Genomic DNA-based hMSH2 and hMLH1 Mutation Screening in 32 Eastern United States Hereditary Nonpolyposis Colorectal Cancer Pedigrees


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ABSTRACT

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant cancer syndrome characterized by early age of onset colorectal cancer (mean, 45 years) as well as endometrial, urinary tract, and upper gastrointestinal adenocarcinomas. The HNPCC phenotype has been shown to segregate with germline mutations in the human homologues of the DNA mismatch repair genes MSH2, MLH1, PMS1, and PMS2. However, the majority of published DNA mismatch repair gene mutation surveys associated with HNPCC kindreds report multiple levels of preference, including 2p and 3p chromosomal linkage analysis and the evaluation of microsatellite instability of proband colorectal cancers prior to mutation analysis. For this reason, the concise contribution of each of the known DNA mismatch repair genes to the HNPCC phenotype remains unknown. We report the results of a genomic DNA-based analysis of hMSH2 and hMLH1 germline mutations in 32 unrelated Eastern United States HNPCC kindreds. These families were selected for study on the basis of phenotype only. We identified three hMSH2 and six hMLH1 mutations in eight families, for a positive mutation rate of 25%. Two mutations were identified in one of the families. Four of the mutations detected have not been reported in the literature previously. One of the mutation-positive families is African American; the others were of European-American ancestry. These results provide a clarification of the contribution of hMSH2 and hMLH1 to the HNPCC phenotype and suggest that in the majority of Eastern United States HNPCC kindreds selected by phenotype alone, the molecular genetic basis for the disease remains unknown.

INTRODUCTION

HNPCC was first described in 1913 (1), and it remains the most common of the recognized inherited colorectal cancer syndromes (2, 3). HNPCC is characterized by early age of onset colorectal cancer (mean, 45 years) and an increased frequency of extracolonic neoplasms, including endometrial, urological, and upper gastrointestinal cancers (2-4). Inheritance is autosomal dominant (2), and penetrance is generally acknowledged to be high (5). To standardize the classification of HNPCC kindreds and facilitate genetic linkage analysis, the International Collaborative Group on HNPCC, meeting in Amsterdam in 1991, issued a set of phenotypic requirements for HNPCC families. The “Amsterdam criteria” stipulate that HNPCC families should include three members diagnosed with histologically verified colorectal cancer in two successive generations. One of the three individuals must be a first-degree relative of the other two, and one of the three affected individuals must be diagnosed prior to 50 years of age (6). These clinical criteria were used in linkage studies of HNPCC to chromosomes 2p and 3p (7, 8). Subsequently, five human homologues of the yeast methyl-directed DNA MMR genes have been cloned and implicated in the pathogenesis of HNPCC. These genes include hMSH2 (9, 10), hMLH1 (11, 12), hPMS1, hPMS2 (13), and the GT binding protein (14).

The elucidation of these genetic elements offers the prospect of presymptomatic genetic testing for members of high-risk families. Identification of the specific germline DNA MMR gene mutation associated with the disease phenotype in such families would allow clarification of which members are truly at increased risk and greatly facilitate clinical surveillance. The use of colonoscopy for colorectal cancer surveillance has been reported to significantly reduce the rate of colorectal cancers in at-risk members of HNPCC kindreds (15), and the International Collaborative Group on HNPCC has published clinical surveillance guidelines for HNPCC families that include periodic colonoscopy (16).

Published studies suggest that the majority of DNA MMR gene mutations associated with HNPCC occur in the hMLH1 and hMSH2 genes (9-14, 17-32). The incidence of MMR gene mutations in HNPCC kindreds reported in these series varies considerably, ranging from 22% (18) to 86% (21). Those series reporting the highest mutation carrier rates acknowledge possible ascertainment bias due to selection factors that include screening proband tumors for the microsatellite phenomenon (23), prior linkage and/or mutation analysis (17, 18, 22, 23), and utilization of study populations characterized by the widespread distribution of a small number of founder mutations (21). Therefore, it is inappropriate to combine data from these various studies to estimate carrier frequencies in the general or selected populations.

