Novel \textit{hMLH1} and \textit{hMSH2} Germline Mutations in African Americans With Colorectal Cancer

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Context Germline mutations of the DNA mismatch repair (MMR) genes \textit{hMLH1} and \textit{hMSH2} have been shown to cosegregate with the colorectal cancer phenotype in multiple hereditary nonpolyposis colorectal cancer (HNPCC) pedigrees. However, the frequency of these mutations among African American patients with colorectal cancer is unknown.

Objective To investigate the frequency of germline alterations of the DNA MMR genes \textit{hMLH1} and \textit{hMSH2} among African Americans affected by HNPCC and early-age onset colorectal cancer.

Design, Setting, and Patients Forty unrelated African American HNPCC and early-age onset colorectal cancer patients (8 women, 3 men) were identified from the cancer registry at a National Cancer Institute–designated referral center, 11 of whom were available for and agreed to study participation from January 1997 to February 1998. The mean age of the subjects was 44 years. An additional 50 age- and sex-matched African Americans without personal or family history of colorectal, endometrial, ovarian, urinary tract, or upper gastrointestinal tract malignancy were also studied as a polymorphism control population. In all subjects, genomic DNA was amplified by polymerase chain reaction for all \textit{hMLH1} and \textit{hMSH2} exons and screened using single-strand conformation polymorphism (SSCP) analysis. Samples demonstrating significant SSCP shifts underwent automated nucleotide sequencing analysis.

Main Outcome Measure Frequency of \textit{hMLH1} and \textit{hMSH2} germline alterations in the affected and control subjects.

Results Germline \textit{hMLH1} and \textit{hMSH2} mutations were detected in 3 (27%) of the African American colorectal cancer probands studied. Each mutation was novel. Two \textit{hMLH1} (an A→T transversion at codon 26 and a GG→AT substitution across codons 177 and 178) mutations and 1 \textit{hMSH2} mutation (a C→T transition at codon 389) were identified in 3 female study subjects. Six other \textit{hMLH1} and \textit{hMSH2} alterations were detected but were presumed to be polymorphisms. Neither missense mutation (at codons 26 and 389) was detected in the control population.

Conclusions The results of our analysis support an association between the 3 mutations reported and predisposition to colorectal cancer. Further studies are needed to define DNA MMR gene–associated colorectal cancer in African Americans, an understudied population at increased risk of fatal colorectal cancer.
and 1 of the 3 must be diagnosed prior to 50 years of age. Five human homologues of the DNA mismatch repair (MMR) genes have been cloned and germline mutations in these genes, principally hMLH1 and hMSH2, have been shown to cosegregate with the colorectal cancer phenotype in multiple HNPCC pedigrees. The protein products of these genes have been shown in *Escherichia coli* and yeast to contribute to DNA replication fidelity by the identification and correction of mispaired nucleotides during DNA replication. The human homologues of these genes are believed to function similarly.

The overwhelming majority of reports on MMR gene–associated colorectal cancer are based on highly selected HNPCC pedigrees meeting Amsterdam criteria. However, MMR gene alterations associated with colorectal cancer are not restricted to these highly selected families. The characterization of DNA MMR genes offers the prospect of identifying the specific MMR gene mutation(s) segregating with colorectal cancer in at-risk families and the delineation of family members at increased risk for the disease. The use of colonoscopy for colorectal cancer surveillance has been reported to reduce the rate of colorectal cancer in at-risk members of HNPCC kindreds. The International Collaborative Group on HNPCC and the Cancer Genetics Studies Consortium have published clinical surveillance guidelines for HNPCC families that include periodic colonoscopy.

Although an African American kindred exhibiting the HNPCC phenotype was described in 1987, the world literature is without comment regarding either the extent of the HNPCC phenotype or the carrier frequency of mutations in any of the known MMR genes among African Americans.

In this article we present the results of a preliminary study of the frequency of germline hMLH1 and hMSH2 mutations in 11 unrelated African Americans with histologically confirmed colorectal cancer. Analysis was confined to hMLH1 and hMSH2 for this initial study as alterations in *PMS1*, *PMS2*, and *GTBP* (*hMSH6*) have been infrequently reported among populations studied to date. Early-age onset is associated with heritable solid tumor malignancies at multiple organ sites, including the colon and rectum, which involve multiple genetic elements. The inclusion of individuals with early-age onset without recognized prior family history in our "family" cancer registry reflects our interest in further study of the molecular genetic basis for early-age onset colorectal cancer. Mismatch repair gene–associated early-age onset colorectal cancer without prior family history has been reported, but remains unstudied among African American probands. Of the *hMLH1* and *hMSH2* mutations detected in 3 probands, herein the results pertaining to the Amsterdam criteria family member have been published; however, none of the mutations detected have been reported by other investigators.

