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ORIGINAL REPORT

Immunohistochemistry Identifies Carriers of Mismatch Repair Gene Defects Causing Hereditary Nonpolyposis Colorectal Cancer

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ABSTRACT

Purpose
Hereditary nonpolyposis colorectal cancer (HNPCC) may be caused by mutations in mismatch repair (MMR) genes. The aim of this study was to validate immunohistochemistry and family history as prescreening tools to predict germline mutations in *MLH1, MSH2,* and *MSH6*.

Patients and Methods
Pedigrees from 250 families were extended, cancer diagnoses were verified, and families were classified according to the Amsterdam and the Bethesda criteria. Tumor specimens were examined with immunohistochemistry for the presence of MLH1, MSH2, and MSH6 proteins. Mutation analyses were performed in blood samples from the same patients.

Results
Blood samples from affected index persons in 181 families and tumor specimens from 127 of the affected index persons were obtained. Thirty tumors lacked one or more gene products. Sensitivity of immunohistochemistry to detect mutation carriers was 100%, specificity was 82%, and positive predictive value was 85%. Sensitivities, specificities, and positive predictive values for the Amsterdam criteria were 82%, 8%, and 45%, respectively, and for the Bethesda criteria were 100%, 0%, and 48%, respectively. Distribution of mutations was *MLH1* = 4, *MSH2* = 11, and *MSH6* = 4.

Conclusion
Wide clinical criteria to select HNPCC kindreds, followed by immunohistochemistry of tumor material from one affected person in each family, had high sensitivity and specificity to predict MMR mutations.

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INTRODUCTION

Approximately 10% of colorectal cancers may be inherited.[1] Because colorectal cancer can be cured by early diagnosis and treatment, identifying those people at risk in order to facilitate an early diagnosis became a challenge.[2] A description of early-onset dominantly inherited colorectal cancer (hereditary nonpolyposis colorectal cancer; HNPCC) was adopted.[3] Two to six percent of colorectal cancers are caused by mutations in known mismatch repair (MMR) genes.[4] People carrying mutations develop intestinal polyps at about the same frequency as the general population, but at a younger age.[5] The adenomas are more likely to undergo malignant transformation and to display an accelerated adenoma-to-carcinoma transition, compared with the adenomas seen in the general population.[6,7] The infiltrating cancers may have a better prognosis compared with sporadic colorectal cancers with the same tumor histology.[8,9]

Demonstration of mutations may also give information to select treatment modalities of HNPCC-related tumors, because MMR-deficient tumors seem to be resistant to certain chemotherapeutic agents.[10]

The criteria used to recognize inherited colorectal cancer were previously limited to family history alone. The initial criteria were referred to as the Amsterdam criteria (Amsterdam criteria I).[3] These criteria were later revised to include extracolonic cancers of the endometrium, small bowel, ureter, and renal pelvis (Amsterdam criteria II).[11] Another set of criteria referred to as the Bethesda guidelines were also established.[12]

Tumors caused by MMR gene defects demonstrate microsatellite instability (MSI). However, MSI occurs in 10% to 15% of sporadic cancers and inherited *MSH6* mutations frequently do not cause MSI in the tumors.[13,14]

Germline mutations in the MMR genes are responsible for the predisposition to cancer in a number of HNPCC families.[15-21] The majority of mutations are detected in *MLH1*, *MSH2,* and *MSH6*,[20,22] whereas only a few are detected in *PMS1* and *PMS2*.[22-24] The genes encode protein products that recognize and correct errors that arise when DNA is replicated.[17,25]

According to the two-hit model of carcinogenesis, the second event in addition to the inherited mutation is expected to be an acquired mutation in the normal allele, leaving the gene product of the gene in question absent in the tumor cells.[26] This has been demonstrated by immunohistochemistry.[14,27-31]

In this report, we validate the sensitivity, specificity, and predictive value of immunohistochemistry, compared with various clinical criteria, to select HNPCC kindreds for mutation testing.

PATIENTS AND METHODS

Patients

The patients were referred to The Norwegian Radium Hospital (Norway, Oslo). The patients were included according to their family history of colorectal and other cancers, applying wide clinical criteria.[32] After expansion of the family history and verification of diagnoses, the patients were reclassified in accordance with the Amsterdam and Bethesda criteria.

