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## Screening for the Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer)

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### ABSTRACT

#### BACKGROUND

Germ-line mutations in the mismatch-repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* lead to the development of the Lynch syndrome (hereditary nonpolyposis colorectal cancer), conferring a strong susceptibility to cancer. We assessed the frequency of such mutations in patients with colorectal cancer and examined strategies for molecular screening to identify patients with the syndrome.

#### METHODS

Patients with a new diagnosis of colorectal adenocarcinoma at the major hospitals in metropolitan Columbus, Ohio, were eligible for the study. Genotyping of the tumor for microsatellite instability was the primary screening method. Among patients whose screening results were positive for microsatellite instability, we searched for germ-line mutations in the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes with the use of immunohistochemical staining for mismatch-repair proteins, genomic sequencing, and deletion studies. Family members of carriers of the mutations were counseled, and those found to be at risk were offered mutation testing.

#### RESULTS

Of 1066 patients enrolled in the study, 208 (19.5 percent) had microsatellite instability, and 23 of these patients had a mutation causing the Lynch syndrome (2.2 percent). Among the 23 probands with the Lynch syndrome, 10 were more than 50 years of age and 5 did not meet the Amsterdam criteria or the Bethesda guidelines for the diagnosis of hereditary nonpolyposis colorectal cancer (including the use of age and family history to identify patients at high risk for the Lynch syndrome). Genotyping for microsatellite instability alone and immunohistochemical analysis alone each failed to identify two probands. In the families of 21 of the probands, 117 persons at risk were tested, and of these, 52 had Lynch syndrome mutations and 65 did not.

#### CONCLUSIONS

Routine molecular screening of patients with colorectal adenocarcinoma for the Lynch syndrome identified mutations in patients and their family members that otherwise would not have been detected. These data suggest that the effectiveness of screening with immunohistochemical analysis of the mismatch-repair proteins would be similar to that of the more complex strategy of genotyping for microsatellite instability.

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**L**ARGE-SCALE SCREENING FOR GERM-line mutations that lead to the onset of disease in adulthood is becoming increasingly possible owing to technical advances. Even when screening is technically feasible, however, it does not necessarily follow that it is desirable.<sup>1</sup> Issues that affect screening include the accuracy, sensitivity, and specificity of the test, the benefit to the patient, the possibly negative ramifications of the results, and the cost.<sup>2</sup> One disease discussed as a plausible candidate for screening is the Lynch syndrome (hereditary nonpolyposis colorectal cancer).<sup>1</sup>

The Lynch syndrome is caused mainly by mutations in the DNA mismatch-repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Heterozygosity for a mutation results in susceptibility to the cancer. The Lynch syndrome can be identified on the basis of age at onset and the characteristics of the family history that fulfill the Amsterdam criteria for the diagnosis of hereditary nonpolyposis colorectal cancer.<sup>3,4</sup> The less stringent Bethesda guidelines for this diagnosis have been proposed to help select patients for molecular testing.<sup>5,6</sup> Even among persons attending clinics for those at high risk for cancer, the sensitivity of the criteria is only 40 to 80 percent,<sup>7</sup> and among unselected patients with colorectal cancer, the sensitivity is 50 percent or less.<sup>8,9</sup> Therefore, the strategies for diagnosing the Lynch syndrome need to be improved.

One possible improvement that has been suggested is based on the presence of a germ-line mutation in a mismatch-repair gene. However, because searching for mutations in the four mismatch-repair genes is difficult and expensive, molecular prescreening for microsatellite instability of the patient's tumor has been performed. Microsatellite instability is a hallmark of the Lynch syndrome,<sup>10</sup> occurring in more than 90 percent of tumors.<sup>11</sup> Microsatellite-instability status is commonly determined by analysis of five genetic markers.<sup>12</sup>

Aside from prescreening for the Lynch syndrome, testing for microsatellite instability is important because of its prognostic and possible therapeutic implications. Patients with colorectal cancer whose tumors have high-frequency microsatellite instability have a more favorable prognosis, independently of stage at diagnosis, than do patients without microsatellite instability.<sup>13</sup> Moreover, according to recent reports, patients with high-frequency microsatellite instability do not benefit from adjuvant chemotherapy with fluorouracil.<sup>13,14</sup> Consequently, there is a need to determine the status

of microsatellite instability in large series of patients with colorectal cancer.

We undertook this study with two aims. The first was to determine the proportion of patients with colorectal cancer who have the Lynch syndrome and the feasibility of molecular screening for the syndrome through the study of unselected patients with newly diagnosed colorectal cancer. The second was to compare the effectiveness of two different techniques to prescreen for mismatch-repair deficiency, genotyping for microsatellite instability and immunohistochemical analysis.

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## METHODS

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### PATIENTS

We enrolled 1066 patients with newly diagnosed adenocarcinoma of the colorectum, regardless of age or the presence or absence of a family history of cancer, at six participating hospitals. These hospitals perform the vast majority of operations for colorectal cancer or suspected colorectal cancer in the Columbus, Ohio, metropolitan area (population, 1.5 million). The research protocol and consent form were approved by the institutional review board at each participating hospital, and all patients provided written informed consent. A family medical history, a blood sample of 10 to 20 ml, and a tissue specimen were obtained from each patient.

