

CLINICAL FINDINGS WITH IMPLICATIONS FOR GENETIC TESTING IN FAMILIES WITH CLUSTERING OF COLORECTAL CANCER

JUUL T. WIJNEN, B.S., HANS F.A. VASEN, M.D., PH.D., P. MEERA KHAN, M.D., PH.D.,* AEILKO H. ZWINDERMAN, PH.D., HELEEN VAN DER KLIFT, M.S., ADRI MULDER, B.S., CARLI TOPS, PH.D., PÅL MØLLER, M.D., AND RICCARDO FODDE, PH.D.

ABSTRACT

Background Germ-line mutations in DNA mismatch-repair genes (*MSH2*, *MLH1*, *PMS1*, *PMS2*, and *MSH6*) cause susceptibility to hereditary nonpolyposis colorectal cancer. We assessed the prevalence of *MSH2* and *MLH1* mutations in families suspected of having hereditary nonpolyposis colorectal cancer and evaluated whether clinical findings can predict the outcome of genetic testing.

Methods We used denaturing gradient gel electrophoresis to identify *MSH2* and *MLH1* mutations in 184 kindreds with familial clustering of colorectal cancer or other cancers associated with hereditary nonpolyposis colorectal cancer. Information on the site of cancer, the age at diagnosis, and the number of affected family members was obtained from all families.

Results Mutations of *MSH2* or *MLH1* were found in 47 of the 184 kindreds (26 percent). Clinical factors associated with these mutations were early age at diagnosis of colorectal cancer, the occurrence in the kindred of endometrial cancer or tumors of the small intestine, a higher number of family members with colorectal or endometrial cancer, the presence of multiple colorectal cancers or both colorectal and endometrial cancers in a single family member, and fulfillment of the Amsterdam criteria for the diagnosis of hereditary nonpolyposis colorectal cancer (at least three family members in two or more successive generations must have colorectal cancer, one of whom is a first-degree relative of the other two; cancer must be diagnosed before the age of 50 in at least one family member; and familial adenomatous polyposis must be ruled out). Multivariate analysis showed that a younger age at diagnosis of colorectal cancer, fulfillment of the Amsterdam criteria, and the presence of endometrial cancer in the kindred were independent predictors of germ-line mutations of *MSH2* or *MLH1*. These results were used to devise a logistic model for estimating the likelihood of a mutation in *MSH2* and *MLH1*.

Conclusions Assessment of clinical findings can improve the rate of detection of mutations of DNA mismatch-repair genes in families suspected of having hereditary nonpolyposis colorectal cancer. (N Engl J Med 1998;339:511-8.)

©1998, Massachusetts Medical Society.

THE lifetime risk of colorectal cancer among whites in the Western world is approximately 4 percent. The cause of colorectal cancer is multifactorial, involving hereditary susceptibility, environmental factors, and somatic genetic changes during tumor progression.¹ A family history of colorectal cancer is a clinically significant risk factor and may be found in up to 15 percent of all patients with colorectal cancer.² Hereditary nonpolyposis colorectal cancer, or the Lynch syndrome, is the most common type of familial colorectal cancer and is thought to account for 1 to 5 percent of all cases of the disease.^{3,4} Members of families with hereditary nonpolyposis colorectal cancer are also at risk for tumors at other sites, including the endometrium, stomach, small intestine, brain, hepatobiliary system, urinary tract, and ovary.^{2,5} In addition, multiple synchronous or metachronous cancers develop in about 30 percent of the patients.^{2,5}

Hereditary nonpolyposis colorectal cancer is caused by germ-line mutations in one of five DNA mismatch-repair genes: *MSH2*,⁶ *MLH1*,^{7,8} *PMS1*, *PMS2*,⁹ and *MSH6* (also known as *GTBP*).^{10,11} Of the 126 germ-line mutations reported to date, almost all have been found in *MSH2* and *MLH1*; only 3 have been reported in *PMS1* and *PMS2*.¹² Recently, two germ-line mutations have been found in *MSH6*.^{10,11} Inac-

From the Departments of Human Genetics (J.T.W., P.M.K., H.K., A.M., R.E.), Gastroenterology (H.F.A.V.), and Medical Statistics (A.H.Z.) and the Clinical Genetics Center (C.T.), Leiden University Medical Center, Leiden, the Netherlands; the Foundation for the Detection of Hereditary Tumors, Leiden, the Netherlands (H.F.A.V.); and the Unit of Medical Genetics, Norwegian Radium Hospital, Oslo, Norway (P.M.). Address reprint requests to Dr. Vasen at the Foundation for the Detection of Hereditary Tumors, Rijnsburgerweg 10, Poortgebouw Zuid, 2333 AA Leiden, the Netherlands.