Initially, the first 56 families with putative inherited colorectal cancer were examined.[31] The next 250 consecutive families identified were included in this report. Inclusion criteria for this report were families fulfilling Amsterdam criteria I or II, aggregation of four or more HNPCC-related cancers on one side of the family, patients with very early onset colorectal cancers, and patients with multiple primaries including colorectal or endometrial cancers.

We obtained written consent and blood samples from 181 index persons. We asked for tumor specimens from one or more affected obligate carriers in each family, and obtained formalin-fixed, paraffin-embedded tissue blocks from 189 adenocarcinomas or tubular adenomas from 127 families. The tumors were colorectal cancers (n = 105), colon adenomas (n = 50), endometrial cancers (n = 10), ovarian cancers (n = 7), gastric cancers (n = 4), renal pelvic cancers (n = 2), adenocarcinomas of the breast (n = 4), and others (n = 7). From three index persons, the blocks obtained only contained normal tissue, from 21 families blocks were not available, and from 30 families blocks were not asked for. Tissue blocks were supplied by the pathology departments of 20 Norwegian hospitals.

Overall Strategy

The study was diagnostic with immunohistochemistry applied on all available tissue blocks as part of health services. Available resources did not allow mutation analyses for all families. Abnormal immunohistochemistry and/or a family history strongly indicating an increased probability of harboring an MMR mutation were employed as criteria for mutation analyses. MSI was not available for comparisons in the present report. Ideally, all MMR genes should have been examined,[23,33] but that was outside our means.

Classification of Families

Information on the site and classification of cancers and polyps, and patient age at diagnosis were obtained and verified in the medical files and/or in the National Cancer Registry whenever possible. Families were classified according to the classical Amsterdam criteria (Amsterdam criteria I), the revised Amsterdam criteria (Amsterdam criteria II), and the Bethesda criteria.

Ethics

The medical files verifying diagnoses were obtained with written permission from living patients, or with permission from the descendants of the dead patients. For mutation analyses, informed consent was obtained in writing following genetic counseling. Written informed consent underlay all requests for tumor specimens. If the patient was dead, the living relatives at risk consented. Because all activity reported was approved health care, all information was kept in our medical files.

Immunohistochemistry

Immunohistochemistry of all tumors for the presence of *MLH1, MSH2,* and *MSH6* gene products was performed in one laboratory (Department of Pathology, Ullevål University Hospital, Oslo, Norway). For each case, a formalin-fixed paraffin-embedded tissue block containing tumor tissue and normal adjacent mucosa was sectioned at 3 to 5 μm. The sections were mounted on slides coated with chromalum, fixed in the incubator for 30 to 40 minutes at 56°C, and then dried overnight at 37°C. The slides were deparaffinized twice in xylene, and rehydrated through descending graded alcohols to water. Heat-induced antigen retrieval was accomplished by immersing slides placed in Tissue-Tek slide holders (4465a; Miles Inc Diagnostic Division, Elkhart, IN; rooming 24 slides, empty spaces filled with blank slides) in Tris-EDTA (Trizmabase, Sigma Aldrich, Oslo, Norway. EDTA dinatrium salt, Titriplex III, VWR International, Oslo, Norway) pH 9 in a microwave oven for 2.5 minutes at 750W plus 15 minutes at 160W. After cooling for 15 minutes, the slides were rinsed in running water for 5 minutes and then immersed in Tris buffered saline (DAKOCytomation-triethanolamine buffered saline; TBS S1968; DAKOCytomation Norden A/S, Glostrup, Denmark) with 0.05% Tween 20 detergent before being placed in the machine. Slides were stained in DAKOCytomation Autostainer (DAKOCytomation Norden A/S, Glostrup, Denmark) with antibodies against MLH1 (No. 13271A; PharMingen, San Diego, CA; dilution 1:200), MSH2 (No. 65051A; PharMingen; dilution

1:150), and MSH6 (No. G70220; BD Transduction, Lexington, KY; dilution 1:300). The antibodies were diluted with Antibody Diluent (S 0809; DAKOCytomation Norden A/S, Glostrup, Denmark) and with Tween 20 (S 1966; DAKOCytomation) detergent. TBS Buffer (S 1968; DAKOCytomation) with Tween 20 was used for rinsing. Immunoreactivity was detected using DAKOCytomation EnVision + Systemkit, horseradish peroxide (HRP; mouse 3,3'-diaminobenzidin [DAB]; K400711; DAKOCytomation). The sections were counterstained with Mayer's Hematoxylin (S 3309; DAKOCytomation) for 1 minute, dehydrated through graded alcohols, cleared in xylene, and finally coverslipped in Eukitt (O. Kindler GmbH and Co, Freiburg, Germany).