### SAMPLES

DNA and RNA were extracted from the EDTA-preserved blood samples with the use of standard methods. The histologic features of the tumor were reevaluated by analysis of the paraffin-embedded tissue block, and an area containing tumor cells was marked on the block. The proportion of tumor cells in the material used for the extraction of DNA exceeded 50 percent in 95 percent of the cases and exceeded 40 percent in the remaining cases. In addition, an area containing no tumor cells (i.e., normal tissue) was marked on the block. Material from the tumor and normal tissue was obtained by microdissection.

### MICROSATELLITE INSTABILITY

To determine the microsatellite instability of the tumor, we ascertained the genotypes using five or six polymorphic markers (BAT25, BAT26, D2S123, D5S346, and D18S69 or D17S250 or both) in tumor tissue and unaffected tissue. Results of testing for microsatellite instability were considered positive

when an allele was present in the tumor but not in the unaffected tissue. High-frequency microsatellite instability was defined as instability shown by two or more markers, and low-frequency microsatellite instability as instability shown by only one marker; if none of the markers showed instability, the result was considered to be negative.

#### IMMUNOHISTOCHEMICAL STAINING

Immunoperoxidase staining was performed on formalin-fixed tissue.<sup>15</sup> The primary antibodies used were MLH1 (1:10 dilution, Pharmingen), MSH2 (1:200, Oncogene Research Products), MSH6 or GTBP (1:300, Transduction Laboratories), and PMS2 (C20) (1:400, Santa Cruz Biotechnology).

#### DETECTION OF MUTATIONS

To search for germ-line mutations, DNA (obtained from blood or normal colon tissue) was directly sequenced with the use of primers described previously.<sup>8,16,17</sup> The sequencing of the *MLH1*, *MSH2*, and *MSH6* genes covered the promoter regions (*MLH1* and *MSH2* only), exons, and the intronic regions adjacent to all splice sites. For *PMS2*, conversion to haploidy (GMP Genetics) and Western blot analysis were used in addition to sequencing.<sup>15</sup> In search of potentially missed sequence changes, a proprietary method (Ambry Test, Ambry Genetics) was applied to 150 randomly chosen patients among the 208 patients with microsatellite instability. This method included a scan for mutations in all exons and exon-intron junctions of the *MSH2* and *MLH1* genes with the use of temporal temperature gradient electrophoresis, after which suspect regions were sequenced. All disease-causing mutations, but no additional ones, were confirmed with the use of this technique. We used multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland) to search for large deletions in the *MLH1* and *MSH2* genes.<sup>18,19</sup>

Missense changes in the mismatch-repair genes are common and often pose a formidable problem of interpretation,<sup>20,21</sup> because these changes do not necessarily affect the function of the protein. To determine the clinical significance of these changes, we relied on multiple lines of evidence and experiments (described in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). When we obtained no clear indication of the pathogenicity of a missense change or of a neutral mutation, we classified it as a variant of uncertain significance for the purpose of this study. However, probands and family members were care-

fully counseled with regard to the uncertainty of the interpretation and to its consequences.

