Frequent Microsatellite Instability and Mismatch Repair Gene Mutations in Young Chinese Patients With Colorectal Cancer

Tsun Leung Chan, Siu Tsan Yuen, Lap Ping Chung, Judy W. C. Ho, Kedo Y. M. Kwan, Annie S. Y. Chan, Joanna C. Y. Ho, Suet Yi Leung, Andrew H. Wyllie

Background: The incidence of colorectal cancer in persons under 46 years of age is substantially higher in Hong Kong than in Scotland and many other countries. Consequently, we examined whether there is a hereditary predisposition for colorectal cancer in this Southern Chinese population.

Methods: We investigated the incidence of microsatellite instability (MSI) at 10 DNA sites in 117 colorectal cancer specimens from Chinese patients of various ages. Those tumors with new alleles at 40% or more of the sites investigated were identified as highly unstable MSI (MSI-H). In young patients, we also searched for germline mutations in three mismatch repair genes (hMSH2, hMLH1, and hMSH6). Results: The incidence of MSI-H varied statistically significantly with age, being observed in more than 60% of those younger than age 31 years at diagnosis and in fewer than 15% of those age 46 years or older. In 15 patients (<46 years old) whose colorectal cancers showed MSI-H, eight possessed germline mutations in either hMSH2 or hMLH1. When mutations in hMSH6 were included, more than 80% of Chinese colorectal cancer patients younger than 31 years had germline mutations in mismatch repair genes. We found a novel germline missense mutation in hMSH6 in a 29-year-old man whose tumor showed no MSI. Two patients had a 4-base-pair insertion in exon 10 causing a truncated protein; this insertion is a common polymorphism with a population allele frequency in Chinese of 5.6%.

Conclusions: Our results indicate that germline mutations in mismatch repair genes contribute substantially to the pathogenesis and high incidence of colorectal cancer in young Hong Kong Chinese. However, because young Chinese and Caucasians show similar proportions of colorectal cancers with MSI-H, despite the higher incidence in the former, additional factors may underlie the high susceptibility of young Chinese to colorectal cancer. [J Natl Cancer Inst 1999;91:1221–6]

The incidence of colorectal carcinoma varies greatly throughout the world. In general, it is high in the Western developed countries, low in developing countries, and intermediate but rising rapidly in urban societies of Eastern Asia, such as Japan, Singapore, and Hong Kong.

We recently drew attention to the fact that there is an excess of patients with colorectal cancer, as high as fourfold, in the younger age groups (<46 years old) in Hong Kong compared with Scotland and other data from predominantly Caucasian countries (1). This high incidence in the young Hong Kong (Southern) Chinese was present 20 years ago and has not changed, despite the rapid rise in the overall incidence of colorectal cancer in recent years. This recent increase is entirely attributable to classical, late-onset (>50 years old) patients. Because of these data, we began to search for a genetic basis that would explain this high incidence of colorectal cancer in the young Hong Kong Chinese population.

Patients with constitutional (or germline) mismatch repair defects are prone to colorectal and certain other cancers at a young age (2,3). Mutations in the hMSH2 or hMLH1 gene are consistently associated with cancers that show microsatellite instability (MSI), characterized by the expansion or deletion of small repeat sequences during DNA replication (4–11). A small number of reports (12,13) document constitutional mutations of hMSH6: Tumors in these individuals may also show MSI. In contrast, tumors from mice genetically engineered with a homozygous null MSH6 gene mutation do not exhibit MSI (14). Patients with constitutional mutations in mismatch repair genes (hMSH2, hMLH1, and hMSH6) sometimes have strong family histories of colorectal cancer that fit the Amsterdam criteria (15) for hereditary nonpolyposis colon cancer (HNPPC) syndrome. However, this characteristic is not invariable (16). Analysis of the data in the Hereditary Gastrointestinal Cancer Registry in Hong Kong did not reveal a high proportion of families fitting the Amsterdam criteria.