Scoring of the tumor staining was performed by pathologists (I.M.B.-L., S.A.) without any knowledge of patients' family history or results of mutation analyses. Staining of tumors was evaluated using normal epithelial cells, stromal cells, or lymphocytes in the same slide as controls. The percentage of nuclear staining was graded as follows: complete absence of detectable nuclear staining (0), positive staining in less than 30% of the tumor cells (1+), positive staining in 30% to 60% of the tumor cells (2+), or positive staining in more than 60% of the tumor cells (3+).

Mutation Analysis

Twenty-three index persons who had a family history strongly indicating HNPCC, and an additional 17 persons with tumors with loss of MLH1, MSH2, or MSH6 protein expression were subjected to mutation analyses. In addition, eight index persons were subjected to mutation analyses for various clinical reasons. Sequencing of the *MLH1* and *MSH2* genes in these 48 index persons were bought from Myriad Genetics Inc (Salt Lake City, UT) as part of our health service.

All index persons without mutation demonstrated by sequencing and with a lack of MMR protein expression (n = 19) were subjected to analyses for large rearrangements in the *MLH1* and *MSH2* genes as previously described (MGC–Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands).[34] The remaining index persons who were lacking gene products of *MSH2* and/or *MSH6* genes (n = 11) were subjected to mutation analysis of the *MSH6* gene by sequencing (Center of Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway). We did not test for large rearrangements in *MSH6*.

Sensitivity and Specificity to Predict MMR Mutations

Sensitivity, specificity, and predictive values were calculated for the Amsterdam criteria I/II, the Bethesda guidelines, and results of immunohistochemistry respectively, versus the results of the mutation testing.

RESULTS

Classification of Families

Of the 181 affected persons (affecteds) with blood samples available, 38 met the Amsterdam criteria I/II and 88 met the Bethesda criteria. Forty-eight index persons were subjected to mutation analyses. Thirty of these index persons belonged to families fulfilling the Amsterdam criteria I/II and 43 belonged to families fulfilling the Bethesda criteria. All mutation-positive families had affecteds with early-onset cancers (Table 1).

Immunohistochemistry

One hundred eighty-nine tumors from 127 families were examined. One hundred fifty-one tumors from 97 of these families showed normal expression of all three proteins. Ninety-five tumors had a score of 3+ for all proteins, 37 tumors had a score of 3+ or 2+ for all three proteins, and 19 tumors had a score of 1+ in at least one of the three proteins and 2+ or 3+ in the remaining proteins. Lack of protein expression was found in 38 tumors from 30 families. The distribution among families was: isolated lack of MLH1 (n = 9), isolated lack of MSH2 (n = 1), isolated lack of MSH6 (n = 3), combined lack of MSH2/MSH6 (n = 16), and combined lack of MLH1/MSH6 (n = 1). Infiltrating cancers in each family had similar staining patterns, and the results were collapsed to one observation per family for further calculations. All exceptions are mentioned separately in the Discussion section. Results and clinical details from families harboring tumors with abnormal staining pattern are listed in Table 2. Three examples of immunostaining with antibodies against MLH1, MSH2, and MSH6 are given in Figure 1.

Table 1. Classification of the Families

	Amsterdam Criteria I and II			Bethesda Guidelines	
	AMS I	AMS I and AMS II	Others	Positive	Negative
All families with blood samples (n = 181)	29	38	143	88	93
All families performed IHC (n = 127)	26	35	92	76	51
Families performed IHC and protein present (n = 97)	18	20	77	51	46
Families performed IHC and protein absent (n = 30)	8	15	15	25	5
Families subjected to mutation analyses (n = 48)*	22	30	18	43	5
Mutation-negative families and protein present (n = 16)	13	14	3	16	0
Mutation-negative families and protein absent (n = 11)	0	2	9	7	4
Mutation-positive families (n = 19)	8	13	6	18	1

Abbreviations: AMS, Amsterdam criteria; IHC, immunohistochemistry.
*Tumor blocks available (n = 46); not available (n = 2).

