Germline BRCA2 Gene Mutations in Patients with Apparently Sporadic Pancreatic Carcinomas

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Abstract

Germline mutations in BRCA2 predispose carriers to the development of breast, ovarian, and a variety of other cancers. The original localization of the BRCA2 gene was aided by its homozygous deletion in a pancreatic carcinoma; indeed, an excess of pancreatic carcinoma has been seen in some BRCA2 cancer families. To determine the involvement of BRCA2 in pancreatic carcinomas, we screened for BRCA2 alterations in an unscreened panel of 41 adenocarcinomas of the pancreas (30 pancreatic adenocarcinoma xenografts and 11 pancreatic carcinoma cell lines). Of the 15 (27%) that had allelic loss at the BRCA2 locus, 4 (9.8%) had abnormalities in the second allele upon screening of the entire BRCA2 gene by in vitro synthesized protein assay. Three of the four mutations were considered germline in origin (7.3% overall; two were confirmed in normal tissue, and one was the 6174delT mutation from the pancreatic carcinoma cell line CAPAN-1, for which normal tissue was unavailable). The identification of two 6174delT mutations in this series prompted us to evaluate the prevalence of this mutation in an overlapping consecutive series of 245 patients who underwent pancreatoduodenectomy for adenocarcinoma of the pancreas. Sequence analysis of this limited region of the gene identified two additional mutations: (a) one additional germline 6174delT mutation (2 of 245, 0.8% overall); and (b) a second nearby germline 6158insT mutation. One of the patients with a germline mutation had a single relative with breast cancer, and another had a single relative with prostate cancer. None had a family history of pancreatic cancer.

The incidence of germline BRCA2 mutations in apparently sporadic pancreatic cancer may be at least as high as in breast or ovarian cancer. Our results suggest that some familial risks for carcinoma will be evident only through a population-based application of gene screening techniques because a low disease penetrance of the germline mutations in some families often evades clinical suspicion.

Introduction

Pancreatic ductal adenocarcinoma is the fifth leading cause of cancer death in the United States (1). Pancreatic adenocarcinoma has a unique genetic profile with somatic mutations of the K-raf (90% or more of tumors; Refs. 2 and 3) and p53 genes (50–70% of tumors; Ref. 4) and genetic inactivation of the p16 (approximately 80% of tumors; Ref. 5) and DPC4 genes (50% of tumors; Ref. 6). It is estimated that 5–10% of patients with pancreatic carcinoma may have an inherited predisposition to developing the disease (7–9). In familial melanoma, persons inheriting a mutant allele of p16 are at increased risk of developing pancreatic carcinoma, although such mutations account for only a small proportion of familial pancreatic carcinoma (10, 11). Patients with pancreatic carcinoma who carry a germline p16 mutation generally have a family history of malignant melanoma (11). Germline mutations of the von Hippel Lindau gene, germline defects in mismatch repair genes, and hereditary relapsing chronic pancreatitis are also thought to account for a minute proportion of familial pancreatic carcinoma (7, 12).

Stratton and coworkers recently reported the identification of the breast cancer susceptibility gene BRCA2 (13), aided by the prior identification of a homozygous deletion at the BRCA2 locus (14) in a pancreatic carcinoma (15, 16). The BRCA2 coding sequence is 10.4 kb and contains 26 exons, but the nature of its tumor suppressor function is unknown (17). In some BRCA2 breast cancer families, mutation carriers with pancreatic carcinoma have been noted (18–21). Here, we investigated a series of tumor-derived and constitutional DNA from patients with pancreatic carcinoma for mutations in the BRCA2 gene. We found BRCA2 alterations in 4 of 41 (9.8%) cancers and the 6174delT mutation in 2 of 245 (0.8%) pancreatic carcinoma patients.

