HNPCC-Associated Small Bowel Cancer: Clinical and Molecular Characteristics

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Background & Aims: The risk for small bowel cancer (SBC) is significantly increased in hereditary nonpolyposis colorectal cancer (HNPCC). HNPCC-associated SBCs are poorly characterized. *Methods:* Thirty-two SBCs were characterized according to clinical, pathologic, and germline mutation data. Histomorphologic characteristics, microsatellite instability (MSI) testing, mismatch repair (MMR) protein expression, and frameshift mutations of 7 coding mononucleotide repeats were investigated in 17 SBCs. *Results:* Median age at diagnosis was 39 years. Fifty percent of SBCs were located in the duodenum. The Amsterdam criteria were fulfilled in 50% of patients; 45% of patients had no personal history of previous malignancies. Two patients had a positive family history for SBC. Pathogenic germline mutations were identified in 81%; high MSI was detected in 95% and loss of MMR protein expression in 89% of cases. *TGFBR2*, *BAX*, *MSH3*, *MSH6*, *ACVR2*, *AIM2*, and *SEC63* frameshift mutations were detected in 69%, 59%, 59%, 35%, 82%, 56%, and 56%, respectively. An expansive growth pattern of the tumor border and an intense intratumoral lymphocytic infiltrate were present in 75%, respectively. *Conclusions:* HNPCC-associated SBC often manifests at a young age and may be the first disease manifestation. Endoscopy may detect 50% of tumors. Considering recent data on gastric cancer, we propose endoscopic screening of mutation carriers starting at 30 years of age because clinical criteria cannot define a high-risk group. In addition, our study shows that histopathologic criteria, MSI, and MMR immunohistochemistry are often similar to these features in HNPCC.

Hereditary nonpolyposis colorectal cancer (HNPCC; MIM 114500) is the most common colorectal cancer (CRC) susceptibility syndrome with an autosomal dominant mode of inheritance, with incomplete penetrance accounting for 2%–5% of all CRCs, and is characterized by familial clustering of CRC, early age of disease onset, predominantly right-sided CRC, and an excess of synchronous and metachronous CRC. Individuals affected with this hereditary cancer predisposition are also at an increased risk for developing endometrial, small bowel, stomach, hepatobiliary, ovarian, and urinary tract cancers as well as brain and skin tumors.[1–4] In 1991 and 1999, the Amsterdam criteria 1 and 2, respectively, were established by the International Collaborative Group on HNPCC, allowing standardized recruitment of families for collaborative studies.[5,6]

HNPCC has been linked to DNA mismatch repair (MMR) gene defects. To date, pathogenic germline mutations in the MMR genes *MLH1* (MIM 120436; GenBank accession no. AH003234), *MSH2* (MIM 120435; GenBank accession no. AH003235), *PMS2* (MIM 600259; GenBank accession no. U13696), and *MSH6* (MIM 600678; GenBank accession no. AH005068) have been described (for

Abbreviations used in this paper: CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability high; MSI-L, microsatellite instability low; SBC, small bowel cancer.

0016-5085/05/$30.00
doi:10.1053/j.gastro.2004.12.051

review, see Peltomaki and Vasen[7] and Liu et al[8]). The majority of germline mutations have been identified in *MLH1* and *MSH2* (>85%),[8,9] whereas *MSH6* mutations were detected in <10% and *PMS2* mutations are rare.

Small bowel cancer (SBC) is rare, accounting for <5% of all gastrointestinal malignancies.[10,11] Patients with hereditary CRC syndromes, such as familial adenomatous polyposis, Peutz–Jeghers syndrome, and HNPCC, have a significantly increased risk for SBC. HNPCC-associated SBC was first reported by Love[12] and later by Lynch et al.[13] The lifetime risk of SBC in patients with HNPCC has been estimated to range from 1% to 4%, resulting in a relative risk of more than 100,[1] and was reported to be higher in *MLH1* mutation carriers compared with *MSH2* mutation carriers.[14] Recently, Vasen et al reported a cumulative lifetime risk for SBC of 7% for *MLH1* mutation carriers,[15] whereas most of the other extracolonic HNPCC-associated tumors tended to be more common in *MSH2* mutation carriers. Recent studies suggest that SBC may be less common in Finnish patients with HNPCC compared with other studies from the Netherlands and France.[1,14–17]