Table 2. Results of Staining for *MLH1*, *MSH2*, and *MSH6* Gene Products From Families Harboring Tumors With Abnormal Staining Pattern and/or a Mutation

Patient No.	Tumor/Age at Diagnosis	Immunohistochemistry			Mutated Gene	Mutation	Fulfilment of Amsterdam criteria		Fulfilment of Bethesda Guidelines
		MLH1	MSH2	MSH6			AMS I	AMS II	
271-2	C59	2	0	2	*MSH2*	Q76X	No	No	Yes
470-1	C24	3	0	0	*MSH2*	IVS10 + 1G > A	No	Yes	Yes
470-2	E42	3	1	1	Nt		No	Yes	Yes
498-7	E52	0	3	Nt	*MLH1*	Q62X	Yes	Yes	Yes
684-4	C68	0	1	2	—	—	No	No	No
853-1	C54	3	0	0	*MSH2*	596delN(1786del3)	No	Yes	Yes
874-3	C42	0	1	1	*MLH1*	Q62X	Yes	Yes	Yes
933-3	C76	0	3	3	—	—	No	No	No
971-1	O35	3	0	0	*MSH2*	IVS11 + 2T > A	Yes	Yes	Yes
971-1	T44	3	3	3	*MSH2*	IVS11 + 2T > A	Yes	Yes	Yes
1117-1	C31	2	0	0	—	—	No	No	Yes
1133-1	C62	3	3	3	Nt		No	No	Yes
1133-2	C63	2	2	2	Nt		No	No	Yes
1133-4	C78	3	1	0	—*		No	No	Yes
1151-1	C74	2	2	1	Nt		Yes	Yes	Yes
1151-3	U63	1	0	0	*MSH6*	3804_3805insA	Yes	Yes	Yes
1151-3	T73	3	3	3	*MSH6*	3804_3805insA	Yes	Yes	Yes
1211-3	C35	2	0	0	*MSH2*	IVS5 + 3A > T	Yes	Yes	Yes
1211-5	O56	1	0	0	Nt		Yes	Yes	Yes
1211-7	C57	3	0	0	Nt		Yes	Yes	Yes
1211-9	E50	1	0	0	Nt		Yes	Yes	Yes
1642-37	O46	1	0	0	*MSH2*	IVS5 + 3A > T	No	Yes	Yes
1642-37	Cx46	1	0	0	*MSH2*	IVS5 + 3A > T	No	Yes	Yes
1294-3	E62	0	1	1	—	—	No	No	No
1316-1	C37	Nt	Nt	Nt	*MSH2*	IVS13 + 8insC†	No	Yes	Yes
1407-1	C44	3	3	3	Nt		No	No	Yes
1407-2	C63	1	1(0)‡	0	—		No	No	Yes
1414-2	C48	2	0	0	—		No	Yes	Yes
1467-2	G62	0	2	2	—		No	Yes	Yes
1532-1	C31	0	3	2	*MLH1*	IVS15 + 1G > C	No	Yes	Yes
1651-4	C57	2	0	0	*MSH6*	S156X	No	No	Yes
1661-1	C37	1	0	0	*MSH2*	G759X	No	No	Yes
1661-6	C55	3	0	0	Nt		No	No	Yes
1704-1	C33	0	3	3	*MLH1*	Q62X	Yes	Yes	Yes
1718-2	C65	3	0	0	*MSH2*	Exon 1-6del	No	No	Yes
1718-17	T43	3	3	3	Nt		No	No	Yes
1773-1	C51	2	0	0	*MSH2*	IVS6 + 1G > A	No	No	Yes
1773-17	U189	2	0	0	Nt		No	No	Yes
1826-3	E64	3	2	0	Nt		No	No	No
1826-5	E47	3	2	0	*MSH6*	R1331X	No	No	No
1956-9	E67	0	1	0	—		No	Yes	Yes
1956-9	C86	3	3	3	—		No	Yes	Yes
1956-10	C78	3	3	3	Nt		No	Yes	Yes
2013-6	C53	2	0	0	*MSH2*	IVS12 − 1G > C	Yes	Yes	Yes
2107-16	O46	3	0	0	*MSH2*	Exon 2-7del	Yes	Yes	Yes
2115-3	E46	2	0	0	*MSH6*	3195_3199delC	No	Yes	Yes
2297-19	E44	2	0	0	—	—	No	No	No
2621-2	C77	0	3	3	—	—	No	No	No

Abbreviations: AMS, Amsterdam criteria; C, colorectal cancer; E, endometrial cancer; Nt, not tested; T, tubular adenoma; U, urinary tract cancer; O, ovarian cancer; Cx, cervical cancer.