Other authors were Fred Menko, M.D., Ph.D. (University Hospital Vrije Universiteit, Amsterdam, the Netherlands), Babs Taal, M.D., Ph.D. (Netherlands Cancer Institute, Amsterdam, the Netherlands), Fokko Nagengast, M.D., Ph.D., and Han Brunner, M.D., Ph.D. (Nijmegen University Hospital, Nijmegen, the Netherlands), Jan Kleibeuker, M.D., Ph.D., and Rolf Sijmons, M.D. (University Hospital Groningen, Groningen, the Netherlands), Gerrit Griffioen, M.D., Ph.D., Annette Bröcker-Vriends, M.D., Ph.D., Egbert Bakker, Ph.D., and Inge van Leeuwen-Cornelisse, B.S. (Leiden University Medical Center, Leiden, the Netherlands), Anne Meijers-Heijboer, M.D., Dick Lindhout, M.D., Ph.D., and Martijn Breuning, M.D., Ph.D. (Erasmus University, Rotterdam, the Netherlands), Jan Post, M.D. (Clinical Genetics Center Utrecht, Utrecht, the Netherlands), Cees Schaap, M.D. (Clinical Genetics Center Maastricht, Maastricht, the Netherlands), Jaran Apold, M.D., Ph.D. (Haukeland University Hospital, Bergen, Norway), Ketil Heimdal, Ph.D. (Norwegian Radium Hospital, Oslo, Norway), Lucio Bertario, M.D., Ph.D. (Istituto Nazionale Tumori, Milan, Italy), Marie Luise Bisgaard, M.D. (Danish Hereditary Nonpolyposis Colorectal Cancer Registry, Hvidovre Hospital, Hvidovre, Denmark), and Petr Goetz, M.D., Ph.D. (Charles University, Prague, Czech Republic).

*Deceased.

tivation of any one of these genes causes widespread genomic instability characterized by the expansion or contraction of short, repeated sequences of DNA (microsatellites).¹³⁻¹⁵ This phenomenon, known as microsatellite instability, is thought to be responsible for the rapid accumulation of somatic mutations in oncogenes and tumor-suppressor genes that have crucial roles in the initiation and progression of tumors.¹⁶⁻¹⁸

In a recent study of the risk of cancer in 19 families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis, we found that the inheritance of a mutated copy of *MSH2* or *MLH1* is associated with an 80 percent risk of colorectal cancer by the age of 80 years, as compared with a risk of about 4 percent in the general population. Female members of such families also have a 40 to 50 percent risk of endometrial cancer, as compared with a risk of less than 2 percent in the general population. Moreover, carriers of an *MSH2* mutation have a higher risk of extracolonic cancers than carriers of an *MLH1* mutation.¹⁹ A study of mutation carriers identified among patients with an early onset of colorectal cancer confirmed the high risk of endometrial cancer in female family members and found that males had a higher risk of colorectal cancer than females; the risk of colorectal cancer among female carriers was only 30 percent.²⁰

In 1990, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer proposed a set of clinical diagnostic criteria (later termed the Amsterdam criteria) to provide uniformity in collaborative studies. For families to be classified as having hereditary nonpolyposis colorectal cancer, at least three members in at least two successive generations must have colorectal cancer, with at least one case diagnosed before the age of 50 years; one of the affected members should be a first-degree relative of the other two; and familial adenomatous polyposis should be ruled out.²¹ Germ-line mutations of *MSH2* and *MLH1* occur with approximately equal frequency in kindreds who meet the Amsterdam criteria (approximately 25 percent in each case),²²⁻²⁷ whereas such mutations were found in only 10 percent of the kindreds that did not meet the criteria.^{28,29}

The detection of pathogenic mutations in families with hereditary nonpolyposis colorectal cancer has made presymptomatic diagnosis possible in persons who may be at risk. Screening for mutations, however, is time consuming and expensive because of the heterogeneity of the mutations in DNA mismatch-repair genes.^{12,25,26,29} Moreover, the number of kindreds with an apparent familial clustering of this common cancer is potentially high. Little is known about the clinical risk factors that best predict the presence of *MSH2* or *MLH1* mutations.^{28,30} To address this issue, we used denaturing gradient gel electrophoresis (DGGE) with guanosine and cytidine

extensions (GC clamping)^{31,32} to analyze the *MSH2* and *MLH1* genes in 184 unrelated kindreds with familial clustering of colorectal and other cancers. Using logistic-regression analysis, we devised a method for evaluating the probability of an *MSH2* or an *MLH1* mutation that is based on several risk factors.

METHODS

Subjects

A total of 184 kindreds participated in the study. The results of mutation analysis and assessments of the risk of cancer in some of these families have been reported previously.^{19,25,26,29} Sixty-seven families were recruited through the Foundation for the Detection of Hereditary Tumors in the Netherlands. These kindreds were referred to the foundation from all parts of the Netherlands because they were suspected of having an inherited form of colorectal cancer. Another 56 Dutch families and 56 Norwegian families were enrolled by clinicians or clinical genetics centers. Also included were three Italian families, one Danish family, and one Czech family. Members of these families attended the clinics because of concern about familial risk factors for colorectal cancer.

Data on family history were collected by genetic fieldworkers or clinical geneticists. Pedigrees were traced backward and laterally as far as possible. In addition, information was collected on the type, site, and number of cancers; the age at diagnosis; and the pathological characteristics of the individual tumors in each of the affected persons. The pedigrees of most families included at least three generations. Eighty-seven percent of the cases of cancer were confirmed by medical or pathology reports or both.

