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Mismatch Repair Genes on Chromosomes 2p and 3p Account for a Major Share of Hereditary Nonpolyposis Colorectal Cancer Families Evaluable by Linkage

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Summary

Two susceptibility loci for hereditary nonpolyposis colorectal cancer (HNPCC) have been identified, and each contains a mismatch repair gene: MSH2 on chromosome 2p and MLH1 on chromosome 3p. We studied the involvement of these loci in 13 large HNPCC kindreds originating from three different continents. Six families showed close linkage to the 2p locus, and a heritable mutation of the MSH2 gene was subsequently found in four. The 2p-linked kindreds included a family characterized by the lack of extracolonic manifestations (Lynch I syndrome), as well as two families with cutaneous manifestations typical of the Muir-Torre syndrome. Four families showed evidence for linkage to the 3p locus, and a heritable mutation of the MLH1 gene was later detected in three. One 3p-linked kindred was of Amerindian origin. Of the remaining three families studied for linkage, one showed lod scores compatible with exclusion of both MSH2 and MLH1, while lod scores obtained in the other two families suggested exclusion of one HNPCC locus (MSH2 or MLH1) but were uninformative for markers flanking the other locus. Our results suggest that mismatch repair genes on 2p and 3p account for a major share of HNPCC in kindreds that can be evaluated by linkage analysis.

Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common form of hereditary colon cancer (Lynch et al. 1993). A systematic search for linkage led to the identification of a susceptibility locus on 2p in two large kindreds, one from Canada and one from New Zealand (Peltomäki et al. 1993*a*). Another HNPCC locus was mapped to the short arm of chromosome 3 by linkage in two Swedish families (Lindblom et al. 1993).

Instability at short tandem repeat sequences has been demonstrated in a subset of sporadic colorectal and other tumors (Ionov et al. 1993; Peltomäki et al. 1993*b;* Risinger et al. 1993; Thibodeau et al. 1993) and in most tumors from both 2p- and 3p-linked HNPCC families (Aaltonen et al. 1993, 1994; Lindblom et al. 1993). This instability reflects defective function of mismatch repair proteins encoded by the genes responsible for HNPCC (Parsons et al. 1993). To date, two mismatch repair genes—MSH2 (*Mut S homolog 2*) on chromosome 2p (Fishel et al. 1993; Leach et al. 1993) and MLH1 (*Mut L homolog 1*) on chromosome 3p (Bronner et al. 1994; Papadopoulos et al. 1994)—have been cloned and characterized. It is likely that HNPCC is in general associated with hereditary defects in mismatch repair genes (Papadopoulos et al. 1994). However, little is known about either the proportion of HNPCC related to the 2p- and 3p-linked genes or whether families linked to 2p versus 3p show distinct phenotypic features.

To address these questions we analyzed 13 HNPCC kindreds for linkage to markers flanking the HNPCC loci on chromosomes 2p and 3p. The results provide preliminary evidence that susceptibility genes on chromosomes 2 and 3 are responsible for a major share of HNPCC. No clear-cut clinical differences were noted between 2p- and 3p-linked families.

Subjects, Material, and Methods

HNPCC Kindreds

To assess the feasibility of obtaining reliable linkage results in the numerous HNPCC pedigrees known to us, we performed simulated linkage analyses based on actual family structure and availability of DNA samples in each fam-

