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Reduced Frequency of Extracolonic Cancers in Hereditary Nonpolyposis Colorectal Cancer Families with Monoallelic *hMLH1* Expression

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Summary

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disease caused by mutations in one of at least four different DNA mismatch repair genes, *hMLH1, hMSH2, hPMS1,* and *hPMS2.* Phenotypically, HNPCC is characterized by the early onset of colorectal cancers and various extracolonic cancers. Depending on the presence or absence of extracolonic tumors, HNPCC has been divided into two syndromes (Lynch syndrome I and Lynch syndrome II), but, so far, no correlation to distinct genotypes has been demonstrated. In this study, we present a frequent *hMLH1* intron 14 founder mutation that is associated with a highly reduced frequency of extracolonic tumors. The mutation disrupts the splice donor site and silences the mutated allele. Tumors exhibited microsatellite instability, and loss of the wild-type *hMLH1* allele was prevalent. We propose that the mutation results in a milder phenotype, because the mutated hMLH1 protein is prevented from exerting a dominant negative effect on the concerted action of the mismatch repair system.

Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disease characterized by the early onset of colorectal cancers and a broad spectrum of extracolonic cancers, particularly in the endometrium, the urinary tract, and the small intestine (Watson and Lynch 1993). HNPCC is caused by mutations in one of at least four different DNA mismatch repair genes, *hMLH1, hMSH2, hPMS1,* and *hPMS2* (Fishel et al. 1993; Leach et al. 1993; Bronner et al. 1994; Nicolaides et al. 1994; Papadopoulos et al. 1994), which, in concert, correct single base-pair errors and short mismatched loops (Fishel and Kolodner 1995).

Diagnosis of HNPCC is based on a characteristic family history, the so-called Amsterdam criteria (Vasen et al. 1991), and on the identification of a mutation in one of the four mismatch repair genes. Mutations are found mainly in *hMLH1* and *hMSH2,* and, with the exceptions of a frequent mutation in intron 5 of *hMSH2* and two Finnish *hMLH1* founder mutations in exons 6 and 16, they are distributed widely among all exons in *hMLH1* and *hMSH2* (Froggatt et al. 1995; Nyström-Lahti et al. 1995; Liu et al. 1996). Clinical studies have indicated that HNPCC is a heterogeneous entity (Lynch et al. 1988). Depending on the presence of extracolonic tumors, HNPCC may be divided into two syndromes, which usually are referred to as Lynch syndrome I and Lynch syndrome II, but, so far, no distinct genotypes have been assigned to these two phenotypes. Accordingly, HNPCC gene carriers cannot be offered individual clinical and prognostic guidelines but are advised to follow the same lifelong clinical surveillance program aimed at detecting both colonic and extracolonic tumors.

In this study, we present a frequent *hMLH1* intron 14 splice-donor mutation that is associated with a highly reduced frequency of extracolonic tumors. In contrast with other splice-site mutations, this mutation silences the affected allele. We propose that the mutation results in a distinct phenotype, because the mutated hMLH1 protein is prevented from exerting a dominant negative effect on the concerted action of the mismatch repair system.

Patients, Material, and Methods

Patients

The Danish HNPCC register comprises 28 families that fulfil the Amsterdam criteria (Vasen et al. 1991): that is, (1) families should exhibit three histologically verified cases of colorectal cancer, of which at least one

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should be diagnosed before the patient is 50 years of age; (2) there should be affected individuals in two generations, and one of these individuals should be a first-degree relative to the other two; and (3) familial adenomatous polyposis should be excluded. DNA was available from 21 of the families. Data concerning date of birth, date of death, date of operation(s), type(s) of cancer(s), and graduation of the cancer(s) are included in the register. Table 1 summarizes the clinical features and the haplotyping of the 9 families with splice-site mutations, compared in this study.

DNA and RNA Isolation

DNA was extracted from whole blood by use of a QIAamp blood kit or a PureGene DNA isolation kit, in accordance with the manufacturer's instructions. RNA was isolated from Epstein-Barr virus–immortalized lymphocytes or from deparaffinized tissue sections of normal colon tissue, as described in previous studies (Chirgwin et al. 1979; Chomczynski and Sacchi 1987).

Mutation Analysis

Mutations in *hMLH1* and *hMSH2* were examined by PCR amplification of individual exons, followed by direct sequencing. M13-tailed primers covering the exons and the splice-site junctions were employed, as described in previous studies (Kolodner et al. 1994, 1995). Direct sequencing of reverse-transcription (RT) PCR products and genomic PCR products was performed in an ABI 377 DNA sequencer (Applied Biosystems) by use of dye-terminator or dye-primer cycle sequencing, according to the manufacturer's manual. All mutations were confirmed by analysis of two independently collected blood samples and were sequenced in both directions. *hMLH1* RNA was amplified by RT-PCR. cDNA was generated by use of random hexamers and 1 μg total RNA template. Amplification of a 1,220-nucleotide *hMLH1* cDNA fragment extending from position −36 upstream of the AUG codon to codon 394 was performed with primers 5′-GCATCTAGACGTTTC-CTTGGC-3′ and 5′-CATCAAGCTTCTGTTCCCG-3′. Amplification of 1,219 base pairs covering codons 326–731 was performed with primers 5′-GGGGTGCAG-CAGCACATCG-3′ and 5′-GGAGGCAGAATGTGT-GAGCG-3′.