All participating family members gave informed consent, and when their blood samples were collected, they were informed of the possibility of genetic counseling if a pathogenic mutation was identified. The protocol for presymptomatic testing of DNA used by the Dutch clinical genetics centers usually involves three sessions. The issues discussed during the first session include the reasons for DNA testing, the clinical features of hereditary nonpolyposis colorectal cancer, the mode of inheritance of the syndrome, and the DNA-testing procedure. In session 2, the consequences of the test results and the options for treatment in the case of a positive result are discussed, and blood samples are taken. The results of the DNA test are disclosed during session 3. All sessions are conducted by a clinical geneticist with the support of a psychologist or a social worker who is also responsible for the patient's follow-up.

Ninety-two families met the Amsterdam criteria for hereditary nonpolyposis colorectal cancer. Among the 92 kindreds that did not meet the criteria, the majority (48 kindreds) had fewer than the minimal number of three family members with colorectal cancer. In 17 families, colorectal cancer was diagnosed in all affected patients after the age of 50 years, in 11 families only one generation was affected, and in 6 families the affected patients were not first-degree relatives. In the remaining 10 families more than one of the criteria were not met.

Mutation Analysis

Isolation of genomic DNA, amplification with the polymerase chain reaction (PCR), mutation analysis with DGGE, and determination of the nucleotide sequences were performed as previously described.^{25,26,29,33} In short, the general strategy was to amplify by PCR each of the 16 exons of *MSH2* and 19 exons of *MLH1* in a single affected member in each family and to analyze these products by GC-clamped DGGE.³² Exons with altered patterns of migration on DGGE were sequenced to determine the molecular nature of the variant. When variants were detected, the investigations were extended, when possible, to the rest of the family to verify the segregation of the nucleotide change with the disease phenotype.^{25,26,29}

Statistical Analysis

We used univariate and multivariate analyses to examine possible associations between specific clinical features and the presence of an *MSH2* or *MLH1* mutation. For univariate analysis, the Pearson chi-square test was used to identify associations. The following variables were examined within each kindred: the mean age at diagnosis of colorectal cancer, whether or not the Amsterdam criteria were met, the number of family members with colorectal cancer, the number with endometrial cancer, the presence of a patient with other cancers related to hereditary nonpolyposis colorectal cancer, and the presence of a patient with multiple synchronous or metachronous cancers in a family. All these variables were also used in the multivariate analysis. Using logistic regression with backward selection, we calculated the logarithm of the odds of having a deleterious mutation as a linear function of the variables that were significant in the multivariate analysis. To determine the probability of an *MSH2* or *MLH1* mutation (p), we used the following equation:

$$\log(p/1+p) = \alpha + \beta_1 V_1 + \beta_2 V_2 + \dots + \beta_k V_k,$$

where $V_1, V_2,$ and $\dots V_k$ are the variables being considered and $\alpha, \beta_1, \beta_2,$ and $\dots \beta_k$ are the usual weighted regression values estimated from the data. Eleven of the 184 families were excluded from these analyses, 6 because of the presence of missense mutations of unknown clinical significance and 5 because data relative to the age at diagnosis were not available.

RESULTS

Mutation Analysis

A total of 47 disease-causing mutations were identified in 184 families (26 percent). Of these mutations 19 were in *MSH2* and 28 in *MLH1*. Tables 1, 2, 3, and 4 show the frequency of mutations in *MSH2* and *MLH1* according to the number of patients with colorectal or endometrial cancer in a kindred, the average age at diagnosis of all colorectal cancers, the number of family members with multiple cancers, and the types of extracolonic cancers, respectively. In addition, six missense mutations of unknown clinical significance were found. Three of these mutations were in *MSH2* (Ala→Thr at codon 305, Phe→Val at codon 447, and Ala→Thr at codon 834), and three were in *MLH1* (Gln→Lys at codon 62, Asn→Ser at codon 64, and Val→Met at codon 716). Although these mutations were not found in 100 control subjects (including 50 with polyposis), the results of an examination of the cosegregation of the alterations with the disease phenotype were inconclusive. Therefore, we decided to exclude these missense mutations from the statistical analyses.

Statistical Analysis

In the univariate analysis, several factors were strongly associated with the presence of mutations in *MSH2* or *MLH1*: younger age at diagnosis of colorectal cancer ($P < 0.001$), fulfillment of the Amsterdam criteria ($P < 0.001$), a higher number of patients with colorectal cancer in a family ($P < 0.001$), the presence of endometrial cancer ($P < 0.001$), a higher number of patients with endometrial cancer in a family ($P < 0.001$), the presence of small-bowel cancer ($P < 0.05$), the presence of multiple colorectal

TABLE 1. FREQUENCY OF *MSH2* AND *MLH1* MUTATIONS ACCORDING TO THE NUMBER OF PATIENTS WITH CANCER WITHIN A FAMILY.

