et al. ¹⁰	15 (94%) ^a	16 (100%)	7 (44%)	nd	38
Pinol et al. ³⁴	4 (57%)	7 (100%)	nd	nd	11
Hampel et al. ³⁷	5 (38%)	13 (100%)	3 (23%)	5 (38%) ^b	26
Cunningham et al. ³³	4 (133%)	3 (100%)	nd	nd	7
Weighted proportion^c	32%	39%	14%	15%	
Salovaara et al. ³⁵	17	1	nd	nd	18 ^d
Aaltonen et al. ³⁸	9	2	nd	nd	11 ^c

^aNumber of observations (proportion relative to number of *MSH2* mutations).

^bThree additional deleterious *PMS2* mutations identified after publication (H Hampel, personal communication).

^cAfter accounting for the total of all weights, so the sum of the proportions would be 100%.

^dStudy not in the analysis because of the high frequency of an *MLH1* founder mutation in Finland.

tation/deletions/rearrangements identified, however, we have chosen to represent the distribution of MMR gene mutations among those currently identifiable (i.e., add up to 100%).

All four studies used in determining the distribution of MMR genes are of lower quality (Level 3 or 4). Only one of them identified *PMS2* mutations, and the estimated proportion of mutations in this gene is provided with the least confidence. Overall, the quality of evidence is inadequate given the small numbers involved. The distribution of MMR genes would be more important if MSI were to be the preliminary test, as the sensitivity has been shown to be lower for *MSH6* mutations. The distribution is less important if IHC is the preliminary test, as the sensitivity is constant over the range of MMR genes. Fewer data are available for the performance of either test to detect *PMS2* mutations.

Testing for the *BRAF V600E* mutation

Somatic *BRAF* mutations have been detected in various cancers, including melanoma and CRC. About 90% of the mutations in the *BRAF* gene are accounted for by a transversion at nucleotide position 1799 (1799T>A) identified as V600E. The majority of CRCs with *BRAF V600E* mutations are associated with MSI positive results. Nearly always, a deleterious MMR gene mutation (i.e., Lynch syndrome) is *not* present when the *BRAF V600E* mutation is identified. There is a strong relationship between the V600E mutation and hypermethylation of the *MLH1* MMR gene. *MLH1* is by far the most common MMR gene to be associated with absent IHC staining, because of the high proportion of these tumors with associated hypermethylation. Researchers have hypothesized that performing *BRAF* testing on tumors with absent *MLH1* staining might identify a group that is nearly entirely composed of sporadic CRC that would not benefit from *MLH1* sequencing. This would result in important cost savings, as *BRAF* testing is relatively inexpensive in comparison to direct sequencing of *MLH1*.

Sensitivity and specificity of *BRAF* mutation testing

Among the subpopulation of newly diagnosed CRC patients that has absent *MLH1* staining, two mutually exclusive groups can be defined: (1) Lynch syndrome (those with a MMR gene mutation) and (2) sporadic cancer (those without a MMR gene mutation). In this subpopulation, the V600E *BRAF* mutation identifies sporadic cancer (not Lynch syndrome). Sensitivity, therefore, is defined as the proportion of sporadic cancers as-

sociated with the *BRAF* mutation. Specificity is defined as the proportion of Lynch syndrome cases without the *BRAF* mutation. In this scenario, specificity needs to be very high so that individuals with Lynch syndrome will be offered *MLH1* sequencing and not be categorized as having sporadic cancers.

Four published studies provide some information on the sensitivity and specificity of *BRAF* testing for sporadic cancer among individuals with absent *MLH1* staining. All are relatively small studies with important deficiencies. The results are summarized in Table 6.

- Wang et al., 2003, reported on 293 patients with CRC who were selected because of their high risk of having Lynch syndrome. Of the 293, 170 tumors were MSI-stable, and the subset of these patients whose tumor was tested for IHC all showed present *MLH1* and/or *MSH2* staining. All absent IHC stains were in patients with MSI-high test results. Among the 60 tumors with absent *MLH1* staining, 15 had germline mutations identified. One of these was a missense mutation that was not likely to have been deleterious, and this patient was found to have the *BRAF* mutation. No *BRAF* mutations were found among the remaining 14 Lynch syndrome patients, yielding a specificity of 14/14 or 100% (95% CI, 77–100%). Among the remaining 45 patients with sporadic cancers, 34 had the *BRAF* mutation, yielding a sensitivity of 76% (95% CI, 60–87%). Although the two populations were well defined, all individuals were considered to be at high risk of Lynch syndrome and, therefore, may not represent the findings from the general population.
- Loughrey et al., 2007, reported on a subset of 500 CRC cases referred for suspicion of HNPCC to a cancer center in Australia. Tumors from 68 of these cases were identified with either a MSI-high or negative IHC stain, and there was also sufficient tumor tissue for *BRAF* mutation testing. *MLH1* staining was absent in 40 of the tumors, and all were MSI-high. Ten of these had a germline *MLH1* mutation identified (Lynch syndrome), and none had the *BRAF* mutation (specificity 10/10, 100%, 95% CI, 69–100%). Only 23 of the remaining 30 tumors with absent *MLH1* staining had been sequenced for germline *MLH1* mutations. None had a mutation identified (i.e., sporadic cancer), and 11 of these had the *BRAF* mutation identified (sensitivity 48%, 95% CI, 27–69%). Although the two

Table 6 Sensitivity and specificity of *BRAF* mutation testing to identify sporadic colorectal cancer among all CRC cases with absent IHC staining for MLH1

Study	Sensitivity ^a (%)	Specificity ^b (%)
Wang et al. ¹²⁴	34/45, 76%	14/14, 100%
Loughrey et al. ¹²⁵	11/23, 48%	10/10, 100%
Kambara et al. ¹²⁶	35/46, 76%	18/18, 100%
All^c	80/114, 68% (95% CI 50–82%)	42/42, 100% (95% CI 92–100%)
Columbus, Ohio ^d	25/36, 69.4%	8/8, 100%
All	103/146, 69% (95% CI 57–79%)	50/50, 100% (95% CI 93–100%)

^aProportion of individuals with sporadic CRC with the *BRAF* V600E mutation. Summary estimates computed using a random effects model.

^bProportion of individuals with Lynch syndrome without the *BRAF* V600E mutation. Confidence interval computed using the binomial distribution.

^cThese studies recruited patients at high risk of Lynch syndrome.

^dPersonal communication Heather Hampel and Dr. Albert de la Chapelle; derived from a population-based cohort.

populations were well defined, all individuals were considered to be at high risk of Lynch syndrome and, therefore, may not represent the findings from the general population.