Haplotyping

Haplotyping was performed with microsatellites D2S2378, D2S2156, D2S2369, D2S2316, D2S123, D2S2292, D2S2251, D3S1283, D3S1266, D3S1609, D3S3512, D3S1611, D3S1298, and D3S3521 (Dib et al. 1996). The marker sets were intragenic or were flanking *hMSH2* or *hMLH1*, spanning 6.5 cM or 11.2 cM, respectively. The products were separated on an ABI 377 sequencer and were analyzed with GeneScan Software 2.02 (Applied Biosystems). The frequency of the alleles in the general population was analyzed by the examination of 36 healthy subjects of Danish origin.

Assessment of Microsatellite Instability (MIN) and Loss of Heterozygosity (LOH)

Tissue sections were deparaffinized with Histoclear (National Diagnostics) and then with 96% alcohol, after which DNA were isolated as described in a previous study (Strauss et al. 1994). The eight dinucleotide-repeat markers that were examined are as follows: D2S123, D2S2156, D3S3512, D3S1611, D3S1298, D5S82, D5S346, and D17S1881 (Nakamura et al. 1988; Spirio et al. 1991; Dib et al. 1996). The oligonucleotides were labeled with fluorescent dyes (6-FAM, TET, and HEX [Applied Biosystems]), and the products were separated on an ABI 377 sequencer and were analyzed with GeneScan Software 2.02 (Applied Biosystems). A tumor was considered to exhibit MIN when allele alterations were observed in at least two markers. LOH was calculated as described in a previous study (Canzian et al. 1996).

Results

A Frequent hMLH1 *Splice-Donor Mutation*

In 5 of the 21 Amsterdam-positive families, we detected a splice-donor mutation in intron 14 of *hMLH1* (fig. 1). The mutation is a combined 7-bp deletion and 4-bp insertion that leads to the exchange of the obligatory thymidine at position +2 and to the exchange of conserved purines at positions +3 to +5, in the splice donor site. Following amplification of a 1,219-bp cDNA fragment encompassing exons 11–19, only the wild-type allele was detected (fig. 2*A*). Sequencing of the RT-PCR product from both lymphocytes and normal colon tissue showed that the cDNA exhibited the normal splice junction from exon 14 to exon 15. In order to confirm that the mutated allele was not expressed, we examined the relative expression of an A→G isoleucine 219–to-valine polymorphism in exon 8 of *hMLH1* (fig. 2*B*) (Tannergård et al. 1995). Whereas the two alleles were detected in DNA, the A allele was almost completely undetectable in RNA. Since the A allele segregated with the intron 14 mutation (see table 2), we conclude that the mutation silences the allele.

Two other families exhibiting a previously characterized A→T splice-donor mutation at position +3 of intron 5 in *hMSH2* (Liu et al. 1994; Froggatt et al. 1995) and two families with an *hMLH1* A→T mutation at position −2, relative to the 3′ splice site, in intron 15 (Wijnen et al. 1996) also were detected among the Amsterdam-positive families. In contrast with the intron 14 mutation, the mutated alleles were expressed. The *hMLH1*

Table 1

Clinical Features of the Affected Individuals in the Amsterdam-Positive Families with Splice-Site Mutations

Mutation and Family-Individual	No. of Colorectal Cancers	Age(s) at Surgery (Years)	Location of Extracolonic Cancer(s) (Age[s] at Diagnosis, in Years)	Haplotype Analysis Performed
hMLH1 intron 14:				
12-02	1	...		
12-04	1	37		
12-08	2	37, 37		
12-14	1	40		
12-16	2	35, 39		X
18-06	1	74		
18-08	1	31		
18-10	1	37		
18-14	1	39		
18-22	1	56		
18-26	1	31		X
21-02	1	...		
21-06	2	50, 53		X
21-08	1	33		X
21-12	1	45		
21-16	3	49, 51, 58	Endometrium (33)	
21-26	4	55, 61, 64, 65	Ampulla of vater (54)	
21-32	1	37		X
31-02	2	55, 66		
31-04	1	57		X
31-10	1	33		X
41-04	1	...[a]		
41-08	1	48		
41-10	2	42, 42		
41-20	2	33, 33		X
hMLH1 intron 15 or *hMSH2* intron 5:				
3-06	4	33, 39, 41, 51	Jejunum (29)	
3-08	2	40, 50	Bladder (51)	
3-16	1	25		X
5-06	...	...	Small intestine (37)	
5-12	1	57		
5-20	3	47, 52, 55	Jejunum (56)	X
5-22	3	37, 42, 48	Endometrium (45)	
5-26	1	33		
5-28	1	51		
5-32	1	37		X
5-38	2	32, 32		X
42-02	3	37, 52, 55	Urinary tract (69, 69)	
42-06	...	...	Pancreas (48)	
42-12	1	46		
42-14	1	27		X
111-02	1	52	Lower limb (Osteosarcoma) (80)	X
111-04	2	56		
111-34	1	55	Endometrium (53)	
111-50	...	...	Ovary, ileum (36, 45)	
111-54	1	13		
111-56	1	42		

NOTE.—All persons are proven gene carriers or obligate gene carriers, since they have passed the gene to their children. Furthermore, 20 nonaffected individuals and 3 spouses, from the families, were haplotyped.