Materials and Methods

Patient Population and Tissue Samples. Normal and tumor specimens were obtained from pancreatic exocrine adenocarcinomas resected at the Johns Hopkins Hospital and from 10 commercially available cell lines. The institutional committee on clinical investigation reviewed and approved the collection of the tissue samples for genetic analysis, and written consent for research to be performed on resected tissues was obtained from patients before surgery. Two groups of pancreatic tumor patients were studied. The first group included 41 pancreatic carcinomas (30 pancreatic carcinoma xenografts and 11 pancreatic carcinoma cell lines) and 2 biliary carcinoma cell lines (27% that had allelic loss at the BRCA2 locus, 4 (9.8%), and 22 among the 245 pancreatic cancer patients). Thirty-one pancreatic tumors from the first group (all xenografts and 45%) were part of a second group of 245 pancreatic cancers that were screened for the 6174delT mutation. These 245 pancreatic cancers were part of a series of 41 patients who underwent pancreatoduodenectomy between May 1992 and May 1996 at the Johns Hopkins Hospital for suspected pancreatic carcinoma. Screening for the 6174delT mutation was performed on 281 (for whom frozen tissue samples were available) of 317 patients. The 281 patients included 245 with pancreatic carcinomas and 36 others (13 with biliary tract carcinomas, 8 with duodenal/ampullary carcinomas, 12 with other carcinomas, 2 with chronic pancreatitis, and 1 with a pancreatic pseudocyst). PCR and sequencing of the segment of the BRCA2 gene for the 6174delT mutation was performed on normal tissues in the majority of cases; in a few cases in which normal tissue was unavailable, tumor xenograft DNA was used.

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3 The abbreviations used are: LOH, loss of heterozygosity; IVSP, in vitro synthesized protein.
One BRCA2 carrier with pancreatic carcinoma participated in a survey conducted by the Familial Pancreatic Tumor Registry (http://www.med.jhu.edu/pancreas/index.htm) at Johns Hopkins, allowing us to contact other members of this family and obtain consent for blood to be drawn from consenting family members for genetic analysis.

Pancreatic carcinoma xenografts and cell lines were established as described, and tumor and normal tissues were stored frozen (4). DNA was isolated from blood samples taken from relatives of the patient enrolled in the registry. cDNA was prepared from RNA obtained from the xenografts of primary carcinomas (22).

LOH Analysis. LOH at the BRCA2 region was determined using the microsatellite markers D13S260, D13S171, D13S267, and mSB489G-3C11 as described (23). For the pancreatic cancer xenografts and the PL-45 cell line, allelic loss was determined by comparing tumor with normal DNA. For the commercial cell lines, presumptive LOH was deemed present if one allele was present for all four highly polymorphic microsatellite markers.

IVSP Assay. The IVSP assay was performed as described (24). PCR primers were designed from the BRCA2 coding sequence (17). Exons 2–9 (one PCR) and 12–26 (three PCRs) were screened using cDNA as template. Exons 10 (one PCR), 11 (six PCRs), and 27 (one PCR) were screened using DNA as template. Together, these PCR products spanned over 99% of the coding region of the BRCA2 gene and virtually all splice donor/acceptor sites within the coding region. The primers used in this study are available (http://www.path.jhu.edu/brca2).

Sequencing. Before sequencing, PCR reactions were incubated with exonuclease I and shrimp alkaline phosphatase (Amersham) according to the manufacturer's recommendations. The 6174delT deletion was determined by cycle sequencing of a 133-bp PCR product that spanned nucleotide 6174. Sequencing of PCR products was performed in microtiter plates as recommended by the manufacturer (Epicentre Technologies, Madison, WI). All mutations were confirmed at least once using independent PCR products.

