Peel et al. (1) have reported a low frequency of mismatch repair (MMR) gene defects in a population-based series of colorectal cancers. Previously, we determined the incidence of hereditary nonpolyposis colorectal cancer (HNPCC) by family history of colorectal cancer in a population-based series of colorectal cancers (2).

We now describe a molecular study to estimate the incidence of MMR mutations in an extended series of 1329 patients. Since 1981, all patients with colorectal cancer (n = 1329) presenting to the referring hospital have been asked about a family history of cancer in first- or second-degree relatives. The family trees were classified as HNPCC according to the strict Amsterdam criteria (3), the Amsterdam II criteria, the modified Amsterdam criteria, or the Bethesda criteria (4).

Case patients classified by the Amsterdam criteria were analyzed for MLH1 and MSH2 mutations by single-strand conformation polymorphism analysis. Individuals fulfilling other criteria were investigated for tumor microsatellite instability (MSI) (5) and for MLH1/MSH2 immunostaining in most cases. Germline MSH2 and MLH1 mutations were sought in case patients with MSI or abnormal immunostaining.

A family history of colorectal cancer in first-degree relatives was present in 134 (10%) of 1329 patients, although only three (0.2%) case patients had a family history of colorectal cancer consistent with the strict Amsterdam criteria (3). A further 16 case patients had two or more first-degree relatives with colorectal or other HNPCC cancers (Amsterdam II criteria), and another 17 case patients had at least one affected first-degree relative where the index case patient or the relative was diagnosed with colorectal cancer at age less than 55 years (the modified Amsterdam criteria). There were 29 patients with colorectal cancer that was diagnosed at age less than 45 years; of these patients, five (17%) had an affected first-degree relative (which included two of the three HNPC families). Two patients had double primary tumors that involved the colon and another HNPC cancer but did not fulfill any HNPC criteria. Germline DNA was available for two of three HNPC patients eligible for mutation analysis, and 62 patients were eligible for tumor studies. Germline mutations were identified in both HNPC patients (Table 1). Tumor DNA was available for 49 of 62 case patients eligible for tumor studies, and three of these case patients demonstrated MSI. One of these patients (patient 2, Table 1) fulfilled Bethesda and Amsterdam II criteria, and mutational analysis revealed a truncating mutation in exon 15 of MLH2. The remaining two patients aged less than 45 years (patients 1 and 3 in Table 1) and a further patient with low-level MSI and absent MLH1 tumor immunostaining did not have a detectable mutation. Thus, MSI was identified in five (10%) of 51 tumors [four (16%) of 24 of those patients diagnosed at age <45 years] analyzed, and three of these patients had a germline MLH1 or MSH2 mutation.

We observed a very low frequency (0.2%) of HNPCC by strict Amsterdam criteria. It is recognized that the Amsterdam criteria are relatively specific but have only moderate sensitivity for HNPCC diagnosis (4). Germline mutations in MSH2 and MLH1 account for most HNPCC gene mutations, and only a small proportion of patients with germline MMR mutations will have negative tumor MSI studies. We detected germline MLH1 or MSH2 mutations in only three case patients; a further case patient satisfied the Amsterdam criteria, but no DNA was available for analysis. Only three of 48 early-onset or familial case patients with available tumor DNA had tumor MSI, and one of these had a germline mutation. Even if we allow for the various causes of false-negative results, our data further support the concept that non-HNPC familial aggregations of colorectal cancer are unlikely to have an MMR mutation and the frequency of HNPCC in colorectal cancer case patients outside founder populations is approximately 1% (1,6,7).

### Table 1. Patients with mutations in MLH1 and MSH2, of 1329 patients with colorectal cancer

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at diagnosis of first colorectal cancer, y</th>
<th>Age at diagnosis of second colorectal cancer, y</th>
<th>Family history of cancer</th>
<th>Microsatellite instability</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>46</td>
<td>Father, colorectal cancer; brother, colorectal cancer</td>
<td>High</td>
<td>MLH1 K618X</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>62</td>
<td>Father, gastrointestinal cancer; sister, gastrointestinal cancer</td>
<td>High</td>
<td>MSH2 2634+2T→C</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td></td>
<td>Father, colorectal cancer; grandfather, colorectal cancer</td>
<td>High</td>
<td>MLH1 R226L</td>
</tr>
</tbody>
</table>

### References


NOTES

Affiliations of authors: D. G. Evans, Department of Medical Genetics, St. Mary’s Hospital, and Centre for Cancer Epidemiology, Christie Hospital, Manchester, U.K.; C.-L. Wu, I. Hansen, Department of Medical Genetics, St. Mary’s Hospital; S. Walsh, C. Robinson, R. Kingston, Department of Clinical Studies, Trafford General Hospital, Manchester; L. Verma, E. R. Maher, Section of Medical and Molecular Genetics, University of Birmingham, The Medical School, Edgbaston, U.K.