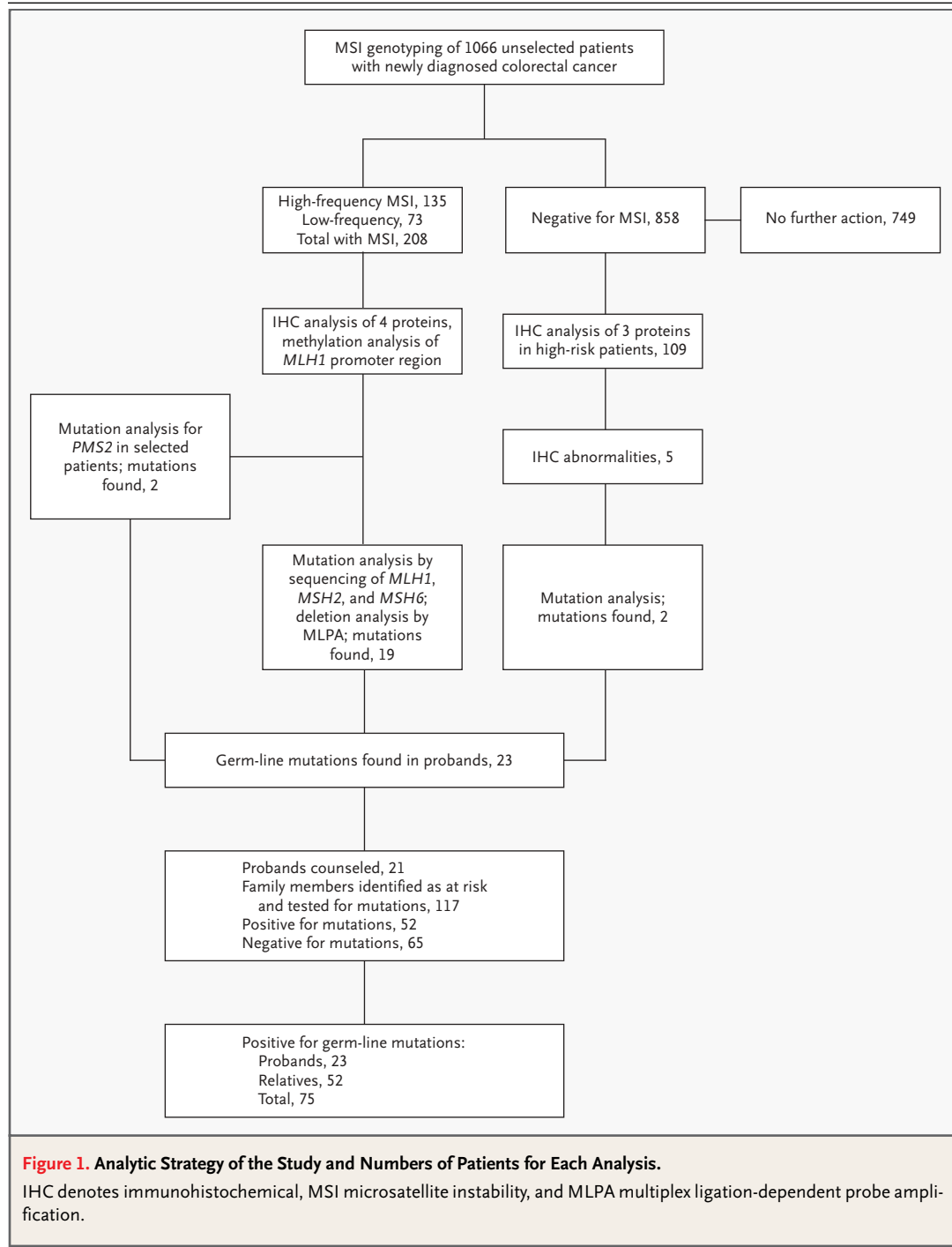
#### METHYLATION OF THE PROMOTER REGION OF *MLH1*

A region in the 5' part of the promoter region (i.e., the H region) was studied for methylation with the use of the methylation-specific polymerase chain reaction.<sup>22</sup> Another region closer to exon 1 (i.e., the D region) was studied with the use of combined bisulfite restriction analysis.<sup>23,24</sup>

#### ANALYTIC STRATEGY

Of the 1066 tumors analyzed for microsatellite instability, only 208 that were considered positive were scrutinized further (Fig. 1). All tumors considered positive underwent sequencing of the *MLH1*, *MSH2*, and *MSH6* genes, MLPA deletion analysis of *MLH1* and *MSH2*, immunohistochemical analysis for the four mismatch-repair proteins, and methylation analysis of the *MLH1* promoter region. Selected tumors that showed the presence of the MLH1 protein and the absence of the PMS2 protein were analyzed for mutations in the *PMS2* gene (Table 1 in the Supplementary Appendix).

In addition, in an effort to identify tumors deficient in mismatch-repair proteins that had not been found in the analysis for microsatellite instability, tumors that were negative for microsatellite instability from patients who were at high risk for the Lynch syndrome were studied with the use of immunohistochemical analysis for the MLH1, MSH2, and MSH6 proteins. Of the 858 tumors negative for microsatellite instability, 109 were selected for these additional studies, because the patients were at high risk on the basis of one or more of the following criteria: a diagnosis of colorectal cancer before the age of 50 years, a diagnosis of synchronous or metachronous colorectal or endometrial cancer, and the presence of a first-degree relative with colorectal or endometrial cancer diagnosed at any age. Of the 109 patients, 5 patients (4.6 percent) had an abnormal result on immunohistochemical analysis and therefore underwent both a second microsatellite-instability analysis and sequencing of the suspect gene. In four of these five patients, the tumors were extremely mucinous, and two of these four tumors had high-frequency microsatellite instability when a different area of the tumor with higher cellularity was studied. In these two patients, germ-line mutations were found. We describe these two cases as missed by the analysis for microsatellite instability.



RESULTS

**PATIENTS**

From April 1999 through August 2004, 1581 patients were enrolled in the study. At the time of the data analysis, 1066 of these patients had undergone molecular evaluation. The mean age among

these patients was 62.9 years; 90 percent reported that they were white, and 8 percent reported that they were black, percentages that reflected those in the 2000 report of the U.S. Census Bureau for Ohio.<sup>25</sup> Slightly less than half the patients (45 percent) were female.