Although both DNA- and RNA-based methods have been used for MMR gene mutation screening in HNPCC, optimal mutation detection in HNPCC remains controversial (24). Several authors have raised concerns about RNA-based MMR gene analysis, citing significant numbers of false-positive hMSH2 deletion variants (5) and hMLH1 alternative transcript results of questionable clinical significance (24).

In this study, we present the results of a genomic DNA-based analysis of germline hMLH1 and hMSH2 mutations in 32 unrelated Eastern United States HNPCC pedigrees with diverse ethnic and racial backgrounds. We report the identification of three hMSH2 and six hMLH1 mutations in eight HNPCC kindreds, one of which is an African-American family, and seven of which are of European-American ancestry. Four of the nine mutations have not previously been reported in the literature (9-14, 17-32). The kindreds studied for this report were selected by clinical and family history data only. In addition, the results of a genomic DNA-based hMSH2 polymorphism study of 58 individuals unrelated to the study kindreds is reported, as well as hMLH1 and hMSH2 polymorphic alterations detected during the evaluation of the 32 pedigrees studied.
MATERIALS AND METHODS

Patients. Thirty-two HNPCC pedigrees registered with the Roswell Park Familial Cancer Registry (4) and the Vermont Cancer Center Familial Cancer Program were selected for study on the basis of family and clinical history data. Thirty-one of the 32 kindreds studied met the Amsterdam criteria described above. One family failed to meet the criteria, in that all of the affected individuals were deceased before a diagnosis could be made. At least one affected individual from each kindred was studied, with preference given to the youngest affected members of each pedigree when more than one affected individual was available for study. Genomic DNA isolation was performed by standard organic extraction methods. All blood specimens were collected after institutional review board-approved consent was obtained.

DNA Isolation. Venous blood samples were obtained from members of study kindreds affected with colorectal adenocarcinoma. At least one affected individual from each kindred was studied, with preference given to the youngest affected members of each pedigree when more than one affected individual was available for study. Genomic DNA isolation was performed by standard organic extraction methods. All blood specimens were collected after institutional review board-approved consent was obtained.

Amplification of Genomic DNA and SSCP Analysis. PCR fragments were generated from 50 ng of genomic DNA in a 50-ml mixture containing: 200 µM dATP, dTTP, and dGTP; 20 µM dCTP; 1.0–2.5 mM MgCl2; 20 pmol of each primer; 0.5 units of Taq polymerase; 7 nm [α-33P]dCTP and ddH2O to a total of 50 µl. Reaction buffers, PCR cycle numbers, and annealing temperatures were optimized for each of the 16 hMSH2 and 19 hMLH1 exons. PCR primers for hMLH1 and hMSH2 are depicted in Table 1. Primer selection reflects every effort to design primers that incorporate sufficient intron-exon boundary such that all known splice sites are included. We analyzed genomic DNA from 38 individuals representing 32 unrelated HNPCC kindreds for germline alterations in the human homologues of the DNA MMR genes MSH2 and MLH1. Thirty-one of the 32 pedigrees studied satisfied the Amsterdam criteria described above. At least one affected individual was studied from each pedigree. Thirty-one study kindreds were of European-American ancestry, and one family was African American. The eight families in which MMR gene mutations were detected included seven European-American and one African-American family. In addition, an hMSH2 polymorphism survey of 58 individuals unrelated to the study kindreds and without history or evidence of cancer was conducted.

SSCP was used to screen all coding exons, including all exon-intron borders and known splice sites (Table 1). Samples exhibiting altered SSCP migration patterns were subjected to direct nucleotide sequencing.

