Germ Line Mutations of Mismatch Repair Genes in Hereditary Nonpolyposis Colorectal Cancer Patients with Small Bowel Cancer: International Society for Gastrointestinal Hereditary Tumours Collaborative Study

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Abstract

Purpose: The aim of study was to determine the clinical characteristics and mutational profiles of the mismatch repair genes in hereditary nonpolyposis colorectal cancer (HNPCC) patients with small bowel cancer (SBC).

Experimental Design: A questionnaire was mailed to 55 members of the International Society for Gastrointestinal Hereditary Tumours, requesting information regarding patients with HNPCC-associated SBC and germline mismatch repair gene mutations.

Results: The study population consisted of 85 HNPCC patients with identified mismatch repair gene mutations and SBCs. SBC was the first HNPCC-associated malignancy in 14 of 41 (34.1%) patients for whom a personal history of HNPCC-associated cancers was available. The study population harbored 69 different germline mismatch repair gene mutations, including 31 mutations in MLH1, 34 in MSH2, 3 in MSH6, and 1 in PMS2. We compared the distribution of the mismatch repair mutations in our study population with that in a control group, including all pathogenic mismatch repair mutations of the International Society for Gastrointestinal Hereditary Tumours database (excluding those in our study population). In patients with MSH2 mutations, patients with HNPCC-associated SBCs had fewer mutations in the MutL homologue interaction domain (2.9% versus 19.9%, P = 0.019) but an increased frequency of mutations in codons 626 to 733, a domain that has not previously been associated with a known function, versus the control group (26.5% versus 2.8%, P < 0.001).

Conclusions: In HNPCC patients, SBC can be the first and only cancer and may develop as soon as the early teens. The distribution of MSH2 mutations found in patients with HNPCC-associated SBCs significantly differed from that found in the control group (P < 0.001).

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Hereditary nonpolyposis colorectal cancer (HNPCC), the most common form of hereditary colorectal cancer, is inherited as an autosomal dominant trait with incomplete penetrance. HNPCC is associated with germ line mutations in the mismatch repair genes, which include MLH1 (MIM #120436), MSH2 (MIM #120435), PMS2 (MIM #600259), and MSH6 (MIM #600678; refs. 1, 2). About 90% of the identified germline mutations in the mismatch repair genes are found in two genes, MLH1 and MSH2 (2).

HNPCC accounts for 2% to 5% of all colorectal cancers and is characterized by early age of onset, predominantly right-sided colorectal cancer, frequent development of synchronous and metachronous colorectal cancer, and extracolonic malignancies (3–7), including endometrial, small bowel, ovary, ureter, and renal pelvic carcinomas. Individuals affected with HNPCC are also at an increased risk for developing hepatobiliary, brain, and skin tumors (8, 9).

In the general population, small bowel cancer (SBC) is relatively rare, accounting for <2% of all gastrointestinal cancers (10). However, in HNPCC patients, the lifetime risk for SBC reportedly ranges from 1% to 4%, which is >100 times the risk in the general population (8). In the Korean Hereditary Tumor Registry, SBCs were found in six patients from three families among 116 families with HNPCC or suspected HNPCC registered. Three different germ line mismatch repair gene mutations were identified in these six patients, all located on the PMS2/MLH3/PM/S1 interaction domain of the MLH1 gene.

In an effort to gain insight into the associations among HNPCC, SBC, and germ line mismatch repair gene mutations, we collaborated with the International Society for Gastrointestinal Hereditary Tumours to conduct a retrospective study of the clinical characteristics of SBC in HNPCC patients and their correlations with various mismatch repair genotypes.

Materials and Methods

**Patients.** A questionnaire was mailed to 55 members of the International Society for Gastrointestinal Hereditary Tumours from 21 countries, requesting information regarding HNPCC patients in their registries who were diagnosed as having SBC. The following clinical data were requested: age at diagnosis of SBC and other malignant disease, gender, personal history of HNPCC-associated malignant disease, family history of SBC, and various characteristics of SBC, such as histologic type, histologic grade, and tumor location. Mutation information was also requested, including identification of the mutated mismatch repair gene, the location of mutation, the nucleotide alteration, and its predicted effect on protein synthesis.

Seventeen members from 13 countries answered the questionnaire. We were able to collect information on 87 patients with SBCs from 78 HNPCC families (68 HNPCC families and 10 suspected HNPCC families). Patients were included in this study only when a pathogenic germline mutation in the mismatch repair gene was identified. Two patients were excluded from the present study, one from the United Kingdom with mutations in both APC and MSH2 and one from the Dutch HNPCC Registry in whom ileal adenocarcinoma was diagnosed at the age of 61 but for whom mismatch repair mutation data were not available. Thus, the study population consisted of 85 patients with identified germ line mismatch repair mutations and a total of 90 SBCs.