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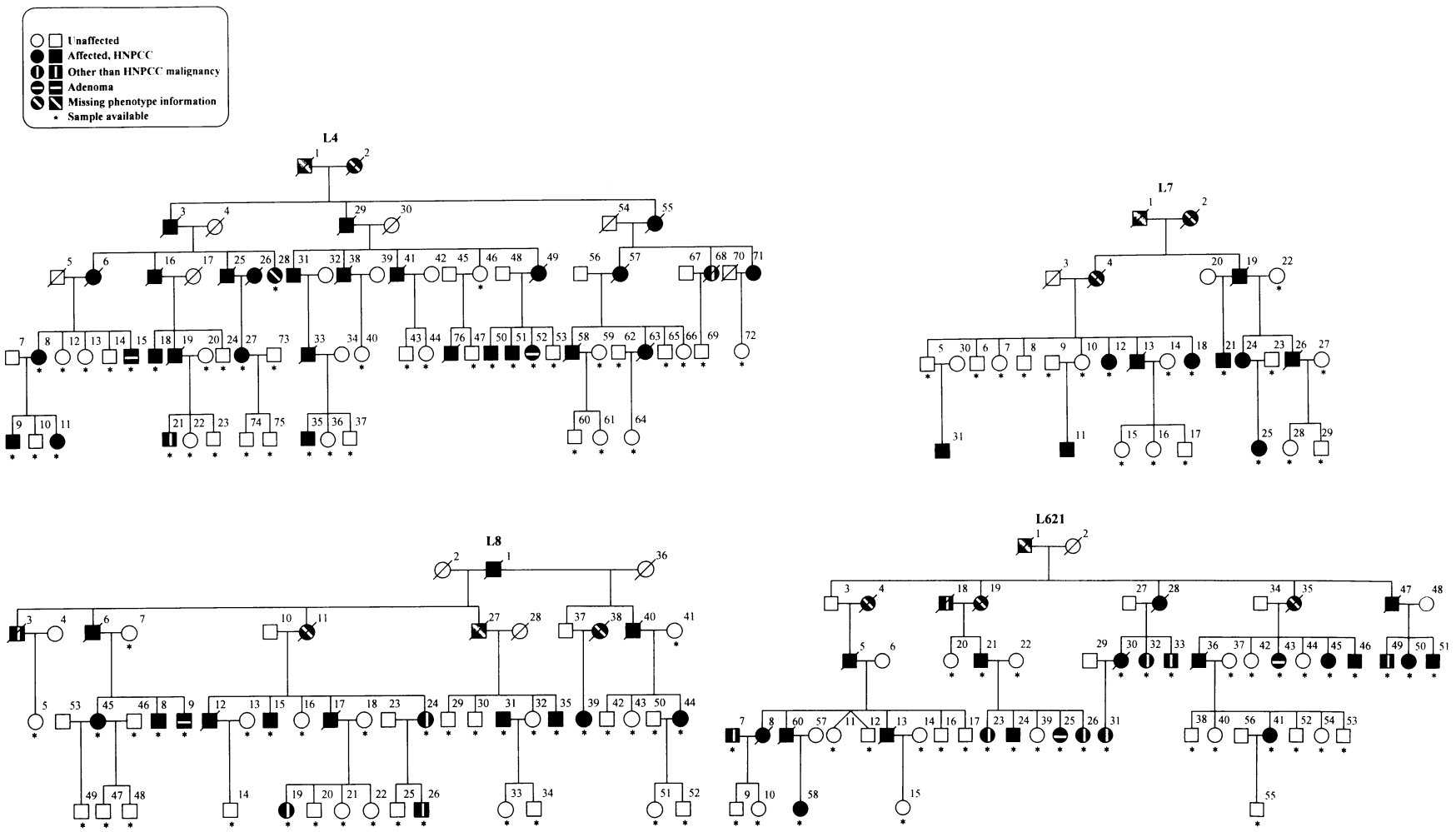
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Affected, HNPCC
Other than HNPCC malignancy
Adenoma
Missing phenotype information
Sample available
L4
L7
L8
L621

