High Prevalence of BRCA1 and BRCA2 Germline Mutations with Loss of Heterozygosity in a Series of Resected Pancreatic Adenocarcinoma and Other Neoplastic Lesions

Aimee L. Lucas1,2, Reena Shakya7, Marla D. Lipsyc2,3, Elana B. Mitchell2,3, Sheila Kumar2,8, Caroline Hwang2,9, Liyong Deng4, Catherine Devoe2,3, John A. Chabot2,5, Matthias Szabolcs6, Thomas Ludwig7, Wendy K. Chung2,4, and Harold Frucht2,3

Abstract

Purpose: Pancreatic ductal adenocarcinoma (PDAC) is associated with the breast ovarian cancer syndrome (BRCA1/BRCA2) mutations. It is unknown if this association is causal.

Experimental Design: This is a single-site study of patients who underwent surgical pancreatic tumor resection and self-identified as Ashkenazi Jewish. DNA from normal pancreatic tissue was genotyped for the three Ashkenazi Jewish BRCA1/2 founder mutations BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT, and loss of heterozygosity (LOH) was determined by sequencing DNA from microdissected tumor. When additional tumor tissue was available, p53 immunohistochemistry (IHC) was conducted.

Results: Thirty-seven patients underwent surgery for PDAC, seven for intraductal papillary mucinous neoplasm (IPMN), and 19 for other diseases. A high prevalence of BRCA1/2 mutations was found in the surgical cohort (12/63; 19.0%; \( P < 0.001 \)), PDAC cohort (8/37; 21.6%; \( P < 0.001 \)), and IPMN cohort (2/7; 28.6%; \( P = .01 \)) compared with published control mutation frequency. A high prevalence of BRCA1 185delAG (8.1%; \( P < 0.001 \)) and BRCA2 6174delT (10.8%; \( P < 0.001 \)) in Ashkenazi Jewish patients with PDAC was shown. BRCA1/2 LOH was found in 2 of 4 BRCA1-associated PDACs and 3 of 4 BRCA2-associated PDACs. Positive p53 IHC was found in 5 of 8 BRCA1/2 PDACs.

Conclusions: We show a high prevalence of BRCA1/2 mutations with LOH in an Ashkenazi Jewish cohort of surgically resected PDAC and neoplastic lesions, suggesting that these germline mutations are causal in selected individuals. Clin Cancer Res; 19(13); 3396–403. ©2013 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of death from malignancy in the United States, with 43,920 new diagnoses and 37,390 deaths in 2012 (1). With near equivalent incidence and mortality, cure can only be achieved with surgical resection of an early-stage lesion. Premalignant disease stages, such as intraductal papillary mucinous neoplasm (IPMN), may be detected with noninvasive and minimal-invasive techniques, providing an opportunity for screening and surveillance of at-risk populations. Approximately 10% of PDAC has a hereditary component (2), and screening this population has a potential impact on disease mortality (3–12). Certain patient populations, such as those with hereditary pancreatitis (13), Peutz-Jeghers syndrome (14), familial atypical multiple mole melanoma (12, 15, 16), Lynch syndrome (17, 18), and the breast ovarian cancer syndrome (BRCA1 and BRCA2 mutations; refs. 19–23) are at the highest risk of PDAC. BRCA2 mutations are the most commonly identified germline mutations in families with PDAC. Even a family history of PDAC without the above-described syndromes has been shown to increase risk, suggesting a unique familial pancreatic cancer syndrome that may be related to the partner and localizer of BRCA2 (PALB2) gene or other PDAC susceptibility genes (24).