- Kambara et al., 2004, reported on 18 cases of HNPCC (likely to be Lynch syndrome, but the report did not identify a mutation in all instances) and 46 cases with “sporadic cancers” whose tumors were classified as both MSI-high and MLH1 IHC stain negative. The 46 cases were classified as sporadic, because of a negative family history and age of onset of 56 years or later. Among the 18 cases of reported HNPCC, none of the tumors carried the *BRAF* mutation. If these were considered to have Lynch syndrome, the specificity is 100% (95% CI, 81–100%). Among the 46 sporadic cases of CRC, 35 carried the *BRAF* mutation (76% sensitivity, 95% CI, 61–87%). Neither of these two groups was properly defined.
- Jensen et al., 2007, reported on 262 consecutive CRC cases (low risk of Lynch syndrome) that were tested for MSI and IHC expression. In such a group, eight individuals might be expected to have Lynch syndrome. All tumors were also tested for the *BRAF* mutation. Two hundred twenty-three of the tumors (85%) had stable or low MSI test results and positive staining for both MLH1 and MSH2, and all were negative for the *BRAF* mutation. It was assumed that none of these patients had Lynch syndrome. Tumors from all of the remaining 39 patients were MSI-high, with 32 also having an MLH1 IHC negative stain and an identified *BRAF* mutation. Tumors from the remaining seven patients did not have a *BRAF* mutation, meaning that a MMR gene mutation was likely to be present; four had negative MLH1 stains, two had negative MSH2 stains, and one was negative for both. This study cannot provide any direct estimate of *BRAF* mutation testing specificity, as none of the individuals had been sequenced for MMR gene mutations. However, the results from this population-based study could easily be consistent with the sensitivity and specificity estimates derived from the previous three studies that were in a high-risk population.
- Additional supporting information on the very high *BRAF* mutation testing specificity (proportion of Lynch syndrome not having the *BRAF* mutation) can be obtained from other published studies that did not perform IHC testing. For example, if a study reports that none of 40 Lynch syndrome patients were found to have the *BRAF*

mutation, then the specificity (as defined earlier) must have been 100%, even though the actual number of tumors with absent MLH1 staining is not known. In seven studies, no *BRAF* mutations were found among 105 cases of Lynch syndrome with deleterious *MLH1* mutations. Some of these studies, however, enrolled only subjects whose tumors were MSI-high (most of whom would have had absent stains) and/or had a positive family history. Regardless, this provides some additional support that the specificity approaches 100%. These studies also tended to enroll subjects at high risk of Lynch syndrome and, therefore, may not represent the findings in the general population.

Gray data supporting the estimates of *BRAF* sensitivity and specificity

Additional recruitment and molecular studies were performed after the publication of a population-based study of CRC and Lynch syndrome in the Columbus, OH catchment area (personal data provided by Dr. Albert de la Chapelle and Ms. Heather Hampel). Among a population-based cohort of 500 newly diagnosed CRC cases, 483 individuals had their tumor tested via IHC for absence of MLH1, MSH2, MSH6, and PMS2 protein. Of these 483 individuals, 71 (14.7%) had absent staining (abnormal result); 56 also were MSI-high. In 48 of the 71 tumors (58%, 95% CI, 44–64%), MLH1 staining was absent. Sequencing of exon 15 of the *BRAF* gene (which includes the V600E mutation) was successful for 39 of the 48 (81%) tumors. Two had insufficient tissue, and seven failed (causes for the failures were not reported). Three of the 39 individuals were identified as having Lynch syndrome associated with an *MLH1* mutation. All three were negative for the *BRAF* mutation. As part of an earlier recruitment, five additional tumors from patients with germline *MLH1* mutations were identified and tested for the *BRAF* mutation; no *BRAF* mutations were found. Thus, the specificity of *BRAF* mutation testing in this series, overall, was 8/8 (100%, 95% CI, 63–100%). Of the remaining 36 “sporadic” cancers, 25 had the *BRAF* mutation, yielding a sensitivity of 69% (95% CI, 52–84%). Identification of Lynch syndrome is close to complete in this series. Sequencing and multiplex ligation-dependent probe amplification testing were performed on all patients with an abnormal IHC result and/or an MSI-high result. All remaining individuals were tested for the two most common large MMR gene deletions, but none were identified. In this population-based cohort, the sensitivity of 69% and specificity of 100% are nearly identical to the sum-

mary estimates of the literature from high-risk populations (68% and 100%, respectively, from Table 6).

All of the published studies are in high-risk populations and are assigned a quality rank of 2 or 3. However, the results are homogeneous. For this reason, we sought gray data to provide evidence that the published estimates would be applicable in the general population. Given the consistency between the published and gray data, the quality of evidence for sensitivity and specificity is adequate.

Is methylation testing of the MLH1 promoter region useful?

This supplemental evidence review did not involve a formal search or statistical summary concerning the literature on methylation testing. The literature suggests, however, that *BRAF* V600E mutation testing and methylation testing of the MLH1 promoter region among CRC cases with absent MLH1 protein might avoid similar numbers of sequencing tests with little loss in Lynch syndrome detection.^{37,39–43}

Benefits and harms to probands and relatives identified with lynch syndrome

Should informed consent be applied to all individuals with CRC before MSI or IHC testing?

Informed consent issues were not discussed in the original evidence report. The process of identifying the subset of individuals with Lynch syndrome from among those with CRC is most appropriately accomplished by a judicial, stepwise application of informed consent.⁴⁴ Two possible options can be considered for informing CRC patients about testing for Lynch syndrome: (1) counsel everyone at the outset, or (2) perform MSI or IHC testing as part of routine practice without consent and reserve counseling for those whose test results indicate that mutation testing be considered. The first option assures that choices are possible for every aspect of the testing process. However, most (97% or 98%) of these patients would receive counseling about Lynch syndrome that is not relevant for their situation, and these same patients would worry about a medical condition that they would be found not to have. The second option does not allow choices about MSI or IHC testing, but instead limits the focus of attention to those with abnormal MSI or IHC results. This is because counseling is directed only at CRC patients with positive preliminary test results, including the 2–4% of patients whose cancers are due to a mutation in a MMR gene. In addition, those with MSI or IHC test results that indicate consideration of mutation testing retain the ability to decide about the most critical choices—definitive mutation testing and transmitting information to other family members.

There are some who feel that IHC testing is analogous to genetic testing, because individuals with results indicating the absence of MSH2 and/or MSH6, or PMS2 staining in their tumor are highly likely to have a germline mutation in these genes. However, patients with abnormal IHC results are not obliged to pursue genetic testing unless they choose to do so, and there are patients with absence of these proteins in their tumors who do not have an identifiable mutation in the corresponding gene. This could be compared to performing estrogen receptor (ER), progesterone receptor (PR), and *HER-2/neu* (a gene that plays a key role in the regulation of normal oncogene cell growth) testing on breast cancer patients. It is known that patients with triple negative (ER–, PR–, and her-2/neu–) breast cancers have a high likelihood of carrying a *BRCA1* gene mutation; however, informed consent is not needed for these tests, as they affect prognosis and management.⁴⁵ There are

clear data indicating that MSI status affects the prognosis for CRC patients, but not management.^{46,47} It has been proposed that a waiver of consent to perform MSI and IHC screening be considered for individuals with CRC. Although it is beyond the scope of this review to make recommendations regarding questions of informed consent for MSI and IHC testing, it is hoped that this presentation of evidence from both perspectives may help to inform the decision making of those in health policy domains, where these issues must ultimately be weighed.

How is management of CRC influenced when an individual is known to have Lynch syndrome?