[a] Patient died at age 52 years.

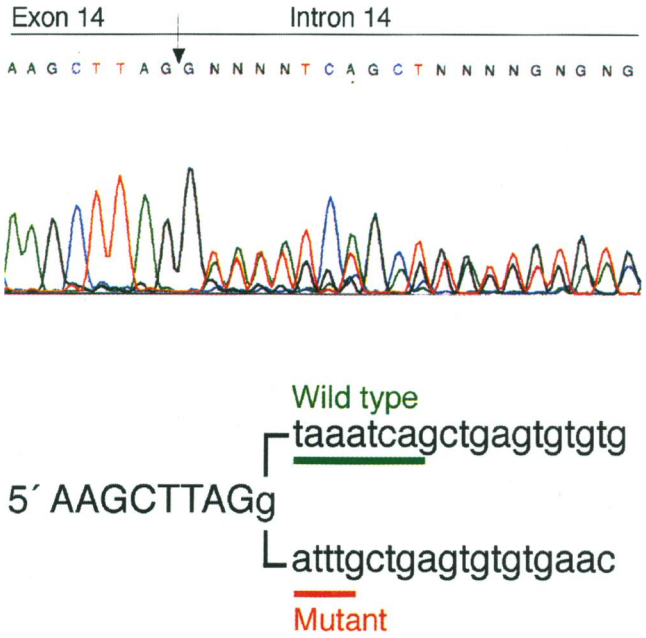

Figure 1 Sequence of the frequent *hMLH1* splice-site mutation. Exon 14 in *hMLH1* was amplified from genomic DNA and was sequenced. *Top,* Electropherogram of the splice-donor mutation. *Bottom,* Sequence of the wild-type and mutant alleles.

intron 15 mutation leads to a so-called in-frame deletion of exon 16, and the *hMSH2* intron 5 mutation leads to an in-frame deletion of exon 5, in the mature transcript (Liu et al. 1994; Froggatt et al. 1995; Wijnen et al. 1996).

Haplotyping

Genealogical data showed that the families with the *hMLH1* intron 14 mutation and the families with the *hMLH1* intron 15 mutation were unrelated within the last four generations. Around the turn of the century, however, they lived within relatively narrow geographic areas, on the Island of Sealand and in a municipality in northern Jutland (fig. 3). Haplotyping, with five markers spanning a total of >7.4 cM, of the families exhibiting the *hMLH1* intron 14 mutation showed that the diseased gene segregated with the same haplotype in all affected individuals (n = 8; table 2) and in two gene carriers, indicating that the families were related. The haplotype comprised the intragenic D3S1611 marker and the three flanking markers D3S1609, D3S3512, and D3S1298. Analysis of the A→G isoleucine 219–to-valine polymorphism in exon 8 (Tannergård et al. 1995) showed that the mutated allele segregated with isoleucine, in all families. When haplotyping was extended to span a total of >11.2 cM, by application of two additional telomeric markers, D3S1283 and D3S1266, and of one centromeric

