Title: High Prevalence of BRCA1 and BRCA2 Germline Mutations With Loss of Heterozygosity In a Series of Resected Pancreatic Adenocarcinoma and Other Neoplastic Lesions.

Authors and Affiliations: Aimee L. Lucas1,2, Reena Shakya3, Marla D. Lipsyc2,4, Elana B. Mitchel2,4, Sheila Kumar2,5, Caroline Hwang2,6, Liyong Deng7, Catherine Devoe2,4, John A. Chabot2,8, Matthias Szabolcs9, Thomas Ludwig3, Wendy K. Chung2,7, and Harold Frucht2,4

1. Icahn School of Medicine at Mount Sinai, Henry D. Janowitz Division of Gastroenterology, New York, New York
3. Ohio State University, Department of Molecular & Cellular Biochemistry, Columbus, Ohio
4. Columbia University School of Medicine, Division of Digestive and Liver Diseases, New York, New York
5. Stanford University School of Medicine, Division of Gastroenterology and Hepatology, Palo Alto, California
6. Keck School of Medicine at the University of Southern California, Division of Gastroenterology and Liver Diseases, Los Angeles, California
7. Columbia University School of Medicine, Department of Pediatrics and Medicine, New York, New York
8. Columbia University School of Medicine, Department of Surgery, New York, New York
9. Columbia University School of Medicine, Department of Pathology, New York, New York

Running Title: BRCA1/2 With Loss of Heterozygosity in Pancreatic Lesions

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Corresponding Author:
Aimee L. Lucas, MD MS
Assistant Professor of Medicine
Icahn School of Medicine at Mount Sinai
Henry Janowitz Division of Gastroenterology
One Gustave L. Levy Place, Box 1069
New York, New York 10029
Telephone: 212-241-0101
Facsimile: 646-537-8647
Email: aimee.lucas@mssm.edu

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This research details investigation of \textit{BRCA1} and \textit{BRCA2} germline mutations in a series of surgically resected human pancreatic adenocarcinoma, intraductal papillary mucinous neoplasms, and other neoplastic lesions at a single institution. This study demonstrates a high prevalence of both \textit{BRCA1} and \textit{BRCA2} germline mutations along with loss of heterozygosity in this population. These data suggest that both \textit{BRCA1} and \textit{BRCA2} may contribute to the development of pancreatic cancer through a loss of heterozygosity mechanism. Ongoing research will better define the genetic contribution of \textit{BRCA1} and \textit{BRCA2} mutations to pancreatic cancer, as well as clinical features of germline \textit{BRCA1}- and \textit{BRCA2} –associated pancreatic adenocarcinomas. This may allow for pancreas cancer prevention and early detection in high-risk individuals.
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 (AJ). DNA from normal pancreatic tissue was genotyped for the three AJ BRCA1/2 founder mutations BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT), and loss of heterozygosity (LOH) determined by sequencing DNA from microdissected tumor. When additional tumor tissue was available, p53 immunohistochemistry (IHC) was performed.

Results: 37 patients underwent surgery for PDAC, 7 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, or 19.0%, p<0.001), PDAC cohort (8/37, or 21.6%, p<0.001), and IPMN cohort (2/7 or 28.6%, p=.01) compared to published control mutation frequency. A high prevalence of BRCA1 185delAG (8.1%, p<0.001) and BRCA2 6174delT (10.8%, p<0.001) in AJ PDAC patients was demonstrated. BRCA1/2 LOH was found in 2/4 BRCA1-associated PDACs and 3/4 BRCA2-associated PDACs. Positive p53 IHC was found in 5/8 BRCA1/2 PDACs.

Conclusions: We demonstrate a high prevalence of BRCA1/2 mutations with LOH in an AJ cohort of surgically resected PDAC and neoplastic lesions, suggesting these germline mutations are causal in select individuals.
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 IPMN, may be detected with noninvasive and minimally 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 the potential to have an 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)[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 which may be related to the partner and localizer of BRCA2 (PALB2) gene[24] or other PDAC susceptibility genes.