Testing for Lynch syndrome is becoming more commonplace, and some institutions have begun screening using IHC or MSI on biopsy or surgical specimens. Also, rectal cancer patients often receive neoadjuvant chemotherapy and radiation therapy, allowing time for counseling and genetic testing before surgery and/or chemotherapy. As a result, surgical and oncological care of newly diagnosed CRC patients may be influenced by knowledge that a patient has Lynch syndrome. Subtotal colectomy with ileorectal anastomosis is recommended as a reasonable choice to be presented to patients with Lynch syndrome as the preferred surgery at the time of diagnosis with CRC. Although favored, it has not been proven superior to segmental resection with follow-up colonoscopic surveillance (category of evidence III, Grade C), based on a nonexperimental, descriptive study,⁴⁸ insufficient evidence,⁴⁹ and a 2006 multisociety evidence review of risk-reducing surgeries in inherited cancers.⁵⁰ Patients with rectal cancers should be offered proctocolectomy with ileal pouch anal anastomosis or anterior proctosigmoidectomy with primary reconstruction.⁵⁰

Persons with CRC and Lynch syndrome have a 16% risk for developing a second primary CRC within 10 years of their first diagnosis.³⁸ This influences the above recommendation for subtotal colectomy, instead of segmental resection with follow-up colonoscopy. A decision analysis study found that subtotal colectomy, in Lynch syndrome patients younger than 47 years, who have been diagnosed with CRC, leads to a 1–2.3 year increase in life expectancy.⁵¹ This was not adjusted based on quality of life, because there were no data available on quality of life differences after these surgeries among individuals with Lynch syndrome. Among patients with sporadic CRC, segmental resection has been shown to result in better quality of life than subtotal colectomy, but this may be offset by the need for frequent, lifelong colonoscopy and fear of CRC among persons with Lynch syndrome.⁴⁸ Surgical risks of subtotal colectomy with ileorectal anastomosis are discussed later.

No alteration in chemotherapeutic management is currently recommended in CRC patients with Lynch syndrome. Both laboratory and clinical studies, though few in number, find that MSI-high tumors are resistant to fluorouracil (5-FU)-based chemotherapy and are more sensitive to CPT11 (irinotecan).^{52,53} Prospective clinical trials are needed, however, before altering the care of CRC patients with Lynch syndrome. If this finding is confirmed, MSI or IHC testing (as a surrogate for MSI) will likely become standard in the pathologic evaluation of all CRCs.

The reviews discussed above^{48–50} were not available at the time the original evidence report was performed. However, no prospective trial results for clinical management options for Lynch syndrome patients have been published since that report's completion.

Table 7 Number of family members at risk for Lynch syndrome and the proportion who underwent genetic counseling and testing

Study	No.		x/y	No.(%) relatives	
	Probands (y)	Relatives (x)		Counseled	Tested
Aktan-Collan et al. ¹²⁷	Unknown	286	Unknown	113 (39)	112 (99)
Hampel et al. ³⁷	21	234 ^a	11	119 (51)	117 (98)
Hadley et al. ¹²⁸	54	111	2.1	57 (51)	56 (98)
Aktan-Collan et al. ⁵⁴	36	446	12	347 (78)	334 (96)
Stanley et al. ¹²⁹	1	96	96	41 (43)	39 (95)
Lerman et al. ¹³⁰	4	208	52	92 (44)	84 (91)
Codori et al. ¹³¹	118	505	4.2	104 (21) ^b	96 (92)
Total (95% CI)	234	1886		873 (52) (34–69)	732 (95) (93–97)

^aPersonal communication, H. Hampel.

^bAn additional 129 were pending counseling—not included in the summary counseling rate.

How many family members of individuals with Lynch syndrome might be identified, counseled, and choose to be tested?

Table 7 summarizes studies that report the number of relatives at risk for Lynch syndrome, the number receiving genetic counseling, and the number undergoing genetic testing. One thousand eight hundred eighty-six relatives of 234 individuals with Lynch syndrome (eight per proband) were identified. This is likely to be an overestimate of the numbers of relatives actually approached as part of a screening program, as some studies targeted large families for study. If the two studies that targeted large families are removed, the remaining four studies provide estimates ranging from 2.1 to 12 first-degree relatives approached. The wide discrepancy is likely due to the amount of effort and resources, and the technique applied to identifying, contacting, and approaching relatives. Genetic counseling, for example, may only be offered at a central site requiring patient travel, or counselors might travel to family events such as reunions to maximize availability. Although not reported, there would likely be at least as many second-degree relatives.

Among the total number of 1886 relatives identified, 873 or 52% (95% CI, 37–66%) were counseled. The resulting analysis showed heterogeneity ($Q = 130, P < 0.001$), mainly due to the later study by Aktan-Collan et al.⁵⁴ With this study removed, the consensus estimate is 46% (95% CI, 41–50%). Among those receiving counseling, 732 or 95% (95% CI, 93–97%) underwent genetic testing. These results were homogeneous ($Q = 12, P = 0.06$). Gene testing offers possible benefits and harms to blood relatives of individuals with Lynch syndrome, whether the results are positive or negative. Those relatives found to carry a MMR gene mutation are encouraged to comply with increased surveillance (e.g., colonoscopy).⁵⁵ Those who do not carry a MMR gene mutation may derive psychological benefits (see next section) and can follow surveillance recommendations for the general population.

Although the original evidence report summarized factors that might affect the acceptance of genetic testing, it did not estimate the number of family members of Lynch syndrome patients that could be identified, nor did it estimate the uptake of genetic counseling and testing by family members.

What information exists about insurance concerns?

Few publications report data on worry about insurance and/or employment due to MMR gene mutation testing results. In one study, 90 women were referred for predictive genetic testing (MMR or *BRCA1/2*).⁵⁶ One woman (1%, 95% CI, 1–6%) chose not to pursue genetic testing, because of concerns regarding future insurance and employment implications. Another study invited patients who met either the Amsterdam or the Bethesda criteria, and their families, to participate in genetic counseling and testing after MMR gene testing became available.⁵⁷ Of the 23 patients and family members who chose to participate, 18% were concerned about problems with insurance and/or employers, compared with 33% of the 48 nonparticipants who returned a questionnaire.

This question was not directly addressed by the original evidence report. Both studies summarized above, however, were included. The insurance concern cited in the study by Arver et al.⁵⁶ was part of the report (original evidence report—Table 26). The study by Keller et al.⁵⁷ was summarized in Extra Table 1 in the original evidence report, however, no mention was made regarding insurance concerns. In May 2008, the Genetic Information Nondiscrimination Act was signed into law. This bill is designed to protect Americans against discrimination based on their genetic information when it comes to health insurance and employment.

What are the future cancer risks (penetrance) among carriers of a MMR mutation?