Eighteen hMSH2 and hMLH1 genetic alterations were detected in our analysis of the 32 HNPCC families and 58 individuals unrelated to the study kindreds (Tables 2 and 3, respectively). Analysis of the 32 HNPCC families detected nine germline mutations in eight unrelated HNPCC families (Table 2). The remaining nine alterations are presumed to be polymorphisms (Table 3), including all of the genetic alterations detected in the 58 individual polymorphism survey.

Pathogenic Mutations. Three hMSH2 mutations were detected in three unrelated families (Table 2). Family 240 carried a C-to-I substitution at codon 406 in exon 7. This mutation results in a change from Arg406 to STOP (CGA→TGA). A second hMSH2 mutation, in family 59, is the result of an A-to-T transversion resulting in a change from Lys309 to STOP. A third hMSH2 mutation was detected in family I10 (Fig. 1). This family carries a C-to-I substitution at codon 406 in exon 7. This mutation results in a change from Arg406 to STOP (CGA→TGA). A second hMSH2 mutation, in family 59, is the result of an A-to-T transversion resulting in a change from Lys309 to STOP. A third hMSH2 mutation was detected in family I10 (Fig. 1). This family carries a C-to-T substitution that is presumed to be polymorphisms (Table 3), including all of the genetic alterations detected in the 58 individual polymorphism survey.

RESULTS

We analyzed genomic DNA from 38 individuals representing 32 unrelated HNPCC kindreds for germline alterations in the human homologues of the DNA MMR genes MSH2 and MLH1. Thirty-one of the 32 pedigrees studied satisfied the Amsterdam criteria described above. At least one affected individual was studied from each pedigree. Thirty-one study kindreds were of European-American ancestry, and one family was African American. The eight families in which MMR gene mutations were detected included seven European-American and one African-American family. In addition, an hMSH2 polymorphism survey of 58 individuals unrelated to the study kindreds and without history or evidence of cancer was conducted.

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Table 1 hMSH2 and hMLH1 primer sequences for PCR amplification

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sense</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5'-CTGCAAGGAGAAGAAGGAGCT-3'</td>
<td>5'-GCTCCCTCCCGACACCCG-3'</td>
</tr>
<tr>
<td>2</td>
<td>5'-ATATTCCTCACCTGTGATTATA-3'</td>
<td>5'-CTTCCTCCCGACACCCG-3'</td>
</tr>
<tr>
<td>3</td>
<td>5'-AGTCCTCCAGAGATTTGTT-3'</td>
<td>5'-CGTGATCTTCTGCTGTTGAG-3'</td>
</tr>
<tr>
<td>4</td>
<td>5'-TTTCTATCTCTCCTCTATA-3'</td>
<td>5'-TGGTAGAGCCATCACTAAAGG-3'</td>
</tr>
<tr>
<td>5</td>
<td>5'-GGGGTGTTTCTCTCTCTCTCT-3'</td>
<td>5'-GGCACTTCTCTCTCTCTCT-3'</td>
</tr>
<tr>
<td>6</td>
<td>5'-AAATATCTGCTTCTCTCTCT-3'</td>
<td>5'-CTCGTTCTCTCTCTCTCT-3'</td>
</tr>
<tr>
<td>7</td>
<td>5'-GTGTTCTCTCTCTCTCTCT-3'</td>
<td>5'-GTTGTCTCTCTCTCTCTCT-3'</td>
</tr>
<tr>
<td>8</td>
<td>5'-CTGAAAGGAGAAGAAGGAGCT-3'</td>
<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
</tr>
<tr>
<td>9</td>
<td>5'-CTTCTCTCTCTCTCTCTCT-3'</td>
<td>5'-GTTGTCTCTCTCTCTCTCT-3'</td>
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<td>10</td>
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<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
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<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
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<td>14</td>
<td>5'-CTGAAAGGAGAAGAAGGAGCT-3'</td>
<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
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<td>15</td>
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<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
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<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
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<td>19</td>
<td>5'-CTGAAAGGAGAAGAAGGAGCT-3'</td>
<td>5'-CGTGATCTTCTCTCTCTCT-3'</td>
</tr>
</tbody>
</table>