**Statistical analysis.** Sex (male versus female), location of SBC (duodenum versus distal to duodenum), affected mismatch repair gene (MLH1 versus MSH2), and the first HNPCC-associated cancer (SBC versus non-SBC) were considered to be binary variables for determining the characteristics of our SBC patients. The age at diagnosis of SBC was analyzed as a continuous variable. The various variables were analyzed using the \( \chi^2 \) and Fisher’s exact tests. All statistical tests were two sided and done with SAS programs (version 8.01, SAS Institute, Inc., Cary, NC). \( P \leq 0.05 \) was considered statistically significant. For analysis of mismatch repair gene mutation distribution patterns, all mismatch repair gene mutations (excluding those found in our experimental group) registered in the International Society for Gastrointestinal Hereditary Tumours database (http://www.insight-group.org/) were used as the control group.

Results

**Clinical characteristics.** Eighty-five individuals from 78 HNPCC families were identified as having germ line mismatch repair gene mutations and a total of 90 SBCs. Of these patients, 51 were male (60.0%). The mean age at diagnosis of SBC was 48 years (range, 11–81 years). Nine patients (10.6%) developed SBC before the age of 30; the patient with the youngest age of diagnosis was a male from Canada, who was diagnosed with HNPCC-associated SBC at the age of 11. Sixty of the 85 patients (70.6%) met the Amsterdam criteria; 11 patients did not meet the Amsterdam criteria, and information on the Amsterdam criteria status was unavailable in the remaining 14 patients.

Four patients had metachronous SBCs. Three patients each had one metachronous SBC that developed at 7, 9, 13 years, respectively, after the index SBC. One patient from Australia had two metachronous SBCs that developed in the jejunum and ileum 21 years after the index SBC in the duodenum. Histologically, the SBC types were as follows: 74 adenocarcinomas (82.2%), 2 carcinoid tumors (2.2%), and 14 not specified (15.6%). The localization of the reported SBC was unknown in 17 patients. The remaining 68 patients had 72 localized SBCs; the duodenum and jejunum were equally affected (31 of 72, 43.1% in each case), and ileal SBCs were seen less frequently (10 of 72, 13.9%).

Personal history regarding other malignant disease was available for 41 patients, in which 51 colorectal cancers developed in 27 cases. SBC was the first HNPCC-associated malignancy in 14 of the 41 patients (34.1%), and 9 of the 41 patients (22.0%) had SBC as their only HNPCC-associated cancer at the time of data collection. In the 14 patients wherein SBC was the first identified HNPCC-associated cancer, colorectal cancers developed in only 3 (21.4%). The other HNPCC-associated malignancies identified in the 41 patients included 5 endometrial cancers, 4 renal pelvis/ureter cancers, 3 ovarian cancers, and 1 pancreatic cancer.

**Mismatch repair mutations.** Sixty-nine different germline mismatch repair gene mutations were identified in the study population; 31 mutations were identified in the MLH1 gene (44.9%), 34 in the MSH2 gene (49.3%), 3 in the MSH6 gene (4.3%), and 1 in the PMS2 gene (1.4%). Thirty-one different MLH1 mutations were identified in 42 patients, including 25 truncation mutations (80.6%) and 6 missense mutations (19.4%). Thirty-four different MSH2 mutations were identified in 38 patients, including 28 truncation mutations (82.4%) and 6 missense mutations (17.6%). Two truncation mutations and one missense mutation were identified in MSH6, and one truncation mutation of PMS2 was identified in two patients from the same family.

We determined the frequency of mutations in each functional domain of the MLH1 gene, including the ATPase domain,
the MutS homologue interaction domain, and the PMS2/MLH3/PMS1 interaction domain. Seven of 31 mutations were found in the ATPase domain, 9 in the MutS homologue interaction domain, and 15 in the PMS2/MLH3/PMS1 interaction domain. Similarly, we analyzed the distribution of MSH2 mutations in the DNA-binding domain, the MSH3/MSH6 interaction domain, the MutL homologue interaction domain, and a region that had no known function (codons 626-733; ref. 11). Fourteen of 34 mutations were found in the DNA binding domain, 10 in the MSH3/MSH6 interaction domain, 1 in the MutL homologue interaction domain, and 9 in a region that had no known function (codon 626-733). We then compared the distribution of MLH1 and MSH2 mutations in our study population with those in a control group derived from the International Society for Gastrointestinal Hereditary Tumours database. As shown in Table 1, the distribution of MLH1 mutations did not differ between the experimental and control groups (P = 0.44), but the distribution of MSH2 mutations found in HNPPC patients with SBCs was significantly different from that in the control group (P < 0.001). Patients with SBCs had less frequent mutations in the MutL homologue interaction domain (2.9% versus 19.9%, P = 0.019, Fisher’s exact test), but more frequent mutations in codons 626 to 733 (26.5% versus 2.8%, P < 0.001, Fisher’s exact test). There were no significant differences in the mutational frequencies of the DNA-binding and MSH3/MSH6 interaction domains of MSH2. In Table 1, we determined the frequency of mutations in each functional domain of the MLH1 and MSH2 genes and compared this distribution with those in a control group derived from the International Society for Gastrointestinal Hereditary Tumours database. Thus, the data are presented in terms of the number of different germline mutations not families or individual cases.