*Only tested for *MSH6*.

†Interpreted as a genetic variant not likely to be associated with disease.

‡Tested for *MLH1* and *MSH2* due to weak staining.

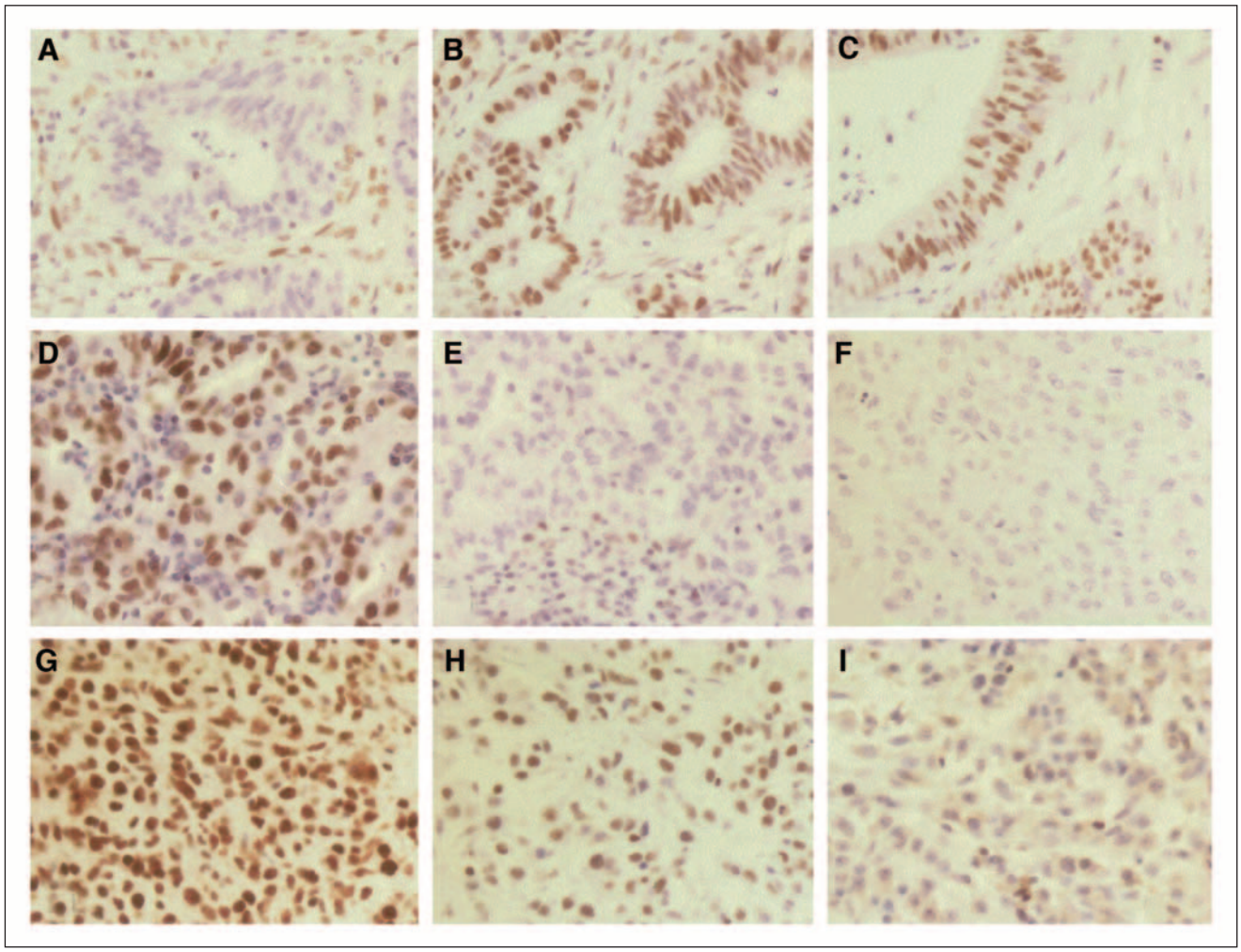

Fig 1. Results of immunostaining in colon cancer (*MLH1* mutation): (A) MLH1 nuclear staining absent, (B) MSH2 retained, and (C) MSH6 retained; in ovarian cancer (*MSH2* mutation): (D) MLH1 nuclear staining retained, (E) MSH2 absent, and (F) MSH6 absent; in endometrial cancer (*MSH6* mutation): (G) MLH1 nuclear staining retained, (H) MSH2 slightly reduced, and (I) MSH6 absent.