Only 1 previous study has particularly addressed HNPCC-associated SBC.[18] This study was based on a questionnaire mailed to 9 participating centers in the United States, Canada, Portugal, Finland, Italy, and Israel. The study design had important limitations due to selection bias and potential influences of different geographic origins and terminologies. To date, no studies on molecular or pathologic features of HNPCC-associated SBC have been reported. Even for sporadic SBC, only a few studies with a limited number of patients have been reported regarding few molecular alterations.[19–27]

The aim of this study was to characterize HNPCC-associated SBC according to clinical, histomorphologic, and molecular variables.

Patients and Methods

Patients

Patient data were retrieved from the database of the German HNPCC Consortium including 1986 families meeting the inclusion criteria, which are based on the Amsterdam and Bethesda criteria.[5,6,28] Details of the study design are reported elsewhere[29] (Engel et al, manuscript in review). All patients gave written informed consent. The study was approved by the local ethic committee of each participating clinical center.

The clinical and pathologic data of all index patients with epithelial tumors of the small bowel were extracted from the database. Patients were included in this study if at least one of the following criteria was fulfilled: (1) a pathogenic MMR germline mutation was identified in the family or (2) the Amsterdam criteria 1 or 2 were fulfilled (with the exception of the age criterion) or (3) one of the classic Bethesda criteria 2–4 was fulfilled in conjunction with the detection of microsatellite instability high (MSI-H) in 1 of the patient's tumors. We identified 32 patients with epithelial small bowel neoplasms fulfilling at least one of these criteria. Germline mutation analysis was performed in each center, and germline mutation data were extracted from the central database. Definitions for the classification of germline mutations as pathogenic, unclassified variant, or polymorphism are reported elsewhere.[30] In 1 patient, germline mutation testing was not performed but a pathogenic *MLH1* mutation was identified in another family member and the tumor tissue of the SBC lacked *MLH1* expression. We therefore considered this patient to have a pathogenic *MLH1* mutation for the purpose of this study.

Methods

All available paraffin-embedded tumor tissues (n = 16) were analyzed at the Institute of Pathology in Bochum. In 1 additional case, tumor and normal DNA were provided. In 4 additional cases, microsatellite instability (MSI) and/or MMR immunohistochemistry was performed previously in one of the centers but tissue was no longer available. These previous results were included in the analysis.

Histomorphology

H&E-stained sections of 16 SBCs were evaluated by a pathologist for histologic grade, subtype, growth pattern, and peritumoral and intratumoral lymphocytes, which were features previously reported to be highly characteristic for CRCs with MSI-H phenotype[31–39] and which were finally integrated in the clinical criteria of HNPCC.[6,10] For quantification of intratumoral and peritumoral lymphocytes, immunohistochemical CD3 staining was performed.[39] The peritumoral infiltrate was classified semiquantitatively as none, discrete, or intense. The intratumoral number of CD3 lymphocytes was calculated relative to the total number of nuclei. A cutoff value of 10% was chosen. The growth pattern of the tumor border was classified as infiltrative or expansive forming a pseudocapsule (pushing border).

MSI Testing and Analysis of Frameshift Mutations in Coding Mononucleotide Repeats

Tumor and normal tissue were microdissected by a pathologist. Tumor cell cellularity was at least 70%. In one case, DNA from peripheral blood leukocytes was used as normal DNA. DNA was isolated with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Microsatellite markers *BAT-25*, *BAT-26*, *D5S346*, *D17S250*, *D2S123*, and *BAT-40* were analyzed as described previously.[41] The markers included the National Institutes of Health reference panel according to the international guidelines for the evaluation of MSI in CRC.[42] Tumors were classified as MSI-H if at least 2 of the 5 markers of the reference panel showed instability. Tumors were classified as low-level microsatellite unstable (MSI-L) if one marker displayed instability. Mononucleotide repeats in the

Table 1. Clinical Characteristics and MMR Germline Mutations of HNPCC-Associated SBC