Forty-four SBCs developed in 42 patients with germline mutations of the MLH1 gene: 16 in the duodenum (36.4%), 11 in the jejunum (25.0%), 5 in the ileum, (11.4%), and 12 in unspecified locations (27.3%). Forty-one SBCs developed in 38 patients with MSH2 mutations: 13 in the duodenum (31.7%), 18 in the jejunum (43.9%), 4 in the ileum (9.8%), and 6 in unspecified locations (14.6%). There were no significant differences in the locations of SBCs between patients with mutations in MLH1 and MSH2 (P = 0.208, Fisher’s exact test).

**Discussion**

SBC is relatively rare in the general population, with an incidence of reportedly <10 cases per one million people annually (12). However, patients with Crohn’s disease and hereditary colorectal cancer syndromes, such as HNPCC, familial adenomatous polyposis, and Peutz-Jegher syndrome, have a 50- to 300-fold higher risk of developing SBC versus the general population (13–15). However, although it is accepted that HNPCC patients are at an increased risk for developing SBC, it has proven difficult for a single investigator or institution to collect a large number of HNPCC patients with SBCs for analysis. Thus, we conducted an international collaborative study.

To our knowledge, only two previous studies have focused on HNPCC-associated SBC. Rodrguez-Bigas et al. reported 49 SBCs from 42 HNPCC patients, based on a questionnaire mailed to members of the International Collaborative Group on HNPCC, which includes individuals from the United States, the Netherlands, Denmark, Finland, Australia, Italy, Portugal, Canada, and Israel (13). Recently, a study from the German HNPCC Consortium reported 32 SBCs from 31 unrelated HNPCC patients, detailing both the pathologic features and the clinical characteristics (16). Similar to the study by Rodrguez-Bigas et al., the present work was based on a questionnaire mailed to participating centers from 13 countries worldwide. Thus, it is possible that our findings may be biased by differences among the participating registries in terms of patient selection, terminologies, and policies regarding management for HNPCC patients and at-risk family members. However, our patient population is the largest group of HNPCC patients with SBCs reported to date, and all enrolled patients had identified germ line mismatch repair gene mutations, allowing us to examine possible genotype-phenotype correlations.

HNPPC patients reportedly develop cancers at earlier ages than seen in sporadic cancer patients. The mean age at

**Table 1. Distribution of the mismatch repair mutations in HNPCC patients with SBCs**

<table>
<thead>
<tr>
<th>A. MLH1 gene (P = 0.44, ( \chi^2 ) test)</th>
<th>ATPase domain (codon 31-151)</th>
<th>MutS homologue interaction domain (152-327)</th>
<th>PMS2/MLH3/PMS1 interaction domain (491-742)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study*</td>
<td>7 (22.6%)</td>
<td>9 (29.0%)</td>
<td>15 (48.4%)</td>
<td>31 (100%)</td>
</tr>
<tr>
<td>Controls 1</td>
<td>58 (28.2%)</td>
<td>73 (35.4%)</td>
<td>75 (36.4%)</td>
<td>206 (100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. MSH2 gene (P &lt; 0.001, Fisher’s exact test)</th>
<th>DNA binding domain (codon1-346)</th>
<th>MSH3/MSH6 interaction domain (codon 378-625)</th>
<th>Region including Walker A (codon 626-733)</th>
<th>MutL homologue interaction domain (codon 734-895)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study*</td>
<td>14 (41.2%)</td>
<td>10 (29.4%)</td>
<td>9 (26.5%)</td>
<td>1 (2.9%)</td>
<td>34 (100%)</td>
</tr>
<tr>
<td>Controls 1</td>
<td>55 (39.0%)</td>
<td>54 (38.3%)</td>
<td>4 (2.8%)</td>
<td>28 (19.9%)</td>
<td>141 (100%)</td>
</tr>
</tbody>
</table>

*The data are presented in terms of the number of different germ line mutations and not families or individual cases.