To clarify the situation in Hong Kong, we analyzed the incidence of MSI and mutations in hMSH2, hMLH1, and hMSH6 genes in a series of Hong Kong Chinese patients with colorectal cancer. We sought to identify germline mutations in hMSH2, hMLH1, and hMSH6 in the young patients with tumors showing instability in a high proportion (>40%) of the microsatellite sites investigated. We designated these tumors as highly unstable MSI (MSI-H). We also searched for germline mutations in hMSH6 in young patients who had tumors in which the microsatellites appeared completely stable (microsatellite stable) or revealed instability at less than 40% of the sites investigated (low-level microsatellite instability [MSI-L]). Moreover, we collected the family history of patients younger than 46 years of age. The data suggest that germline mutation in these three mismatch repair genes have a substantial but perhaps not exclusive role in determining the susceptibility of young Chinese to colorectal cancer.

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See “Notes” following “References.”

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PATIENTS, MATERIALS, AND METHODS

Patients and Specimens

We studied 117 colectomy specimens from patients with adenocarcinoma at Queen Mary Hospital, Hong Kong, from 1991 through early 1997. The following patients were selected: 1) all patients (total, 59 patients) for whom frozen tumor blocks were available and the patients’ age at diagnosis was less than 46 years and 2) a similar number of patients (total, 58 patients) whose age at diagnosis was 46 years or older. In the latter group, the selection of patients was such that about equal numbers of patients were selected from each decade of life from 46 years onward. Overall, 72 specimens were from male Chinese patients and 45 specimens were from female Chinese patients. The patients ranged in age from 24 through 87 years. We received the specimens unfixed on ice from the operating theaters and took representative blocks of tissue from both the tumor and the normal mucosa. These blocks were snap-frozen in liquid nitrogen and stored at −70 °C. Other representative blocks from these specimens were fixed in buffered 10% formalin and embedded in paraffin for routine histologic examination.

The paraffin and frozen sections, prepared from stored blocks, were assessed by light microscopy. Only blocks with tumor tissue occupying more than 70% of the section area were used to prepare DNA. DNA was also extracted from the normal mucosa of each patient. DNA was extracted by standard protocols that included proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation.

A detailed family history was taken from all patients younger than 46 years old during interviews with patients and relatives. The interviewer (K. Y. M. Kwan) had undergone training at the Polyposis Registry (St. Mark’s Hospital, London, U.K.). Venous blood was drawn, with informed consent, from the patients and from some of the available family members for extraction of DNA and RNA. This study was approved by the Ethics Committee of Queen Mary Hospital.

Analysis of MSI

The MSI analysis was performed as previously described (17,18). In summary, segments of genomic DNA at 10 sites including known microsatellite sequences were amplified by the polymerase chain reaction (PCR). Twenty-five to 30 cycles of PCR were performed in 10 µL that contained 50–100 ng of DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5–3 mM MgCl₂, 2–4 pmol of each primer, 0.1 µL of deoxyctydine 5'-[α-32P]triphosphate (6000 Ci/mmol; 10 µCi/µL), all four deoxynucleoside triphosphates (each at 50 µM), and 0.5 U of Taq polymerase (Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD). The annealing temperatures were 52 °C–60 °C. Radiolabeled PCR products were subjected to electrophoresis in 6% polyacrylamide–32% formamide–5.0 M urea gels at 70 W for 2–3 hours. The gels were then fixed, dried, and autoradiographed. The following 10 loci were used: D2S123, D3S1067, D5S382, D5S346, TP53 (or p53), D18S58, DCC (deleted in colon cancer), BAT-26, BAT-40, and TGFβRII (transforming growth factor-β receptor II). For each tumor, at least five loci were analyzed, including both dinucleotide and mononucleotide loci. Tumors in which at least 40% of the microsatellite loci analyzed showed altered electrophoretic mobility relative to the corresponding normal tissue (Fig. 1) were designated MSI-H. Tumors with some (but <40%) loci showing electrophoretic shift were designated MSI-L. All other tumors were designated microsatellite stable.