Sex (M/F)	22/10 (69% male)
Age (*y*)	
Median	39.0
Range	15–73
Inclusion criteria	
Amsterdam criteria 1	14 (44%)
Amsterdam criteria 2	2 (6%)
Amsterdam criteria 1 (without fulfillment of the age criterion)	1 (3%)
Bethesda criteria 2	14 (44%)
Bethesda criteria 3	1 (3%)
Family history of SBC	2/32 (6%)
Germline mutation	
Pathogenic	22/27 (81%)
MLH1	13 (59%)
MSH2	8 (37%)
MSH6	1 (4%)
Unclassified variant	5/27 (19%)
MLH1	3
MSH2	1
MSH6	1

coding sequence of *TGFBR2*, *ACVR2*, *BAX*, *MSH3*, *MSH6*, *AIM2*, and *SEC63L* were analyzed for frameshift mutations using the same protocol. Primer sequences are available on request.

Immunohistochemistry of MMR Proteins

Immunohistochemical staining of MMR proteins (MLH1, MSH2, and MSH6) was performed as previously described.[41] Staining was only considered informative when there was normal nuclear staining in adjacent nonneoplastic cells. Staining of <1% of tumor cells was considered as loss of expression of an MMR protein, and 10%–100% of stained tumor cells were considered as positive staining for MMR proteins. Staining of 1%–10% of tumor cells for MMR proteins was not observed.

Results

Clinical Data

Thirty-one unrelated patients with 32 SBCs were identified. One patient had 2 synchronous SBCs (patient 9). In addition, 1 patient with a duodenal adenoma was identified (see supplementary table online at http://www.ruhr-uni-bochum.de/meduni-kkh/koloinfo.htm). Twenty-two patients (69%) were men. The median age at diagnosis of the SBC was 39 years (mean, 44.2 ± 13.4 years). Only 1 SBC was diagnosed before the age of 30 years (15 years; patient 10). Sixteen patients (50%) were from families that met the Amsterdam criteria, whereas 15 patients met at least 1 of the classic Bethesda criteria 2, 3, or 4. One patient failed to meet the classic or revised Amsterdam or Bethesda criteria but fulfilled the Amsterdam 1 and 2 criteria with the exception of the age criterion. A family history of SBC was present in 2 patients (6%). Clinical characteristics of the patients are summarized in Table 1.

SBC was part of the first clinical manifestation of HNPCC in 14 patients (45%) (Table 2). Among them, SBC was the only site of malignancy in 6 patients so far. In 4 patients, the SBC was the first neoplasm, later followed by other HNPCC-related malignancies during follow-up. In 4 cases, the SBC was synchronously detected with other HNPCC-related malignancies (all were CRC) as the first manifestation of disease. Seventeen patients (55%) had a history of previous HNPCC-related malignancies before diagnosis of the small bowel neoplasm; in 14 of 17 cases, the previous diagnosis was a CRC. In one case, the chronologic order of CRC and SBC could not be determined retrospectively.

The localization of SBCs was unknown in 3 cases. The remaining cases showed a clearly decreasing frequency from the duodenum to the ileum (Table 3). There were 24 adenocarcinomas, one adenoma, and one carcinoid tumor. Seven tumors were not classified histologically. In most of these cases, the diagnoses were made more than 20 years ago and pathologic reports only stated "malignant epithelial neoplasia" without histologic subtyping and TNM classification. Of note, the carcinoid tumor displayed MSI-H and loss of MLH1 expression and occurred in a carrier of an *MLH1* germline mutation. There were 5 T2, 11 T3, and 10 T4 tumors. Seventeen tumors had no lymph node metastasis, whereas 8 had lymph node metastasis (7 had N1 and 1 had N2). Including the clinical course of patients with incomplete staging information, we classified 20 patients as having localized disease and 8 patients as having regional or disseminated disease.

Five patients had died after a median follow-up of 48 months after the detection of SBC. Two patients died from SBC, 2 patients died from other metachronous cancers, and 1 patient died from postoperative complications. The overall 10-year survival rate was 87% (Figure 1). In patients with regional or disseminated disease and sufficient follow-up information (>24 months), 2 of 5 patients (40%) died from SBC. In contrast, none of 16

Table 2. Chronological Order of Tumor Manifestations in 31 Patients With HNPCC With SBC or Small Bowel Adenoma

SBC as first malignancy (45%)	
SBC only	6
SBC synchronous to other HNPCC-associated tumor	4
SBC followed by other HNPCC-associated tumor	4
SBC after other HNPCC-related malignancy (55%)	
After CRC	14
After other tumors than CRC	3