The study included patients who were seen by

**Table 1. Characteristics of 23 Patients with Colorectal Cancer and Deleterious Mutations in a Mismatch-Repair Gene.**

Patient No.	Sex	Age*	Site of Colon Cancer	Criteria Met (First-Degree Relatives Only)†		Microsatellite-Instability Status  <i>no. of positive markers/ total no. of markers</i>	Gene	Nucleotide Change‡	Mutation§
				Amsterdam Criteria	Bethesda Criteria				
1	F	52	Descending	Yes	Yes	4/5¶	<i>MLH1</i>	c.298C>T	p.Arg100X
2	F	46	Ascending	No	Yes	5/5	<i>MLH1</i>	c.790+1G>A	Skip exon 9
3	M	39	Rectosigmoid	No	Yes	5/5	<i>MLH1</i>	c.1192C>T	p.Gln398X
4	F	46	Ascending	No	Yes	4/5	<i>MLH1</i>	c.1489delC	p.Arg497GlnfsX11
5	F	47	Ascending	No	Yes	5/5	<i>MLH1</i>	c.1778_1779delCA	p.Pro593ArgfsX16
6	F	38	Cecum	No	Yes	2/6	<i>MSH2</i>	c.425C>G	p.Ser142X
7	M	45	Rectosigmoid	No	Yes	4/5	<i>MSH2</i>	c.586delC	p.Pro196GlnfsX18
8	F	63	Rectum and sigmoid	No	Yes	5/5	<i>MSH2</i>	c.942+3A>T	Skip exon 5
9	M	58	Transverse and rectosigmoid	Yes	Yes	4/6	<i>MSH2</i>	c.942+3A>T	Skip exon 5
10	M	34	Ascending	No	Yes	4/5¶	<i>MSH2</i>	c.942+3A>T	Skip exon 5
11	M	41	Cecum	No	Yes	5/5	<i>MSH2</i>	c.942+3A>T	Skip exon 5
12	F	33	Transverse and cecum	No	Yes	4/5	<i>MSH2</i>	c.942+3A>T	Skip exon 5
13	M	51	Cecum	No	No	4/5	<i>MSH2</i>	c.1147C>T	p.Arg383X
14	M	70	Rectum	No	Yes	4/6	<i>MSH2</i>	c.1906G>C	p.Ala636Pro
15	M	57	Splenic flexure	No	No	5/5	<i>MSH2</i>	c.2648dupA	p.Ile883AsnfsX16
16	M	45	Transverse	No	Yes	4/5	<i>MSH2</i>	g.26399108_26483640del84533 (NT_022184)	Deletion of exons 1–7
17	M	33	Ascending	No	Yes	5/5	<i>MSH2</i>	g.26488048_26491055del3008 (NT_022184)	Deletion of exon 8
18	F	46	Transverse	Yes	Yes	4/5	<i>MSH2</i>	g.26446264-?_26514134+? (NT_022184)	Deletion of exons 1–11
19	M	87	Rectum	No	Yes	3/5	<i>MSH6</i>	c.3155_3156delAG	p.Glu1052ValfsX13
20	M	67	Ascending	No	No	2/5	<i>MSH6</i>	c.3261delC	p.Phe1088SerfsX2
21	M	56	Sigmoid	No	No	4/5	<i>MSH6</i>	c.3956_3959delAAGC	p.Ala1320GlnfsX6
22	M	82	Sigmoid	No	No	5/5	<i>PMS2</i>	Unknown	—
23	M	23	Transverse	No	Yes	4/5	<i>PMS2</i>	c.2192_2196delTAACT	p.Leu731CysfsX3

\* The mean age of the patients was 50.4 years, and the median age was 46 years.

† Data on the Amsterdam criteria for a diagnosis of hereditary nonpolyposis colorectal cancer are from Vasen et al.,<sup>3,4</sup> and data on the Bethesda guidelines for this diagnosis are from Rodriguez-Bigas et al.<sup>5</sup> and Umar et al.<sup>6</sup>

‡ Abbreviations are in accordance with the current nomenclature.<sup>26</sup> The letter “c” indicates that the numbering starts with the first nucleotide of the complementary DNA.

§ The letter “p” refers to the numbering of the amino acids in the protein.

¶ The results of testing for microsatellite instability were originally found to be negative but showed high-frequency status when the test was repeated on a different area of the tumor after an abnormal immunohistochemical finding.

|| No protein from the affected allele was detected on Western blot analysis of a haploid converted clone.<sup>15</sup>

approximately 110 physicians. Only 144 patients who were approached declined to participate in the study, with a range of 23 to 70 patients declining at each of the sites. The main reasons given for declining to participate were lack of interest, no family members who were at risk, not wanting to “wor-

ry about genetics” or involve family members, and concern about putting insurance coverage at risk.

**MICROSATELLITE INSTABILITY**

High-frequency microsatellite instability was detected in 135 of the 1066 tumors analyzed (12.7

percent), and low-frequency microsatellite instability was detected in 73 tumors (6.8 percent). No mutations were found in patients whose tumors had low-frequency microsatellite instability.

#### IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical analysis had an excellent sensitivity for the detection of tumors with high-frequency microsatellite instability: 123 of 132 tumors that showed high-frequency microsatellite instability (3 analyses failed because of insufficient tumor material on the slides) also showed abnormalities on immunohistochemical analysis for at least one protein (93.2 percent sensitivity; 95 percent confidence interval, 88.9 to 97.4 percent). The sensitivity of immunohistochemical analysis to pinpoint the affected gene in tumors with deleterious mutations was high: MSH2 was detected in 11 of 12 tumors, the *MLH1* gene in 4 of 5, MSH6 in 3 of 3, and PMS2 in 2 of 2. In contrast to high-frequency microsatellite instability, low-frequency microsatellite instability showed rare abnormalities on immunohistochemical analysis — staining showed abnormalities in only 10 of 70 tumors.

#### PROBANDS WITH THE LYNCH SYNDROME

There were 23 probands with a deleterious mutation (Table 1). None of these patients had previously received a diagnosis of the Lynch syndrome. Mutations in *MSH2* (13 patients) were more common than mutations in *MLH1* (5 patients), *MSH6* (3 patients), and *PMS2* (2 patients). Five probands had the A→T mutation of intron 5 in the donor splice site of *MSH2*, which is the most commonly recurring mutation in a mismatch-repair gene in humans.<sup>27,28</sup> Six of the patients had had a metachronous tumor when they were younger, and three had synchronous primary colorectal cancers. Among the probands, the mean age at diagnosis was 50.4 years (range, 23 to 87). Had testing been limited to those less than 50 years of age — a commonly applied criterion<sup>6</sup> — 10 of the 23 probands would not have been detected. Of the 23 probands, 3 fulfilled the Amsterdam criteria for the diagnosis of the Lynch syndrome,<sup>3,4</sup> and an additional 15 fulfilled the Bethesda guidelines for testing.<sup>5,6</sup> Five of the 23 probands fulfilled none of these criteria.