NO. OF PATIENTS WITH CANCER	AMSTERDAM CRITERIA MET		AMSTERDAM CRITERIA NOT MET	
	NO. OF FAMILIES	NO. (%) WITH MUTATIONS	NO. OF FAMILIES	NO. (%) WITH MUTATIONS
Colorectal cancer				
1	0	0	16	1 (6)
2	0	0	37	2 (5)
3	20	9 (45)	21	1 (5)
4	17	6 (35)	9	1 (11)
5	18	7 (39)	6	1 (17)
6	14	6 (43)	3	0
7	10	7 (70)	0	0
8	4	1 (25)	0	0
9	3	1 (33)	0	0
10	3	2 (67)	0	0
11	1	0	0	0
12	2	2 (100)	0	0
Total	92	41 (45)	92	6 (7)
Endometrial cancer				
0	58	17 (29)	65	1 (2)
1	15	9 (60)	21	5 (24)
2	9	7 (78)	4	0
3	6	4 (67)	1	0
4	2	2 (100)	1	0
5	1	1 (100)	0	0
6	1	1 (100)	0	0

TABLE 2. FREQUENCY OF *MSH2* AND *MLH1* MUTATIONS ACCORDING TO THE AVERAGE AGE AT DIAGNOSIS OF COLORECTAL CANCER OF ALL PATIENTS WITHIN A FAMILY.*

AVERAGE AGE AT DIAGNOSIS OF COLORECTAL CANCER (YR)	AMSTERDAM CRITERIA MET		AMSTERDAM CRITERIA NOT MET	
	NO. OF FAMILIES	NO. (%) WITH MUTATIONS	NO. OF FAMILIES	NO. (%) WITH MUTATIONS
<35	5	4 (80)	6	1 (17)
35-39	13	9 (69)	7	2 (29)
40-44	21	14 (67)	8	0
45-49	20	9 (45)	16	1 (6)
50-54	15	3 (20)	12	2 (17)
55-59	13	2 (15)	14	0
≥60	5	0	24	0
Total	92	41 (45)	87†	6 (7)

*The mean age at diagnosis of colorectal cancer was 42 years in the families with deleterious mutations that met the Amsterdam criteria and 44 years in families with mutations that did not meet the Amsterdam criteria; the respective ages in the families without mutations were 50 and 54 years.

†Five families for which data on the age at diagnosis were not available were excluded.

cancers in a single member of a family ($P < 0.001$), and the presence of concomitant colorectal and endometrial cancer in a patient ($P < 0.001$) (Table 5). No significant association was found between *MSH2* or *MLH1* mutations and tumors of the stomach, brain, urinary tract, ovary, or hepatobiliary system

TABLE 3. FREQUENCY OF *MSH2* AND *MLH1* MUTATIONS IN FAMILIES WITH SYNCHRONOUS AND METACHRONOUS CANCERS.

NO. OF PATIENTS	AMSTERDAM CRITERIA MET		AMSTERDAM CRITERIA NOT MET	
	NO. OF FAMILIES	NO. (%) WITH MUTATIONS	NO. OF FAMILIES	NO. (%) WITH MUTATIONS
Multiple colorectal cancers in a single patient				
0	62	22 (35)	75	3 (4)
1	18	9 (50)	11	1 (9)
2	7	5 (71)	6	2 (33)
3	5	5 (100)	0	0
Total	92	41 (45)	92	6 (7)
Concomitant colorectal and endometrial cancer in a single patient				
0	78	28 (36)	83	5 (6)
1	9	8 (89)	8	1 (12)
2	2	2 (100)	1	0
3	3	3 (100)	0	0
Concomitant colorectal cancer and an associated cancer in a single patient*				
0	73	31 (42)	86	6 (7)
1	15	8 (53)	6	0
2	3	1 (33)	0	0
3	0	0	0	0
4	1	1 (100)	0	0

*Associated cancers were cancers of the stomach, small intestine, brain, urinary tract, ovary, and the hepatobiliary system.

TABLE 4. FREQUENCY OF *MSH2* AND *MLH1* MUTATIONS ACCORDING TO THE TYPES OF EXTRACOLONIC CANCERS WITHIN A FAMILY.

CANCER SITE	AMSTERDAM CRITERIA MET		AMSTERDAM CRITERIA NOT MET	
	WITH CANCER	WITHOUT CANCER	WITH CANCER	WITHOUT CANCER
	no. of families (% with mutation)			
Endometrium	34 (71)	58 (29)	31 (16)	61 (2)
Stomach	16 (63)	76 (41)	13 (0)	79 (8)
Small bowel	8 (75)	84 (42)	2 (0)	90 (7)
Brain	10 (60)	82 (43)	3 (0)	89 (7)
Urinary tract	12 (58)	80 (43)	10 (0)	82 (7)
Ovary	12 (50)	80 (44)	13 (8)	79 (6)
Hepatobiliary system	6 (33)	86 (45)	6 (0)	86 (7)

or with the presence in a single family member of concomitant colorectal cancer and a tumor related to hereditary nonpolyposis colorectal cancer.

In the multivariate analysis, only a younger age at diagnosis of colorectal cancer within a family ($P < 0.001$), fulfillment of the Amsterdam criteria ($P < 0.001$), and the presence of endometrial cancer ($P < 0.001$) were independent risk factors (Table 5). Therefore, we analyzed the probability of detecting

a deleterious mutation in *MSH2* or *MLH1* using logistic regression as a function of the mean age at diagnosis of colorectal cancer in a family, the presence or absence of endometrial cancer, and fulfillment or nonfulfillment of the Amsterdam criteria. The results, expressed as the log odds ratios and their 95 percent confidence intervals, are shown in Figure 1.