Results

BRCA2 Mutation Analysis in Pancreatic Carcinomas. LOH determination at chromosome 13q12 revealed LOH (only one allele present for each microsatellite marker tested; Ref. 25) in 9 of 31 (29%) pancreatic carcinoma xenografts, none of 2 biliary tract carcinomas, and 6 of 10 pancreatic carcinoma cell lines (AsPC-1, BX-Pc3, CAPAN-1, Panc-1, CFPAC1, and Su8686). IVSP analysis of the complete coding sequence of the BRCA2 gene revealed inactivation of BRCA2 in 4 of these 15 (26.7%) pancreatic carcinomas (4 of 41 overall, 9.8%). Three BRCA2 mutations (7.3%) of the 41 patients were considered germline in origin. A germline 6174delT (codon 1977) was found in an Ashkenazi Jewish patient with pancreatic carcinoma (see Figs. 1 and 2). All three children and one brother were carriers of the mutant allele. A second brother declined genetic testing. The brother with the BRCA2 mutation developed prostate carcinoma in his sixties, whereas the three children (one daughter and two sons) are presently asymptomatic and in their forties. The cell line CAPAN-1 had the same 6174delT deletion and might reasonably be assumed to be a germline mutation reflecting a recurrent population allele (26). The third mutation was a germline 2458insT mutation (codon 751). Interestingly, this patient was one also of Ashkenazi Jewish descent, and the mother of this patient had breast carcinoma (tissue was not available for analysis). The fourth BRCA2 inactivation from this tumor series was the previously reported homozygous deletion (DPC1/2) in a patient with pancreatic carcinoma (15). Further characterization of this deletion as part of this study confirmed that the deletion indeed involved the BRCA2 gene. The published markers 886s239 and 886s186 represented sequences from exon 2 and intron 24 of the BRCA2 gene, respectively (17). LOH analysis using the marker mSB489G-3C11 (courtesy of A. Kamb, Myriad Genetics, Salt Lake City, Utah), which is located within the homozygous deletion, revealed that two alleles were present in the germline of this patient, indicating that the homozygous deletion was somatically acquired.

Population-based Screening of Pancreatoduodenectomy Specimens for the 6174delT Mutation. A 133-bp PCR product that contained the 6174 nucleotide was sequenced from DNA prepared from normal tissue obtained from 281 pancreatoduodenectomy specimens (245 with pancreatic cancer and 36 others). A germline 6158insT mutation (codon 1977) and one additional germline 6174delT mutation were found in patients of non-Jewish descent, giving an overall prevalence of 2 of 245 (0.8%) pancreatic cancers for the 6174delT mutation. In addition, CAPAN-1 had the same mutation but was not considered part of the consecutive series. There were no mutations among the 36 patients who underwent pancreatoduodenectomies and did not have pancreatic cancer. In neither of the two cases identified in this screen was there a family history of pancreatic, breast, or ovarian carcinoma. Interestingly, the germline 6158insT mutation was not accompanied by loss of the other allele. The second allele may have been inactivated by point mutation in another region of the BRCA2 gene, a mode of inactivation of a second allele reported to be common for the APC gene (27) and also noted for the BRCA2 gene (28). The ages of presentation of the patients with germline BRCA2 mutations (68, 69, 74, and 75 years) were similar to the mean age of the patients in this series (64 years). A summary of the results is provided in Fig. 3.

Discussion

Germline BRCA2 mutations represent the most common inherited predisposition to pancreatic carcinoma identified to date. Pancreatic
GERMLINE BRCA2 MUTATIONS IN PANCREATIC CANCER

A. Methods

<table>
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<th>Tumors</th>
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<tr>
<td>41 pancreatic adenocarcinomas (30 xenografts, 11 cell lines)</td>
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<tr>
<td>15 with LOH (37% of 41)</td>
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<tr>
<td>Assessment of mutation origin (in-vitro synthesized protein assay—&gt; sequencing of tumors)</td>
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<tr>
<td>4 mutations (27% of 15)</td>
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<tr>
<td>homozgyous deletion (1 case, somatic)</td>
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<tr>
<td>6174delT (2 cases, germline*) (1 case, germline)</td>
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<tr>
<td>2458insT</td>
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B. Limited gene sequence (nucleotides 8106 to 6214 of normal DNA)