In Vitro Synthesized Protein Assay

In vitro synthesized protein assays to screen for truncation mutations in hMSH2 and hMLH1, the two mismatch repair genes, were performed as previously described (18). These assays were performed for all young patients (≤46 years) with MSI-H tumors and from whom blood leukocytes had been collected for DNA extraction. In brief, 3 µg of total RNA was reverse transcribed in a reaction mixture containing 20–200 ng of random hexamers or oligo(dT), 20 U to 30 cycles of PCR were performed in 10 µL of the first-strand complementary DNA (cDNA) mixture, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 3–5 mM MgCl₂, 0.5 pmol of each primer, all four deoxynucleoside triphosphates (each at 200 µM), and 2.5 U of Taq polymerase (Life Technologies, Inc.). Both hMSH2 and hMLH1 were amplified in two overlapping segments that were between 1.2 and 2.0 kilobases long. The left-hand primers of each segment were tagged with a T7 promoter sequence and a translation initiation site. The products were then subjected to in vitro transcription and translation by use of the Linked T7 transcription-translation system (Amer sham Corp., Little Chalfont, U.K).

DNA or cDNA Sequencing Analysis

When truncated protein products were detected by an in vitro synthesized protein assay, genomic sequencing of the regions of interest was performed to detect the site and nature of the underlying mutations. Because missense mutations are common in hMLH1 but are not detectable by an in vitro synthesized protein assay, we sequenced the whole hMLH1 cDNA from all young patients who had MSI-H tumors. Mutations found were confirmed by genomic sequencing. We designed primers for PCR amplification that spanned individual exons of hMSH2 and hMLH1 genes including intron–exon boundaries. Similarly, primers were designed to amplify overlapping regions of hMLH1 cDNA. Sequences of the primers are available from the authors on request. The PCR products were then purified by use of the High Pure PCR product purification kit (Boehringer Mannheim GmbH, Mannheim, Germany). Direct sequencing was performed by a manual sequencing method by use of Sequenase Version 2.0 (Amersham Corp.) or by automated sequencing by use of the dRhodamine terminator cycle sequencing kit (The Perkin-Elmer Corp., Norwalk, CT). Both forward and reverse primers were used in sequencing, following the manufacturer’s protocols. For manual sequencing, the sequencing products were denatured at 80 °C for 5 minutes and subjected to electrophoresis through 6% polyacrylamide–urea gels at 70 W for 2–3 hours. The gels were then fixed, dried, and exposed to autoradiographic films. For automated sequencing, the sequencing products were analyzed by an automated sequencer (Applied Biosystems, Foster City, CA).

Single-Strand Conformation Polymorphism and Direct DNA Sequencing Analysis of hMSH6

Mutational analyses for hMSH6 were performed by use of blood leukocytes or normal colonic tissue from the following three groups of young patients: 1) all young patients (≤46 years) who had MSI-H tumors but who did not have germline mutations in hMSH2 or hMLH1, 2) all young patients (≤46 years) who had MSI-L tumors, and 3) all patients 40 years old or younger who had microsatellite stable tumors. In all patients, individual exons of hMSH6 including intron–exon boundaries were amplified by PCR. The sequences of the primers are available on request from the authors. The PCR products were denatured in the presence of NaOH and EDTA at 50 °C for 15 minutes. Six microliters of the denatured PCR product was snap-cooled, mixed with 3 µL of formamide dye, and loaded onto MDE™ gels (FMC BioProducts, Rockland, ME). The gels were subjected to electrophoresis at 6–10 W overnight and silver stained, as described by the Promega Corp. (Madison, WI) protocol. Products with abnormal mobility were sequenced as described above.
**Statistical Analysis**

A binomial response model with age as the explanatory variable was used to assess the prevalence of MSI with age. The statistical analysis was done by the statistical package GLIM (19). We also used the \( \chi^2 \) test with the Yates correction. All \( P \) values are two-sided.

