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.

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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.[6,40] 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