BRCA1 and BRCA2 are tumor suppressor genes that cause breast and ovarian cancer through a loss of heterozygosity (LOH) mechanism. The prevalence of BRCA1/2 mutations in all patients with PDAC remains unknown, although epidemiologic studies and case series suggest the prevalence is between 5% and 19% (19, 20, 25–27). The penetrance of BRCA1/2 for PDAC is incomplete, and a
This research details investigation of BRCA1 and BRCA2 germline mutations in a series of patients with surgically resected human pancreatic adenocarcinoma, intraductal papillary mucinous neoplasms, and other neoplastic lesions at a single institution. This study shows a high prevalence of both BRCA1 and BRCA2 germline mutations along with loss of heterozygosity in this population. These data suggest that both BRCA1 and BRCA2 may contribute to the development of pancreatic cancer through a loss of heterozygosity mechanism. Ongoing research will better define the genetic contribution of BRCA1 and BRCA2 mutations to pancreatic cancer, as well as clinical features of germline BRCA1 and BRCA2–associated pancreatic adenocarcinomas. This may allow for pancreas cancer prevention and early detection in high-risk individuals.

**Materials and Methods**

**Subjects**

All patients who underwent pancreatic resection at the Pancreas Center of Columbia University Medical Center between 2003 and 2011 with frozen pancreatic tissue and good-quality pancreas DNA banked were eligible for this study. Genetic and clinical data were linked through a unique study identification number. De-identified clinical and demographic information including ethnicity and religion was extracted from our Pancreas Center Database. This study was approved by the Columbia University Institutional Review Board.

The prevalence of the BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT mutations in the control population was calculated from a previously described PDACs. To determine if LOH was present in Ashkenazi Jewish surgical cohort of patients with PDAC, the IPMN and PDAC lesions were microdissected from 10-μm sections that were stained with eosin only were microdissected by laser-capture (Zeiss PALM Microbeam IV) to maximize for purity of tumor DNA. Fresh-frozen tissue was used whenever possible. For 1 patient, only formalin-fixed paraffin embedded (FFPE) tissue was available. When PDAC was seen to arise out of IPMN, the IPMN and PDAC lesions were microdissected separately.

**BRCA1 and BRCA2 genotype and loss of heterozygosity**

PCR products were generated using forward (F) and reverse (R) primers as follows: BRCA1 185delAG, forward: 5′-ATTATCTGCTCTTGCTTTG, reverse: 5′-AAGGTCAATT-GTGTCTCCACTTCCATTG, 160-bp product; BRCA1 5382insC, forward: 5′-TGCCGGTCCTCCACCTCAITG, reverse: 5′-TGAGG-ATGGAAGATGAAAA, 315-bp product; and BRCA2 6174delT, forward: 5′-CACCCTGATGTTAGTGG, reverse: 5′-GACCTGTCGTCAAGTTCG, 240-bp product to genotyping the 2 founder BRCA1 (185delAG and 5382insC) and 1 BRCA2 (6174delT) mutations. PCR products were sequenced by dyeoxy sequencing on an ABI capillary sequencer according to the manufacturer’s instructions and analyzed with Sequencher 4.7 software (Genecodes). For patients heterozygous for one of the BRCA1/2 founder mutations, microdissected tumor DNA was assessed for LOH by generating PCR products for the corresponding germline mutation. LOH at the BRCA1 or BRCA2 locus was determined by the absence of the wild-type allele at the site of the germline mutation.

**Immunohistochemistry**

Immunohistochemical staining for p53 was conducted on frozen tumor tissue sections with mouse monoclonal antibody against human p53 (Santa Cruz, SC-98). Briefly, frozen tissue sections were fixed in cold methanol for 10 minutes, blocked in serum-free protein block reagent (Dako) for 1 hour, and incubated overnight with the primary antibody. Following incubation with a biotinylated anti-mouse secondary antibody (Vector Labs), Elite ABC reagent (Vector Labs, M.O.M. Immunodetection kit) and 3′-diaminobenzidine substrate were used to develop peroxidase activity.
Table 1. Patient demographic information