Table 3. Histopathologic Characteristics of HNPCC-Associated Small Bowel Tumors

	n	%
Localization		
Duodenum	16	47
Jejunum	10	29
Ileum	4	12
Not specified	3	—
Histology		
Adenocarcinoma	24	92
Carcinoid	1	4
Adenoma	1	4
Not specified	7	—
T stage		
T0	1[a]	4
T1	0	0
T2	5	19
T3	11	41
T4	10	37
Not specified	6	—
N stage		
N0	17	68
N1	7	28
N2	1	4
N3	0	0
Not specified	8	—
M stage		
M0	16	84
M1	3	16
Not specified	14	—
UICC stage		
0	1	6
I	4	22
II	7	38
III	3	17
IV	3	17
Not specified	15	—
Clinical stage		
Local	20	71
Regional/distant	8	29
Unknown	5	—

NOTE. Based on data extracted from the central database.
[a]Tubular adenoma with low-grade dysplasia.

patients with localized disease and a minimal follow-up of 24 months died from SBC ($P = .0476$; Fisher exact test, 2 tailed).

Molecular Data (MSI Testing, MMR Immunohistochemistry, and Germline Mutations)

MSI testing was performed in 21 tumors. Twenty tumors showed the MSI-H phenotype, and one tumor showed the MSI-L phenotype. The sensitivity was 100% for *BAT-25* and *BAT-40*, 95% for *BAT-26*, 94% for *D17S250*, 88% for *D2S123*, and 85% for *D5S346*. The case with an *MSH6* germline mutation showed instability only at 2 of 3 mononucleotide repeats (*BAT-25*, *BAT-40*), whereas dinucleotide repeats showed no instability. Based on our definition, the tumor was classified as MSI-L because it showed instability of only one of 5 markers of the National Institutes of Health reference panel (Table 4).

MMR immunohistochemistry was performed in 18 cases (Table 4). Eight patients had an isolated loss of MLH1 expression, whereas 1 case with a pathogenic *MLH1* germline mutation showed an additional loss of MSH6 expression. Eight of those patients had a pathogenic *MLH1* germline mutation, and 1 patient had an unclassified variant. Six tumors had a loss of MSH2 expression; 5 of them also had a combined loss of MSH6 expression, whereas 1 case was not investigated for MSH6 expression. Five of those 6 cases had a pathogenic *MSH2* germline mutation, whereas the remaining had an unclassified variant in the *MSH2* gene. The tumor with MSI-L phenotype showed an isolated loss of MSH6 expression, and sequencing revealed a pathogenic *MSH6* germline mutation. Two MSI-H SBCs revealed normal expression of MLH1, MSH2, and MSH6, suggesting a germline mutation in a different MMR gene (eg, *PMS2*). Indeed, 1 of these 2 tumors (patient 11) was previously investigated for PMS2 expression in one of the centers and showed an isolated loss of PMS2 expression. Using 3 antibodies (MLH1, MSH2, and MSH6), the immunohistochemical analysis had a sensitivity of 89% and a specificity of 100% for predicting MSI in HNPCC-associated SBC.

Pathogenic germline mutations were identified in 22 (81%) of 27 patients who underwent genetic testing (Table 1). Thirteen mutations were located in *MLH1*, 8 in *MSH2*, and 1 in *MSH6*. In addition, unclassified variants were identified in 5 patients (19%; 3 *MLH1*, 1 *MSH2*, and 1 *MSH6*). In the remaining 5 patients, genetic testing was not performed. The mutation distribution of patients with SBC was not significantly different from the overall mutation distribution of all mutation-positive individuals in the database (data not

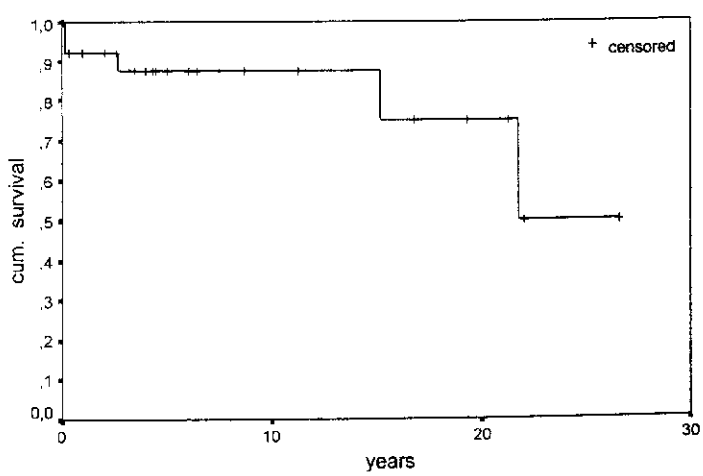

Figure 1. Overall survival of HNPCC-associated SBC.