<table>
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<tr>
<th>Tumors</th>
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<tr>
<td>281 pancreatic-duodenectomy patients, 4 yr consecutive series</td>
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<tr>
<td>245 pancreatic adenocarcinoma patients (31 previously analyzed as tumors, above)</td>
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<tr>
<td>36 patients with other GI tract cancers</td>
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<tr>
<td>3 other diagnoses</td>
</tr>
<tr>
<td>6174delT (in 1 new case, 1 known case from tumor study above, both germline)</td>
</tr>
<tr>
<td>6158insT (1 new case, germline)</td>
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<td>no mutations</td>
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**Fig. 3. Overview of the BRCA2 mutational survey in pancreatic cancer. A, analysis of pancreatic adenocarcinomas for BRCA2 mutations. B, screening of a consecutive series of patients with pancreatic cancer for the germline 6174delT mutation. *, CAPAN-1 is presumed to have a germline 6174delT mutation (see text).**

cancer, the fifth leading cancer killer in the United States, may have an inherited predisposition in approximately 5–10% of patients (7–9). We identified germline BRCA2 mutations in 7.3% of patients with adenocarcinoma of the pancreas. Indeed, our mutational survey may have underestimated the inactivation of BRCA2 in pancreatic carcinoma. We initially screened for BRCA2 inactivation using LOH, but both we and others have demonstrated BRCA2 mutations in cancers without LOH (28). In addition, the in vitro protein truncation assay would overlook any missense mutations. The detected prevalence of germline BRCA2 mutations in our patients with apparently sporadic pancreatic carcinoma (7.3%) thus compares well with that seen for apparently sporadic breast (<4%) or ovarian carcinoma (<4%; Refs. 29–32).

Remarkably, a family history clinically suggestive of an inherited predisposition to cancer was not seen among the BRCA2 carriers with pancreatic carcinoma. None had a family history of pancreatic cancer. Only one BRCA2 carrier with pancreatic carcinoma had a family history of breast cancer, which was present in only one family member. This is despite the fact that the 6174delT mutation is strongly implicated in the development of breast cancer, and the two other mutations identified would produce truncated proteins biochemically similar to those reported from well-defined breast cancer families (26). In our study, small family size (particularly the small number of females in the families), may explain in part the paucity of cancers in close relatives. However, several carriers over the age of 70 years have been reported who did not have cancer (18). Certainly, relative to that seen for breast carcinoma, the penetrance for pancreatic carcinoma is low. Phelan et al. (19) found only 4 pancreatic carcinomas as compared to 48 breast carcinomas among 8 families with germline mutations of the BRCA2 gene. Similarly, Thorlacius et al. (18) noted 100 breast carcinomas and 11 pancreatic carcinomas in 21 families carrying the 999del5 mutation, and Couch et al. (20) found no pancreatic carcinomas in 11 families with 36 male and female breast carcinomas.

The age of onset of pancreatic carcinoma in BRCA2 carriers seems variable. The mean age of the four patients with pancreatic carcinoma in our study with germline BRCA2 mutations was 72 years. In contrast, Phelan et al. (19) observed an earlier onset of pancreatic cancer (mean age, 53 years) in four cases carrying germline BRCA2 mutations. A low penetrance and a rather high but variable age of onset for pancreatic carcinoma in BRCA2 carriers suggests that other genetic or environmental cofactors (such as smoking or radiation exposure) may modify the risk of developing pancreatic carcinoma in individual carriers. Indeed, there is evidence that an accumulation of genetic events are required for the development of pancreatic carcinoma. Ultimately, long-term follow-up of BRCA2 families, identified from unbiased population-based gene screening, will be required to assess the age-dependent risks of pancreatic and other carcinomas and possibly the influence of other genetic and environmental factors in BRCA2 carriers.