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>37 (58.7)</td>
</tr>
<tr>
<td>Female</td>
<td>26 (41.3)</td>
</tr>
<tr>
<td>White</td>
<td>63 (100)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>67.4; range 44–86; SD 10.4</td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>31 (49.2)</td>
</tr>
<tr>
<td>Former</td>
<td>23 (36.5)</td>
</tr>
<tr>
<td>Current</td>
<td>6 (9.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (4.8)</td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>34 (54.0)</td>
</tr>
<tr>
<td>Light/social</td>
<td>20 (31.7)</td>
</tr>
<tr>
<td>Heavy</td>
<td>6 (9.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (4.8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50 (79.4)</td>
</tr>
<tr>
<td>Yes</td>
<td>13 (20.6)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Neoadjuvant chemotherapy</td>
<td>9 (14.3)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>21 (33.3)</td>
</tr>
</tbody>
</table>

Statistical analysis

Categorical variables were analyzed by χ square or Fisher exact test, as appropriate. Continuous variables were compared using Student t test or analysis of variance (ANOVA). All P-values are two-sided and tested at an alpha level of 0.05. Statistical analysis was conducted using SAS 9.2.

Results

A total of 645 unique patients underwent pancreatic resections between January 2003 and July 2011. Four hundred and thirty-two patients had an adequate sample of normal-frozen pancreas available in the tumor bank; DNA was successfully extracted from 392 specimens. Of those, 63 patients (16.1%) self-identified as Ashkenazi Jewish and were included in the analysis. All patients were self-described as being of white race (Table 1). Thirty-seven (58%) patients were male with the mean age of 67.4 years (range 44–86 years, SD 10.4 years). Thirty-one (49.2%) were never-smokers, 23 (36.5%) were former smokers, 6 (9.5%) were current smokers, and 3 (4.8%) had an unknown smoking status. No alcohol use was found in 34 (54%) patients, 20 (31.7%) were light drinkers, 6 (9.5%) were heavy drinkers, and 3 (4.8%) had no data on alcohol consumption. Thirteen (20.6%) patients reported a history of diabetes at the time of diagnosis. Final pathology revealed 37 patients with PDAC, 11 with neuroendocrine tumors, 7 with IPMN, and 8 with other cystic and neoplastic lesions (Table 2).

A high prevalence of both BRCA1 and BRCA2 mutations was shown in the entire Ashkenazi Jewish cohort, the Ashkenazi Jewish PDAC only cohort, and the Ashkenazi Jewish IPMN only cohort as compared with published Ashkenazi Jewish population controls. (Table 3). Twelve of 63 (19.0%) patients in the total Ashkenazi Jewish surgical cohort were found to carry an Ashkenazi Jewish BRCA1 or BRCA2 founder mutation (P < 0.001). Eight of 37 (21.6%) Ashkenazi Jewish patients with PDAC were found to carry BRCA1/2 germline founder mutations (P < 0.001). Four of 37 (10.8%) Ashkenazi Jewish patients with PDAC were found to have a BRCA1 mutation (P < 0.001). When individual BRCA1 mutations were examined, 3 of 37 (8.1%) patients with PDAC were found to harbor the BRCA1 185delAG mutation (P < 0.001), and 1 of 37 patients with (2.7%) PDAC carried the BRCA1 5382insC mutation (P = 0.14). Four of 37 (10.8%) Ashkenazi Jewish patients with PDAC were found to carry the BRCA2 6174delT founder mutation; this was also significantly increased over population prevalence (P < 0.001). Upon pathology review of a BRCA1 185delAG specimen with clinical diagnosis PDAC, histology confirmed a duodenal adenocarcinoma and this patient was excluded from the PDAC analysis group. Another patient with a neuroendocrine tumor carried a BRCA2 6174delT mutation.