Table 4. Correlation of MSI Status, MMR Protein Expression, and MMR Germline Mutations of HNPCC-Associated SBC

	MSI testing			Pathogenic germline mutation	
	MSI-H	MSI-L	Not done	Gene	n
MMR immunohistochemistry					
MLH1	9[a]	—	—	*MLH1*	8
MSH2	6[b]	—	—	*MSH2*	5
MSH6	—	1	—	*MSH6*	1
Normal	2[c]	—	—	—	—
Not done	3	—	—	—	—
Pathogenic germline mutation					
Pathogenic	15	1	6	NA	NA
Unknown variant	3	—	2	NA	NA
Not done	2	—	3	NA	NA

NA, not applicable.
[a]One tumor had an additional loss of MSH6 expression.
[b]Five had an additional loss of MSH6 expression, and one was only investigated for MLH1 and MSH2 expression and tissue was not available anymore for MSH6 staining.
[c]One was investigated for PMS2 expression and had an isolated loss of PMS2 expression.

shown). Two patients (with 3 SBCs) had the same *MLH1* germline mutation (c.1489_1490insC).

Frameshift Mutation in Coding Mononucleotide Repeats

We analyzed 17 SBCs for frameshift mutations of coding mononucleotide repeats in the coding sequence of 7 genes (Table 5). Frameshift mutations in *TGFBR2*, *BAX*, *MSH3*, *MSH6*, *ACVR2*, *AIM2*, and *SEC63* were identified in 69%, 59%, 59%, 35%, 82%, 56%, and 56% respectively. Considering these 7 genes, the average number of frameshift mutations per SBC was 4.1. We compared the frequency of frameshift mutations in tumors with MLH1 versus MSH2 deficiency (based on germline mutations and immunohistochemical deficiency) and could not detect a statistical difference for any of the 7 genes ($P > .05$; Fisher exact test, 2 tailed).

Histopathology

We analyzed 16 SBCs for histopathologic criteria (Table 5). Five SBCs (31%) were well differentiated, 1 (6%) was moderately differentiated, and 10 (62%) were poorly differentiated. Of the latter tumors, 2 SBCs (13% of all SBCs) were mucinous adenocarcinomas. In 4 cases (25%) the tumor border showed an infiltrative growth pattern, whereas 12 SBCs (75%) had an expansive growth pattern ("pushing border") (Figure 2). A high number of peritumoral lymphocytes was detected in 6 cases (37%). In 12 SBCs (75%), a dense intratumoral lymphocytic infiltration (>10% of cells) was observed (Figure 3).

Discussion

Patients with HNPCC are at high risk of developing CRC. Moreover, these patients are prone to develop certain extracolonic cancers as well. The relative risk of SBC is 100 compared with the general population.[1] So far, HNPCC-associated SBCs are poorly characterized. SBCs represent 2.1% of all malignancies in mutation-positive HNPCC individuals (Goecke et al, manuscript submitted), and MSI-H SBCs (n = 20) account for 2.6% of all MSI-H cancers (n = 779) in our database. We analyzed 32 patients with HNPCC (31 SBCs and one duodenal adenoma) according to clinical, histomorphologic, and molecular variables. Moreover, we analyzed a subset of available tumors for the presence of MSI, MMR protein expression, and coding microsatellite frameshift mutations. Our study provides important insights into the characteristics of SBC in patients with HNPCC.