3799
does not meet all of the Amsterdam criteria; however this mutation was carried by two affected brothers, both diagnosed with colorectal cancer prior to age 50. One of the brothers developed metachronous colorectal cancer, bladder cancer, and transitional cell ureteral cancer. The father of these brothers died an accidental death at age 50 with a history of a rectal polyp and a jejunal carcinoid (carrier status unknown). The mother is alive at age 73 without history or evidence of gastrointestinal malignancy and does not carry the mutation.

hMLH1 mutations were detected in five unrelated families (Table 2). Family 241 carries a deletion of G at codon 497, resulting in a frameshift and subsequent STOP at codon 514. Family 166 carries an A→T transversion at codon 26, resulting in a substitution of phenylalanine for leucine in exon 2 (families 241 and 166). Although both misense and nucleotide substitution are nonpolar amino acids, the bulk ring structure of the phenylalanine could alter protein structure and function. However, this same kindred also demonstrated a 3-bp deletion (CTT) at codon 749, resulting in the loss of a serine in exon 6 (family 59). Thus, each of the observed hMLH1 mutations is likely to contribute significantly to the observed cancer predisposition in the corresponding families.

A review of 35 hMLH1 mutations in 22 publications (9–14, 17–32) indicates that 15 (42%) were single-nucleotide substitutions. Nine (60%) of the 15 substitutions observed were C→T transitions. An A→T transversion mutation in hMLH1 was noted in only one of the 14 reports reviewed (17). In that instance, the alteration was reported at the splice donor site of exon 5. Hence, the A→T transversion in hMLH1 we report here (family 59) appears to be novel. The exon

Table 3 Polymorphisms detected in studies of 58 individuals without personal or family history of colon cancer and 32 HNPCC pedigrees

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMSH2</td>
<td>Intron 1</td>
<td>C→G</td>
<td>7 of 57 (12%)</td>
</tr>
<tr>
<td></td>
<td>Intron 2</td>
<td>Variable T deletion</td>
<td>3 of 58 (5%)</td>
</tr>
<tr>
<td></td>
<td>Exon 2</td>
<td>A→G</td>
<td>1 of 38 (2%)</td>
</tr>
<tr>
<td></td>
<td>Exon 4</td>
<td>LYS→ARG</td>
<td>10-bp deletion of A</td>
</tr>
<tr>
<td></td>
<td>Exon 5</td>
<td>G→A</td>
<td>1 of 31 (3.2%)</td>
</tr>
<tr>
<td></td>
<td>Exon 6</td>
<td>Gly→Ser</td>
<td>2 of 56 (3.6%)</td>
</tr>
<tr>
<td></td>
<td>Exon 7</td>
<td>G→C</td>
<td>3 of 38 (8%)</td>
</tr>
<tr>
<td></td>
<td>Intron 9</td>
<td>T→A</td>
<td>5 of 54 (9%)</td>
</tr>
<tr>
<td></td>
<td>Intron 12</td>
<td>C→T</td>
<td>9 of 48 (19%)</td>
</tr>
<tr>
<td>hMLH1</td>
<td>Intron 4</td>
<td>TT deletion</td>
<td>3 of 38 (8%)</td>
</tr>
</tbody>
</table>

Nonpathogenic Sequence Variations. SSCP screening followed by direct nucleotide sequencing demonstrated eight hMSH2 sequence variations in a group of 58 individuals without known personal or family history of colon cancer. All of these alterations are presumed to be polymorphisms (Table 3). An additional hMLH1 alteration, presumed to be a polymorphism, detected during our analysis of the 32 HNPCC families is also listed in Table 3. The nonpathogenic hMSH2 sequence alterations observed (Table 3) include single base substitutions involving all 4 nucleotides. In contrast, the pathological hMSH2 sequence alterations involve T substitutions only (Table 2).