Given these results, we calculated the predicted probability of detecting *MSH2* or *MLH1* mutations in individual families (p) with the following equation:

$$p = e^L / (1 + e^L),$$

where e is the exponential function and L is the log odds. Using the equation described in the Methods section, we calculated the log odds ratios with the following formula:

$$L = 1.4 + [-0.1]V_1 + 1.7 V_2 + 2.4 V_3,$$

where V_1 is the mean age at diagnosis of colorectal cancer of all affected members of a family; V_2 equals 1 if at least one member of the family has endometrial cancer and equals 0 otherwise; and V_3 equals 1 if the family meets the Amsterdam criteria and equals 0 otherwise (Table 5). For example, the estimated probability of detecting a deleterious mutation in a family that met the Amsterdam criteria and in which the mean age at the diagnosis of colorectal cancer was 40 years is 48 percent (95 percent confidence interval, 31 to 65 percent). If this family also includes a patient with endometrial cancer, then the estimated probability is 83 percent (95 percent confidence interval, 68 to 92 percent). The optimal use of the equation requires a detailed family history and knowledge of all cases of cancer in the family.

To assess which variables are significant predictive factors in the absence of the Amsterdam criteria, we repeated the multivariate analysis after excluding these criteria. In this case, a younger age at the diagnosis of colorectal cancer and a higher number of patients with colorectal or endometrial cancer are independent risk factors. The predicted probability of detecting *MSH2* or *MLH1* mutations in individual families can be calculated with the other coefficients reported in Table 5.

DISCUSSION

A recent review of 126 different mutations of DNA mismatch-repair genes from 202 kindreds with hereditary nonpolyposis colorectal cancer¹² confirmed that *MSH2* and *MLH1* are responsible for the majority of cases of hereditary nonpolyposis colorectal cancer. The mutations are scattered along the coding regions of both genes, with some clustering in exon 12 of *MSH2* and exon 16 of *MLH1*. The majority of *MSH2* mutations are chain-terminating, whereas both nonsense and missense mutations are found in *MLH1*. The heterogeneity of the *MSH2* and *MLH1* mutations implies that both genes need

TABLE 5. RESULTS OF UNIVARIATE AND MULTIVARIATE ANALYSES.

VARIABLE	INCLUSION OF AMSTERDAM CRITERIA		MULTIVARIATE ANALYSIS, BACKWARD SELECTION	
	UNIVARIATE ANALYSIS	MULTIVARIATE ANALYSIS	INCLUSION OF AMSTERDAM CRITERIA	EXCLUSION OF AMSTERDAM CRITERIA
	log odds ratio ±SE			
Age at diagnosis of colorectal cancer within a family (for each year of increasing age)	-0.11±0.02*	-0.12±0.03*	-0.10±0.03*	-0.09±0.05†
Fulfillment of Amsterdam criteria	2.47±0.47*	2.78±0.75*	2.45±0.54*	
No. of patients with colorectal cancer in a family (for each additional affected member)	0.37±0.08*	-0.09±0.54	Eliminated	0.27±0.11*
Endometrial cancer in at least one family member	1.69±0.36*	1.05±0.82	1.66±0.46*	Eliminated
No. of patients with endometrial cancer in a family (for each additional affected member)	0.86±0.19*	0.28±0.53	Eliminated	0.75±0.23‡
Small-bowel cancer in at least one family member	1.44±0.60†	0.86±0.95	Eliminated	Eliminated
Stomach cancer in at least one family member	0.22±0.21	-0.26±0.40	Eliminated	Eliminated
Brain tumor in at least one family member	0.47±0.46	-0.43±0.71	Eliminated	Eliminated
Urinary tract cancer in at least one family member	0.30±0.29	-0.19±0.42	Eliminated	Eliminated
Ovarian cancer in at least one family member	0.05±0.37	0.19±0.53	Eliminated	Eliminated
Hepatobiliary-system cancer in at least one family member	-0.16±0.45	-0.82±0.93	Eliminated	Eliminated
Multiple colorectal cancers in a single patient	1.37±0.37*	-0.21±0.62	Eliminated	Eliminated
Concomitant colorectal cancer and endometrial cancer in a single patient	1.75±0.47*	1.10±0.90	Eliminated	Eliminated
Constant (α)		2.39±1.61	1.43±1.37	1.83±1.22

*P<0.001. †P<0.05. ‡P<0.01.