Our data show that SBC in patients with HNPCC develops at a young age (median, 39 years). This is 10 years earlier than previously reported for HNPCC-associated SBC[18] and more than 20 years earlier than for sporadic SBC, similar as for HNPCC-associated CRC.[43] However, only 1 patient in our study developed SBC before the age of 30 years. This patient (patient 10) had a very unusual history of malignancies (glioblastoma at ages 6 and 15 years; cecal, jejunal, and ureteral cancer at age 15 years). The tumor analysis showed MSI-H along with normal expression of MLH1, MSH2, and MSH6. Therefore, this is not a typical patient with HNPCC.

Approximately 70% of patients in our study were male. This confirms similar observations reported by

Table 5. Frameshift Mutations of Mononucleotide Repeats in the Coding Sequence of 7 Genes and Histopathologic Characteristics of HNPCC-Associated SBC

	No. of SBCs (%)
Coding microsatellites	
TGFBR2	11/16[a] (69)
ACVR2	14/17 (82)
BAX	10/17 (59)
MSH3	10/17 (59)
MSH6	6/17 (35)
AIM2	9/16[a] (56)
SEC63L	9/16[a] (56)
Grading	
G1	5/16 (31)
G2	1/16 (6)
G3	10/16[b] (62)
Tumor border pattern	
Expansive ("pushing border")	12/16 (75)
Invasive	4/16 (25)
Intratumoral lymphatic infiltration	
None	0/16 (0)
Discrete (<10%)	4/16 (25)
Intensive (>10%)	12/16 (75)
Peritumoral lymphatic infiltration	
None	0/16 (0)
Discrete	10/16 (62)
Intense	6/16 (37)

[a]One case each was not informative.
[b]Two cases were mucinous adenocarcinomas.

Rodriguez-Bigas et al.[18] This proportion is slightly higher than reported in the Surveillance, Epidemiology, and End Results (SEER) population tumor registry of the United States for SBC.[41] However, this distribution was not significantly different from the overall sex distribution of mutation-positive individuals in our database (data not shown).

Only 50% of the patients in our study fulfilled the Amsterdam 1 and/or 2 criteria, whereas in a previous study more than 80% of patients fulfilled the Amsterdam criteria.[18] This difference may be caused by different selection methods of the registries in recruiting patients. The other major characteristic of patients in our study was the development of multiple HNPCC-associated malignancies. However, diagnosis of SBC was part of the first tumor manifestation in these patients in 45% (SBC only, SBC synchronous to other tumors, and SBC later followed by other tumors). Only 6% of patients had a positive family history of SBC at the time of data extraction, whereas Rodriguez-Bigas et al reported a positive family history in 17% of patients. The mutation detection rate in our series of patients was 81%, which is higher than the frequency among patients from the German HNPCC registry with MSI-H tumors (56%),[30] indicating that the occurrence of an SBC in a family with suspected HNPCC increases the likelihood of identifying an MMR gene germline mutation. In addition, we found sequence variants of unknown significance in all remaining patients investigated for germline mutations. At least some of those unclassified variants are likely to be pathogenic but need further functional testing.

In contrast to previously reported data of a nearly even anatomic distribution for HNPCC-associated SBC,[18] we found that HNPCC-associated SBCs show a decreasing gradient from the duodenum to the ileum, reflecting the distribution in sporadic SBC,[43,44] and may therefore be detectable by endoscopic surveillance in the majority of cases. Most of the tumors were adenocarcinomas; however, one tumor was a carcinoid in a proven *MLH1* germline mutation carrier. The tumor tissue showed MSI-H and loss of MLH1 expression. To our knowledge, this is the first evidence that carcinoids may be causally linked to HNPCC. One previous study reported the occurrence of 3 carcinoids but did not investigate for MSI or loss of MMR protein expression.[18]

Previous studies have reported a relatively favorable prognosis for HNPCC-associated SBC[18] or MSI-H SBC.[22] Most of the patients in our study were diagnosed with localized disease, which is also reflected in the excellent overall survival in our series. However, this may also be caused by selection bias, because most patients were referred to the centers to evaluate a suspected HNPCC syndrome, thus potentially selecting for survivors. Only 2 patients died from SBC in our series (one patient had primary metastatic disease, and the other had a T3N2 SBC and was diagnosed with liver metastases 1 year later). In contrast, there are 2 patients primarily diagnosed with metastatic disease who were surviving at 24 and 53 months, respectively, at the time of data extraction.