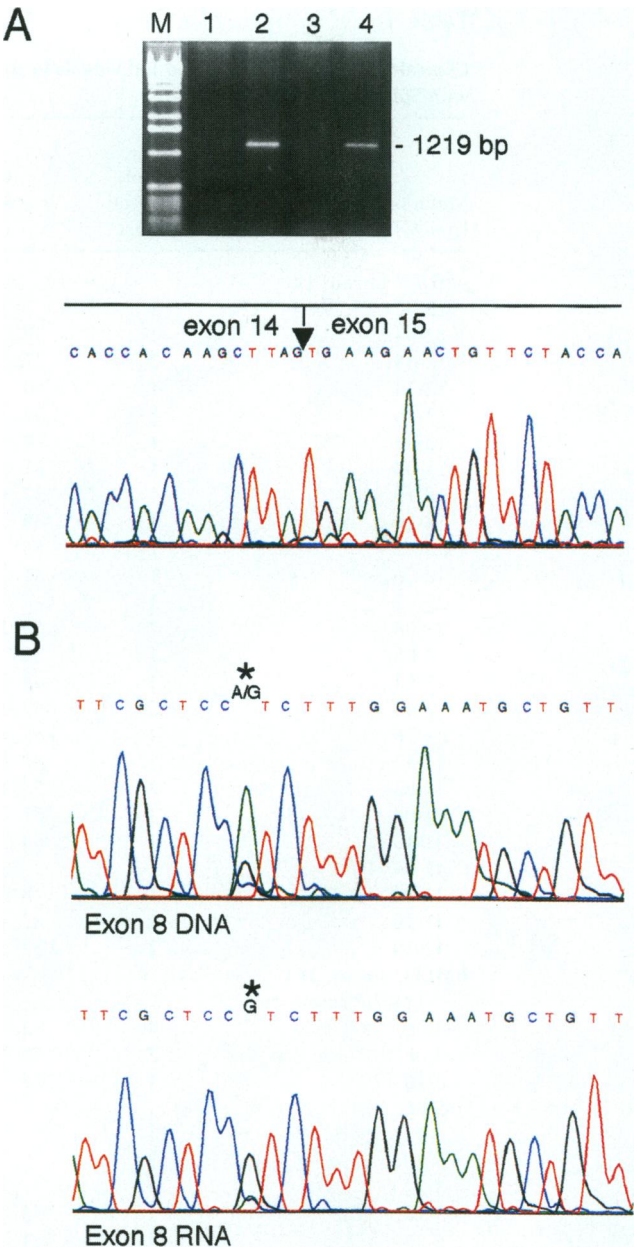

Figure 2 Silencing of the *hMLH1* intron 14 mutant allele. *A,* RT-PCR analysis of mRNA from an *hMLH1* intron 14 mutation carrier (lane 2) and a normal subject (lane 4) are shown. Control samples in which RT was omitted are shown (lanes 1 and 3). cDNA was amplified with primers, generating a 1,219-bp fragment encompassing the region from codon 326 in exon 11 to codon 731 in exon 19 and was examined by agarose-gel electrophoresis. The electropherogram shows the normal splice junction from exon 14 to exon 15, in the cDNA fragment. *B,* Sequence of exon 8 in *hMLH1*, in DNA (*top*) and RNA (*bottom*). DNA was amplified with intron primers, as described in Patients, Material, and Methods, and cDNA was generated with primers extending from position −36 upstream from the AUG codon to codon 394. The PCR products were sequenced with fluorescent-dye terminators. The position of the exon 8 A→G polymorphism is indicated by an asterisk (*).

Table 2

Haplotypes of Affected Individuals from Families with *hMLH1* and *hMSH2* Mutations

Marker/ Exon[a]	Distance (cM)	Haplotype of *hMLH1* Intron 14–Mutation Family[b] 12	21	31	18	41	Frequency of Founder Alleles[c] Danish	CEPH	Haplotype of *hMLH1* Intron 15–Mutation Family[b] 3	5	Frequency of Founder Alleles[c] Danish	CEPH
D3S1283	.1						.30	.34	158	158	.30	.34
D3S1266	2.6						.17/.25[d]	.14/.19[d]	289	289	.29	.26
D3S1609	6.8	257	257	257	257	257	.33	.34	253	253	.60	.48
D3S3512	.0	133	133	133	133	133	.35	.52	141	141	.04	.13
Exon 8[e]	.0	A	A	A	A	A	.69	...	...	...	...	...
D3S1611	.6	268	268	268	268	268	.03	.05	252	252	.20	.21
D3S1298	1.1	210	210	210	210	210	.09	.11	...	...	...	...
D3S3521							.03/.19[d]	.06/.19[d]	281	281	.34	.29

Marker	Distance (cM)	Haplotype of *hMSH2* Intron 5–Mutation Family[b] 42	111[f]
D2S2378	3.3		
D2S2156	.0		
D2S2369	.0		
D2S2316	.0		
D2S123	2.7	213	213
D2S2292	.5		
D2S2251			

[a] Markers are listed in order.
[b] Haplotypes with preserved alleles are within the unshaded region, and haplotypes with distinct alleles are within the shaded region.
[c] Determined from analysis of 72 chromosomes from 36 Danish controls and of 56 CEPH chromosomes.
[d] The first frequency is for the allele shared by families 12, 21, and 31, and the second frequency is for the allele shared by families 18 and 41.
[e] The nucleotide at polymorphic site 219 is indicated.
[f] Haplotype of each allele is shown, since it was not possible to determine which haplotype segregated with the mutation.

marker, D3S3521, two recombination events resulting in two different haplotypes were apparent, in three and in two of the families. The gene frequencies also were determined in 36 unrelated controls (table 2). The likelihood of finding, by chance, one of the two different haplotypes in a non–gene carrier was found to be 3.3×10^{-7} for one haplotype and 3.1×10^{-6} for the other. When the five markers shared by all families were considered, the likelihood was 2.2×10^{-4}. The two families with the *hMLH1* intron 15 mutation exhibited an identical haplotype over the examined 11.2-cM DNA strand containing *hMLH1*. The likelihood of this haplotype was 1.4×10^{-4}. In the two families (42 and 111) with *hMSH2* intron 5 mutations, the haplotype segregating with the mutated allele was known only in family 42. However, the two families differed in 13 of 14 possible alleles, and it could therefore be concluded that they were unrelated.