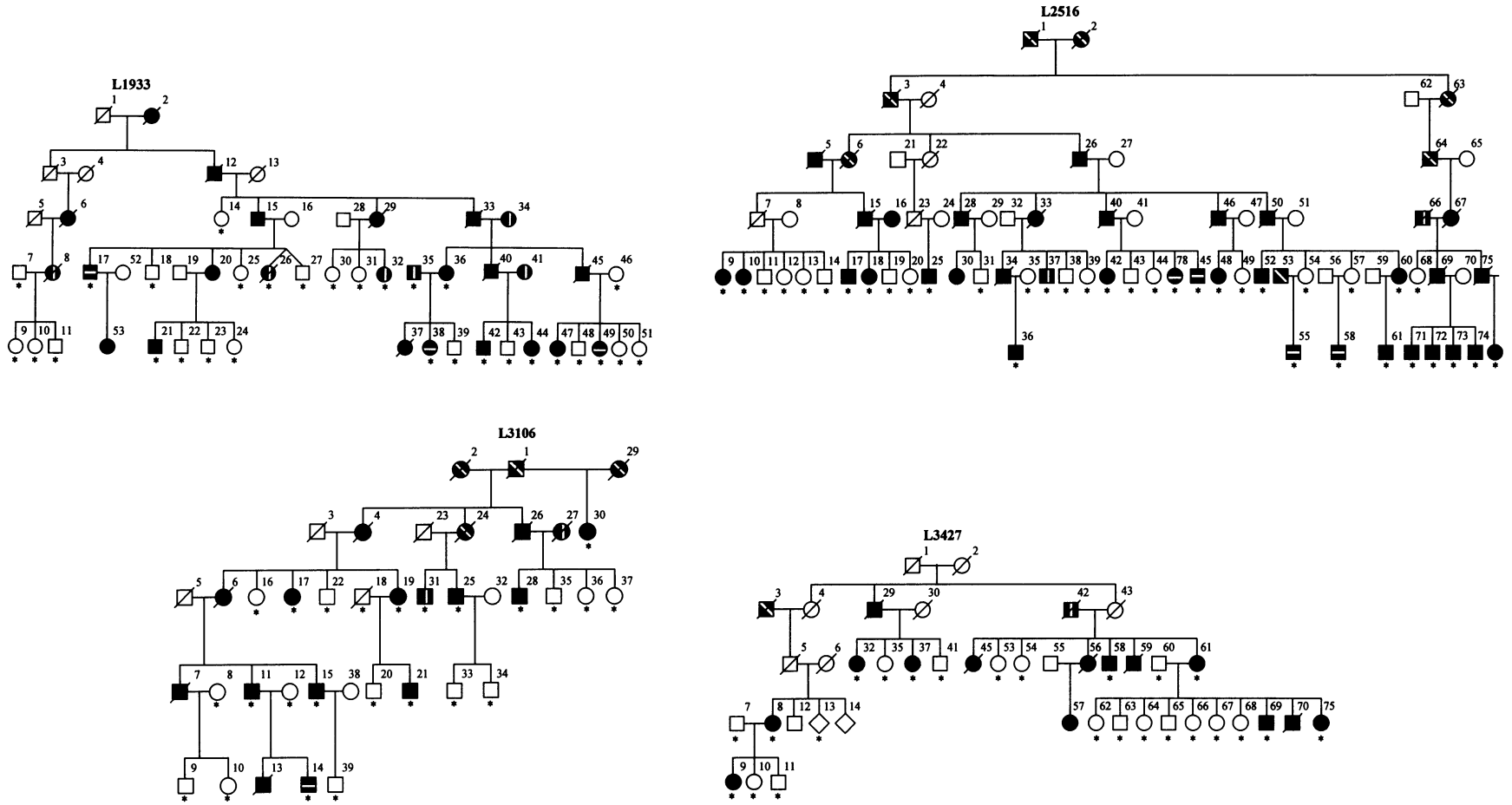

Figure 1 Pedigrees of eight HNPCC families studied, representing different clinical and genetic subcategories (see text). The symbols are as follows: Squares denote males; circles denote females; and a diagonal slash denotes that individual is deceased.

ily. Thirteen families met the criteria of potentially yielding maximum pairwise lod scores >2 at a recombination fraction (θ) of $\leq .05$, with a marker having four alleles, each having a frequency of .25. Ten families (L4, L7, L8, L621, L1933, L2516, L3106, L3427, B1, and B2) were from the United States. The pedigrees of eight of these families are shown in figure 1. For more detailed clinical information, consult the references listed in table 1. The remaining three families included in the linkage analysis were from Canada (C), New Zealand (J), and Finland (F2). The pedigrees and the linkage status of the latter kindreds have been reported elsewhere (Peltomäki et al. 1993*a*; Nyström-Lahti et al. 1994).

Microsatellite Markers and Conditions for PCR

We studied four microsatellite markers from 2p and seven microsatellite markers from 3p. The 2p markers were (distal to proximal) D2S119, AFM337yh5, D2S123, and D2S147 (Weissenbach et al. 1992; Leach et al. 1993). MSH2 maps between AFM337yh5 and D2S123, ~2 cM proximal to the former marker (Leach et al. 1993). The 3p markers were (distal to proximal) D3S1283, D3S1619, D3S1561, D3S1611, D3S1298, D3S1260, and D3S1029 (Jones et al. 1992; Weissenbach et al. 1992; Nyström-Lahti et al. 1994). Marker D3S1611 is contained in an intron of the MLH1 gene (Papadopoulos et al. 1994).