Relevant findings from studies mentioned in this section are summarized in Table 8. Recent data suggest a colon cancer risk ranging between 22% and 58% by age 70 for individuals with a MMR mutation.^{58,59} Based on all available data, reasonable estimates for penetrance by age 70 might be 45% for men, and 35% for women. These penetrance estimates are lower than previously thought. One likely explanation is that more recent studies tend to be population based. Earlier studies focused on families with many affected members, where risk might be intensified by other genetic and environmental influences. A review in 2002 exemplifies the earlier thinking about penetrance.⁶⁰ It documented a consistent finding among seven studies that risk for colon cancer by age 70 years is higher among male *MLH1* and *MSH2* mutation carriers (80%) than

Table 8 Risk of cancers by age 70 among relatives with Lynch syndrome

Study	Number evaluated	<i>MLH1/MSH2</i>		<i>MLH1/MSH2/MSH6/ PMS2</i>		<i>MSH6</i>
		Males	Females	Males	Females	Males + females
Colorectal cancer (males and females)						
Vasen et al. ⁶⁶	382	92 ^a	68 ^{a,b}			
Dunlop et al. ¹³²	1563	100	54			
Aarnio et al. ⁶⁴	156	74	30			
Vasen et al. ⁶³	3222	69	54			
Hampel et al. ⁶⁵	373	69	52			
Quehenberger et al. ⁵⁸	2392	27	22			
Jenkins et al. ⁵⁹	97			45	38	
Wagner et al. ⁶²	80					32 ^a
Buttin et al. ⁶¹	59					58
Endometrial cancer (females only)						
Aarnio et al. ⁶⁴	Not given		60			
Vasen et al. ⁶⁶	Not given		50 ^b			
Vasen et al. ⁶³	Not given		31 ^b			
Hampel et al. ⁶⁵	183		54			
Quehenberger et al. ⁵⁸	Not given		32			
Wagner et al. ⁶²	53					52 ^a
Buttin et al. ⁶¹	Not given					64 ^a

^aTo age 80 yr.^bEstimated from data given.

among females (40%). By contrast, a cohort study of colon cancer cases diagnosed before age 45 years, reported in 2006, cites a lower risk for colon cancer among men (45%), but still finds a reduced risk among women (38%).⁵⁹ This study included *MLH1*, *MSH2*, *MSH6*, and *PMS2* mutation carriers and suggested that CRC risk may be 10% lower for *MSH6* and *PMS2* mutation carriers than for *MLH1* and *MSH2*. This finding is supported by two studies that show the risk for colon cancer in carriers of *MSH6* mutations to be 58% and 32% by age 80, respectively.^{61,62} Reanalysis of data that adjusted for the ascertainment of cases collected from a previous study⁶³ showed that the cumulative risk for CRC among *MLH1* or *MSH2* mutation carriers is 27% for men and 22% for women, by age 70 years.⁵⁸ There is a great deal of inconsistency among data pertaining to this question. Because of the heterogeneity of population sampling, MMR genes tested and other variables, it is not possible to create a single summary estimate. There are, however, two consistent findings. First, in all studies, females have a lower risk of developing CRC than their male counterparts, usually by 20–40%. Second, there is a clear trend toward lower penetrance estimates when studies are population based. Using these two findings, reasonable estimates for penetrance by age 70 might be 45% for men, and 35% for women. It should be noted that our analyses have been focused primarily upon cohort, and generally on more recent studies, that attempt to avoid the “high-risk family” biases.

The risk for other Lynch syndrome–related cancers (e.g., endometrial, stomach, and brain) has been estimated to be 22%

for men and 34% for women, up to age 70 years.⁵⁹ The gender difference is mainly due to the risk of endometrial cancer in women, and it may be possible to impact the incidence by screening and/or risk-reducing activities (discussed later). The proportion of female *MLH1/MSH2* mutation carriers that develops endometrial cancer by age 70 has been reported in five studies summarized in Table 8.^{58,63–66} The estimates range from around 30% to 60%. The proportion of female *MSH6* mutation carriers that develops endometrial cancer by age 80 seems to be similar, at 52% and 64%.^{61,62} These estimates seem high compared with the overall rates of Lynch syndrome–related cancers. A recent methodological study⁶⁷ suggests that the studies on the risk of endometrial cancer among relatives may also suffer from the same bias of strong family history discussed for CRC. Overall, the penetrance for endometrial cancer by age 70 in the general Lynch syndrome population may be as low as 20–25%.

What are the recommended frequency, uptake, and risks of CRC surveillance among carriers of a MMR mutation?

A recent literature review evaluated how predictive testing affects surveillance/screening behaviors.⁶⁸ Uptake of colonoscopy for MMR mutation carriers was high in six^{69–75} of seven studies, ranging from 53%⁷⁶ to 100% (top half of Table 9). Five studies reported the uptake of colonoscopy within 1–2 years after receiving genetic test results,^{69–71,74,76} the other two studies reported the uptake of colonoscopy since receiving their

Table 9 Proportion of relatives with Lynch syndrome who comply with surveillance

Study	No. carriers	No. (%) having
Colorectal cancer surveillance		
		<u>Colonoscopy</u>
Hadley et al. ⁷⁶	17	9 (53)
Collins et al. ⁷⁰	21	15 (71)
Halbert et al. ⁷¹	22	16 (73)
Johnson et al. ⁷²	7	7 (100)
Ponz de Leon et al. ⁷⁴	23	18 (78)
Claes et al. ⁶⁹	36	33 ^a (100)
Wagner et al. ⁷⁵	42	37 (88)
Total (95% CI)	168	135 (79)^a (67–87%)
Endometrial cancer surveillance		
		<u>TVU/biopsy</u>
Wagner et al. ⁷⁵	29	20 (69)
Collins et al. ⁷⁰	17	9 (53)
Total (95% CI)	46	29 (63)^b (46–75%)

^aThree mutation carriers were under age 25 and, therefore, were not recommended to have a colonoscopy.

^bThree mutation carriers were under age 25 and, therefore, were not recommended to have endometrial cancer surveillance.

genetic test results.^{72,75} Overall, the average uptake rate was 79% (95% CI, 67–87%) with some evidence of heterogeneity ($Q = 14, P = 0.03$). This is due to the study of Hadley et al. (53%).⁷⁶ If that study were to be removed, the uptake rate would be 81% (95% CI, 72–87%) with no evidence of heterogeneity

($Q = 8, P = 0.2$). Two recent reviews regarding clinical management of individuals with Lynch syndrome^{48,49} reached similar conclusions regarding colonoscopy surveillance for CRC and endometrial cancer. Colonoscopy is recommended every 1–2 years, beginning at ages 20–25.^{48,49,77,78} The 1–2 year frequency is graded level of evidence IIIC.⁴⁸ Individuals with *MSH6* mutations are recommended to begin colonoscopy at age 30, given the lower colon cancer risks associated with mutations in this gene.⁴⁹

Colonoscopy risks include adverse events related to the bowel preparation. The most common events are nausea, abdominal pain, and dizziness, which seem to be comparable between the two most common bowel cleansing agents, sodium phosphate (NaP) and polyethylene glycol. Biochemical changes associated with NaP are largely asymptomatic, but require caution in patients with cardiovascular or renal impairment. Significantly more patients are able to complete the NaP preparation than that for polyethylene glycol.⁷⁹ There have been rare serious or fatal events from the bowel preparation.⁸⁰

The most common serious, adverse events associated with the colonoscopy procedure (Table 10) are bleeding (1.1/1000, 95% CI, 0.8–1.4), perforation (3.3/1000, 95% CI, 2.3–4.6), and death (0.08/1000, 95% CI, 0.05–0.14).^{81–92} Factors associated with increased risk for adverse events are female gender, increasing age, and history of surgery, or diverticular disease.

Is there evidence that routine colonoscopy among carriers of a MMR mutation improves outcome?