DISCUSSION

This study presents the results of a genomic DNA-based hMSH2 and hMLH1 germline mutation analysis in 32 Eastern United States HNPCC Pedigrees. These families were selected on the basis of clinical and family history alone and were not screened for 2p or 3p chromosome linkage prior to mutation analysis. Colorectal or extra-colonic neoplasms from members of these kindreds were not analyzed for microsatellite instability prior to or during the study, and none of the 32 pedigrees studied were subjected to prior MMR gene mutation analysis.

We detected germline mutations in eight unrelated HNPCC families. Three families carried altered hMSH2 genes, whereas five carried hMLH1 mutations. One of the hMSH2 mutations (pedigree 59) and three of the hMLH1 mutations (pedigrees 241 and 166) detected in our study are novel (9–14, 17–32). All of the hMSH2 mutations were single-bp substitutions resulting in STOP codons. Two of the hMLH2 mutations detected were C→T substitutions resulting in STOP codons in exons 7 and 12, respectively (families 240 and 110). A third hMSH2 mutation is an A→T transversion resulting in a STOP codon in exon 6 (family 59). Thus, each of the observed hMSH2 mutations is likely to contribute significantly to the observed cancer predisposition in the corresponding families.
distribution of these 35 published mutations is similar to the distribution of hMSH2 mutations detected in our study (Fig. 2, top).

Fig. 2 (bottom) depicts the exon distribution of 66 hMLH1 mutations cited in 22 published reports (9—14, 17—32). The six hMLH1 mutations reported in our study are more heterogeneous in location and type than the hMSH2 mutations described above. Three of the six involve nucleotide deletions, two involve single-bp substitutions, and the sixth is a 2-bp substitution.

The G deletion in codon 497 identified in family 241, resulting in a frameshift and subsequent STOP at codon 514, has not been reported previously (9—14, 17—32). This mutation was detected in an affected member of a HNPCC pedigree diagnosed with three metachronous colorectal cancers prior to age 50, the first at age 39. All of the remaining four colorectal cancers diagnosed in this family (in four separate individuals) occurred prior to age 41, including one at age 22 and a second at 19 years of age. It is intriguing to speculate that this previously unreported mutation could be associated with a consistently early age of onset colorectal cancer, with high penetrance, and is reminiscent of specific APC (altered in polyposis coli) gene mutations associated with particularly aggressive familial adenomatous polyposis phenotypes (34).

The two syntenic sequence alterations we found in the hMLH1 gene carried by members of family 166 are of particular interest, because this pedigree is of African-American origin. Both alterations were carried by two affected siblings, and neither alteration has been reported previously (9—14, 17—32). A review of the literature indicates no specific reference to the identification of any MMR gene mutations in a kindred of African-American origin affected by HNPCC (9—14, 17—32). We note with interest the early report of Ionov et al. (32) of ubiquitous somatic mutations in simple repeat DNA sequences in colorectal cancer in which mutations in somatic simple repeat DNA sequences were identified in the colorectal tumors of seven African-American patients and nine European-American patients.

A fourth hMLH1 mutation was identified in family 163, in which an A→G substitution at the 5′ splice site of exon 7 can be predicted to lead to abnormal splicing of this exon. The affected mutation carrier in this pedigree was diagnosed with three metachronous colorectal cancers at 29, 46, and 62 years of age. This alteration has been reported previously by Tannergard et al. (19) in a Swedish pedigree, in which the earliest age of colorectal cancer diagnosis was 30 years of age.

The fifth hMLH1 mutation we found, the 2-base substitution AAG→GAG at codon 618 in family 55, has also been observed by Maullion et al. (26), who reported an identical alteration in a French family without evidence of extra colonic malignancy. We observed renal cell carcinoma, lymphoma, and leukemia in the family we studied that carries this mutation.