Extracolonic Cancers in HNPCC Kindreds

A comparison of the tumor spectrum of the 28 Amsterdam-positive families from the Danish HNPCC register with that of the general population showed, in agreement with the results of previous studies, that HNPCC families frequently acquire tumors in the endometrium and the urinary tract, in addition to colorectal cancer (fig. 4) (Vasen et al. 1990; Watson and Lynch 1993). HNPCC generally is considered to be heterogeneous, since certain HNPCC families exhibit few extracolonic tumors. We therefore compared the frequency of extracolonic cancers in the five families with the intron 14 founder mutation with that of the other Amsterdam-positive families in the register (table 3). The median age for diagnosis of the first colorectal cancer was comparable in the two groups. However, since only 2 of 25 affected individuals suffered from an extracolonic cancer, the affected individuals in the families with the

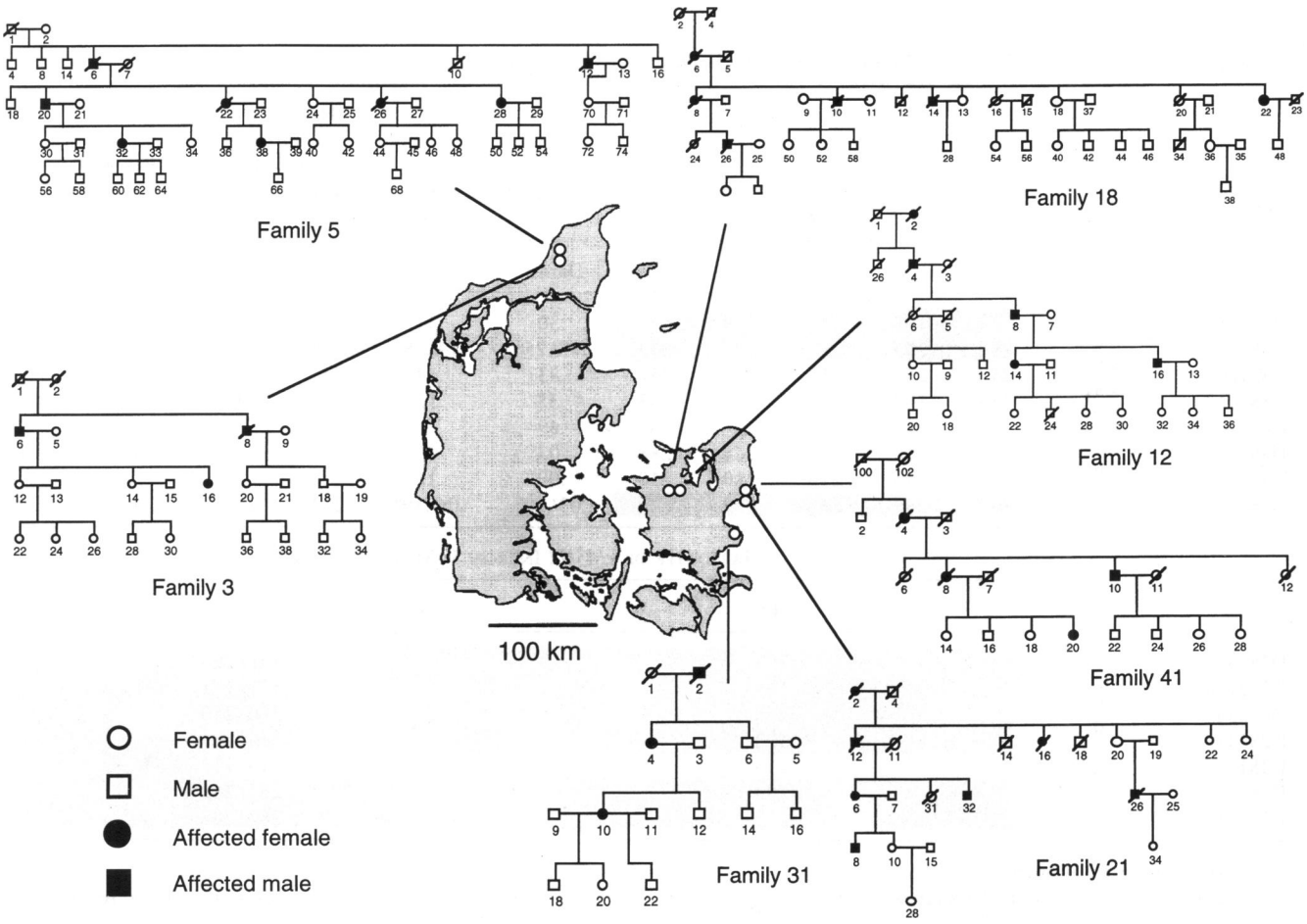

Figure 3 Pedigrees and origins of Danish families with either *hMLH1* intron 14 or *hMLH1* intron 15 founder mutations

intron 14 mutation were found to have significantly fewer extracolonic tumors ($P < .05$) than those in the Amsterdam-positive families. The first patient had endometrial cancer, by the age of 33 years, and three successive colorectal cancers, and the second patient had cancer of the ampulla of vater, by the age of 54 years, and four colorectal cancers. In the four families with the *hMLH1* intron 15 and *hMSH2* intron 5 splice-site mutations, almost 50% of the affected individuals had extracolonic tumors. This frequency not only was significantly higher ($P < .01$) than that found in families with the intron 14 mutation, but it also was higher ($P < .05$) than that found in the collection of Amsterdam-positive families.