Although there are no randomized trials to document whether systematic surveillance is effective in reducing Lynch syndrome–related morbidity and mortality, one long-term study from Finland (begun in 1982) reports follow-up on 252 family members (age 20–66 years) of 22 index cases.⁹³ Initially, the families were selected on the basis of family history, and 133 chose

Table 10 Adverse events related to colonoscopy

Study	Population	Procedures	Complications per 1000		
			Perforation	Bleeding	Deaths ^a
Cobb et al. ⁸¹	Medical center	43,609	0.32	—	—
Gatto et al. ⁸²	Medicare sample over 65 yr	39,286	1.96	—	—
Levin et al. ⁸³	Kaiser Permanente	16,318	0.90	4.8	0.06
Rathgaber et al. ⁸⁴	Community GI practice	12,407	0.16	—	0.00
Korman et al. ⁸⁵	45 endoscopic surgery centers	116,000	3.00	—	—
Viiiala et al. ⁸⁶	Australian teaching hospital	30,463	1.00	2.1	0.09
Nelson et al. ⁸⁷	VA medical centers	3196	0.00	4.4	0.00
Dafnis et al. ⁸⁸	One Swedish county	6066	1.00	2.0	0.00
Anderson et al. ⁸⁹	U.S. teaching hospital	10,486	1.90	—	0.19
Zubarik et al. ⁹⁰	Georgetown University Hospital	1196	—	21 ^b	—
Eckardt et al. ⁹¹	German gastroenterology practice	2500	0.80	2.4	—
Waye et al. ⁹²	Chapter review 1974–1994	99,539	0.45	4.6	0.06
All (95% CI)		381,066	1.1 (0.8–1.4)	3.3 (2.3–4.6)	0.08 (0.05–0.14)

^aOccurring within 30 days after the procedure (a total of 12 are reported in these 7 studies).

^bThis study used patient reports rather than physician reports, and is a referral center for high risk patients. It is not included in the summary estimate.

colonic examination (initially at a 5-year interval, and then at 3-year intervals); 78 chose to forego colon cancer screening and 41 were not initially contacted. After 14.5 years, 8 of 133 of those who chose screening (6%) and 19 of 119 (16%) of those who chose not to be screened had developed CRC. Mutation testing became available in 1996–1998, and all of the colon cancers were found to have developed in family members who carried a mutation—8 of 44 (18%) and 19 of 46 (41%), respectively. There were no CRC deaths among those who chose colon cancer screening, and nine among those who chose not to be screened. Overall deaths were 10 (8%) in those choosing screening, and 26 (22%) in those choosing not to be screened. This study reports a 62% reduction in risk for CRC and a significant reduction in CRC-associated mortality among family members of index Lynch syndrome cases who were found to carry a mutation. All results are computed using an “intention to treat” analysis. Crossover clearly occurred, with a proportion of individuals initially choosing to forgo surveillance later agreeing. This would likely result in an underestimation of the actual reduction in CRC risk among the surveillance group.

Further evidence for the efficacy of surveillance was reported recently.⁹⁴ In this cohort study, 2788 members from 146 Lynch syndrome families in the Netherlands were followed to assess mortality caused by CRC. The standardized mortality ratio for CRC showed a 70% decrease over three time periods: 1960–1974, 1975–1989, and 1990–2004 ($P < 0.001$). When comparing the subjects who did ($n = 897$) or did not ($n = 1073$) have surveillance colonoscopies, a significant difference in standardized mortality ratio was observed (6.5 vs. 23.9, respectively; $P < 0.001$). Evidence for efficacy of periodic colonoscopy is graded as level IIb.⁴⁸

Is risk-reducing colectomy recommended for carriers of a MMR gene mutation?

Risk-reducing colorectal resection is generally not recommended for MMR gene mutation carriers who do not have CRC (or a Lynch syndrome–related cancer).^{49,50} Subtotal colectomy with ileorectal anastomosis has an overall 30-day mortality rate of 0.9% and an overall 30-day morbidity rate of 26.0%. Morbidity is explained by the following complications: anastomotic leak (6.5%), small bowel obstruction (14.4%), fistula (2.8%), and anastomotic stricture (1.4%).⁹⁵ Fistula and anastomotic stricture are significantly more common in patients having surgery for Crohn disease, so these risks would be lower in patients with Lynch syndrome. Median stool frequency is 3/day 1 year after surgery and does not change with longer follow-up. Among patients with familial adenomatous polyposis, the quality of life is significantly poorer than that of the general population after total colectomy with ileorectal anastomosis ($P < 0.001$).⁹⁶

The difficulty of this surgery and concerns about quality of life after surgery, combined with the efficacy of colonoscopic surveillance, leads patients to rarely choose this option. Only one study was found that assessed the uptake of risk-reducing colectomy.⁷⁰ Of 32 MMR mutation carriers, none had undergone this risk-reducing surgery 12 months after receiving their mutation status. One mutation carrier indicated an intention to do so. It could be discussed as an alternative to regular colonoscopy, given the high cancer risk, concern about the safety of (or compliance with) repeated colonoscopy, and in the setting of high patient anxiety.⁶⁸

Is endometrial cancer surveillance recommended for women carrying a MMR gene mutation?

More than 75% of women with a MMR gene mutation who develop endometrial cancer will be diagnosed at Stage I. However, a proportion of these women with Stage I disease will still develop metastatic disease at a later time. This is based on documentation that 10–15% of women in the general population with sporadic early stage tumors will die from metastatic disease.^{49,97} Transvaginal ultrasound is not highly effective at identifying endometrial cancers in women with Lynch syndrome.^{98–100} Endometrial biopsy, however, is effective at identifying both premalignant and malignant lesions.⁹⁹ Two reviews dealing with this aspect of Lynch syndrome management reached similar conclusions. Endometrial cancer surveillance, including transvaginal ultrasound in combination with endometrial aspiration biopsy, should be performed every 1–2 years, beginning at age 30–35.^{48,49} The level of evidence for this surveillance guideline is IIIC.⁴⁸ Transvaginal ultrasound is relatively noninvasive and inexpensive.⁹⁸ In a large study using endometrial biopsy with an aspiration method, 96% of patients found the pain acceptable.¹⁰¹ A recent study suggests hysteroscopy with biopsy may be a feasible method to screen for endometrial cancer among women with Lynch syndrome ($n = 11$) and women who meet Amsterdam II criteria ($n = 46$).¹⁰² Of 91 attempted hysteroscopies (10 failed), 2 endometrial carcinomas were detected. Two studies performed outside the United States assessed uptake for endometrial cancer surveillance among women. In both studies, increased adherence to surveillance was noted after genetic testing (bottom of Table 9).^{70,75} Overall, 63% of women complied with endometrial cancer surveillance (95% CI, 46–75%).

Is risk-reducing hysterectomy with bilateral salpingo-oophorectomy recommended for women carrying a MMR gene mutation?

In a recent retrospective study, 61 of 315 women with MMR gene mutations chose risk-reducing surgery.¹⁰³ The entire cohort was followed for approximately 10 years. No cases of endometrial or ovarian cancer developed in women who had risk-reducing surgery, whereas 33% of women who did not have surgery developed endometrial cancer and 5.5% developed ovarian cancer. The two recent reviews discussed in previous sections also addressed this aspect of Lynch syndrome management and were in agreement that risk-reducing hysterectomy and bilateral salpingo-oophorectomy are not recommended,^{48,49} but should be presented as an option.