The sixth hMLH1 mutation reported in our study (family 42), a three-nucleotide deletion of GAA results in an in-frame deletion of one of three lysines coded at codons 616—618 in exon 16. This family did not demonstrate any extracolonic malignancy. Three similar, but not identical, alterations resulting in a lysine deletion at 618 have been reported. Hamilton et al. (20) reported an inframe deletion of AAG at 618 in a pedigree characterized by a Turcot’s phenotype (primary brain tumor and multiple colorectal adenomas). Liu et al. (23) also reported a deletion of AAG in a pedigree not related to the family cited in the Hamilton et al. report (20). Liu et al. (23) did not observe any extracolonic neoplasms in the family they reported. Wijnen et al. reported an in-frame deletion of one of the three lysines at codons 616—618 in one Dutch and one Italian pedigree. Although no central nervous system malignancies were reported in the Wijnen et al. pedigrees, both families demonstrated extracolonic malignancies.

Additional clinical evaluation of the eight families with mutations reported in this study reveals some interesting features that are relevant to genotype-phenotype correlation. The mean age of earliest colorectal cancer onset in the HNPCC families with hMSH2 mutations we studied was 42 years. In the families carrying hMLH1 mutations, the mean age of earliest colorectal cancer onset was 34 years. One of the three families with an hMSH2 mutation demonstrated a rectal cancer, whereas four of the five families with hMLH1 mutations exhibited rectal cancers. All of the hMSH2 mutation families demonstrated extracolonic malignancies. Three of the five families with hMLH1 mutations demonstrated extracolonic malignancies. Two of the three families with hMSH2 mutations reported urinary tract malignancies, whereas one of the five families with hMLH1 mutations reported a renal cell malignancy. The three lymphomas and one leukemia reported in these eight families all occurred in hMLH1 mutation families.

The eight nonpathogenic hMSH2 sequence variations we observed (Table 3) include single-bp substitutions involving all four nucleotides. This contrasts with the pathogenic hMSH2 sequence variations we observed (Table 2), which involved T substitutions only. Interestingly, two-thirds of the pathogenic hMSH2 single-base substitutions reported in the literature involve T substitutions (9—14, 17—32). Consistent with our findings are two reports (18, 21) citing nonpathogenic hMSH2 alterations in which none of the single-bp substitutions observed involved thymine. These observations indicate that, although T substitutions constitute the majority of pathogenic hMSH2 single-bp substitutions, they occur infrequently in nonpathogenic alterations of the gene. This suggests that the underlying mechanism(s) responsible...
for single-nucleotide substitutions in pathogenic and nonpathogenic hMSH2 sequence alterations may be different and remain poorly understood.

Our results are based on genomic DNA analysis only. Multiple recent HNPCC-associated MMR gene surveys also report primary analysis based on genomic DNA (18, 19, 22). RNA-based RT-PCR and protein truncation testing have been used for MMR gene mutation analysis in some studies, but this strategy is less common (24). Two recent reports raise concern about the use and interpretation of RNA-based RT-PCR due to the possibility of alternative transcripts. Using RT-PCR, Xia et al. (5) reported an hMSH2 exon 13 transcript deletion variant in “more than 90%” of 54 unrelated individuals tested. This variant was detectable in lymphocytes only. The variant was not detectable in 13 associated colorectal cancers, and Western blot analysis of lymphocyte-derived protein from these same individuals demonstrated a single protein product corresponding in size to the predicted wild-type protein.

More recently, Kohonen-Corish et al. (24) reported the presence of multiple alternative transcripts from RT-PCR analysis that “appeared unrelated to the hereditary defect.” This was found to be especially prevalent in RNA isolated from peripheral blood and less prevalent when RNA from lymphocyte cell lines was used. Kohonen-Corish et al. concluded that RT-PCR- and protein truncation testing-based mutation analysis in HNPCC should be interpreted with caution and, ideally, confirmed in at least two patients per family or from genomic DNA.

A number of studies have reported higher fractions of hMSH2 and hMLH1 mutations in HNPCC cohorts. We agree with Tannergard et al. (19), who observed that HNPCC-associated MMR gene mutations published to date have been identified in selected kindreds, which complicates estimations of the real frequency of hMLH1 and hMSH2 mutations in HNPCC families.