1 Controls were assembled from the database of the International Society for Gastrointestinal Hereditary Tumours (http://www.insight-group.org/).
diagnosis of SBC in our study population was 48 years, which is consistent with the age reported in two previous studies (13, 17), but is ~10 years older than the age of diagnosis reported for German patients (16). We found that SBCs occurred at similar ages at which diagnosis of colorectal cancer and endometrial cancers were made in our HNPCC patients, which is consistent with a previous report (18). Our data also indicated that some HNPCC-associated SBCs could occur at a very early age. Over 10% of our patients were diagnosed with SBCs before the age of 30, with two cases occurring as early as 11 and 17 years of age. These findings suggest that clinicians managing HNPCC patients and at-risk family members should be alert for unusually early manifestations of SBC. The patient from Italy in the study population, who was diagnosed with colorectal cancer with mutations in the mismatch repair gene. PM2S2 genetic testing identified the nonsense 1951C>T (Q643X) and the missense 161C>T (S46I) mutations, and these two mutations were located in two different alleles of the patient, with the nonsense mutation inherited from his mother and the missense mutation probably inherited from his father (19). Although it remains to be defined, correlation between biallelic mutations and very young cancer might be probable.

Studies have shown that both sporadic (20) and HNPCC-associated (13, 16) SBCs are more common in males than females, with the latter showing a reported 3:2 ratio of males to females. In the present study, 60% of our enrolled patients were male, yielding a 3:2 ratio of males to females that is relatively consistent with the previous findings. When we analyzed patients grouped with respect to MLH1 and MSH2 mutations, male patients predominated in both subgroups, and there was no significant difference in the gender ratio.

In terms of histology, an early study reported adenocarcinoma as the only HNPCC-associated colorectal cancers (17), but more recent studies showed that 4% to 6% of HNPCC-associated SBCs are carcinoid tumors (13, 16). Consistent with the latter reports, most of the SBCs in our study population were adenocarcinomas (82.2%), but 2 carcinoid tumors (2.2%) were also identified. With regard to localization of HNPCC-associated SBCs, the previous study in a German population reported that half of HNPCC-associated SBCs developed in the duodenum (16), whereas Rodriguez-Bigas et al. reported an almost uniform distribution of SBCs throughout the small bowel (13). In the present study, data on tumor location were available in 72 of 90 SBCs (80.0%). Most of the tumors with identified locations occurred in the proximal small bowel, with ileal cancers occurring at a lower frequency. The duodenum and jejunum were equally affected, and endoscopic surveillance could have detected 43% of the HNPCC-associated SBCs identified in our patients. There were no significant differences in tumor location between patients with mutations in MLH1 versus MSH2, and in some families, SBCs developed in different locations among family members having identical germline mismatch repair mutations. Thus, it does not seem as though the genotype can be used to predict tumor location.

Our data indicated that SBC could be the first and only cancer in HNPCC patients. Furthermore, although the lifetime cumulative risk of colorectal cancer is about 80% in HNPCC patients, colorectal cancer developed less frequently (3 of 14, 20.4%) in patients in whom SBC had occurred as the first cancer. This result might be influenced by several factors, including the sample size and the death of patients from other cancers before development of colorectal cancer. However, the presence of patients having only SBC without colorectal cancer in our study population suggests that a family history should be obtained from all patients newly diagnosed as having SBCs.

Although our study was not designed to compare the incidence of SBC among HNPCC patients with mutations in the different mismatch repair genes, our study population contained similar numbers of individuals with mutations in MLH1 and MSH2 (31 and 34, respectively). Vasen et al. (21) reported that the lifetime risk of cancer at any site was significantly higher for MSH2 mutation carriers versus MLH1 mutation carriers. The same report indicated that MSH2 mutation carriers had higher risks for developing cancer of the stomach, ovary, and brain, but not SBC. Consistent with our findings, Vasen et al. reported that of the 16 SBC patients in their study, 9 had mutations in MLH1 and 7 had mutations in MSH2 (21). Peltomaki et al. reported that extracolonic cancers were more common in MSH2 mutation carriers, but their study included only two ileal cancers, which developed in two patients with mutations in MLH1 (22). Because of the relatively rarity of SBCs in HNPCC patients, it is difficult to accurately estimate the incidence of SBC in carriers of specific mismatch repair gene mutations.

In conclusion, we herein examined the mutational spectrum in HNPCC patients with SBCs, examined the frequency of mutations in each functional domain of the MLH1 and MSH2 genes, and showed for the first time that the distribution of MSH2 mutations in HNPCC patients with SBC significantly differed from that of a control group. Patients with SBCs had less frequent mutations in the MutL homologue interaction domain but more frequent mutations in a region without known function (codons 626–733). This result suggests that mutational spectrums might differ among patients having different sets of cancers.

Conclusions

SBC can be the first and only cancer in HNPCC patients and may develop as soon as the early teens. The duodenum and jejenum are commonly and equally affected, with ileal SBC found less frequently. Patients with SBCs have fewer mutations in the MutL homologue interaction domain but more frequent mutations in codons 626 to 733, a domain of previously unknown function.

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Clinical Cancer Research

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