DNA extracted from blood (Kunkel et al. 1977) was amplified by PCR in the following final reaction conditions: 1 × PCR buffer (10 mM Tris, pH 8.8; 1.5 mM $MgCl_2$; 50 mM KCl; and 0.1% Triton X-100); 200 μM each of dGTP, dATP, and dTTP; 2.5 μM dCTP; 0.7 μCi of α^{32}P-dCTP, 3,000 Ci/mmol; 10 ng of each primer (one or two primer pairs at a time); 30 ng of genomic DNA template; and 0.3 units of DynaZyme thermostable DNA polymerase (Finnzymes) in a volume of 10 μl. The samples were cycled 27 times at 94°C for 30 s, 55°C for 75 s, and 72°C for 15 s, plus, after the last cycle, 72°C for 6 min. Electrophoresis was done by using 6% polyacrylamide gels containing 7.7 M urea. After electrophoresis the gels were fixed in 10% acetic acid for 15 min and were dried and exposed to X-ray film.

Linkage Analysis

We used programs of the LINKAGE program package (Lathrop et al. 1984). In pairwise linkage analyses, individuals with colorectal or endometrial carcinoma or other cancers considered to belong to the HNPCC tumor spectrum (notably, cancer of the stomach, hepatobiliary system, small intestine, kidney and ureter, and ovary; Mecklin and Järvinen 1991; Lynch et al. 1993) were regarded as affected. Patients with colorectal adenoma or cancers not considered to be part of the HNPCC spectrum were treated as having an unknown status. Phenotypes for HNPCC were coded as affected, with an autosomal dominant mode of inheritance and four liability classes (penetrances) according to the age at the time of observation (healthy) or at the time of diagnosis. Penetrances for heterozygotes were set as .15 at age ≤30 years (liability class 1), .40 at age 31–45 years (liability class 2), .70 at age 46–60 years (liability class 3), and .90 at age ≥61 years (liability class 4). The frequency of the HNPCC gene was set as .001. Phenocopies were introduced, with frequencies set at 1%, 3%, and 5% in liability classes 2, 3, and 4, respectively. We used allele frequencies, calculated by the program ILINK, from HNPCC kindreds including five to eight of the present families.

Results

When markers from chromosome 2p were used, kindreds L4, L8, and L621 showed pairwise lod scores of ≥2 in support of linkage (table 2). Additionally, the lod scores for L3106 were positive, although nonsignificant, when marker AFM337yh5, which was the closest marker studied relative to MSH2 (see table 2), was used. A germ-line mutation resulting in a predicted coding change from arginine to a stop codon and segregating with HNPCC was subsequently found in family L8, as reported elsewhere (Leach et al. 1993). A heritable mutation of MSH2 has also been detected in family L3106 (B. Vogelstein, unpublished data). Genetic linkage to MSH2 and a heritable mutation in this gene have previously been demonstrated in kindreds C and J (Leach et al. 1993; Peltomäki et al. 1993*a*).

The MSH2 locus was excluded by lod scores < -2 in families L7, L1933, L2516, L3427, and B1 (table 2). In family B2 the lod scores for 2p markers were uninformative. These families were then tested for linkage to the MLH1 locus. Table 3 shows two-point lod scores for markers D3S1619, D3S1561, D3S1611, and D3S1298, of which D3S1611 is an intragenic marker (see Subjects, Material, and Methods). Lod-score values >2 that were compatible with linkage were obtained in L7, L2516, and L3427. The highest pairwise lod scores were shown by family L2516 (4.93 at $\theta = .01$, for D3S1561). Later, family L7 revealed a heritable 4-bp deletion predicted to result in a frameshift and substitution of new amino acids, while kindred L2516 showed an insertion mutation (Papadopoulos et al. 1994). Linkage and mutation findings showing the involvement of MLH1 in family F2 have been published (Nyström-Lahti et al. 1994; Papadopoulos et al. 1994).

Finally, the lod scores in kindred L1933 were <-2, the conventional threshold for rejection of linkage, for both 2p and 3p markers (tables 2 and 3). Families B1 and B2 were informative for markers flanking one HNPCC locus only. Close linkage to MSH2 was rejected in B1 by clearly negative lod scores for AFM337yh5 and D2S123, while the results obtained with 3p markers—D3S1561 in particular—suggested exclusion of MLH1 in B2.