Two of the 7 (28.6%) Ashkenazi Jewish patients who underwent surgical resection for IPMN also carried mutations in BRCA1 or BRCA2 (P = 0.01). One patient carried a BRCA1 185delAG mutation (P = 0.05) and the other carried a BRCA2 6174delT mutation (P = 0.08). Further analyses were limited by sample size and tumor tissue availability. LOH was shown in 2 of 4 BRCA1-associated PDACs and 3/4 BRCA2-associated PDACs (Table 4, Fig. 1). Interestingly, one of the BRCA2-associated PDACs was found to arise from an IPMN. The IPMN was microdissected separately from the PDAC, and the IPMN revealed partial LOH, whereas the PDAC showed complete LOH. Direct sequencing of the BRCA1 5382insC PDAC revealed reversion to a
wild type at the $BRCA1\ 5382\text{insC}$ locus within the tumor (Fig. 1). This primary tumor was resected from a patient who did not receive neoadjuvant chemotherapy. DNA from multiple normal pancreas and PDAC tumor blocks was re-extracted and run on the AmpFLSTR Identifiler Kit (Applied Biosystems) to confirm that the samples belonged to the same subject and were not due to an error in sample acquisition. DNA was repeatedly extracted from the normal and tumor pancreas blocks and confirmed the presence of the $BRCA1$ mutation in the normal pancreas, but wild-type sequence in the tumor.

Nuclear staining for $p53$ was found in 5 of 8 $BRCA1/2$ carriers with PDAC (Fig. 2 and Table 4). No differences in disease stage, histology, or overall survival (OS) were noted between $BRCA1/2$ carriers and noncarriers, as well as $BRCA1/2$ carriers with and without LOH.

The mean age of diagnosis for Ashkenazi Jewish patients with PDAC who were found to carry a $BRCA1/2$ mutation was 63.8 years, compared with 70.0 years for noncarriers ($P = 0.15$). The mean age at diagnosis for Ashkenazi Jewish patients with PDAC with LOH was 59.4 years compared with 71.0 years for those without LOH ($P = 0.15$). $BRCA1$ carriers with LOH were diagnosed at a mean age of 60 compared with 69 for the $BRCA1$ carriers without LOH; $BRCA1\ 185\text{delAG}$ carriers with LOH were diagnosed at a mean age of 60, whereas the patient without LOH was diagnosed at age 85. For $BRCA2\ 6174\text{delT}$ carriers, the patients with PDAC with LOH were diagnosed at a mean age of 59, compared with a diagnosis at age 75 for the patient without LOH. Statistical analysis of these groups showed no significant difference but is limited by sample size.

### Table 3. Increased prevalence of $BRCA1$ and $BRCA2$ mutations

<table>
<thead>
<tr>
<th>A.</th>
<th>Prevalence (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi Jewish population (1)</td>
<td>120/5,318 (2.3)</td>
<td>Reference</td>
</tr>
<tr>
<td>Ashkenazi Jewish cohort</td>
<td>12/63 (19.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ashkenazi Jewish PDAC</td>
<td>8/37 (21.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ashkenazi Jewish IPMN</td>
<td>2/7 (28.6)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

B. | Ashkenazi Jewish population prevalence (%) | Ashkenazi Jewish PDAC cohort prevalence | Ashkenazi Jewish IPMN cohort prevalence |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All $BRCA1$ Mutations</td>
<td>61/5,318 (1.15)</td>
<td>4/37 ($P &lt; 0.001$)</td>
<td>1/7 ($P = 0.08$)</td>
</tr>
<tr>
<td>$BRCA1\ 5382\text{insC}$</td>
<td>20/5,318 (0.38)</td>
<td>1/37 ($P = 0.14$)</td>
<td>N/A</td>
</tr>
<tr>
<td>$BRCA1\ 185\text{delAG}$</td>
<td>41/5,318 (0.77)</td>
<td>3/37 ($P &lt; 0.001$)</td>
<td>1/7 ($P = 0.05$)</td>
</tr>
<tr>
<td>$BRCA2\ 6174\text{delT}$</td>
<td>59/5,318 (1.11)</td>
<td>4/37 ($P &lt; 0.001$)</td>
<td>1/7 ($P = 0.08$)</td>
</tr>
</tbody>
</table>

NOTE: A, a high prevalence of Ashkenazi Jewish $BRCA1/2$ founder mutations was shown in the entire Ashkenazi Jewish surgical cohort (19%; $P < 0.001$), the Ashkenazi Jewish PDAC cohort (18.9%; $P < 0.001$), and the Ashkenazi Jewish IPMN cohort (28.6%; $P = 0.01$). B, a high prevalence of individual $BRCA1$ and $BRCA2$ mutations was shown in the Ashkenazi Jewish PDAC cohort and the Ashkenazi Jewish IPMN cohort.