**RESULTS**

**Microsatellite Analysis and Statistics**

Of the 59 tumors from patients whose age at diagnosis was younger than 46 years, 19 were MSI-H and four were MSI-L. Of those tumors from patients whose age at diagnosis was 46 years old or older, eight were MSI-H and seven were MSI-L. The profile of microsatellite instability in all 27 MSI-H tumors is shown in Table 1. The incidence of MSI-H had a striking association with the age of the patients at diagnosis (Table 2). The model logit \( (p) = 1.08-0.61 \) age provides a sufficient fit to the data in Table 2 \( (X_{1}^2 = 2.79) \). In other words, the age factor can be used to explain the differences in the incidence rate of MSI-H tumors in the different age groups. The age effect is statistically significant \( (P<.001) \), with a 95% confidence interval of \(-0.25\) to \(-0.96\). The interpretation of the suggested model is that each upward step in the age group decreases the logit by \(-0.61\) (i.e., multiplies the odds on the prevalence of MSI-H by \(0.55\)).

Thus, of tumors in patients whose age at diagnosis was younger than 36 years, 62.5% showed MSI-H; however, in patients whose age at diagnosis was 36 years or older, only about 17% showed MSI-H \( (P<.001; \chi^2 \text{ test with Yates correction}) \). The difference in incidence of MSI-H was also statistically significant when those younger than 46 years were compared with those 46 years old or older \( (32\% \text{ versus } 14\%; P = .032; \chi^2 \text{ test with Yates correction}) \). Of the 19 patients with MSI-H tumors diagnosed before age 46 years, five had one relative with colorectal or endometrial cancer and three had more than one relative with colorectal or endometrial cancer. These family histories were analyzed with reference to the Bethesda guidelines \( (20) \) and are highlighted in Table 1. Three patients \( (2, 4, \text{ and } 19) \) also developed metachronous high-grade gliomas and were included in our earlier studies of early-onset glioma and Turcot’s syndrome \( (18,21) \).

**Table 1. Profile of microsatellite instability (MSI) and clinical data from patients with highly unstable MSI (MSI-H) tumors**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Bethesda guidelines with reference to family history of cancer</th>
<th>Germline mismatch repair gene mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S123</td>
<td>% loci mismatch repair gene</td>
<td></td>
</tr>
<tr>
<td>D3S1067</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSS82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSS346</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D18S58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAT-26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAT-40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGFBR1I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*BG1 = individuals with family histories of colorectal cancer that meet Amsterdam criteria; BG2 = individuals with two hereditary nonpolyposis colon cancer (HNPPC)-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers (including endometrial, ovarian, gastric, hepatobiliary, or small bowel cancer or transitional cell carcinomas of the renal pelvis or ureter); BG3 = individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPPC-related extracolonic cancer and/or a colorectal adenoma (one of the cancers diagnosed at age younger than 45 years and the adenoma diagnosed at younger than 40 years); ND = not done/not analyzable; NA = not available; M = male; F = female.

**Table 2. Incidence of highly unstable microsatellite instability (MSI-H) in patients of various age groups**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>No. of tumors</th>
<th>No. of tumors with MSI-H (%)</th>
<th>Germline mutation in mismatch repair genes (No. of patients)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;31</td>
<td>6</td>
<td>4 (67)</td>
<td>5</td>
</tr>
<tr>
<td>31–35</td>
<td>10</td>
<td>6 (60)</td>
<td>2 (1 NA)</td>
</tr>
<tr>
<td>36–40</td>
<td>21</td>
<td>4 (19)</td>
<td>1 (2 NA)</td>
</tr>
<tr>
<td>41–45</td>
<td>22</td>
<td>5 (23)</td>
<td>1 (1 NA)</td>
</tr>
<tr>
<td>&gt;45</td>
<td>58</td>
<td>8 (14)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*NA = not analyzed; ND = not done.
Germline Status of hMSH2 and hMLH1

Of the 19 patients with MSI-H tumors who were diagnosed before age 46 years, blood leukocytes from 15 patients were available for analysis. In six of these patients, an *in vitro* synthesized protein assay identified truncated hMSH2 proteins and direct DNA sequencing of the corresponding regions identified the causative mutations (Fig. 2; Table 3). *In vitro* synthesized protein data did not reveal any truncated hMLH1 protein products in these 15 patients.