Correspondence to: Professor D. Gareth Evans, St. Mary’s Hospital, Regional Genetic Service, Hathersage Rd., Manchester, M130H, U.K. (e-mail: gevans@central.cmlht.nwct.nhs.uk).

RESPONSE

We are pleased that the data presented by Evans et al. support our concern about the adequacy of the Amsterdam criteria in the characterization of the HNPCC syndrome. Evans et al. refer to their study of 1329 consecutive colorectal cancer case patients, in which they found that 10% of the case patients had a positive family history of colorectal cancer in first-degree relatives (1), compared with 20% in our study of a population-based cohort of 1134 colorectal cancer case patients (2).

Evans et al. also classified a subgroup of their patient population as “hereditary” on the basis of family history of colorectal cancer consistent with the Amsterdam criteria to determine the proportion of HNPCC families. Evans et al. confirm our observation that the frequency of HNPCC families identified by the Amsterdam criteria is much lower than was previously predicted (5%) and that the HNPCC syndrome is likely to account for less than 1% of all colorectal cancer. They observed a very low frequency (0.2%) by using the strict Amsterdam criteria, compared with the frequency that we reported (0.9%) by using population-based series. Unless molecular characterization is applied, we believe that the Amsterdam criteria are not sufficient to identify HNPCC syndrome families fully.

In addition to our 13 families who fit the strict Amsterdam criteria, we identified a subgroup of colorectal cancer case patients (n = 48) who have a family history of colorectal cancer either consistent with the Bethesda criteria or who have several but not all of the features of the Amsterdam criteria. Using a 16-marker panel including the National Cancer Institute workshop reference set, we tested all 61 probands for MSI as the gold standard to obtain measures of sensitivity and specificity of the Amsterdam criteria. In this subgroup, we found 13 probands that had three or more of the 16 replication-error markers positive for instability. As shown in Table 1, the specificity of the Amsterdam criteria using MSI positivity with three or more markers is 46.2% and the estimated specificity is 85.4%. These data indicate that using the Amsterdam criteria to identify families with the HNPCC syndrome will result in failure to identify many families (25.5%–80.8%). Furthermore, 61.1%–78.8% of the families who are non-HNPCC-affected families will be falsely identified as having the syndrome. We consider these results to be very preliminary. Further molecular genetic characterization using MSH2, MLH1, MSH6, and other MMR genes is necessary. As shown by our data from nine Amsterdam criteria probands with both tissue and blood samples available, 55% of the families identified according to the strict Amsterdam criteria were MSI positive but only 44% also had mutations in MSH2 or MLH1.

Both studies reported a large number of families now identified as “Amsterdam II,” “modified Amsterdam,” or “Bethesda” that do not meet the strict criteria for Amsterdam but remain suspicious for the HNPCC status. Systematic molecular characterization of hereditary colorectal cancer using population-based cases is essential, particularly in view of the results of studies such as ours, in which large series of colorectal cancer cases are analyzed but the current criteria lack the necessary validation against a gold standard using molecular genetic studies. We believe it is time for multiple centers internationally to join resources and pool data from such families in order to characterize the HNPCC syndrome epidemiologically, clinically, and molecularly.

HODA ANTON-CULVER
DAVID J. PEEL
ARGYRIOS ZIOGAS

REFERENCES


NOTES

Affiliation of authors: Epidemiology Division, Department of Medicine, University of California, Irvine.

Correspondence to: Hoda Anton-Culver, Ph.D., Epidemiology Division, Department of Medicine, 224 Irvine Hall, University of California, Irvine, CA 92697–7550 (e-mail: hantoncu@uci.edu).

<table>
<thead>
<tr>
<th>Amsterdam criteria</th>
<th>MSI positive</th>
<th>MSI negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>41</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>48</td>
<td>61</td>
</tr>
</tbody>
</table>

Sensitivity = 46.2% (95% confidence interval = 19.2 to 74.5)
Specificity = 85.4% (95% confidence interval = 72.2 to 93.9)