**METHODS**

Forty African Americans with histologically confirmed colorectal cancer enrolled in the Roswell Park Family Cancer Registry were selected for study on the basis of family and clinical history alone. Eleven individuals, 8 women and 3 men, were available for and consented to study participation. Among the 29 nonparticipants, 9 had died since their diagnosis, 10 were no longer accessible by telephone or US mail, and 10 declined to participate when contacted. The age and sex distribution, family history, and stage at diagnosis characteristics of the nonparticipants were similar to those of participants. One study proband was a member of an Amsterdam criteria HNPCC pedigree. The remaining 10 study participants were diagnosed prior to 50 years of age and had no prior family history of the disease. An additional 50 age- (within 2-year age range) and sex-matched African American adults (composed of individuals who were hospital employees and members of the community) without personal or known family history of colorectal, endometrial, ovarian, urinary tract, or small bowel cancer were included as a polymorphism control population. Seventy-five of 102 individuals initially agreed to participate in the control group; however, 25 were excluded because of positive personal or family cancer history. The additional 27 individuals declined to participate at the time of initial invitation. Twenty control subjects received a modest financial incentive for participation. The remaining 30 participants were unpaid volunteers.

After obtaining institutional review board approval and subject informed consent, venous blood samples were obtained from each study subject. The Roswell Park Cancer Institute Division of Surgical Oncology employs 2 full-time genetic counselors who are available to all study subjects.

Genomic DNA isolation was performed using standard organic extraction methods. Polymerase chain reaction fragment generation, single-strand conformation polymorphism analysis, and direct nucleotide sequence analysis were performed as described previously. All exons of each gene were analyzed for sequence variation in all study subjects. The Fisher exact test was used for comparisons.

**RESULTS**

Of the 11 probands studied, a total of 9 *hMLH1* and *hMSH2* alterations were detected (Table 1 and Table 2). These included 3 mutations detected in 3 unrelated study probands (Table 1). One carrier proband (166) was a member of an Amsterdam criteria HNPCC family and the remaining 2 mutation carriers were diagnosed prior to 50 years of age without prior family history. Each of the carriers were women. The remaining 6 alterations are presumed to be polymorphisms (Table 2). Proband 166 was diagnosed as having metachronous rectal and colon cancers at ages 47 and 49 years, respectively. This proband carried an A→T transversion resulting in a missense mutation characterized by
One of the 50 control subjects carried the hMLH1 codon 718 C→T transition. The control subject, a 40-year-old woman, reported that her father died of an uncertain malignant disease at 40 years of age and her father’s mother died of a presumed abdominal malignancy at 45 years of age.

**COMMENT**

Ethnic variations in both the prevalence of the HNPCC phenotype and carrier frequencies of germline hMLH1 and hMSH2 mutations in selected HNPCC families have been extensively described; however, the world literature is without comment on these points in regard to the African American population. This article presents the results of a preliminary study of germline hMLH1 and hMSH2 mutations in African American patients with colorectal cancer that includes a member of an Amsterdam criteria HNPCC family and 10 unrelated individuals diagnosed prior to age 50 years without known prior family history.

Three of the 11 unrelated probands tested carried 3 MMR gene mutations. Two of these alterations were detected in hMLH1, 1 in hMSH2. Two of the 3 mutations detected are missense in character; in previous reports, approximately 50% of hMLH1 and 25% of hMSH2 mutations associated with HNPCC were missense alterations, however, multiple authors have acknowledged the role low-penetrance mutations may play in colorectal cancer predisposition, and MMR gene–associated colorectal cancer in early-age onset individuals without prior family history has been documented. Significantly, in these reports, the MMR gene alterations observed are missense in character. Regarding the substitution of phenylalanine for isoleucine at hMLH1 codon 26 in proband 166, although both isoleucine and phenylalanine are nonpolar amino acids, the bulky ring structure of the phenylalanine is likely to contribute to altered protein structure and function. In addition, we have published evidence for microsatellite instability in the colorectal tumors of this patient, supporting the pathologic significance of the hMLH1 alteration detected. Tissue from the rectal cancer of proband 404 also demonstrated microsatellite instability. Tumor tissue from proband 449 was not available for study. None of the missense mutations reported in this study (Table 1) have been detected in prior polymorphism studies, and they are not recorded in the Mutation/Polymorphism Data Base of the International Collaborative Group on HNPCC. The results of our study support an association between each of the missense mutations reported and colorectal cancer predisposition.

Probands 404, 410, and 460 carried an identical exon 19 alteration at codon 718 (Table 2). This codon 718 alteration results in the substitution of a polar-uncharged tyrosine for a positively charged histidine. Although the number of hMLH1 exon 19 mutations reported is second only to the number reported for exon 16, the codon 718 alterations detected in this study have not been reported. We note with interest that the charge-alteration substitution at codon 718 occurs at a highly conserved codon that is located within the PMS1 interactive domain of the hMLH1 gene. Fisher exact test com-

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**Table 1. Mismatch Repair (MMR) Gene hMLH1 and hMSH2 Mutations in African American Probands With Colorectal Cancer**