A second study examined preventive behaviors 1 year after genetic testing for MMR mutations. There were 21 female mutation carriers and 48 females with no mutations. When the study began, five of the women reported having had a hysterectomy, and two reported having had an oophorectomy. In the 12 months after receipt of the genetic test results, none of the women had chosen to have a hysterectomy. Two of the women who reported having had a hysterectomy at baseline chose to have a bilateral oophorectomy. No information is given on mutation status of the women who chose risk-reducing surgery.⁷⁰ A larger series in a surveillance study from Finland found that 34% (59 of 175) of women with Lynch syndrome underwent a hysterectomy.⁹⁹ Of these, 43 (72%) elected surgery, because of the finding of a premalignant lesion on screening, simultaneous laparotomy for another reason, or for risk reduction.

Risks from hysterectomy (Table 11) vary depending on the surgical technique (abdominal, laparoscopic, and vaginal) but

Table 11 Complications from hysterectomy

Study	Procedures	Complication (%)			
		Infection	Bleeding	Organ injury	Death
Abdominal hysterectomy					
Meltomaa et al. ¹⁰⁶	516	24.4	5.4	0.6	0
Makinen et al. ¹⁰⁵	5875	10.5	4.0	0.8	0.02
Olsson et al. ¹⁰⁷	72	33			
Garry et al. ¹⁰⁴	292	16.1	3.4	2.0	0
All					
Laparoscopic hysterectomy					
Meltomaa et al. ¹⁰⁶	66	3.0	9.1	0.7	0
Makinen et al. ¹⁰⁵	2434	9.0	4.7	2.8	0.04
Olsson et al. et al. ¹⁰⁷	71	27			
Garry et al. ¹⁰⁴	584	14.7	6.0	3.2	0
Garry et al. ¹⁰⁴	336	10.7	7.5	1.2	0
All					
Vaginal hysterectomy					
Meltomaa et al. ¹⁰⁶	66	3.0	9.1	0.7	0
Makinen et al. ¹⁰⁵	2434	9.0	4.7	2.8	0.04
Olsson et al. ¹⁰⁷	71	27			
All					

include infection (3–33%), bleeding (3.4–9.1%), organ injury (0.6–3.2%), and rarely death (none observed to 0.04%).^{104–107} Because of the significant heterogeneity, summary estimates for complication rates were not computed. A meta-analysis of 27 trials with 3643 participants found that “return to normal activities” was quicker for vaginal compared with abdominal hysterectomy with weighted mean differences of 9.5 days (95% CI, 6.4–12.6), and for laparoscopic compared with abdominal hysterectomy with weighted mean differences of 13.6 days (95% CI, 11.8–15.4).¹⁰⁸ There were no significant differences in return to normal activities between laparoscopic versus vaginal hysterectomy. There were more urinary tract injuries with laparoscopic than with abdominal hysterectomy (odds ratio 2.61, 95% CI, 1.22–5.6), but no other intraoperative visceral injuries showed a significant difference between surgical approaches. One study measured subjective outcomes by questionnaire survey at 4–6 weeks and 1 year after hysterectomy.¹⁰⁶ Although subjective complaints (including abdominal pain, urinary incontinence, menopausal symptoms, and genital prolapse) increased significantly at the 1-year questionnaire ($P < 0.001$), 95% of patients reported being satisfied with the procedure.

Risks from bilateral salpingo-oophorectomy are numerous and it is beyond the scope of this article to summarize all of them. Two recent reviews on this topic have been published.^{109,110} Risk-reducing oophorectomy in premenopausal women induces the sudden onset of menopause. Effects may include a higher percentage of adipose tissue and lower muscle mass, adverse changes in multiple cardiovascular risk factors, higher risk of myocardial infarction, higher rates of atherosclerosis, increased risk of mortality, short-term memory declines, dementia, macular degeneration, increased risk of bone frac-

tures and osteoporosis, sexual dysfunction and loss of desire, skin changes, and urogenital atrophy.

What surveillance is recommended for other Lynch syndrome-associated cancers among MMR gene mutation carriers?

There are no studies addressing surveillance for other less common Lynch syndrome-associated cancers, so the recommendation below, extracted from the two recent reviews, is based on expert opinion. Persons with Lynch syndrome should stay in contact with their physicians and report signs or symptoms immediately (one review recommends annual visits beginning at age 21). There are no data regarding the efficacy or compliance with this screening recommendation.

- **Gastric cancer surveillance:** Gastroduodenoscopy is recommended in persons with Lynch syndrome every 1–2 years, beginning at age 30–35 years, if gastric cancer runs in their family, or if they are from a country with a high incidence of gastric cancer.⁴⁸ A modified recommendation is that this procedure be offered periodically, taking into consideration that there is no evidence that persons with Lynch syndrome in a family where gastric cancer has been diagnosed are at higher risk for that cancer than if there were no gastric cancer cases in the family.^{49,111}
- **Urinary tract cancer surveillance:** Although there are data which indicate that urine cytology is not an effective method to screen for urinary tract cancers in patients with Lynch syndrome, currently it is recommended as an inexpensive and noninvasive surveillance modality every 1–2

years beginning at age 25–35.^{48,49,112} Abdominal ultrasound for urinary tract cancers is also suggested at the same interval.⁴⁸

Anxiety and psychosocial issues

What is the psychosocial impact of mutation testing for family members (worry, anxiety, depression, and benefits)?

Data pertaining to psychosocial outcomes of genetic counseling and testing have been looked at by comparing outcomes between carriers and noncarriers of MMR gene mutations, and changes in outcomes over time. Changes in distress among mutation carriers seem to be short term, and there is no indication of adverse effects of genetic testing.^{56,69,113–116} Unaffected noncarriers derive psychological benefits, such as short- and long-term decreases in colon cancer worry, general anxiety, and depression,³¹ other benefits include removal of uncertainty, assurance that offspring are not at high risk, avoidance of intensive surveillance, and removal of the threat of discrimination.¹¹⁷ Table 12 summarizes differences in outcomes at selected time intervals after genetic testing between mutation carriers and noncarriers.

The original evidence report noted the changes in psychosocial outcomes over time, and the differences in outcomes between mutation carriers and noncarriers. The study with the longest follow-up (3 years)¹¹³ was not available for this report. The evidence report also discussed the efficacy of pretest genetic counseling for informing family members of potential risks and benefits of testing. Psychological benefits of genetic testing were not reviewed.

Other options for preliminary testing

- *Family history:* The original evidence report discusses the use and limitations of both the original and revised versions of the Amsterdam criteria and the Bethesda guidelines. Both of these tools use personal and family history to either diagnose HNPCC (which some call Lynch syndrome), or to predict which patients with CRC are likely to

have a MMR gene mutation. The Amsterdam criteria have a lower sensitivity for detecting Lynch syndrome, ranging from 54% to 91%.¹ A series of 1066 patients with newly diagnosed CRC, regardless of age and family history, was recently studied.³⁷ Of the 23 patients identified with Lynch syndrome, 3 (13%) met the Amsterdam criteria and 18 (78%) met the Bethesda guidelines. These data show that using family history as a screening test for Lynch syndrome will likely miss a significant proportion of cases. This finding should not be construed as suggested that family history is not associated with CRC or that collecting a family history might not be valuable in the general population. Only that with the existing infrastructure, the use of routine family history is not supported by sufficient evidence to form a first-line test for Lynch syndrome among individuals with newly diagnosed CRC.