#### PEDIGREE ANALYSIS AND MUTATIONAL STATUS OF RELATIVES

Of the 23 probands with colorectal cancer and deleterious mutations, to date 21 have received ge-

**Table 2. Results of Testing for the Proband's Mutation among Relatives of 21 Probands.**

Relationship	Tested	Positive	Negative
First degree	54	25	29
Second degree	22	10	12
Third degree and beyond	41	17	24
Total	117*	52†	65

\* Of the 119 relatives who received counseling and were offered testing, 2 chose not to undergo testing.

† Of these 52 relatives, 14 had previously had a cancer related to the Lynch syndrome and 38 were unaffected at the time of testing. None of the 52 relatives had previously received a diagnosis of the Lynch syndrome.

netic counseling. As part of the counseling, each of the 21 probands was offered confirmation of the results in our study through testing of a new blood sample in a laboratory approved according to the standards of the Clinical Laboratory Improvement Act. Genetic counseling and testing were offered at no cost to all relatives who were found to be at risk. Among a total of 117 relatives who received counseling and testing, test results were positive for the mutation among 52 relatives and negative among 65 relatives (Table 2). Of the 52 relatives with positive test results, 14 had had a cancer related to the Lynch syndrome and 38 were unaffected at the time of testing (2 of these 38 relatives subsequently received a diagnosis of cancer).

#### METHYLATION OF THE *MLH1* PROMOTER REGION

Although the power of immunohistochemical staining to detect hereditary nonpolyposis colorectal cancer is high for the *MSH2* and *MSH6* proteins, staining for *MLH1* and its heterodimer partner *PMS2* is often lost in sporadic colorectal cancer due to hypermethylation of the *MLH1* promoter region.<sup>29</sup> Among the 135 tumors with high-frequency microsatellite instability, 106 showed methylation of the *MLH1* promoter region — the H region, the D region, or both. None of the 23 patients with a germ-line mutation, including 7 with mutations in *MLH1* or *PMS2*, showed methylation in the D region (described in the Supplementary Appendix).