MIN and LOH

MIN and LOH at the *hMLH1* locus were examined with eight microsatellite markers, in seven paraffin-embedded colorectal tumors from four of the founder families. Three markers were located at the *hMLH1* locus (D3S3512, D3S1611, and D3S1298), whereas the remaining markers were located at the *hMSH2* (D2S123 and D2S2156), *APC* (D5S82 and D5S346), and *p53* (D17S1881) loci (fig. 5). All tumors exhibited MIN in more than four of the markers. LOH at the *hMLH1* locus, defined as a >40% signal reduction of one allele (Canzian et al. 1996), was observed in three tumors. In all cases, the loss occurred in the *hMLH1* wild-type allele. In the remaining four tumors, LOH could not be determined, owing to the occurrence of MIN. We conclude that the founder mutation is associated with MIN and that loss of the wild-type *hMLH1* allele is a frequent event in the tumors.

Discussion

In this study, we demonstrate a frequent founder mutation in *hMLH1*, which leads to an attenuated HNPCC phenotype characterized by a highly reduced frequency of extracolonic tumors. The *hMLH1* intron 14 mutation was found in ~25% of the Danish Amsterdam-positive HNPCC kindreds and was localized to the splice donor site

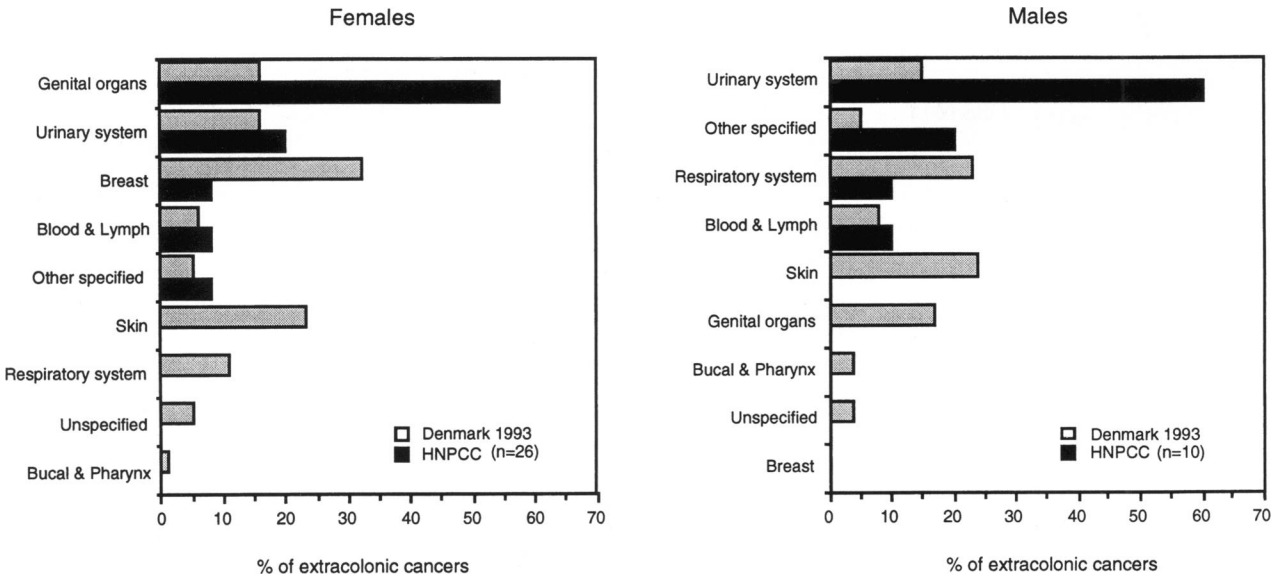

Figure 4 Distribution and frequency of extracolonic cancers in HNPCC patients. Proportional distribution of extracolonic cancers in different organ systems in females and males from HNPCC families and in the Danish population of 1993 (Storm et al. 1993). The HNPCC material included 36 neoplasms collected over the period 1910–1996. The grouping called "Other specified" included tumors in the eye and lachrymal glands, brain and nervous system, thyroid, endocrine glands, and bone and connective tissue.

of intron 14. Basically, the mutation is a combined 4-bp substitution and 3-bp deletion, in which the first four nucleotides are complementary to the wild-type sequence. In this way, five of the six highly conserved nucleotides in the splice junction are changed. In contrast with other splice-site mutations that result in exon skipping, intron retention, or use of cryptic donor/acceptor sites, the *hMLH1* intron 14 splice-donor mutation silences the mutant allele. Down-

Table 3

Frequency of Extracolonic Tumors in Affected Individuals with the *hMLH1* Intron 14 Mutation, Compared with the Remaining Amsterdam-Positive Families of the HNPCC Register and with Affected Individuals with an *hMLH1* Intron 15 or an *hMSH2* Intron 5 Mutation

	Families with *hMLH1* Intron 14 Splice-Site Mutations	Families with *hMLH1* Intron 15 or *hMSH2* Intron 5 Splice-Site Mutations	Amsterdam-Positive Families
Median age (range) at first colorectal cancer diagnosis [years]	39 (31–74)	38 (14–58)	42 (14–81)
No. of individuals with colorectal cancer	23	11	80
No. of individuals with colorectal and extracolonic cancer or extracolonic cancer only	2[a]	10[b,c]	34

NOTE.—Affected individuals are defined as gene carriers or as obligate gene carriers. The number of extracolonic cancers in the different groups were compared by use of Fisher's exact test.