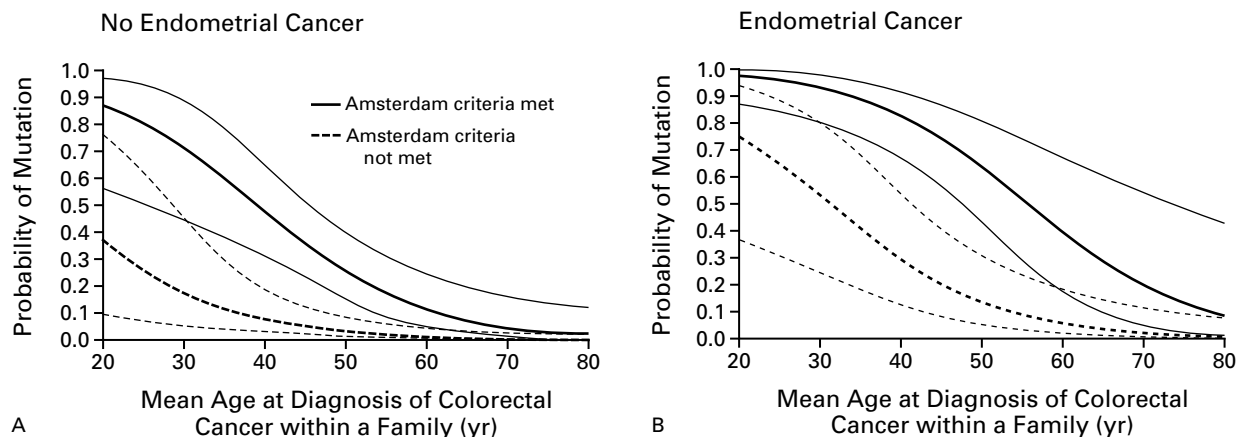


Figure 1. Estimated Probability of a Deleterious *MSH2* or *MLH1* Mutation as a Function of the Mean Age at Diagnosis of Colorectal Cancer within a Family and Whether the Amsterdam Criteria Were Met in Families with No Members with Endometrial Cancer (Panel A) and in Families with at Least One Member with Endometrial Cancer (Panel B). Values are the log odds ratios; the thin lines indicate the 95 percent confidence intervals.

to be screened exon by exon for an accurate molecular diagnosis of hereditary nonpolyposis colorectal cancer. Such an approach is laborious and expensive. Screening costs might be reduced if clinical factors that predict the outcome of genetic testing could be identified.

In the present study, we found that an early age of onset of colorectal cancer, fulfillment of the Am-

sterdam criteria, and the presence in a kindred of a patient with cancer of the endometrium or small bowel, multiple colorectal cancers, or both colorectal and endometrial cancer are strong predictive factors for the presence of *MSH2* or *MLH1* mutations. For example, if a kindred that met the Amsterdam criteria (mutation-detection rate, 45 percent) also included one patient with endometrial cancer, then the ob-

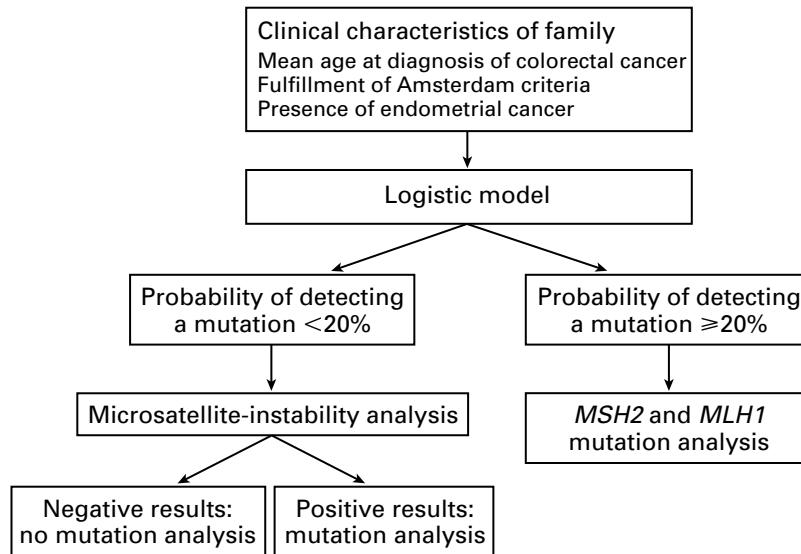


Figure 2. Strategy of Molecular Analysis in Families Suspected of Having Hereditary Nonpolyposis Colorectal Cancer.

served rate of detection increases to 71 percent. And, in a family that met the Amsterdam criteria and had one member with both colorectal and endometrial cancer, the likelihood of finding the disease-causing germ-line mutation rises to about 90 percent. Multivariate analysis indicated that only a younger age at diagnosis, fulfillment of the Amsterdam criteria, and the presence of endometrial cancer are independent predictive variables. If the Amsterdam criteria are excluded as a variable in the analysis, a younger age at diagnosis of colorectal cancer and a higher number of patients in the kindred with colorectal or endometrial cancer become independent risk factors.

Our logistic model can be used to estimate the probability of detecting a germ-line mutation on the basis of the clinical features of a kindred with familial clustering of colorectal cancer and other tumors related to hereditary nonpolyposis colorectal cancer. The probability of finding an *MSH2* or *MLH1* mutation can be deduced from Figure 1 or calculated with the use of simple software available at the Web site of the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (<http://www.nfdht.nl>). If the predicted probability is low, one might consider performing microsatellite-instability analysis of the DNA of the colon tumor, which gives an indication of whether there is a mutation of a mismatch-repair gene. Patients with positive results can then undergo mutation analysis. Since the cost of microsatellite-instability analysis is about half that of mutation analysis, this type of analysis will be more cost effective than primary mutation analysis as a first step only if the expected proportion of patients with tumors with microsatellite instability is

less than 50 percent. Our preliminary data, which are based on a limited number of tumor samples, indicate that most colorectal tumors from kindreds with hereditary nonpolyposis colorectal cancer that meet the Amsterdam criteria show microsatellite instability, whereas the opposite is true for tumors from families that do not meet the Amsterdam criteria.²⁹ Moreover, recent studies^{20,34} showed that 57 percent of colorectal cancers that were diagnosed in a patient before the age of 35 years had microsatellite instability and that a germ-line mutation was detected in 25 percent of all cases. These observations suggest that the proportion of patients with tumors with microsatellite instability will drop below 50 percent when the probability of a mutation is less than 20 percent. In these cases, microsatellite-instability analysis of tumor DNA should be considered as a first diagnostic step. Figure 2 illustrates our recommended strategy.