### Table 4. Summary of $BRCA1$ and $BRCA2$ mutation carriers with IPMN or PDAC

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Histology</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Tobacco use</th>
<th>Alcohol use</th>
<th>Stage</th>
<th>Vital status</th>
<th>Survival (days)</th>
<th>LOH</th>
<th>$p53$ IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$BRCA1\ 185\text{delAG}$</td>
<td>IPMN</td>
<td>81</td>
<td>M</td>
<td>Former</td>
<td>Mild</td>
<td>—</td>
<td>Alive</td>
<td>1,526</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>$BRCA1\ 185\text{delAG}$</td>
<td>PDAC</td>
<td>67</td>
<td>M</td>
<td>No</td>
<td>Mild</td>
<td>IIB</td>
<td>Alive</td>
<td>528</td>
<td>Complete</td>
<td>Positive</td>
</tr>
<tr>
<td>$BRCA1\ 185\text{delAG}$</td>
<td>PDAC</td>
<td>53</td>
<td>F</td>
<td>Unknown</td>
<td>Unknown</td>
<td>IB</td>
<td>Deceased</td>
<td>1,883</td>
<td>Complete</td>
<td>Negative</td>
</tr>
<tr>
<td>$BRCA1\ 185\text{delAG}$</td>
<td>PDAC</td>
<td>85</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>IIB</td>
<td>Alive</td>
<td>1,635</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>$BRCA1\ 5382\text{insC}$</td>
<td>PDAC</td>
<td>53</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>IIB</td>
<td>Alive</td>
<td>1,005</td>
<td>Wild type</td>
<td>Negative</td>
</tr>
<tr>
<td>$BRCA2\ 6174\text{delT}$</td>
<td>PDAC</td>
<td>61</td>
<td>M</td>
<td>Former</td>
<td>Mild</td>
<td>IIB</td>
<td>Alive</td>
<td>1,744</td>
<td>Complete</td>
<td>Negative</td>
</tr>
<tr>
<td>$BRCA2\ 6174\text{delT}$</td>
<td>IPMN</td>
<td>44</td>
<td>F</td>
<td>Former</td>
<td>No</td>
<td>—</td>
<td>Alive</td>
<td>889</td>
<td>Negative</td>
<td>Unable</td>
</tr>
<tr>
<td>$BRCA2\ 6174\text{delT}$</td>
<td>PDAC</td>
<td>57</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>IIA</td>
<td>Deceased</td>
<td>1,002</td>
<td>Partial</td>
<td>Focal nuclear</td>
</tr>
<tr>
<td>$BRCA2\ 6174\text{delT}$</td>
<td>PDAC</td>
<td>59</td>
<td>F</td>
<td>No</td>
<td>Heavy</td>
<td>IIB</td>
<td>Alive</td>
<td>1,061</td>
<td>Complete</td>
<td>Positive</td>
</tr>
<tr>
<td>$BRCA2\ 6174\text{delT}$</td>
<td>PDAC</td>
<td>75</td>
<td>M</td>
<td>Former</td>
<td>Mild</td>
<td>IIB</td>
<td>Alive</td>
<td>681</td>
<td>Negative</td>
<td>Focal cytoplasmic</td>
</tr>
</tbody>
</table>

Abbreviation: IHC, immunohistochemistry.
CLINICAL CANCER RESEARCH

Lucas et al.