- *Cut off level for age at onset of CRC:* Another alternative strategy might be to offer MSI or IHC testing only to individuals with a newly diagnosed CRC who are under a specific age. For example, it might be possible to offer testing only to new cases whose age at diagnosis was under 70 years, given the known association between Lynch syndrome and early age of onset. This would be an efficient strategy if a high proportion of Lynch syndrome CRC cases occurred before that age (high sensitivity), whereas a relatively high proportion of sporadic CRC cases occurred after that age (high specificity). Not testing this older age group would then save resources while maintaining high detection of Lynch syndrome. A CEA would be required to properly answer this question as it involves a tradeoff between benefits and harms. Such an analysis is beyond the scope of this report.

Economic modeling

Existing economic analyses that included relatives with Lynch syndrome were reviewed and found inadequate.^{118–121} None included IHC or *BRAF* testing as part of the strategy to identify Lynch syndrome, and they did not address varying sensitivities/specificities of MSI and IHC by MMR gene mutation.

Table 12 Psychosocial outcomes in Lynch syndrome relatives compared with relatives without mutations

Psychosocial outcome	Finding statistically significant at							
	2–4 Wks		4–6 Mo		1 Yr		3 Yr	
	Yes	No	Yes	No	Yes	No	Yes	No
Worried about developing cancer	Collins et al. ¹¹³		Collins et al. ¹¹³		Aktan-Collan et al. ¹¹⁵ and Collins et al. ¹¹³		Collins et al. ¹¹³	
Anxiety	Meiser et al. ¹¹⁴ , Arver et al. ⁴⁵⁶ Collins et al. ¹¹³ , and Claes et al. ⁶⁹		Meiser et al. ¹¹⁴ , Collins et al. ¹¹³ , and Arver et al. ⁴⁵⁶		Meiser et al. ¹¹⁴ , Collins et al. ¹¹³ , and Arver et al. ⁴⁵⁶		Collins et al. ¹¹³	
Intrusive/avoidant thoughts	Meiser et al. ¹¹⁴		Meiser et al. ¹¹⁴		Meiser et al. ¹¹⁴			
Depression	Meiser et al. ¹¹⁴ , Collins et al. ¹¹³ , and Arver et al. ⁴⁵⁶		Meiser et al. ¹¹⁴ , Collins et al. ¹¹³ , and Arver et al. ⁴⁵⁶		Meiser et al. ¹¹⁴ , Arver et al. ⁴⁵⁶		Meiser et al. ¹¹⁴ and Collins et al. ¹¹³	

^aIncludes only women with either *BRCA1/2* or MMR mutations.

Table 13 Lynch syndrome: epidemiological parameters, test performance, uptake rates, and direct medical costs

Epidemiological parameters			Costs and uptake rates		
Parameter	Source ^a	Base	Parameter	Source ^a	Base
Newly diagnosed CRC tested	Current	150,000	Average number of 1° and 2° relatives	EO	4
Prevalence of Lynch syndrome (LS)	current	3%	Relatives accepting counseling	Current	52%
Proportion of LS with <i>MLH1</i> mutation	Current	32%	Relatives accepting testing counseling	Current	95%
Proportion of LS with <i>MSH2</i> mutation	Current	39%	Relative with mutation testing	EO	30%
Proportion of LS with <i>MSH6</i> mutation	Current	14%	Cost of offering testing	EO	\$20
Proportion of LS with <i>PMS2</i> mutation	Current	15%	Cost of <i>MLH1</i> sequencing/deletion analysis	M	\$808
Sequencing/MLPA sensitivity of LS	EO	99.5%	Cost of <i>MSH2</i> sequencing/deletion analysis	M	\$683
Sequencing/MLPA (1 – specificity) for LS	EO	0.03%	Cost of <i>MSH6</i> sequencing	M	\$983
MSI sensitivity for <i>MLH1/MSH2</i> mutations	Current	91%	Cost of <i>MSH6</i> deletion analysis	M	\$102
MSI sensitivity for <i>MSH6/PMS2</i> mutations	Current	77%	Cost of microsatellite instability (MSI) testing	M	\$457
MSI specificity	Current	9.8%	Cost of immunohistochemical (IHC) testing	M	\$261
IHC sensitivity for LS	Current	83%	Cost of <i>BRAF</i> V600E mutation testing	M	\$100
IHC specificity for LS	Current	11.2%	Cost of initial counseling	M	\$175
IHC MSH1 absent stain (PMS2 + or –)	PC	70%	Cost of result session (positive result)	M	\$95
IHC MSH2 absent stain (MSH6 + or –)	PC	15%	Cost of approaching 1° relative	SR	\$100
MSH6 absent stain only	PC	10%	Cost of targeted testing in relatives proband	M	\$55
PMS2 absent stain only	PC	5%			
<i>BRAF</i> sensitivity for SC among IHC absent	Current	99.5%			
<i>BRAF</i> specificity for SC among IHC absent	Current	69%			

^acurrent, this review; EO, expert opinion; M, 2007 Medicare reimbursement rates; PC, personal communication H Hampel, data from the Ohio experience; SR, Ramsey et al.¹³³

Many possible testing strategies for identifying Lynch syndrome in a general population are possible using individual (or combinations of) preliminary tests (MSI, IHC, and *BRAF* mutation testing) and diagnostic tests (sequencing/deletion analysis for the three or four MMR genes). The strategies will have varying sensitivities for detecting Lynch syndrome ranging from very high (diagnostic DNA testing for all) to somewhat lower (e.g., MSI testing and then diagnostic testing). These strategies will also have varying specificities and costs associated with implementing the testing strategies. For this reason, we undertook a simple cost-consequences analysis with the primary outcome being the number of probands (newly diagnosed CRC cases) identified with Lynch syndrome. Once diagnosed, these probands with Lynch syndrome could then, theoretically, contact first-degree relatives who could consider targeted testing for the family mutation. Thus, the secondary outcome is the number of probands plus the number of relatives identified with Lynch syndrome. Lastly, the incremental costs associated with each of the strategies can be computed, using the one found to be least costly as the baseline.

The four strategies examined were selected because they demonstrated an important target (e.g., Strategy 1 has the highest possible sensitivity) or because they are used (or proposed to be used) in routine clinical practice. The four strategies formally compared are listed below with a short description. All assume that 150,000 individuals are approached and 100,000 agree to

the first line of testing. After the first line of testing, uptake rates from the literature are used.