<table>
<thead>
<tr>
<th>MMR Gene</th>
<th>Exon</th>
<th>Codons</th>
<th>Nucleotide</th>
<th>Mutation</th>
<th>Earliest Age at Onset, y</th>
<th>Tumor Spectrum</th>
<th>Sex</th>
</tr>
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<tbody>
<tr>
<td>hMLH1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband 166</td>
<td>1</td>
<td>26</td>
<td>ATC→TTC (A→T)</td>
<td>Ile→Phe</td>
<td>47/49</td>
<td>Rectum/Colon</td>
<td>Female</td>
</tr>
<tr>
<td>Proband 404</td>
<td>6</td>
<td>177, 178</td>
<td>TTG GAA→TTA TAA (GG→AT)</td>
<td>Glu→Stop</td>
<td>35/38</td>
<td>Uterus/Rectum</td>
<td>Female</td>
</tr>
<tr>
<td>hMSH2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband 449</td>
<td>7</td>
<td>389</td>
<td>CTT→TTT (C→T)</td>
<td>Leu→Phe</td>
<td>44</td>
<td>Colon</td>
<td>Female</td>
</tr>
</tbody>
</table>

* Indicates isoleucine; Phe, phenylalanine; Glu, glutamic acid; and Leu, leucine.

**Table 2. hMLH1 and hMSH2 Polymorphisms in African American Probands With Colorectal Cancer**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Location</th>
<th>Nucleotide Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMLH1</td>
<td>13</td>
<td>3’ Untranslated region G→A</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Codon 718 C→T</td>
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<tr>
<td></td>
<td>19</td>
<td>3’ Untranslated region CT→CT</td>
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<tr>
<td></td>
<td>19</td>
<td>3’ Untranslated region T→G</td>
</tr>
<tr>
<td>hMSH2</td>
<td>1</td>
<td>3’ Untranslated region C→G</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3’ Untranslated region G→T</td>
</tr>
</tbody>
</table>

* Four individuals, 3 cases (including proband 166) and 1 control, had the codon 718 alteration.

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parative analysis of the frequency of the codon 718 alteration in cases (3/11) vs controls (1/50) yielded a P value of .02. However, these data are not sufficient to interpret the 718 alteration as a disease-causing mutation. In a worst-case scenario of increased initial participation among our 40 cases with the finding of 3 carriers of the 718 alteration, the comparison of 3 of 40 cases vs 1 of 50 controls using the Fisher exact test results in a P value of .32, which is not significant. Because of participation rates, we cannot rule out the possibility of potential bias due to lack of generalizability of the subgroup studied relative to the initial sample. We strongly agree with White\(^5\) that large, well-controlled studies will be required to determine the true pathologic significance of apparently variably penetrant alterations such as the adenomatous polyposis coli gene \(H\)I\(307\)K\(^6\) and possibly the hMLH1 codon 718 alteration we report in this study.

For affected individuals without prior history, explanations for the presence of disease-associated mutations include de novo mutations as well as variable penetrance, nonpaternity, and inadequate family history. Parents of the 3 carriers reported were not available for study, precluding evaluation of de novo mutation and variable penetrance. With regard to mutation detection methods used in this study, although single-strand conformation polymorphism analysis sensitivity is limited to approximately 80% to 85%, it enjoys wide acceptance in screening for MMR gene alterations\(^2\) and possibly the hMLH1 codon 718 alteration we report in this study. However, we cannot rule out the possibility of potential bias due to lack of generalizability of the subgroup studied relative to the initial sample. We strongly agree with White\(^5\) that large, well-controlled studies will be required to determine the true pathologic significance of apparently variably penetrant alterations such as the adenomatous polyposis coli gene \(H\)I\(307\)K\(^6\) and possibly the hMLH1 codon 718 alteration we report in this study.

The results of this study indicate that MMR gene alterations may play a role for both African American Amsterdam criteria HNPCC families and patients with early-age onset colorectal cancer without prior family history. This latter group is of particular clinical relevance as these patients are unlikely to appreciate the potential increased risk of subsequent colorectal cancer for themselves and their parents, siblings, and children.\(^6\) In addition, the characteristics of the mutations we have reported suggest their codon distribution is novel when compared with that of other ethnic and racial groups.\(^9\)-\(^10\),\(^19\)-\(^29\),\(^42\)-\(^46\) These results are reminiscent of recent findings of ethnic variability involving increased frequency among Ashkenazi Jews of specific mutations associated with the BRCA1 and BRCA2 genes\(^6\) and the adenomatous polyposis coli gene.\(^5\)

In summary, these preliminary results suggest further study of the contribution of germline mutations in the MMR genes to the colorectal cancer phenotype in African Americans would enhance our understanding of this disease and facilitate the directing of clinical surveillance to those at high risk. The observation that as much as 50% of the increased mortality sustained by this segment of the population is attributable to a higher frequency of late-stage diagnosis compared with the white population\(^4\) underscores the potential benefit that could be derived from increased clinical surveillance of members of this high-risk group. Current studies in progress in our laboratory include an enlarged survey of MMR gene–associated colorectal cancer among African Americans with the inclusion of MSH6, PMS1, and PMS2 in our proband analysis. We conclude that progress in the understanding of the molecular genetics of MMR gene–associated colorectal cancer will lead to an important contribution to cancer control in this under-studied, high-risk patient population.

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**REFERENCES**

3. Griffin.

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NOVEL hMLH1 AND hMSH2 GERMLINE MUTATIONS


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