Figure 1. BRCA1 185delAG LOH. A, chromatogram showing the wild-type sequence of BRCA1 gene at region of 185delAG deletion. This patient does not carry a germline BRCA1 185delAG mutation. Highlighted nucleotides indicate area to be deleted in heterozygote or LOH. B, chromatogram showing sequence in germline BRCA1 185delAG carrier. This sequence was generated from normal pancreatic DNA. Arrow indicates mutation site. C, chromatogram showing LOH at BRCA1 185delAG. DNA was obtained from microdissected PDAC tumor tissue from the same patient as in B. Arrow indicates mutation site. D, chromatogram showing the wild-type sequence of BRCA1 gene at region of 5382insC mutation. This patient does not carry a germline BRCA1 5382insC mutation. E, representative chromatogram showing sequence in patient with PDAC with germline BRCA1 5382insC mutation. Arrow indicates mutation site. DNA from multiple normal pancreas tissue blocks showed the same sequence. F, chromatogram showing the wild-type sequence at BRCA1 5382insC site in the same patient. Arrow indicates mutation site. Microdissected DNA from multiple tumor blocks showed the same sequence. DNA profiling confirmed that the samples in D and E belong to the same subject.

Discussion

Prior small studies have shown an association between BRCA2, and to a lesser degree BRCA1 (30), germline mutations, and PDAC. Ferrone and colleagues reported on the incidence of the three common BRCA1/2 founder mutations (BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT) in Ashkenazi Jewish patients who underwent PDAC resection and showed an increased prevalence of the BRCA2 6174delT mutation in individuals with surgically resected PDAC but no increase for the BRCA1 185delAG or BRCA1 5382insC mutations (29). More recent studies have reported a high prevalence of BRCA1/2 mutations in families with both pancreas and breast cancer (31), but none has clearly established a higher than expected prevalence of BRCA1 mutations in PDAC. We have also shown that BRCA1/2 carriers presenting to a breast cancer prevention program had infrequent family history of PDAC compared with BRCA1/2 carriers presenting to our PDAC genetics and prevention center, suggesting BRCA1/2 mutations may cooperate with different genetic alterations and environmental exposures in breast cancer as compared with PDAC, leading to different cancer manifestations (34). Furthermore, the risk of PDAC in patients who carry a germline BRCA1/2 mutation is poorly defined. Here, we report an increased prevalence of both BRCA1 and BRCA2 founder mutations in an Ashkenazi Jewish surgical cohort of patients with PDAC and IPMN with LOH in the PDAC at the corresponding BRCA1/2 loci.

Although our study is blinded to whether the patients knew their BRCA1/2 mutation status, the high prevalence of BRCA1/2 mutations could reflect a referral bias to our Pancreas Center of patients with known BRCA1/2 mutations. However, no patients were ascertained for this study through a pancreatic cancer screening program. We are also limited only to patients who underwent surgical pancreatic resection, therefore biasing toward patients who presented at an earlier stage of disease or who were otherwise deemed good surgical candidates. The true prevalence of BRCA1/2 mutations may be lower than 21.6% in Ashkenazi Jewish patients with PDAC across all stages of disease but likely remains above the Ashkenazi Jewish population frequency of 2% to 3% (33, 35). Our study is also limited to those who self-identified as Ashkenazi Jewish in a single institution and examined only for the three common Ashkenazi Jewish founder mutations. Due to the retrospective nature of this study, we do not have data on the overall prevalence of BRCA1/2 mutations in the entire pancreatic cancer registry, but prospective studies using the registry are being undertaken. The overall prevalence of BRCA1/2 mutations in all ethnic groups is estimated at 1:400 to 1:800, but few
studies have examined BRCA1/2 mutation prevalence in diverse ethnic backgrounds (36–39). We hypothesize that germline BRCA1/2 mutations are present and causal in other ethnic backgrounds, although at this time the cost of comprehensive testing for these mutations is prohibitive for this study.