The highest reported rate of HNPCC-associated hMSH2 and hMLH1 mutations appears in a report by Nystrom-Lahti et al. (21), who reported an 86% rate of detection in 35 Finnish families that met all of the Amsterdam criteria. This percentage dropped to 30% in an additional 20 families that did not meet all of the Amsterdam criteria, for an overall rate 64% when the two groups were combined. The authors attribute the high proportion of mutation positive kindreds to the fact that two of the eight mutations detected are widespread among Finnish kindreds due to founder effects (21). Twenty of the total of 34 (58%) mutations cited were one or the other of the two hMLH1 founder mutations. None of our mutation-positive kindreds were of Scandinavian ancestry. None of the eight (total) different mutations reported by Nystrom-Lahti et al. in 55 HNPCC kindreds studied matched the nine mutations we detected in our survey of 32 HNPCC kindreds.

Liu et al. (23) reported identification of a MMR gene mutation in 70% of 48 HNPCC kindreds analyzed for hMSH2, hMLH1, hPMS1, and hPMS2 mutations. The authors report a selection strategy that included screening for microsatellite instability in proband tumor DNA with positive status a prerequisite for mutation analysis (23). Nineteen of the 34 mutations reported had been reported previously. Wijnen et al. (22) reported hMLH1 mutations in 35% of 34 HNPCC families studied, although they acknowledged that families studied were genetically “well characterized and defined,” including linkage analysis to the 3p locus prior to hMLH1 sequence analysis.

Each of these studies (21–23) acknowledges multiple levels of pedigree selection prior to mutation analysis. We have refrained from any molecular genetic characterization of the families included in this study prior to mutation analysis. Our mutation-positive pedigree rate of 25% underscores the potential that exists for the identification of novel genetic elements associated with familial colorectal cancer. We feel this interpretation is supported by the findings of Lewis et al. (35), who reported linkage implicating hMSH2 in only 1 of 10 Utah Pioneer Data Base colorectal cancer pedigrees studied for both
hMSH2 and hMLH1 linkage. Lewis et al. (35) concluded that genetic elements less penetrant than, and distinct from, hMSH2 and hMLH1 remain to be detected and may be responsible for a greater proportion of familial colorectal cancer than the MMR genes already identified. It should also be acknowledged that phenomena independent of DNA sequence alterations may affect MMR gene function. Kane et al. (36) have recently reported a class of colon tumors that do not express hMLH1 protein and do not have mutations in the hMLH1 coding sequence. Lack of hMLH1 protein expression in these tumors correlated with cytosine methylation of the hMLH1 promoter region. Additional studies will be required to determine whether MMR gene promoter methylation is associated with carcinogenesis in HNPCC probands without MMR gene sequence alterations.

In summary, we have presented the results of a genomic DNA-based hMSH2 and hMLH1 mutation analysis of 32 Eastern United States HNPCC pedigrees selected on the basis of clinical and family history only. Three hMSH2 and six hMLH1 mutations were detected in eight unrelated HNPCC families. One of the eight families was African American and demonstrated novel hMLH1 exon 1 and exon 19 mutations in two affected siblings. The proportion of families testing positive for mutations was 25%. This finding suggests that, in the majority of Eastern United States HNPCC kindreds, the molecular genetic basis for the two affected siblings. The proportion of families testing positive for mutations was 25%. This finding suggests that, in the majority of Eastern United States HNPCC kindreds, the molecular genetic basis for the observed phenotype remains unknown. We conclude that these HNPCC kindreds, without hMSH2 or hMLH1 mutations, represent a valuable resource and may provide an opportunity for the identification of novel genetic elements associated with familial colorectal cancer.

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Genomic DNA-based hMSH2 and hMLH1 Mutation Screening in 32 Eastern United States Hereditary Nonpolyposis Colorectal Cancer Pedigrees


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