BRCA1 and BRCA2 are tumor suppressor genes which cause breast and ovarian cancer through a loss of heterozygosity (LOH) mechanism. The prevalence of BRCA1/2 mutations in all PDAC patients remains unknown, although epidemiology studies and case series suggest the prevalence is between 5-19%[19, 20, 25-28]. The penetrance of BRCA1/2 for PDAC is incomplete, and a causal molecular genetic sequence of events for BRCA1/2-associated PDAC has not been elucidated. A clearer understanding of the mechanism of disease in BRCA1/2-associated PDAC is necessary for implementation of targeted screening and prevention trials. In the United States much of the focus of BRCA1/2 mutations has been on
individuals of Ashkenazi Jewish (AJ) ancestry given the presence of 3 common founder mutations. Prior studies in AJ patients have demonstrated an association between \textit{BRCA2} and PDAC\cite{29, 30}, but have not evaluated the mechanism of disease pathogenesis. The association of \textit{BRCA1} and PDAC is less well defined\cite{30-33}. We hypothesized that both \textit{BRCA1} and \textit{BRCA2} play a significant role in the pathogenesis of hereditary PDAC through LOH. We aimed to determine the prevalence of \textit{BRCA1/2} mutations in an AJ surgical cohort of PDAC patients, and determine if LOH was present in \textit{BRCA1/2}-associated PDACs.

\textbf{MATERIALS AND METHODS}

\textbf{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 \textit{BRCA1} 185delAG, \textit{BRCA1} 5382insC, and \textit{BRCA2} 6174delT mutations in the control population was calculated from a previously described cohort of Jewish subjects who were recruited from the Washington, D.C., area\cite{34}. Individuals were recruited from media sources in both the general population and Jewish organizations. 70.4\% of the subjects were female, and 94.3\% of patient had no prior history of breast or ovarian cancers. Subjects completed a questionnaire and provided a blood specimen which was genotyped for the 3 \textit{BRCA1/2} AJ founder mutations. Subjects did not receive their individual test results.
Normal and Tumor DNA Extraction

Genomic DNA from frozen normal resected pancreatic tissue adjacent to the pancreas lesion was extracted utilizing a modified protocol (Qiagen, REPLI-g Mini kit). The concentration was determined by Nanodrop photospectrometer. For tumor DNA, a representative hematoxylin and eosin stained section was reviewed with an experienced pancreatic pathologist. Tumor cells from 10 μm sections stained with eosin only were microdissected by laser-capture (Zeiss PALM Microbeam IV, Jena, Germany) to maximize for purity of tumor DNA. Fresh frozen tissue was utilized whenever possible. For one 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

Polymerase chain reaction (PCR) products were generated using forward (F) and reverse (R) primers as follows: BRCA1 185delAG, forward: 5'-ATTATCTGCTCTTCGCGTTG, reverse: 5'-AAGGTCATTCTGTTCCATTGC, 160-bp product; BRCA1 5382insC, forward: 5'-TGTCTGCTCCACTTCCATTG, reverse: 5'-TTGGGATGGAAGAGTGAAAAA, 315-bp product; and BRCA2 6174delT, forward: 5'-CACCTTGATGATTTAGTTGGAA, reverse: 5'-TGAGCTGTCTGATGATGTTGGAA, 240-bp product to genotype the two founder BRCA1 (185delAG and 5382insC) and one BRCA2 (6174delT) mutations. PCR products were sequenced by dideoxy sequencing on an ABI capillary sequencer (Carlsbad, CA) according to the manufacturer's instructions and analyzed with Sequencher 4.7 software (Genecodes, Ann Arbor, MI). 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 absence of the wild-type allele at the site of the germline mutation.
Immunohistochemistry

Immunohistochemical staining for p53 was performed 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 10min, 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, 3’-diaminobenzidine substrate were used to develop peroxidase activity.

Statistical Analysis

Categorical variables were analyzed by chi-square or Fisher’s exact test, as appropriate. Continuous variables were compared using Student’s t-test or analysis of variance (ANOVA). All p-values are two-sided and tested at an alpha level of .05. Statistical analysis was performed using SAS 9.2 (Cary, North Carolina).

RESULTS

A total of 645 unique patients underwent pancreatic resections between January 2003 and July 2011. 432 patients had 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 AJ and were included in the analysis. All patients were self-described white race (Table 1). Thirty seven (58%) patients were male; the mean age was 67.4 years (range from 44 to 86 years, standard deviation 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%) of patients, 20 (31.7%)
were light users, 6 (9.5%) were heavy drinkers, while 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 neuroendocrine tumors, 7 IPMNs, and 8 other cystic and neoplastic lesions (Table 2).