- **Strategy 1:** All newly diagnosed CRC cases (probands) are offered diagnostic testing (sequencing and deletion/large rearrangement analysis for *MSH2*, *MLH1*, and *MSH6*). The order of MMR gene testing is selected because of both costs (*MSH6* testing is most expensive) and prevalence (mutations in *MSH6* are less common).
- **Strategy 2:** All newly diagnosed CRC cases are offered MSI testing using at least three mononucleotide repeats. Inclusion of three mononucleotide repeats may be an important component in improving the sensitivity of MSI testing, and assuming that most laboratories would be using the NCI standard of five markers (which already includes two mononucleotide repeats), the target of three could be met with a panel of six markers. Those with an MSI-high result are offered diagnostic testing of the three MMR genes in the same order as Strategy 1.
- **Strategy 3:** All newly diagnosed CRC cases are offered IHC testing (for all four MMR genes) and two thirds agree. Those with one or more absent stains are offered diagnostic testing for specific MMR genes, depending on which MMR proteins are absent.
- **Strategy 4:** The same as Strategy 3, except all individuals with absent *MLH1* staining are then offered *BRAF* muta-

Table 14 Cost-consequence model describing the costs of identifying Lynch syndrome among a cohort of 150,000 newly diagnosed colorectal cancer (CRC) cases and among the relatives of probands with Lynch syndrome

Outcome measure	Strategy 1 ^a		Strategy 2 ^b		Strategy 3 ^c		Strategy 4 ^d	
	Number	Costs	Number	Costs	Number	Costs	Number	Costs
Considering only the probands								
Inform newly diagnosed CRC cases	150,000		150,000	3.0	150,000	3.0	150,000	3.0
CRC cases accepting testing	100,000		100,000		100,000		100,000	
Detectable Lynch syndrome (LS)	3000		3000		3000		3000	
MSI testing (six or more markers)	ND		100,000	68.7	ND		ND	
IHC testing (all four MMR genes)	ND		ND		100,000	26.1	100,000	26.1
<i>BRAF</i> V600E mutation testing	ND		ND		ND		8775	0.5
Counseling probands	150,000	26.1	12,060	2.1	13,354	2.3	6405	1.1
DNA sequence/deletion test of one gene	296,609	252.3	43,195	28.0	17,576	12.3	11,262	7.8
Lynch syndrome detected	2537		2198		2105		2097	
Lynch syndrome detection rate (%)	84.6		73.3		70.2		69.9	
False positive LS	144		16		6		4	
Counseling putative LS	2681	0.3	2198	0.2	2111	0.2	2101	0.2
Total costs		279		104		45.9		40.7
Average cost per LS detected		\$111,825		\$47,268		\$21,315		\$18,863
90% Confidence interval		\$83,028–148,040		\$33,266–64,241		\$14,385–29,441		\$12,945–26,111
Considering the probands								
Relatives approachable	10,727	1.1	8856	0.9	8445	0.8	8406	0.8
Relatives accepting counseling	5578	1.0	4605	0.8	4392	0.8	4371	0.8
Relatives having targeted testing	5299	0.3	4375	0.2	4172	0.2	4152	0.2
Relatives w/mutation (counseling)	1504	0.1	1303	0.1	1248	0.1	1243	0.1
Additional costs		2		2		2		2
Considering the probands and their relatives								
Total LS detected	4041		3501		3353		3340	
Average costs per LS detected		\$71,869		\$30,705		\$14,163		\$12,600
90% Confidence interval		\$49,172–100,711		\$20,162–43,632		\$9,184–20,359		\$8,301–17,968
Total program costs		\$281,000,000		\$104,000,000		\$46,000,000		\$41,000,000
Incremental costs per LS detected	\$348,000	(\$244k–\$504k)	\$312,000	(82% of the time)	\$398,000	(\$180–\$940k)		Referent

^aAll individuals have *MSH2*, *MLH1*, and *MSH6* genes sequenced and tested for large deletions and rearrangements.^bAll tumors tested for microsatellite instability; those with MSI-high offered testing for three MMR genes.^cAll tumors tested for absence of MLH1, MSH2, MSH6, and PMS2 protein; MMR gene testing offered as appropriate.^dSame as Strategy 3, except those with MHL1 absent staining have MMR testing only if *BRAF* mutation is missing.

MSI, microsatellite instability; IHC, immunohistochemical; CRC, colorectal cancer; EC, endometrial cancer; MMR, mismatch repair gene; ND, not done.

tion testing and only those without the V600E mutation continue for diagnostic testing. Those with present MLH1 staining (but absent staining for other MMR gene proteins) are treated as in Strategy 3.

- Table 13 shows the information used to inform the economic model; much of it drawn from the current evidence report. The modeling was performed using @RISK v4.5.7 (Palisade Corporation, Ithaca, NY), a Microsoft Excel add-in. All variables, except the 150,000 newly diagnosed cases and 100,000 agreeing to initial testing, were subjected to sensitivity analysis (often assuming a Gaussian distribution centered at the baseline value with a standard deviation equal to 20% of the baseline value). Costs per case of Lynch syndrome detected are provided with 90% confidence intervals based on probabilistic sensitivity analysis with convergence after 5000 iterations. A copy of the model suitable for use with @RISK along with additional information about data used to inform the model (e.g., range of estimates, order of testing) are available from the authors.
- Table 14 shows the results of the cost-consequences analysis for the four example strategies using a population of 150,000 newly diagnosed CRC cases (the approximate number for 2007 in the United States). The rows under Strategy 1 shows the number (and, if appropriate, costs) associated with each activity derived using the baseline estimates. For example, among the 150,000 new CRC cases, 100,000 agree to be tested. Among those 100,000 cases, 3,000 individuals with MMR gene mutations (detectable Lynch syndrome) are estimated to be present. No preliminary laboratory tests are offered. All 150,000 cases need to be provided with information and counseling costing an estimated \$26.1 million. DNA sequencing of the 100,000 cases costs an additional \$252 million for the 296,609 sequencing tests performed (each case needs to be sequenced for each of the three MMR genes, unless a mutation is found first). The total cost is then divided by the number of probands identified with Lynch syndrome to yield the cost per case detected. The reported \$111,825 is the average of the 5000 modeled costs per case detected used for sensitivity analysis and is slightly higher than the baseline estimate. The 90% confidence interval is provided as a measure of the overall reliability of that estimate. Similar analyses are performed for the relatives of probands identified with Lynch syndrome (bottom half of Table 14).
- The number of Lynch syndrome cases detected decreases from Strategies 1 through 4 (2537 of 3000 [85%] to 2097 of 3000 [69.9%]). However, costs per proband diagnosed with Lynch syndrome, costs per proband and relatives diagnosed with Lynch syndrome, and total costs also decrease from Strategies 1 through 4. For example, the cost per proband with CRC detected with Lynch syndrome drops from a high of \$111,825 with Strategy 1 to \$18,863 with Strategy 4. The baseline total costs for the four strategies drop from \$279 million to \$41 million. Whether the tradeoff between lower detection (and lower costs) or higher detection (and higher costs) is worthwhile can be approached by considering the medical benefits and harms via a CEA. Such modeling, however, is beyond the scope of this report.
- One important cost advantage for IHC testing is that the current Medicare reimbursement of \$261 is much less than the corresponding reimbursement for MSI testing (\$457). We reran the simulation with the assumption that the two

tests both cost \$457 and found that much of the apparent advantage of IHC testing is negated. For example, the cost per proband detected for Strategy 2 and Strategy 3 is reported in Table 14 to be \$47,000 vs. \$21,000, but with equal costs of \$457, the costs for the two strategies are much closer, at \$47,000 vs. \$31,000, respectively. This illustrates the care that is needed in interpreting economic analyses.

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