Other groups have suggested that the sequence of p53, Kras, and BRCA1/2 mutations is critical in the progression to PDAC. Rowley and colleagues found that in a murine model of PDAC, p53 mutations were required before BRCA2 LOH, regardless of the co-existence of an activated Kras mutation (40). Skoulidas and colleagues have shown that the addition of a BRCA2 heterozygote to the KPC mouse model (41, 42) was sufficient to promote Kras- and p53-driven tumorigenesis (43). The mechanism and order of somatic mutations in human BRCA1/2-associated tumorigenesis has yet to be studied. We show that p53 mutations are present with both BRCA1 and BRCA2 LOH and may promote pancreatic carcinogenesis, although the order of mutations cannot be determined in this study. In addition, some tumors exhibit complete LOH with p53 staining, and some tumors contain a germline BRCA1/2 mutation without LOH or p53 staining. This suggests that not all PDACs in BRCA1/2 germline carriers are caused by LOH at the BRCA1/2 locus and that they may occur through a mechanism similar to sporadic PDAC.

Skoulidas and colleagues also examined an Icelandic registry for all patients with pancreatic tumors who carried the Icelandic founder mutation BRCA2 999del5 and reported 7 pancreatic tumors (3 acinar tumors and 4 adenocarcinomas) in patients with germline BRCA2 mutations (43). Interestingly, 3 of the 7 tumors in their cohort did not exhibit LOH. In the 4 tumors in which LOH was described, 3 of the tumors were of acinar histology. Acinar tumors of the pancreas are rare, representing less than 1% of primary pancreatic tumors (44), and a review of our database did not reveal any patients who underwent pancreatic resection for acinar tumors. Given the absence of BRCA2 LOH in PDACs, Skoulidas and colleagues proposed an alternative model for the development of pancreatic tumors. They postulated that PDACs develop in the background of a BRCA2 germline mutation if an activating Kras mutation is present along with additional somatic genetic alterations. They further hypothesized that biallelic loss of BRCA2, with an activating Kras mutation and inactivation of p53, may lead to tumors with variant histology such as acinar tumors. Under this model, BRCA2 LOH yields an adenocarcinoma after a prolonged delay, if at all. Whether or not biallelic inactivation of BRCA2 is present in PDACs is critical from a therapeutic perspective, as BRCA2 tumors with LOH may be sensitive to poly-ADP ribose polymerase (PARP) inhibitors (45). Neither the Skoulidas and colleagues study nor the present study fully evaluated the BRCA1/2 genes for other somatic mutations that may lead to biallelic loss. However, we show LOH of both BRCA1 and BRCA2 in some but not all BRCA1/2-associated PDACs, suggesting PARP inhibitors may be useful in selected tumors exhibiting biallelic inactivation of BRCA1 or BRCA2. Further analysis of the remainder of the BRCA1/2 gene in PDACs that do not show LOH may be indicated if therapy with PARP inhibitors is considered.

PDACs may develop in tissue with heterogeneity of somatic genetic changes. While nuclear accumulation of p53 protein is a surrogate marker for the presence of a p53 mutation, our immunohistochemistry (IHC) studies clearly show that the PDAC tumors are genetically heterogeneous. Within a single tumor, different areas show differential positivity for p53 staining. Some areas of tumor are strongly positive for nuclear p53 staining, whereas other regions completely lack staining. Intratumor heterogeneity for p53 mutations has been observed previously when preinvasive and invasive pancreatic lesions of the same patient were compared. Such genetic differences within the same neoplasm explain the variable p53 signal seen by IHC (46).

Interestingly, among our Ashkenazi Jewish BRCA2 germline carriers, one PDAC was clearly identified as arising from an IPMN. The IPMN lesion was microdissected separately from the PDAC, and DNA was extracted separately. The IPMN revealed partial LOH, whereas the PDAC showed complete LOH. This finding may suggest that the transition from a preneoplastic pancreatic lesion such as IPMN to PDAC requires BRCA1/2 LOH in certain individuals. It is noteworthy that similar observations have been reported for PanIN-derived PDAC among BRCA2 mutation carriers (47). Although we feel this is further evidence that germline BRCA1/2 mutations are causal in PDAC tumorigenesis, even with careful microdissection it is likely that the pancreatic tumor DNA is not pure tumor DNA, and this heterogeneity may affect our ability to detect LOH.