Mutation Analysis

Deleterious mutations were demonstrated in 19 index persons (*MLH1*, n = 4; *MSH2*, n = 11, and *MSH6*, n = 4; Table 2). All demonstrated a lack of protein expression of the corresponding gene in the tumors. One index person (patient 1316-1) had a genetic variant in the *MSH2* gene not likely to be associated with disease and was scored as no mutation demonstrated. Blocks were not available for immunohistochemistry.

Three of the mutations occurred in more than one family. Three families with a Q62X mutation in *MLH1* all emerged from the same geographic area. Two families with an IVS5 + 3A > T mutation in *MSH2* were later genealogically demonstrated to be one kindred. One family had an inframe *MSH2* codon 596 deletion that we previously reported[35] in multiple families turning out to be branches of one large kindred.

Sensitivity and Specificity

Sensitivity of immunohistochemistry to detect *MLH1*, *MSH2*, or *MSH6* mutation carriers was 100%, specificity was 82%, positive predictive value was 85%, and negative predictive value was 100%, compared with 100%, 94%, 47%, and 100% in our pilot study.[31] Details are listed in Table 3. The sensitivity of clinical criteria and immunohistochemistry in families with a detected mutation is listed in Table 4. Sensitivity of Amsterdam criteria I to detect mutations was 40%, compared with 39% in the pilot study.[31] Sensitivity of Amsterdam criteria II was 70%, compared with 100% in the pilot study.

DISCUSSION

The results confirmed that immunohistochemistry has high sensitivity, specificity, and positive predictive value to indi-

Table 3. Sensitivities, Specificities, and Predictive Values of Clinical Criteria and IHC to Predict Germline Mutations in MMR Genes in Families With a Calculated Probability for Mutation > 15%*

	Clinical Criteria (%)			
	Amsterdam Criteria I	Amsterdam Criteria I or II	Bethesda Criteria	IHC
Sensitivity	73	82	100	100
Specificity	17	8	0	82
Positive predictive value	44	45	48	85
Negative predictive value	40	33	—†	100

Abbreviations: IHC, immunohistochemistry; MMR, mismatch repair.
*Logistic regression analyses.[36] Families included had been examined with all methods.
†Bethesda criteria identified all families as affected, making negative predictive value not calculable.

cate HNPCC kindreds caused by MMR gene mutations.[31] Immunohistochemistry fulfills the requirements for a screening test: it is cheap, rapid, has high sensitivity and high positive predictive values, it requires readily available material, and the results are reproducible. Immunohistochemistry can point directly to the mutated gene, which reduces the cost for the subsequent mutation analyses. Immunohistochemistry can be used to demonstrate inherited cancer in deceased family members.

The Amsterdam criteria are specific, but insensitive. The Bethesda criteria had high sensitivity, but low specificity. In sum, none of the clinical criteria were suitable tools to identify HNPCC kindreds with MMR mutations for clinical use.

Concurrent loss of MSH2 and MSH6 protein was found and is most likely the result of abrogation of the MutSα complex formed by MSH2 and MSH6 proteins. The complex acts as a mismatch recognition factor and is required to repair base pair and single base insertion/deletion mismatches.[14,31,37,38] It has been shown that the MSH2 protein has two interaction regions with the MSH6 protein, and that the MSH6 protein is unstable in the absence of the MSH2 protein.[39-41] Thus, absence of MSH6 protein may be expected if no normal MSH2 protein is present, and vice versa.[14] Accordingly, no distinction between underlying *MSH2* and *MSH6* defects is possible. Methylation of the *MSH2/MSH6* promotors has not been found, and lack of MSH2 expression due to somatic mutation only is rare.[42,43] Five tumors had loss of expression of MLH1 protein, without demonstrated mutation in the *MLH1* gene. Median patient age at diagnosis was 68 years (range, 62 to 77 years). After the present series were concluded, we have examined tumors from other relatives in three of these families; all demonstrated MLH1 protein. *MLH1*-promotor hypermethylation and lack of expression of this protein has been shown in 10% to 15% of sporadic colorectal cancers, and occurs in approximately 15% of sporadic endometrial cancers as well.[42-44] Methylation and loss of MLH1 protein expression has been shown to be strongly associated with increasing patient age.[45] Thus, aquired alterations may explain our findings of some tumors lacking MLH1 protein without demonstrating germline *MLH1* mutations.