[a] Comparison of the *hMLH1* intron 14–mutation families with the Amsterdam-positive families resulted in $P < .05$.

[b] Comparison of the *hMLH1* intron 14–mutation families with the *hMLH1* intron 15– and the *hMSH2* intron 5–mutation families resulted in $P < .01$.

[c] Comparison of the *hMLH1* intron 15– and the *hMSH2* intron 5–mutation families with the Amsterdam-positive families resulted in $P < .05$.

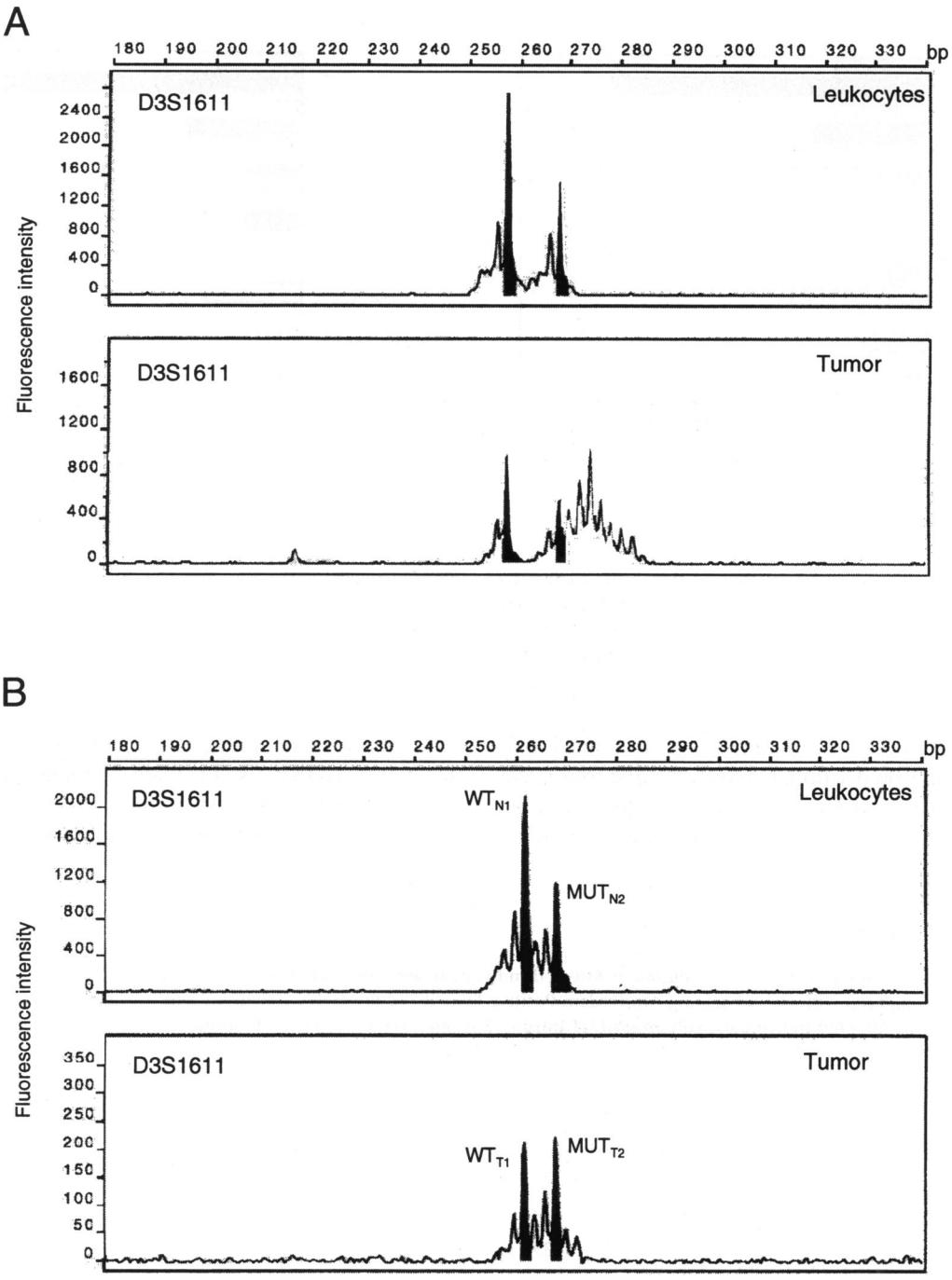

Figure 5 MIN and LOH in colorectal tumors from founder families. *A*, Analysis of locus D3S1611, in leukocytes (*top panel*) and in tumor tissue (*bottom panel*), from patient 41-20. The x-axis indicates the size of the alleles, in bp, and the y-axis indicates the peak heights, in fluorescence units. The positions of the two alleles are indicated by the blackened trace. *B*, Assessment of LOH in locus D3S1611, in tumor tissue from patient 12-16. The wild-type and mutant alleles in normal leukocytes (*top panel*) and the alleles in tumor tissue (*bottom panel*) are shown. LOH was defined as a >40% reduction in the relative intensity of the wild-type allele and was calculated by use of the formula $L = (a_{T2} \times a_{N1}) / (a_{T1} \times a_{N2})$, where a is the area of the peaks (Canzian et al. 1996). When $L > 1.67$, the allele is decreased >40%. In this case, L was 1.92.