The MSI-H phenotype was detected in 5%–45% of unselected SBCs.[22–27] All but 1 SBC in our study showed the MSI-H phenotype. The sensitivity of each microsatellite marker for the detection of MSI-H was similar than previously reported for CRC; therefore, the recommended marker panel is suitable for the evaluation of MSI-H in SBC. Interestingly, the tumor with MSI-L occurred in a patient with an *MSH6* germline mutation and affected only mononucleotide markers, as reported previously as more common in *MSH6*-associated neoplasms.[29,45] It was the only disease manifestation in this male patient so far, and the diagnosis of SBC was made at the age of 70 years, supporting a later onset of disease in *MSH6* germline mutation carriers as reported previously.[29,46–48] All but 2 tumors with MSI-H/MSI-L had a loss of expression of at least one MMR protein (MLH1,

MSH2, MSH6). These 2 cases are candidates for germline mutation testing in other genes (eg, *PMS2*).

Only 3 studies have investigated *TGFBR2* mutations in a total of 15 MSI-H SBCs with a mutation rate ranging from 0% to 67%, with an average for all 3 studies of 27%,[22,26,27] and only one study screened for *BAX* mutations and reported one mutation in 10 SBCs.[22] These frequencies are much lower than reported for CRC. No study has specifically focused on molecular alterations in HNPCC-associated SBC. Recent studies have shown differences in the spectra of frameshift mutation between CRC and gastric and endometrial carcinomas.[49–52] Here we report frameshift mutations in *AIM2*, *SEC63*, *MSH3*, *MSH6*, and *ACVR2* for the first time in SBC. The frequency of frameshift mutations of all 7 genes in our study was not significantly different from results of a recent meta-analysis of sporadic MSI-H CRC and HNPCC-associated CRC,[53] although there was a trend toward a higher frequency of *MSH3* and *ACVR2* mutations in HNPCC-associated SBC. Furthermore, although the differences were not statistically significant, we found a higher frequency of frameshift mutations of *MSH3* and *MSH6* in MLH1-deficient tumors compared with MSH2-deficient tumors, consistent with previous reports in MSI-H CRC.[54] The relatively high frequency of *ACVR2* mutations supports recent studies on the functional relevance of ACVR2 deficiency as a mechanism for inactivation of the *smad* signaling pathway in gastrointestinal cancers.[55–57]

Figure 2. Expansive growth pattern of the tumor border forming a pseudocapsule ("pushing border") (Elastica van Gieson staining).

Figure 3. Dense intratumoral lymphocytic infiltrate shown by immunostaining of CD3 of more than 10% of cells.

In summary, we show here that the median age of diagnosis for SBC is lower than for CRC in HNPCC-affected patients with SBC. Consistent with this, SBC may be the first tumor manifestation in 50% of the patients. Tumors predominantly develop in the duodenum and could therefore theoretically be detected endoscopically. Sex, family history, previous personal cancer history, and the affected MMR gene were not able to define patients at risk for SBC accurately. Most of the tumors were T3 or T4 carcinomas, and one third of tumors had a locally advanced or systemic disease. Based on the fact that most of the tumors could have been theoretically detected endoscopically and based on recent data from our HNPCC study group (Goecke et al, manuscript submitted) and a review of the literature,[58] limiting gastric cancer screening to families with a positive family history does not seem to be justified. Therefore, duodenoscopy or push enteroscopy starting at the age of 30 years might be beneficial for early detection of proximal SBC. In addition, our data show that the pathologist may have an important role in identifying patients with HNPCC-associated SBC, because it has frequent morphologic features such as tumor-infiltrating lymphocytes and a pushing tumor border predicting MSI phenotype in 75%. This has been shown previously for MSI-H CRC (sporadic and HNPCC) and HNPCC-associated endometrial carcinomas[59] and may also apply for HNPCC-associated SBC. In SBCs showing these features, immunohistochemical monitoring of MMR protein expression and MSI testing might provide further evidence for a suspected HNPCC syndrome.

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Received August 14, 2004. Accepted December 9, 2004.

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Supported by a multicenter grant from the Deutsche Krebshilfe (German Cancer Aid).

K.S. and F.E.B. contributed equally to this work.

The authors thank Britta Redecker and Sabine Geiger for excellent technical assistance, Andreea Olaru for helpful discussions, and all of the pathologists who provided tumor blocks and enabled this study.