Reverse transcription-coupled PCR and direct cDNA sequencing of hMLH1 in those patients with no truncated protein products detected by an *in vitro* synthesized protein assay further identified two germline missense mutations in patient 24. These mutations were confirmed by genomic sequencing. The two mutations were located in exons 4 and 9. There was strong presumptive evidence that these two mutations were in the same allele because the two mutations were also found in the patient’s mother (who had a history of colorectal cancer). The mutation in exon 4 has been previously reported to be pathogenic, and its pathogenic role is supported by a recent functional assay (22). To our knowledge, the mutation in exon 9 has not been reported. We screened 105 normal Chinese individuals (108 individuals were initially included, but PCR data from three were not usable) as control subjects and did not find a similar mutation in the germline.

Patient 19 also developed a brain glioblastoma that exhibited MSI-H but did not have detectable truncated protein by an *in vitro* synthesized protein assay. However, he had been included in our earlier studies (18,21) and did have a germline mutation in hMSH2 that was detected by direct genomic sequencing. This missense mutation was also found in one sibling who had colorectal cancer.

Overall, eight patients had a mutation in either hMLH1 or hMSH2; seven patients had a mutation in hMSH2 and one patient had a mutation in hMLH1 in the germline.

Analysis of hMSH6

Single-strand conformation polymorphism and sequencing of individual exons of hMSH6 revealed multiple nucleotide changes (Table 4). The following three changes were located within the coding region: one change that resulted in no amino acid change, one change that was a 4-base-pair (bp) insertion at the codon of the 7th amino acid from the C terminus of hMSH6, and the remaining change that resulted in a single amino acid change (T1284M) in exon 9 (Fig. 3). We screened a number of normal individuals (who had usable PCR results) who had the same 4-bp insertion in one allele, and we concluded, therefore, that this change was a common

![Fig. 2. A) *In vitro* synthesized protein assay of 5' segment of the hMSH2 gene. Truncated products of 53 kd are detected in the germline of patient 18. Lanes: C = normal control; B = blood leukocytes from patient 18. Arrow = wild-type protein; arrowhead = truncated protein product. B) Sequencing results of hMSH2 exon 9 from blood leukocytes of patient 18 revealed a 4-nucleotide deletion at nucleotide 1453 (arrowhead). Lanes: C = normal control; B = blood leukocytes from patient 18; A = adenosine; C = cytidine; G = guanosine; T = thymidine.](http://jnci.oxfordjournals.org/)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gene</th>
<th>Exon</th>
<th>Mutation site*</th>
<th>Family history of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>hMSH2</td>
<td>12</td>
<td>c.1760delG</td>
<td>Nil</td>
</tr>
<tr>
<td>4</td>
<td>hMSH2</td>
<td>11</td>
<td>c.1738G→T(E580X)</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td>hMSH2</td>
<td>3</td>
<td>c.513delG</td>
<td>Positive (the same germline mutation was present in other family members with colorectal cancer)</td>
</tr>
<tr>
<td>18</td>
<td>hMSH2</td>
<td>9</td>
<td>c.1452–1455delAAATG</td>
<td>Positive (one sibling had colorectal cancer but was not available for testing)</td>
</tr>
<tr>
<td>19</td>
<td>hMSH2</td>
<td>3</td>
<td>c.595T→C(C199R)</td>
<td>Positive (the same germline mutation was present in another sister with colorectal cancer)</td>
</tr>
<tr>
<td>23</td>
<td>hMSH2</td>
<td>5</td>
<td>IVS5 + 3A→T†</td>
<td>Positive (other family members with colorectal cancer were not available for testing)</td>
</tr>
<tr>
<td>24</td>
<td>hMLH1</td>
<td>4</td>
<td>[c.350C→T(T117M); c.790C→T(H264Y)]</td>
<td>Positive (the same two germline mutations are present in his mother having colorectal cancer)</td>
</tr>
<tr>
<td>27</td>
<td>hMSH2</td>
<td>9</td>
<td>c.1452–1455delAAATG</td>
<td>Positive (father had colorectal cancer but had died and was not available for testing)</td>
</tr>
<tr>
<td>28</td>
<td>hMSH6</td>
<td>9</td>
<td>c.3851C→T(T1284M)</td>
<td>Nil</td>
</tr>
</tbody>
</table>

*del = deletion.
†Splicing defect resulting in skipping of exon 5.