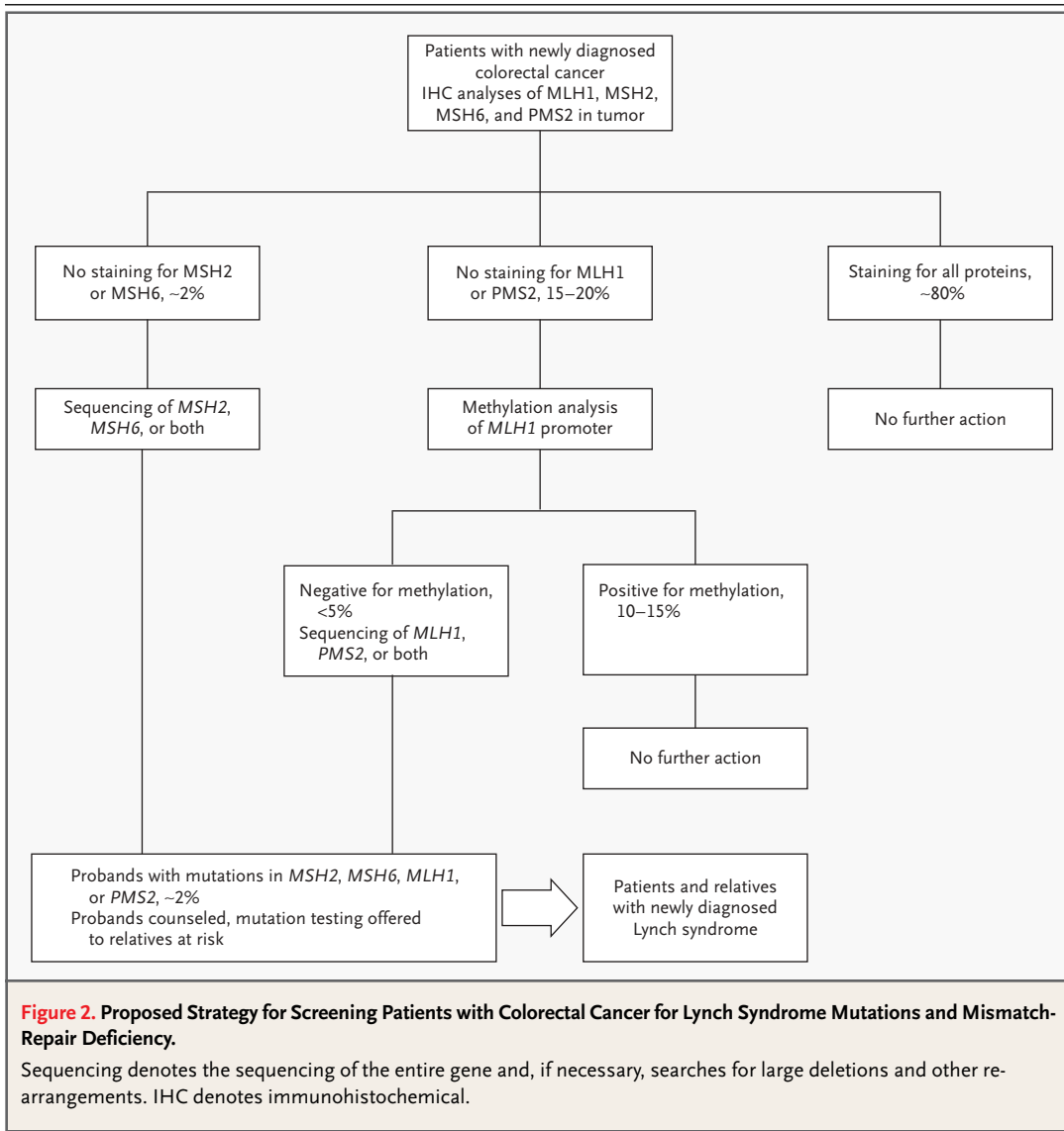
#### DISCUSSION

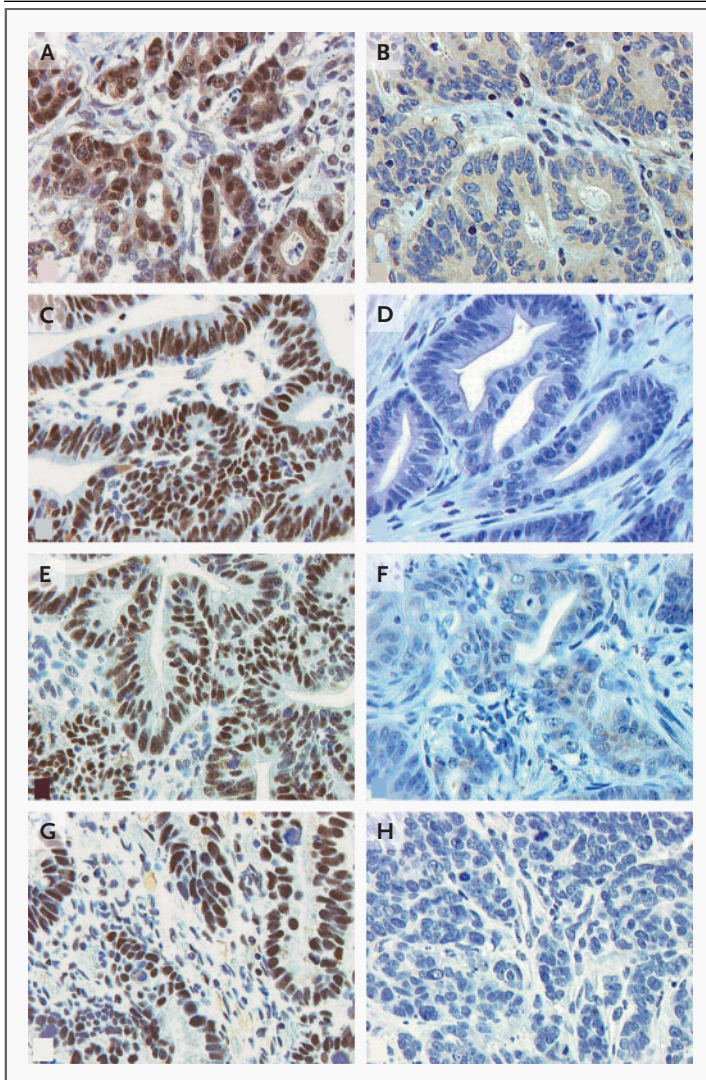
Our study showed that screening large numbers of patients with colorectal cancer is feasible, and in the setting of this clinical trial, screening was well

received by both patients and medical personnel. The study showed that at least 23 of the 1066 patients who underwent testing were found to have a deleterious mutation in a mismatch-repair gene. Thus, the proportion of all colorectal cancers that was due to the Lynch syndrome was at least 2.2 percent. This figure is the highest recorded in the United States and is broadly in accord with the results of several previous studies,<sup>6,8,9,30</sup> whereas others have reported lower estimates.<sup>21,31,32</sup> All numbers, including ours, are underestimates, because none of the methods for prescreening and detecting mutations are 100 percent sensitive; in this study, a few cases might have been missed. Moreover, among

the numerous sequence changes of uncertain interpretation (described in the Supplementary Appendix), some may well be deleterious.

A striking fact was that among the 23 probands with the Lynch syndrome, as many as 10 were more than 50 years of age and only 3 fulfilled the Amsterdam criteria for the syndrome. This finding emphasizes the need to search for the Lynch syndrome outside the typical "high-risk" situation. Extensive counseling of 21 of the 23 probands led to the tracing of pedigrees and family members. Acceptance among family members receiving counseling was excellent, and all but 2 of 119 family members at risk who received counseling chose to accept





**Figure 3. Immunohistochemical Staining for Mismatch-Repair Proteins in Colorectal Adenocarcinoma.**

Panel A shows positive staining for MLH1, Panel B negative staining for MLH1, Panel C positive staining for MSH2, Panel D negative staining for MSH2, Panel E positive staining for MSH6, Panel F negative staining for MSH6, Panel G positive staining for PMS2, and Panel H negative staining for PMS2.

the offer of testing for the proband's mutation, for 52 of whom the results of testing were positive. These people were referred to their own physicians for high-risk surveillance. Moreover, the results of testing of 65 family members at risk were negative for the mutation, and these people were counseled accordingly.