Discussion

Of the 13 families studied, 11 were informative for both 2p and 3p markers. Our analysis shows that six families

Table 1

Characteristics of 10 HNPCC Families from the United States

Family	Origin	No. of Affected Individuals		Mean Age at Diagnosis (years)	Special Characteristics	Reference
		Analyzed	Total			
L4	Dutch	8	35	46.4	Lynch I	Lynch et al. 1988*c*
L7	Amerindian (Navajo)	4	13	38.4	Lynch II	Lynch et al. 1985
L8	Norwegian	7	22	48.1	Lynch II	Lynch et al. 1988*a*
L621	Longtime Americans (English)	9	24	47.9	Muir-Torre/Lynch II	Lynch et al. 1990*b*
L1933	Longtime Americans (English)	5	17	38.4	Lynch II	Lynch et al. 1991*a*
L2516	German, French	16	42	48.9	Lynch II	Lynch et al. 1990*a*
L3106	German, Prussian	8	17	48.2	Lynch II (Muir-Torre)	Lynch et al. 1991*b*
L3427	English	8	14	46.9	Lynch II	Lynch et al., in press
B1	African American	5	11	47.2	Lynch I	Authors' unpublished data
B2	Longtime Americans (English)	3	12	56.5	Lynch I	Authors' unpublished data

(L4, L8, L621, L3106, C, and J) are linked to 2p and that four (L7, L2516, L3427, and F2) are linked to 3p, while one (L1933) is apparently unlinked to either locus. The number of affected individuals per family ranges from 4 to 42. These data suggest that the loci on 2p and 3p account for a major share of HNPCC in kindreds that are large enough to be informative in linkage analysis. However, it is obvious that the present findings need to be confirmed in a larger series of HNPCC families. Importantly, the recent cloning of the MSH2 and MLH1 genes provides the basis for direct mutation analyses, and thus information about the predisposing locus will soon be derived from those numerous families that cannot be tested for linkage.

On the basis of phenotypic features, various subgroups of HNPCC have been distinguished, but it is not known whether any genetic basis exists for such distinctions. Subcategories Lynch syndrome I and II have been proposed, referring to the absence versus presence of extracolonic cancers, endometrial carcinoma in particular (Lynch et al. 1988*b*). Recent clinical studies have failed to support this distinction; furthermore, the validity of the subdivision is influenced by the number of affected individuals per kindred (Hakala et al. 1991; Watson and Lynch 1993). The Muir-Torre syndrome has been proposed to represent a variant form of HNPCC, characterized by sebaceous adenomas, sebaceous carcinomas, and keratoacanthomas that

Table 2

Pairwise Lod Scores for Each Family, When Markers AFM337yh5 (located 2 cM distal to MSH2) and D2S123 (located 6 cM proximal to MSH2) Are Used

	Lod Score at θ =													
	AFM337yh5							D2S123						
Kindred	.0	.01	.05	.1	.2	.3	.4	.0	.01	.05	.1	.2	.3	.4
L4	1.00	.99	.96	.87	.62	.36	.14	2.69	2.73	2.78	2.66	2.12	1.37	.61
L7	.22	.23	.26	.27	.22	.12	.03	−2.60	−2.61	−2.24	−1.48	−.64	−.24	−.05
L8	2.00	1.99	1.90	1.70	1.18	.63	.20	1.74	1.70	1.54	1.33	.94	.56	.23
L621	2.01	1.98	1.88	1.72	1.33	.87	.37	2.64	2.60	2.43	2.18	1.60	.94	.32
L1933	−6.49	−2.98	−1.55	−.94	−.40	−.18	−.07	.29	.30	.34	.35	.30	.18	.06
L2516	−6.08	−4.82	−2.72	−1.70	−.73	−.26	−.04	−1.29	−1.08	−.62	−.33	−.10	−.04	−.01
L3106	.44	.45	.46	.45	.35	.23	.11	−.26	−.16	.09	.25	.31	.20	.05
L3427	−2.47	−1.71	−.83	−.45	−.20	−.15	−.10	−1.21	−1.11	−.79	−.52	−.19	−.03	.02
B1	−2.35	−1.63	−.86	−.49	−.17	−.03	.02	−1.97	−1.79	−1.30	−.92	−.45	−.19	−.05
B2	.20	.20	.18	.15	.10	.05	.02	−1.02	−.82	−.46	−.27	−.10	−.05	−.02