![Table 4. Nucleotide changes in hMSH6](http://jnci.oxfordjournals.org/)

<table>
<thead>
<tr>
<th>Location</th>
<th>Nucleotide (nt) position</th>
<th>Nucleotide change (amino acid)*</th>
<th>Allele frequency, No. of alleles found/No. of alleles examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 5</td>
<td>3306</td>
<td>c.3306T→A</td>
<td>23/68</td>
</tr>
<tr>
<td>Exon 9</td>
<td>3851</td>
<td>c.3851C→T(T1284M)†</td>
<td>0/216</td>
</tr>
<tr>
<td>Exon 10</td>
<td>4064</td>
<td>c.4064→4065insGTCA</td>
<td>11/198</td>
</tr>
<tr>
<td>Intron 1</td>
<td>−36 nt of 5’ exon 2</td>
<td>IVS1−36A→G</td>
<td>2/68</td>
</tr>
<tr>
<td>Intron 5</td>
<td>+11 nt of 3’ exon 5</td>
<td>IVS5 + 11–14delCTTA</td>
<td>1/68</td>
</tr>
<tr>
<td>Intron 7</td>
<td>+28 nt of 3’ exon 7</td>
<td>IVS7 + 28–29insCTTA</td>
<td>1/68</td>
</tr>
<tr>
<td>Intron 9</td>
<td>−24 nt of 5’ exon 10</td>
<td>IVS9−24C→T</td>
<td>1/68</td>
</tr>
</tbody>
</table>

*ins = insertion; IVS = intervening sequence; del = deletion.
†This missense mutation was found in patient 28 whose tumor was microsatellite stable.
that an MSH6 deficiency may have a less consistent association with MSI in patients whose tumors exhibited MSI.

We have gathered evidence regarding the pathogenesis of colorectal cancer in young Hong Kong Chinese population, a subpopulation of particular interest because of its high cancer susceptibility relative to young Caucasians (1). The data show that, in these young people, a high proportion of the colorectal carcinomas show defects in DNA replication fidelity and that MSI is increasingly common in patients with younger ages of onset. Moreover, many of the young colorectal cancer patients have a germline mutation in one of the mismatch repair genes (hMSH2, hMLH1, or hMSH6). In the youngest age group studied (<31 years old), more than 80% have a germline deficiency in hMSH2, hMLH1, or hMSH6.

The data on hMSH6 are of special interest. To our knowledge, this is one of the first systematic studies to reveal multiple nucleotide changes in authentic human tumors (Table 4). Most of these changes are unlikely to be responsible for tumor development because they are intronic changes, conservative changes, or polymorphisms. Our finding of a common polymorphism, the 4-bp insertion at the very end of the last exon of hMSH6, in two patients has in agreement with a previous report of a similar 4-bp insertion at the same location, although with different sequences, in a Japanese population (12). We did find, however, one missense mutation in a 29-year-old man whose tumor did not exhibit MSI at any loci. Although the possibility of this mutation being a rare polymorphism cannot be absolutely excluded, a search of 108 normal Chinese individuals did not show a similar nucleotide change. Furthermore, this mutation alters an amino acid (threonine) that is conserved in MSH6 and MSH2 in yeast, mouse, and human proteins (Fig 4), indicating its potential importance for the function of the protein. To our knowledge, this is the first report of a germline hMSH6 mutation in a human tumor without MSI. Tumors from mice with an engineered deficiency in MSH6 also do not exhibit MSI (14). On the other hand, there are two reports (12,13) of three germline hMSH6 mutations in patients whose tumors exhibited MSI. It is possible that an MSH6 deficiency may have a less consistent association with MSI than a deficiency in other mismatch repair genes.