Our study, as in previous studies, did not show a significant age difference between instances of sporadic and hereditary pancreatic cancer (29). Further studies are under way to more definitively establish the age at which patients with BRCA1 and BRCA2 germline mutations are at increased risk for PDAC. Survival and tumor histology are not different between BRCA1/2-associated tumors and other PDAC, although the sample is underpowered for survival analyses controlled for age and invasion characteristics.

As the association between BRCA1 and BRCA2 may be causal in selected individuals with PDAC, the next logical question is whether all patients carrying germline BRCA1/2 mutations should undergo screening for PDAC. Our group and others have shown that PDAC screening in selected high-risk individuals is successful at detection of early-stage PDAC and high-grade dysplastic lesions in the pancreas (3–12). Genetic testing conducted at our institution for a family history of PDAC has also led to the identification and resection of curable ovarian cancers in BRCA1/2 carriers (3). With the exception of hereditary pancreatitis and Peutz–Jeghers syndrome, current consensus guidelines do not recommend screening high-risk individuals for PDAC before the age of 50 (48, 49). These guidelines, developed on the basis of expert opinion, recommend PDAC screening for BRCA2 mutation carriers with one first-degree relative with PDAC, and for BRCA2 carriers with two non–first-degree relatives with PDAC. Although screening of selected high-risk individuals may
be successful in the identification of these neoplastic lesions, current PDAC screening is expensive and invasive and is likely only to be beneficial in those who are truly at high risk of PDAC. Further study of PDAC prevention and screening based on the presence of a germline genetic mutation is warranted and ongoing.

In summary, we show an increased prevalence of both BRCA1 and BRCA2 mutations in an Ashkenazi Jewish surgical cohort of patients with PDAC and IPMN. We furthermore show LOH of the corresponding BRCA1/2 loci and nuclear p53 staining of selected tumors, suggesting that mutation and/or loss of p53 cooperates with BRCA1/2 mutations in the progression of PDAC. Survival and tumor histology are not significantly different in BRCA1/2-associated tumors compared with other PDACs in our cohort. Further studies are under way to more definitively establish the mechanism and clinical characteristics of BRCA1- and BRCA2-associated PDAC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A.L. Lucas, W.K. Chung, H. Frucht
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Shakya, M.D. Lipsyc, E.R. Mitchel, C. Hwang, L. Deng, C. Devoe, J.A. Chabot, M. Szabolcs, W.K. Chung, H. Frucht
Analysis and interpretation of data (e.g., statistical analysis, biosistics, computational analysis): A.L. Lucas, R. Shakya, M. Szabolcs, T. Ludwig, W.K. Chung, H. Frucht
Writing, review, and/or revision of the manuscript: A.L. Lucas, R. Shakya, M.D. Lipsyc, E.R. Mitchel, S. Kumar, J.A. Chabot, M. Szabolcs, T. Ludwig, W.K. Chung, H. Frucht
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.L. Lucas, M.D. Lipsyc, C. Hwang, C. Devoe, H. Frucht
Study supervision: H. Frucht

Acknowledgments
The authors thank the Muzzi Mirza Pancreatic Cancer Prevention and Genetics Program, the Pancreas Center at Columbia University, Tao Su in the Molecular Pathology Core, Ashley Dikos, MPH, Michelle Miller Chang and Jason Chua in the Muzzi Mirza Program, and the Pancreas Center Data Managers, especially Kimone Crosley, for their contributions.

Grant Support
This study was supported by a grant from the Hirszberg Foundation for Pancreatic Cancer Research (to H. Frucht) and the NIH T32 DK083256 Ruth L. Kirschstein National Research Service Award (to A.L. Lucas).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 24, 2012; revised March 5, 2013; accepted May 2, 2013; published OnlineFirst May 8, 2013.

References


High Prevalence of BRCA1 and BRCA2 Germline Mutations with Loss of Heterozygosity in a Series of Resected Pancreatic Adenocarcinoma and Other Neoplastic Lesions

Aimee L. Lucas, Reena Shakya, Marla D. Lipsyc, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-3020

Cited articles
This article cites 48 articles, 15 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/19/13/3396.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/19/13/3396.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.