A high prevalence of both BRCA1 and BRCA2 mutations was demonstrated in the entire AJ cohort, the AJ PDAC only cohort, and AJ IPMN only cohort as compared to published AJ population controls[34] (Table 3). 12/63 (19.0%) patients in the total AJ surgical cohort were found to carry an AJ BRCA1 or BRCA2 founder mutation (p<.001). 8/37 (21.6%) AJ PDAC patients were found to carry BRCA1/2 germline founder mutations (p<.001). 4/37 (10.8%) AJ patients with PDAC were found to have a BRCA1 mutation (p<.001). When individual BRCA1 mutations were examined, 3/37 (8.1%) PDAC patients were found to harbor the BRCA1 185delAG mutation (p<.001) and 1/37 (2.7%) PDAC patients carried the BRCA1 5382insC mutation (p=0.14). 4/37 (10.8%) AJ PDAC patients were found to carry the BRCA2 6174delT founder mutation; this was also significantly increased over population prevalence (p<.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 seven (28.6%) AJ patients who underwent surgical resection for IPMN also carried mutations in BRCA1 or BRCA2 (p=.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 demonstrated in 2/4 BRCA1-associated PDACs and 3/4 BRCA2-associated PDACs (Table 4, Figure 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 while the PDAC demonstrated complete LOH. Direct sequencing of the BRCA1 5382insC PDAC revealed reversion to a wild-type at the BRCA1 5382insC locus within the tumor (Figure 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 AmpFLSTR Identifiler Kit (Applied Biosystems) to confirm the samples belonged to the same subject and was 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 (Figure 2 and Table 4). No differences in disease stage, histology or overall survival were noted between BRCA1/2 carriers and non-carriers, as well as BRCA1/2 carriers with and without LOH.

The mean age of diagnosis for AJ PDAC patients who were found to carry a BRCA1/2 mutation was 63.8 years, compared to 70.0 years for non-carriers (p=0.15). The mean age at diagnosis for AJ PDAC patients with LOH was 59.4 years, compared to 71.0 years for those without LOH (p=.15). BRCA1 carriers with LOH were diagnosed at a mean age of 60, compared to 69 for the BRCA1 carriers without LOH; BRCA1 185delAG carriers with LOH were diagnosed at a mean age of 60, while the patient without LOH was diagnosed at age 85. For BRCA2 6174delT carriers, the PDAC patients 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 demonstrated no significant difference but is limited by sample size.

**DISCUSSION**

Prior small studies have demonstrated an association between BRCA2, and to a lesser degree BRCA1 [31], germline mutations and PDAC. Ferrone et al. reported on the incidence of
the three common \textit{BRCA1/2} founder mutations (\textit{BRCA1} 185delAG, \textit{BRCA1} 5382insC, and \textit{BRCA2} 6174delT) in AJ patients who underwent PDAC resection, and demonstrated an increased prevalence of the \textit{BRCA2} 6174delT mutation in individuals with surgically resected PDAC but no increase for the \textit{BRCA1} 185delAG or \textit{BRCA1} 5382insC mutations\cite{30}. More recent studies have reported a high prevalence of \textit{BRCA1/2} mutations in families with both pancreas and breast cancer\cite{32}, but none have clearly established a higher than expected prevalence of \textit{BRCA1} mutations in PDAC. We have also demonstrated that \textit{BRCA1/2} carriers presenting to a breast cancer prevention program had infrequent family history of PDAC compared to \textit{BRCA1/2} carriers presenting to our PDAC genetics and prevention center, suggesting \textit{BRCA1/2} mutations may cooperate with different genetic alterations and environmental exposures in breast cancer as compared with PDAC, leading to different cancer manifestations\cite{35}. Furthermore, the risk of PDAC in patients who carry a germline \textit{BRCA1/2} mutation is poorly defined. Here we report an increased prevalence of both \textit{BRCA1} and \textit{BRCA2} founder mutations in an AJ surgical cohort of PDAC and IPMN patients with LOH in the PDAC at the corresponding \textit{BRCA1/2} loci.