Some adenomas from patients with abnormal immunohistochemistry in the corresponding malignant tumor showed the presence of all three proteins (Table 2). One patient (patient 971-1) had an adenoma, diagnosed at 44 years, located in the proximal colon, and the other patient (patient 1151-3) had an adenoma diagnosed at 73 years located in the distal colon; both adenomas were histologically moderate dysplastic (size unknown). We have seen previously that adenomas have shown the presence of all three proteins despite the patient being a mutation carrier.[35] This may indicate that the adenoma formation is not caused by the inactivation of the second allele. Polyps are "normally" seen in adult persons. They may have an increased propensity to become malignant in MMR mutation carriers. The "second hit" may make the polyp malignant.[46] Other studies have shown that small adenomas forming in patients with an MMR germline mutation did not show a loss of expression of the MMR protein, whereas larger adenomas had lost expression.[47] Whatever the reason may be, in this study immunohistochemistry of adenomas was not a reliable tool to predict MMR mutations. Our conclusions are restricted to findings in infiltrating cancers.

Three index persons (patients 1117-1, 1414-2, and 2297-19) had concurrent loss of MSH2/MSH6 proteins in tumor but no mutation detected. After the present series

Table 4. Number of Families With Detected Mutations in MMR Genes That Meet the Recommended Methods for Scoring Family History and IHC

	MMR Mutations							
	MLH1 (n = 4)		*MSH2* (n = 11)		*MSH6* (n = 4)		Total (N = 19)	
	No.	%	No.	%	No.	%	No.	%
Amsterdam criteria I	3	75	4	36	1	25	8	42
Amsterdam criteria I and II	4	100	7	64	2	50	13	68
Bethesda criteria	4	100	11	53	3	75	18	95
IHC, loss of protein	4	100	11	100	4	100	19	100

Abbreviations: MMR, mismatch repair; IHC, immunohistochemistry.

was concluded, we examined ten tumors from two of these families. Each family had one late-onset colorectal cancer lacking expression of MLH1 protein and four more tumors showing presence of all three proteins. Three families without demonstrated mutation (patients 1133, 1407, and 1956) had loss of protein in one tumor, but protein present in other tumors from the same person/family. The results may indicate technical problems for scoring of the immunohistochemical findings in some tumors, and the possible false-negative results of mutation testing in a few families. Genetic linkage may be used to clarify whether or not the lack of MMR protein in tumor is a phenotypic trait segregating together with cancer.

One *MSH2* mutation carrier (patient 470-1) showed loss of MSH2/MSH6 protein in tumor. An untested relative (patient 470-2) with a cancer of the endometrium had a tumor with weak staining (1+) for MSH2/MSH6 protein. Of 152 tumors with protein present in this study, 19 were scored as having nuclear staining in less than 30% of the tumor cells. We have reported previously on scoring problems, particularly in endometrial cancers.[14] Affecteds with tumors that have weak staining (< 10%) for one or two proteins can be considered for mutation analyses.[48]

None of the families with demonstrated mutations were classified as late-onset HNPCC. Distribution of mutations in this study was *MLH1* = 4, *MSH2* = 11, and *MSH6* = 4. The prevalence of *MSH6* mutations might have been underestimated, as the gene was not examined for large rearrangements. In our previous study,[31] the distribution of mutations was *MLH1* = 1, *MSH2* = 4, and *MSH6* = 2. Thus, the distribution of MMR mutations found in our total series so far is 19% *MLH1*, 58% *MSH2*, and 23% *MSH6*. Other studies show an approximately equal frequency of mutations in *MLH1* (≈ 40%) and *MSH2* (≈ 40%) and fewer mutations in *MSH6*.[36,49] We assume the reported discrepancies to be caused by a combination of different selection criteria, true differences between the populations studied, and variations in small numbers.

In conclusion, wide clinical criteria to select HNPCC kindreds, followed by immunohistochemistry of tumor material from one affected in each family, had high sensitivity and specificity to predict MMR mutations.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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