Aaltonen et al.⁴ evaluated the frequency of hereditary nonpolyposis colorectal cancer in Finland by screening colorectal tumors from 509 patients for microsatellite instability and by performing mutation analysis of the tumors with positive results. Tumors from 63 patients showed microsatellite instability, and 10 of these patients had a germ-line mutation of *MSH2* or *MLH1*. Nine of the 10 patients had a first-degree relative with colorectal or endometrial cancer, 7 were under 50 years of age, and 4 had had colorectal or endometrial cancer previously. The authors recommended microsatellite-instability analysis for all patients with colorectal cancer who meet one or more of the following criteria: a family history of colorectal or endometrial cancer, an age at diagnosis of less than 50 years, and a history of multiple colorectal

or endometrial cancers. We suggest calculating the probability of finding a mutation with our model when clinical and family data are available and selecting patients for either microsatellite-instability analysis or mutation analysis on the basis of the outcome.

Our series of 184 families included 17 families in which no *MSH2* or *MLH1* mutations were found. These families met all the Amsterdam criteria except one: a diagnosis of colorectal cancer before the age of 50 years. Earlier studies of similar kindreds with a late onset of colorectal cancer revealed phenotypic differences between these families and those with classic hereditary nonpolyposis colorectal cancer: a predilection for tumors in the left colon, the absence of extracolonic cancers, and a relatively high ratio of adenomas to colorectal cancer.^{35,36} Tests for microsatellite instability in the colorectal tumors of these patients were negative.³⁶ These findings suggest that such families may represent a separate genetic entity. Recently, Laken et al. reported an unusual mutation in the *APC* gene that is responsible for familial clustering of late-onset colorectal cancer among Ashkenazi Jews.³⁷ Moreover, the clinical picture characteristic of attenuated familial adenomatous polyposis³⁸ (later onset and fewer polyps than in typical familial adenomatous polyposis) could easily be misinterpreted as that of hereditary nonpolyposis colorectal cancer. Similar atypical *APC* mutations may also be responsible for a proportion of cases in the 17 families in our study.

The Amsterdam criteria were established to help ensure the uniformity of collaborative studies of hereditary nonpolyposis colorectal cancer.²¹ The high rate at which mutations were detected among kindreds that meet these criteria and the low percentage of mutations in families that do not confirmed the value of these criteria in selecting patients for screening for mutations of mismatch-repair genes.²⁹ However, the Amsterdam criteria have been criticized as being too stringent and for excluding extracolonic cancers known to be associated with hereditary nonpolyposis colorectal cancer. Moreover, it is obviously harder for small families to fulfill the criteria. Our study shows that endometrial cancer and, to a lesser extent, tumors of the small bowel are important predictive risk factors. These findings suggest that the Amsterdam criteria should be modified to include these extracolonic cancers.

In conclusion, we detected mutations in DNA mismatch-repair genes in a minority (26 percent) of 184 kindreds with familial clustering of colorectal cancers suggestive of hereditary nonpolyposis colorectal cancer. Using logistic-regression analysis, we devised a simple method for calculating the likelihood of detecting *MSH2* or *MLH1* germ-line mutations. The majority of the kindreds we studied are representative of families that are often referred to clinical geneticists for evaluation of the risk of hereditary colo-

rectal cancer. An accurate estimate of the probability of *MSH2* or *MLH1* mutations in these families will not only allow a cost-effective strategy for molecular analyses but also help clinicians counsel such families.

This work is dedicated to the memory of Dr. P. Meera Khan.

Supported in part by the Dutch Cancer Society and Praeventiefonds and by a grant (118571/320) from the Norges Forskningsraad (to Drs. Møller and Heimdal).