regulation of the mutant allele by defective splice junctions may be accomplished by retention of the pre-mRNA in incomplete spliceosomes (Legrain and Rosbach 1989) or by nonsense codon–directed mRNA degradation, as described in previous studies (Maquat 1995; Aoufouchi et al. 1996). In agreement with the unusual composition of the mutation, haplotyping shows that all affected kindreds are related. We have not determined when the intron 14 mutation originated, but, in a recent study, Moisio et al. (1996) were able to determine that the origin of a Finnish founder mutation was during the 16th century. In the Finnish kindreds, a distinct haplotype spanning ~12 cM, comprising markers similar to those employed in this study, was preserved without recombinations. In view of the fact that the haplotype in the Danish kindreds was preserved only within 7.4 cM and that two recombination events were observed within markers spanning 11.2 cM, it is likely that the Danish founder mutation is older than the Finnish mutation. The two kindreds sharing the *hMLH1* intron 15 splice-acceptor mutation came from the same confined geographical area and therefore, not surprisingly, also were related. In contrast, the families exhibiting the frequent *hMSH2* intron 5 splice-site mutation clearly were unrelated, as demonstrated in a previous study (Froggatt et al. 1995). It is striking that this mutation is located in connection to 26 successive adenosine residues, which have a strong potential for stacking. During replication, the polymerase is likely to slide on the DNA strand, and, therefore, this site may be subject to frequent mutations.

The collection of Amsterdam-positive families in the Danish HNPCC register demonstrates that ~30% of the affected individuals in HNPCC families develop an extracolonic cancer. In agreement with the results of previous studies (Vasen et al. 1990; Watson and Lynch 1993), extracolonic tumors were found mainly in the urinary tract and the endometrium, which clearly is distinct from the general population, for which tumors in the breast and lung dominate. It was recognized early by Lynch et al. (1988) that the frequency of extracolonic cancer varied among HNPCC families. In this study, the five families with the *hMLH1* intron 14 mutation correspond to Lynch syndrome I, which comprises HNPCC families with few or no extracolonic cancers. The colorectal tumors in these families were not different from other HNPCC tumors, in the sense that they exhibited MIN, and, as demonstrated in a previous study (Hemminki et al. 1994), LOH of markers within or adjacent to *hMLH1* occurred nonrandomly at the wild-type allele.

The phenotypes of patients with *hMLH1* or *hMSH2* mutations have been examined in several studies, but no distinct phenotypes have been apparent, despite the fact that HNPCC mutations are variable and widespread in the different DNA-repair genes (Liu et al. 1996; Vasen et al. 1996). Vasen et al. (1996) specifically compared the risk of endometrial cancer in patients with either *hMLH1* or *hMSH2* mutations and found no difference between the two groups. Since efficient mismatch repair requires the concerted action of *hMSH2*, *hMLH1*, *hPMS2*, and *hMSH6* (Fishel and Kolodner 1995), distinct phenotypes may not depend on whether mutations are found in *hMSH2* or in *hMLH1*. Two different disease-modifying mechanisms, which would operate independently of the involved gene, may be envisaged. The first involves the presence of a linked modifier gene, within 7.4 cM of *hMLH1*, that could compensate for defective DNA repair, in a tissue-specific manner. The second model implies a dosage effect. Rather, we propose that it is the silencing of the mutated allele that is responsible for the milder phenotype, because the mutated hMLH1 protein is prevented from exerting a dominant negative effect on the mismatch repair system. One other *hMLH1* mutation associated with allelic inactivation has been reported by Liu et al. (1996), unfortunately without reference to extracolonic manifestations. In agreement with such a model, the families with the *hMSH2* intron 5 or the *hMLH1* intron 15 splice-site mutations, which result in the expression of a defective protein, all exhibited a high frequency of extracolonic tumors, corresponding to Lynch syndrome II. The number of extracolonic cancers was significantly higher than in the complete collection of Amsterdam-positive families, which is in agreement with the observation that Amsterdam-positive families represent a mixture of families with low, as well as high, frequencies of extracolonic cancers.

In conclusion, we present a frequent *hMLH1* founder mutation that phenotypically corresponds to Lynch syndrome I. Although additional studies of kindreds with other inactivating mutations are needed, the results imply that clinical surveillance could be restricted to colonic examinations in HNPCC gene carriers with monoallelic *hMLH1* expression.

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