Table 3

Pairwise Lod Scores for 3p Markers in Families L7, L1933, L2516, L3427, B1, and B2

	Lod Score at θ =													
	D3S1619							D3S1561						
Kindred	.0	.01	.05	.1	.2	.3	.4	.0	.01	.05	.1	.2	.3	.4
L7	2.88	2.83	2.61	2.33	1.73	1.09	.44	2.80	2.75	2.54	2.26	1.68	1.06	.42
L1933	−3.47	−.22	.55	.75	.65	.33	.04	−2.39	−.47	.11	.27	0.29	.19	.06
L2516	2.23	2.28	2.29	2.12	1.58	.95	.34	4.90	4.93	4.78	4.38	3.29	2.03	.76
L3427	−.15	−.15	−.14	−.12	−.08	−.04	−.01	2.81	2.76	2.56	2.30	1.71	1.07	.43
B1	−.98	−.84	−.47	−.20	.06	.14	.10	.21	.22	.23	.22	.16	.08	.02
B2	−.97	−.88	−.63	−.44	−.22	−.09	−.02	−2.99	−2.50	−1.59	−1.04	−.48	−.19	−.05
	D3S1611							D3S1298						
L7	.19	.18	.15	.12	.06	.02	.00	2.62	2.57	2.35	2.07	1.49	.90	.34
L1933	−.24	−.22	−.15	−.08	−.01	.01	.00	−.01	.11	.32	.40	.39	.29	.15
L2516	.50	.48	.41	.33	.19	.10	.04	2.38	2.38	2.30	2.10	1.51	.83	.26
L3427	.53	.52	.46	.38	.24	.13	.05	2.69	2.65	2.46	2.20	1.64	1.03	.42
B1	.00	.01	.04	.06	.08	.07	.04	.73	.73	.76	.77	.70	.49	.21
B2	−1.02	−.86	−.53	−.35	−.17	−.09	−.04	−1.16	−1.02	−.69	−.47	−.25	−.12	−.04

Note.—The markers (locations) are: D3S1619 (1 cM distal to MLH1), D3S1561 (0 cM to MLH1), D3S1611 (contained in MLH1), and D3S1298 (1 cM proximal to MLH1).

occur in association with internal malignancies typical of HNPCC and increased survival after metastatic spread (Lynch et al. 1981). Six kindreds studied by us were linked to 2p, including a kindred (L4) representing Lynch I syndrome and five kindreds (L8, L621, L3106, C, and J) representing Lynch II syndrome, of which two (L621 and 3106) showed features of the Muir-Torre syndrome. On the other hand, four kindreds (L7, L2516, L3427, and F2) representing Lynch II syndrome were linked to 3p. Although awaiting confirmation by direct mutation analyses, our results suggest that Lynch I and II syndromes, as well as Muir-Torre syndrome, have a shared genetic basis, mainly involving mismatch repair genes on chromosomes 2p and 3p. Tight linkage to D2S123 was recently reported from Great Britain, in two Muir-Torre families (Hall et al., in press), and microsatellite instability was found in tumors from Muir-Torre kindreds (Honchel et al. 1994), further supporting the hypothesis suggested above.

The human MSH2 and MLH1 genes were identified by virtue of their homology to bacterial and yeast mismatch repair genes (Fishel et al. 1993; Leach et al. 1993; Bronner et al. 1994; Papadopoulos et al. 1994). Further genes showing interspecies homology and possibly involved in mismatch repair have already been identified in humans. They include MSH3 on chromosome 5q (Fujii and Shimada 1989; New et al. 1993), PMS1 on chromosome 2 (Papadopoulos et al. 1994), and PMS2 on chromosome 7 (Papadopoulos et al. 1994). It is not yet known whether mutations of these genes are associated with human disease. A minor subset of HNPCC kindreds exists in which cancer susceptibility is apparently not linked to the major loci on 2p or 3p (e.g., kindred L1933 in the present study and one family described by Lindblom et al. 1993). It is possible that the genes on chromosomes 2, 5, and 7—or as yet unidentified genes—underlie HNPCC in these kindreds.

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