REFERENCES

1. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87:159-70.
2. Lynch HT, Smyrk TC, Watson P, et al. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;104:1535-49.
3. Rodriguez-Bigas MA, Boland CR, Hamilton SR, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 1997;89:1758-62.
4. 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.
5. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 1993;71:677-85.
6. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993;75:1215-25.
7. Papadopoulos N, Nicolaides NC, Wei Y-F, et al. Mutation of a *mutL* homolog in hereditary colon cancer. *Science* 1994;263:1625-9.
8. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary nonpolyposis colon cancer. *Nature* 1994;368:258-61.
9. Nicolaides NC, Papadopoulos N, Liu B, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 1994;371:75-80.
10. Akiyama Y, Sato H, Yamada T, et al. Germ-line mutation of the *hMSH6/GTBP* gene in an atypical hereditary nonpolyposis colorectal cancer kindred. *Cancer Res* 1997;57:3920-3.
11. Miyaki M, Konishi M, Tanaka K, et al. Germline mutation of *MSH6* as the cause of hereditary nonpolyposis colorectal cancer. *Nat Genet* 1997;17:271-2.
12. Peltomäki P, Vasen HFA, International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. *Gastroenterology* 1997;113:1146-58.
13. Aaltonen LA, Peltomäki P, Leach FS, et al. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993;260:812-6.
14. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993;363:558-61.
15. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;260:816-9.
16. Lazar V, Grandjouan S, Bognel C, et al. Accumulation of multiple mutations in tumour suppressor genes during colorectal tumorigenesis in HNPCC patients. *Hum Mol Genet* 1994;3:2257-60.
17. Markowitz S, Wang J, Myeroff L, et al. Inactivation of the type II TGF- β receptor in colon cancer cells with microsatellite instability. *Science* 1995;268:1336-8.
18. Parsons R, Myeroff LL, Liu B, et al. Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. *Cancer Res* 1995;55:5548-50.
19. Vasen HFA, Wijnen JT, Menko FH, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* 1996;110:1020-7. [Erratum, *Gastroenterology* 1996;111:1402.]
20. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. *Hum Mol Genet* 1997;6:105-10.
21. Vasen HFA, Mecklin J-P, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum* 1991;34:424-5.
22. Liu B, Parsons R, Papadopoulos N, et al. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med* 1996;2:169-74.
23. Kolodner RD, Hall NR, Lipford J, et al. Structure of the human *MLH1* locus and analysis of a large hereditary nonpolyposis colorectal carcinoma kindred for *MLH1* mutations. *Cancer Res* 1995;55:242-8.

24. Han HJ, Maruyama M, Baba S, Park JG, Nakamura Y. Genomic structure of human mismatch repair gene, hMLH1, and its mutation analysis in patients with hereditary non-polyposis colorectal cancer (HNPCC). *Hum Mol Genet* 1995;4:237-42.
25. Wijnen J, Vasen H, Khan PM, et al. Seven new mutations in *bMSH2*, an HNPCC gene, identified by denaturing gradient-gel electrophoresis. *Am J Hum Genet* 1995;56:1060-6.
26. Wijnen J, Khan PM, Vasen H, et al. Majority of *bMLH1* mutations responsible for hereditary nonpolyposis colorectal cancer cluster at the exonic region 15-16. *Am J Hum Genet* 1996;58:300-7.
27. Weber TK, Conlon W, Petrelli NJ, et al. Genomic DNA-based hMSH2 and hMLH1 mutation screening in 32 eastern United States hereditary nonpolyposis colorectal cancer pedigrees. *Cancer Res* 1997;57:3798-803.
28. Moslein G, Tester DJ, Lindor NM, et al. Microsatellite instability and mutation analysis of *bMSH2* and *bMLH1* in patients with sporadic, familial and hereditary colorectal cancer. *Hum Mol Genet* 1996;5:1245-52.
29. Wijnen J, Khan PM, Vasen H, et al. Hereditary nonpolyposis colorectal cancer families not complying with the Amsterdam criteria show extremely low frequency of mismatch-repair-gene mutations. *Am J Hum Genet* 1997;61:329-35.
30. Pensotti V, Radice P, Presciuttini S, et al. Mean age of tumor onset in hereditary nonpolyposis colorectal cancer (HNPCC) families correlates with the presence of mutations in DNA mismatch repair genes. *Genes Chromosomes Cancer* 1997;19:135-42.
31. Myers RM, Maniatis T, Lerman LS. Detection and localization of single base changes by denaturing gradient gel electrophoresis. *Methods Enzymol* 1987;155:501-27.
32. Fodde R, Losekoot M. Mutation detection by denaturing gradient gel electrophoresis (DGGE). *Hum Mutat* 1994;3:83-94.
33. Fodde R, van der Lijjt R, Wijnen J, et al. Eight novel inactivating germ line mutations at the APC gene identified by denaturing gel electrophoresis. *Genomics* 1992;13:1162-8.
34. Liu B, Farrington S, Peterson GM, et al. Genetic instability occurs in the majority of young patients with colorectal cancer. *Nat Med* 1995;1:348-52.
35. Vasen HFA, Taal BG, Griffioen G, et al. Clinical heterogeneity of familial colorectal cancer and its influence on screening protocols. *Gut* 1994;35:1262-6.
36. Jass JR, Pokos V, Arnold JL, et al. Colorectal neoplasms detected colonoscopically in at-risk members of colorectal cancer families stratified by the demonstration of DNA microsatellite instability. *J Mol Med* 1996;74:547-51.
37. Laken SJ, Petersen GM, Gruber SB, et al. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in *APC*. *Nat Genet* 1997;17:79-83.
38. Spirio L, Olschwang S, Groden J, et al. Alleles of the APC gene: an attenuated form of familial polyposis. *Cell* 1993;75:951-7.

RECEIVE THE *JOURNAL'S* TABLE OF CONTENTS EACH WEEK BY E-MAIL

To receive the table of contents of the *New England Journal of Medicine* by e-mail every Thursday morning, send an e-mail message to:

listserv@massmed.org

Leave the subject line blank, and type the following as the body of your message:

subscribe TOC-L

You can also sign up through our Web site at: <http://www.nejm.org>