While our study is blinded to whether the patients knew their \textit{BRCA1/2} mutation status, the high prevalence of \textit{BRCA1/2} mutations could reflect a referral bias to our Pancreas Center of patients with known \textit{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 \textit{BRCA1/2} mutations may be lower than 21.6\% in AJ PDAC patients across all stages of disease, but likely remains above the AJ population frequency of 2-3\%\cite{34,36}. Our study is also limited to those who self-identified as AJ in a single institution, and examined only for the 3 common AJ founder mutations. Due to the retrospective nature of this study, we do
not have data on the overall prevalence of \textit{BRCA1/2} mutations in the entire pancreatic cancer registry but prospective studies by use of the registry are being undertaken. The overall prevalence of \textit{BRCA1/2} mutations in all ethnic groups is estimated at 1:400 to 1:800, but few studies have examined \textit{BRCA1/2} mutation prevalence in diverse ethnic backgrounds[37-40]. We hypothesize that germline \textit{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 \textit{p53}, \textit{Kras}, and \textit{BRCA1/2} mutations is critical in the progression to PDAC. Rowley \textit{et al.} found that in a murine model of PDAC, \textit{p53} mutations were required prior to \textit{BRCA2} LOH, regardless of the co-existence of an activated \textit{Kras} mutation[41]. Skoulidis \textit{et al.} demonstrated that the addition of a \textit{BRCA2} heterozygote to the KPC mouse model[42, 43] was sufficient to promote \textit{Kras}– and \textit{p53}-driven tumorigenesis[44]. The mechanism and order of somatic mutations in human \textit{BRCA1/2}-associated tumorigenesis has yet to be studied. We demonstrate that \textit{p53} mutations are present with both \textit{BRCA1} and \textit{BRCA2} LOH, and may promote pancreatic carcinogenesis, although the order of mutations cannot be determined in this study. Additionally, some tumors exhibit complete LOH with \textit{p53} staining; some tumors contain a germline \textit{BRCA1/2} mutation without LOH or \textit{p53} staining. This suggests that not all PDACs in \textit{BRCA1/2} germline carriers are caused by LOH at the \textit{BRCA1/2} locus, and may occur via a mechanism similar to sporadic PDAC.

Skoulidis \textit{et al.} also examined an Icelandic Registry for all patients with pancreatic tumors who carried the Icelandic founder mutation \textit{BRCA2} 999del5, and reported 7 pancreatic tumors (3 acinar tumors and 4 adenocarcinomas) in patients with germline \textit{BRCA2} mutations[44]. Interestingly, three of the seven tumors in their cohort did not exhibit LOH. In
the four tumors in which LOH was described, three of the tumors were of acinar histology. Acinar tumors of the pancreas are rare, representing less than 1% of primary pancreatic tumors[45], 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, Skoulidis et al. proposed an alternative model for the development of pancreatic tumors. They postulate 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 hypothesize 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[46]. Neither the Skoulidis et al. study nor the present study fully evaluated the BRCA1/2 genes for other somatic mutations which may lead to biallelic loss. However, we demonstrate LOH of both BRCA1 and BRCA2 in some but not all BRCA1/2-associated PDACs, suggesting PARP inhibitors may be useful in select tumors exhibiting biallelic inactivation of BRCA1 or BRCA2. Further analysis of the remainder of the BRCA1/2 gene in PDACs that do not demonstrate 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 presence of a p53 mutation, our IHC studies clearly demonstrate that the PDAC tumors are genetically heterogeneous. Within a single tumor, different areas demonstrate differential positivity for p53 staining. Some areas of tumor are strongly positive for nuclear p53 staining while 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[47].

Interestingly, among our AJ \( BRCA2 \) germline carriers, one PDAC was clearly identified as arising from an IPMN. The IPMN lesion was microdissected separately from the PDAC, and DNA extracted separately. The IPMN revealed partial LOH while the PDAC demonstrated complete LOH. This 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[48]. While 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 demonstrate a significant age difference between sporadic and hereditary pancreatic cancer [30]. Further studies are underway 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 is no different between \( BRCA1/2 \)-associated tumors and other PDAC, although the sample is underpowered for survival analyses controlled for age and invasion characteristics.