Among investigators searching for inherited predispositions to carcinoma, there is a general expectation that the identification of germline mutations will eventually enable clinicians to identify and appropriately manage the carriers by preventive interventions. However, there are many important and complex clinical and ethical questions raised by the issue of DNA testing for cancer susceptibility. The initial important question is whether to test or not to test. For example, it is unclear which patients, if any, with pancreatic carcinoma should be gene-tested for germline BRCA2 mutations. A subset of medical geneticists, genetic counsellors, ethicists, and lawyers would take a nihilistic approach to DNA testing and, if followed literally, DNA testing would be suspended. Other of these authorities take a more moderate view and would recommend testing but mandate that it be performed on patients with inherited cancer-prone families. Indeed, at least two professional organizations recommend that

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gene testing for familial cancer genes should be limited to the identified high-risk families (33, 34). Still other investigators would test members from certain ethnic or religious groups in which a particular gene is believed to be expressed at high frequency, such as those of Ashkenazi Jewish descent. Our findings suggest, however, that the majority of carriers might be missed by this recommended approach. An inherited predisposition to pancreatic carcinoma may be particularly hard to identify because pancreatic carcinoma generally presents in the seventh or eighth decade of life, a time when deaths from other causes or concomitant disease may mask its presentation in other family members. The expected risk for breast and ovarian carcinoma among the female relatives of pancreatic carcinoma patients might provide an adequate justification for genetic testing in many patients because this risk is not necessarily evident from the family history.

Furthermore, technical and economical considerations presently impede progress in genetic screening of genes such as BRCA2. Two of 245 (0.8%) patients in our series with pancreatic carcinoma had a 6174delT mutation. Initial estimates put the 6174delT allele frequency at approximately 3/1000 Ashkenazi Jewish individuals in the United States (26). Recent evidence, including our series, suggests that this mutation is also present in patients of non-Jewish ancestry (21). It would be technically straightforward to screen Ashkenazi Jewish patients with pancreatic carcinoma for the 6174delT mutation. However, as one of our cases illustrates, there are other BRCA2 mutations in the Ashkenazi population besides the 6174delT mutation. Until the penetrance and allele distributions of BRCA2 mutations, including the influence of different mutations on the risk of developing various carcinomas, are known, it will be difficult to counsel patients regarding their risks for these carcinomas.

Once a mutation is identified in a given individual, the next logical question is how that individual should be screened for carcinoma. Females who are BRCA2 carriers could be offered an appropriate breast cancer screening regimen. But due to the aggressive nature of pancreatic cancer, many BRCA2 carriers might inquire whether screening protocols were available to detect early pancreatic cancer. Although there have been advances in the imaging and treatment of pancreatic lesions, the limited available data suggesting a low penetrance and late age of presentation of pancreatic carcinoma in BRCA2 carriers imply that screening for pancreatic carcinoma will be difficult. Under any reasonable estimate of sensitivity and specificity for such a clinical test, given the anticipated risk of pancreatic cancer in BRCA2 carriers, the number of false-positive tests would be high. Because a positive test would likely require follow-up with an invasive procedure, the implications of a false-positive test in this setting are potentially unacceptable. Despite the fact that the clinical benefits of such early detection are unclear, consideration will need to be given to the possibilities of screening protocols for detecting early pancreatic carcinoma in BRCA2 gene carriers. If magnetic resonance imaging or other nonionizing forms of imaging were to be found useful, they would avoid an additional exposure to radiation. Such considerations and others will have to weigh the morbidity of screening against the fact that most carriers will not develop pancreatic carcinoma.

Our finding of a germline BRCA2 mutation in a relative with prostate carcinoma supports previous suggestions that BRCA2 may be involved in the predisposition to prostate carcinoma (18–20). BRCA2 families may also have an increase in colon carcinoma and other carcinomas (18–21). Additional studies are necessary to clarify the risk of these cancers in BRCA2 carriers to determine the merits of screening for these neoplasms.

Future developments in the technology of mutational analysis and legal protection for the confidentiality for the identified gene carriers may facilitate the identification of BRCA2 families by enabling the screening of consenting patients having pancreatic carcinoma. Additional epidemiological studies of gene carriers need to be conducted to identify the genetic and environmental cofactors for cancer incidence in this at-risk population. Clinicians who manage patients with pancreatic cancer will have to weigh the potential clinical benefits of genetic testing in patients with the disease while recognizing that the identification of such mutations will have considerable impact on the present and future generations of affected families.

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References


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