In the diagnosis of the Lynch syndrome, the key procedure is the sequencing of the mismatch-repair genes for mutations, an undertaking that is

demanding and expensive owing not only to laboratory expenses but also to the need for time-consuming interpretation of the sequence tracings. Typically, at least the *MLH1*, *MSH2*, and *MSH6* genes need to be sequenced when there is no clue to which gene is affected. In large-scale screening, performing this procedure is simply not possible, so pre-screening for mismatch-repair deficiency is necessary and has been widely practiced with the use of genotyping for microsatellite instability.<sup>5,6,8,12</sup>

Both genotyping for microsatellite instability and immunohistochemical analysis require a pathology laboratory and interpretation by experts. Genotyping for microsatellite instability requires a molecular laboratory as well. However, both methods are much less demanding, together amounting to perhaps 1/10 of the effort required for sequencing three genes. Both methods can be used to measure mismatch-repair deficiency, and, given the similar costs of the two methods, the choice between them will depend on such factors as availability and the sensitivity and specificity of the test. Immunohistochemical analysis as the primary screening method (Fig. 2) has several advantages over genotyping for microsatellite instability, in particular the fact that immunohistochemical analysis is available as part of routine services in general pathology laboratories (Fig. 3). Limiting the primary screening method to immunohistochemical analysis would not require the involvement of a molecular genetics laboratory, nor in 80 percent of cases would a blood sample need to be obtained. With the use of the strategy diagrammed in Figure 2, only about 20 percent of patients will need molecular testing, and well less than 10 percent will need to undergo a search for mutations, often in just one gene. Moreover, tumors that do not stain for MLH1 will need to be sequenced for germ-line mutations only if they do not show methylation. This procedure constitutes a considerable savings of time, effort, and cost over the method used in this study.

In our study of 1066 patients, the sensitivity and specificity of genotyping for microsatellite instability, as opposed to immunohistochemical analysis, to detect the Lynch syndrome could have been determined only by performing sequencing, genotyping for microsatellite instability, and immunohistochemical analysis on every sample; but this was not possible, owing to cost constraints. Nevertheless, our results allow us to conclude that the sensitivity of immunohistochemical analysis to detect high-frequency microsatellite instability was

appropriate in 123 of 132 samples (93 percent). This result is in line with that of a large study in which immunohistochemical analysis had a 100 percent sensitivity to detect high-frequency microsatellite instability among 1144 patients with colorectal cancer.<sup>33</sup> Thus, no great loss in sensitivity will occur if molecular prescreening for microsatellite instability is replaced by immunohistochemical analysis.

With regard to sensitivity to detect the Lynch syndrome, our results show that both methods failed to identify 2 of 23 cases. For the two cases not detected with the use of molecular prescreening for microsatellite instability, the initial results were false negative genotyping in DNA obtained from highly mucinous tumors. For the two cases not detected with the use of immunohistochemical analysis (one with a truncating mutation in *MLH1*, the other with a truncating mutation in *MSH2*), there was no obvious explanation for the normal-appearing staining of the respective protein. Given that we could not assess the sensitivity by studying all patients with both methods, the question remains, how many patients with the Lynch syndrome may have been missed among the 749 patients with tumors for which the results of testing for microsatellite instability were negative and that did not undergo immunohistochemical analysis. Only indirect evidence can be invoked. For instance, in studies conducted in Finland, where founder mutations account for more than 50 percent of all diagnoses of the Lynch syndrome, 915 patients with colorectal cancer whose test results were negative for microsatellite instability had no founder mutations on mutation-specific testing.<sup>8,9</sup> We estimate that in our study at most a very small number of patients with the Lynch syndrome were missed.

In the United States, the public health perspective on truly large-scale screening for the Lynch syndrome can be illustrated as follows. On the basis of our data, some 2 percent of the 148,000 patients with newly diagnosed colorectal carcinoma<sup>7</sup>

are carriers of a mutation for the Lynch syndrome (2960 carriers), and among the family members of each proband, 3 more carriers can be detected (8880 carriers). Thus, the proposed screening provides an opportunity to diagnose the Lynch syndrome in 11,840 persons annually in the United States. These numbers are only estimates, and the proposed strategies need to be tested on an even larger scale than the present study. The proposed screening does not primarily target unaffected persons; instead, only probands with cancer are targeted. The search for the Lynch syndrome in these patients may be seen as part of an appropriate clinical workup. The way in which the patient is treated will be profoundly affected in the presence of a diagnosis of the Lynch syndrome. For instance, subtotal colectomy is generally recommended because of the high risk of metachronous tumor,<sup>34</sup> and lifelong surveillance for colorectal cancer, endometrial cancer, and other tumors is indicated.<sup>35</sup> A clear benefit of intensified clinical surveillance is well documented.<sup>36</sup> Testing of family members who are at risk for the syndrome allows triage according to the presence or absence of the mutation.

Finally, patients with tumors with high-frequency microsatellite instability have a better prognosis than those with tumors without microsatellite instability,<sup>13</sup> and they may not benefit from fluorouracil-based adjuvant chemotherapy.<sup>13,14</sup> Thus, determining the mismatch-repair status (with the use of genotyping for microsatellite instability or immunohistochemical analysis) of all patients with colorectal cancer has prognostic implications and may serve as a guide to optimal chemotherapy.

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Dr. de la Chapelle reports holding patents for the human *MSH2* protein and for diagnostic methods involving *MSH2*. Dr. Kuebler reports having served as a paid speaker and consultant for Sanofi.