The mutation database of the International Collaborative Group of HNPCC contains more than 230 germline mutations in hMLH1 or hMSH2; about 40% are located in hMSH2. Our present data show that our study population has a much higher mutation rate in hMSH2 than in hMLH1 (7:1), which differs from studies of other populations. Our strategy of reverse transcription-coupled PCR and cDNA sequencing of hMLH1 allowed us to detect most of the missense and splicing mutations common in this gene. Analysis of more patients from our population is proceeding to confirm this difference in the distribution of germline mutations in these two genes involved in HNPCC.

Although a family history of cancer is evident for some of our young colorectal cancer patients, all but one of these family histories fell short of the Amsterdam criteria. Although the Amsterdam criteria were necessary to define the core clinical HNPCC syndrome, it is now clear that penetrance falls short of 100%. In particular, some patients with an unequivocal germline deficiency in mismatch repair gene develop colorectal cancer late in life. Table 1 documents the eight families with a positive family history of cancer according to the Bethesda guidelines. There are five patients with germline mismatch repair gene mutations whose family histories of cancer satisfy the more relaxed Bethesda guideline 3 but not the Amsterdam criteria.

Patients younger than 36 years of age make up only 1%–2% of the total patients with colorectal cancers in most Caucasian populations (23–25). However, similar data in Hong Kong show that patients younger than 36 years of age account for up to 3%–5% of the total number of patients with colorectal cancer (26,27) and that the population incidence in this age group exceeds, in absolute terms, the incidence in Scotland by some fourfold (1). We have shown that these patients are a particularly important subgroup, with a high chance of harboring germline mutations in the mismatch repair genes. Indeed, 84% of our patients who were younger than 31 years of age carry a germline mutation in one of the mismatch repair genes. This observation has important implications for the management of these young patients. Total colectomy may be the surgical treatment of choice, and a subsequent surveillance program is essential for both the patients and their relatives.

It is of interest to note that the proportions of Hong Kong Chinese and Caucasian patients with young-onset cancer who have MSI-H tumors, germline mutation in mismatch repair genes, and a positive family history of cancer are very similar (16). Therefore, despite the fact that this study has emphasized the numeric importance of mismatch repair deficiency (involving hMSH2, hMLH1, and hMSH6) in the pathogenesis of young-onset cancer in Hong Kong Chinese, there is no evidence that these genes are uniquely responsible for the high susceptibility in Hong Kong Chinese. Other types of genomic instability are also proportionately more common, including those responsible for susceptibility to cancer with evidence of chromosome instability in hMSH2, hMLH1, and hMSH6.

**Fig. 3.** A) Single-strand conformation polymorphism screening of exon 9 of hMSH6 revealed the presence of abnormal band (arrowhead) in the germline of patient 28. Lanes: C = control; N = normal mucosa of patient 28. Arrow = wild-type alleles. B) Direct DNA sequencing of exon 9 of hMSH6 revealed a cytidine (C) to thymidine (T) transition (arrowhead). Lanes: N = normal mucosa of patient 28; G = guanosine; A = adenosine.

**Fig. 4.** Homology comparison between MSH2 and MSH6 from human, mouse, and yeast. The numbering of the amino acid refers to the position in hMSH6. * = threonine at codon 1284 of hMSH6.
instability. The nature of this instability and susceptibility in the Hong Kong Chinese population needs further investigation.

REFERENCES


NOTES

Supported by Committee on Research and Conference grant 337/046/0024, by University Research Committee grants 344/046/0003 and 10202505/13955/21200/302/01 from the University of Hong Kong, and by Croucher Foundation Research grant 394/046/1238. T. L. Chan is a Ph.D. student from the University of Hong Kong.

We thank Dr. Paul Yip, Department of Statistics, The University of Hong Kong, for his advice and help in the statistical analyses of this article and Prof. L. C. Chan and Dr. R. J. Collins for their support through this project.

Presented at the 89th Annual Meeting of the American Association for Cancer Research, 1998, New Orleans, LA.

Manuscript received November 30, 1998; revised May 19, 1999; accepted June 3, 1999.