#### APPENDIX

In addition to the authors, the following investigators participated in this study: The Ohio State University Medical Center, Columbus — C. Ellison, S. Melvin, J. Winston III; the Human Cancer Genetics Program, the James Cancer Hospital and Solove Research Institute at Ohio State University, Columbus — A. Adeli, W. Burak, R. Chadwick, I. Elkhatib, T. Hemingway, K. Jamieson, C. Johnson, J. LaJeunesse, S. Liyanarachchi, P. Rangel, D. Soble, M. Walker, T. Wise, Y. Zhang; Ohio State University Hospital East, Columbus — R. Schlanger; Mount Carmel East Medical Center, Columbus, Ohio — P. Aguilar, D. Hura, J. Keith, B. Kerner, G. Lavalle, C. Taylor, T. Vara, J. Zangmeister; Mount Carmel West Medical Center, Columbus, Ohio — S. DeVictor, L. Hines, M. Lindsey, J. Madhavan, A. Padmanabhan; St. Ann's Hospital, Westerville, Ohio — K. Hamelberg, T. Niemann; Riverside Methodist Hospital (OhioHealth), Columbus, Ohio — B.C. Behrens, S.C. Blair, M. Brimer, C.S. George, W.J. Hicks, J.K. Hofmeister, P.J. Kourlas, J.A. Matyas, J. Mitchell, K.E. Nichols, T.J. Sweeney, R.L. Toscano, W.L. Wheeler, W. Wise, T. Williams; Grant Medical Center (OhioHealth), Columbus, Ohio — S. Miller, T.D. Moore; Ambry Genetics, Irvine, Calif. — C.L.M. Dunlop, A. Kammesheidt, J. Schymick; GMP Genetics, Waltham, Mass. — N. Papadopoulos.

## REFERENCES

- Beaudet AL. Making genomic medicine a reality. *Am J Hum Genet* 1999;64:1-13.
- Motulsky AG. Screening for genetic diseases. *N Engl J Med* 1997;336:1314-6.
- Vasen HFA, Mecklin J-P, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (IGC-HNPCC). *Dis Colon Rectum* 1991;34:424-5.
- Vasen HFA, Watson P, Mecklin J-P, Lynch HT. New clinical criteria for hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. *Gastroenterology* 1999;116:1453-6.
- Rodriguez-Bigas MA, Boland CR, Hamilton SR, et al. National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 1997;89:1758-62.
- Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer and microsatellite instability. *J Natl Cancer Inst* 2004;96:261-8.
- Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;348:919-32.
- Aaltonen LA, Salovaara R, Kristo P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;338:1481-7.
- Salovaara R, Loukola A, Kristo P, et al. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000;18:2193-200. [Erratum, *J Clin Oncol* 2000;18:3456.]
- Aaltonen LA, Peltomäki P, Leach FS, et al. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993;260:812-6.
- Aaltonen LA, Peltomäki P, Mecklin J-P, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 1994;54:1645-8.
- Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248-57.
- Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;349:247-57.
- Carethers JM, Smith EJ, Behling CA, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004;126:394-401.
- Nakagawa H, Lockman JC, Frankel WL, et al. Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. *Cancer Res* 2004;64:4721-7.
- Chadwick RB, Pyatt RE, Niemann TH, et al. Hereditary and somatic DNA mismatch repair gene mutations in sporadic endometrial carcinoma. *J Med Genet* 2001;38:461-6.
- Goodfellow PJ, Buttin BM, Herzog TJ, et al. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. *Proc Natl Acad Sci U S A* 2003;100:5908-13.
- Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* 2002;30:e57.
- Nakagawa H, Hampel H, de la Chapelle A. Identification and characterization of genomic rearrangements of MSH2 and MLH1 in Lynch syndrome (HNPCC) by novel techniques. *Hum Mutat* 2003;22:258.
- Cotton RG, Scriver CR. Proof of "disease causing" mutation. *Hum Mutat* 1998;12:1-3.
- Samowitz WS, Curtin K, Lin HH, et al. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology* 2001;121:830-8.
- Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci U S A* 1998;95:6870-5.
- Deng G, Chen A, Hong J, Chae HS, Kim YS. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. *Cancer Res* 1999;59:2029-33.
- Nakagawa H, Nuovo GJ, Zervos EE, et al. Age-related hypermethylation of the 5' region of MLH1 in normal colonic mucosa is associated with microsatellite-unstable colorectal cancer development. *Cancer Res* 2001;61:6991-5.
- Ohio QuickFacts. Washington, D.C.: Census Bureau, 2005. (Accessed April 11, 2005, at <http://quickfacts.census.gov/qfd/states/39000.html>.)
- den Dunnen JT, Antonarakis SE. Nomenclature for the description of human sequence variations. *Hum Genet* 2001;109:121-4.
- InSiGHT home page. (Accessed April 11, 2005, at <http://www.insight-group.org>.)
- Desai DC, Lockman JC, Chadwick RB, et al. Recurrent germline mutation in MSH2 arises frequently de novo. *J Med Genet* 2000;37:646-52.
- Kane MF, Loda M, Gaida GM, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997;57:808-11.
- Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. *Am J Hum Genet* 2001;69:780-90. [Erratum, *Am J Hum Genet* 2001;69:1160.]
- Percesepe A, Borghi F, Menigatti M, et al. Molecular screening for hereditary nonpolyposis colorectal cancer: a prospective, population-based study. *J Clin Oncol* 2001;19:3944-50.
- Ravnik-Glavac M, Potocnik U, Glavac D. Incidence of germline hMLH1 and hMSH2 mutations (HNPCC patients) among newly diagnosed colorectal cancers in a Slovenian population. *J Med Genet* 2000;37:533-6.
- Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 2002;20:1043-8.
- Burke W, Petersen G, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. *JAMA* 1997;277:915-9.
- Lynch PM. Clinical challenges in management of familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. *Cancer* 1999;86:Suppl:2533-9.
- Järvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2000;118:829-34.

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