Since the association between \( BRCA1 \) and \( BRCA2 \) may be causal in select 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 demonstrated that PDAC screening in select high-risk individuals is successful at detection of early stage PDAC and high-grade dysplastic lesions in the pancreas[3-12]. Genetic testing performed 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 age 50[49, 50]. These guidelines, developed based on expert opinion, recommend PDAC screening for \textit{BRCA2} mutation carriers with one first-degree relative with PDAC, and for \textit{BRCA2} carriers with two non-first degree relatives with PDAC. While screening of select 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 demonstrate an increased prevalence of both \textit{BRCA1} and \textit{BRCA2} mutations in an AJ surgical cohort of PDAC and IPMN. We furthermore demonstrate LOH of the corresponding \textit{BRCA1/2} loci, and nuclear p53 staining of select tumors, suggesting that mutation and/or loss of p53 cooperates with \textit{BRCA1/2} mutations in the progression of PDAC. Survival and tumor histology is not significantly different in \textit{BRCA1/2}-associated tumors compared to other PDACs in our cohort. Further studies are underway to more definitively establish the mechanism and clinical characteristics of \textit{BRCA1}- and \textit{BRCA2}-associated PDAC.

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REFERENCES

Table 1. Patient Demographic Information.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Number (%)</th>
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<tr>
<td>Sex</td>
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<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>
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<tr>
<td>Age (years)</td>
<td>Mean 67.4, range 44-86, standard deviation 10.4</td>
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<tr>
<td>Tobacco Use</td>
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<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>
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<tr>
<td>Alcohol Use</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>34 (54.0)</td>
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<tr>
<td>Light / Social</td>
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<tr>
<td>Heavy</td>
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<td>Diagnosis</td>
<td>Count (Percentage)</td>
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<td>--------------------</td>
</tr>
<tr>
<td>Unknown</td>
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<tr>
<td>Diabetes Mellitus</td>
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<td>No</td>
<td>50 (79.4)</td>
</tr>
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<td>Yes</td>
<td>13 (20.6)</td>
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<td>9 (14.3)</td>
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<td>Adjuvant</td>
<td>21 (33.3)</td>
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Table 2. Final Pathology for Ashkenazi Jewish surgical cohort.

<table>
<thead>
<tr>
<th>Final Pathology</th>
<th>Number (%)</th>
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<tr>
<td>Pancreatic Ductal Adenocarcinoma</td>
<td>37 (58.7)</td>
</tr>
<tr>
<td>Neuroendocrine Tumor</td>
<td>11 (17.4)</td>
</tr>
<tr>
<td>IPMN</td>
<td>7 (11.1)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Ampullary Carcinoma</td>
<td>1 (1.6)</td>
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<tr>
<td>Serous Cystadenoma</td>
<td>2 (3.2)</td>
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<tr>
<td>Mucinous Cystadenoma</td>
<td>1 (1.6)</td>
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<tr>
<td>Other cystic neoplasms, unspecified</td>
<td>2 (3.2)</td>
</tr>
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</table>

A total of 63 AJ patients had frozen normal tissue available in the tumor bank with good quality DNA. 37 patients underwent surgery for PDAC, 7 for IPMN, 11 for neuroendocrine tumors and another 8 patients underwent surgery for assorted cystic and neoplastic disease.
Table 3. Increased Prevalence of *BRCA1* and *BRCA2* mutations.

### 3A.

<table>
<thead>
<tr>
<th></th>
<th>Prevalence (%)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>AJ Population(1)</td>
<td>120/5318 (2.3)</td>
<td>reference</td>
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<tr>
<td>AJ Cohort</td>
<td>12/63 (19.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AJ PDAC</td>
<td>8/37 (21.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AJ IPMN</td>
<td>2/7 (28.6)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### 3B.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>AJ Population Prevalence (%)</th>
<th>AJ PDAC Cohort Prevalence</th>
<th>AJ IPMN Cohort Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>BRCA1</em> Mutations</td>
<td>61/5318 (1.15)</td>
<td>4/37 (p &lt;0.001)</td>
<td>1/7 (p=0.08)</td>
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<tr>
<td><em>BRCA1</em> 5382insC</td>
<td>20/5318 (0.38)</td>
<td>1/37 (p=0.14)</td>
<td>N/A</td>
</tr>
<tr>
<td><em>BRCA1</em> 185delAG</td>
<td>41/5318 (0.77)</td>
<td>3/37 (p &lt;0.001)</td>
<td>1/7 (p=0.05)</td>
</tr>
<tr>
<td><em>BRCA2</em> 6174delT</td>
<td>59/5318 (1.11)</td>
<td>4/37 (p &lt;0.001)</td>
<td>1/7 (p=0.08)</td>
</tr>
</tbody>
</table>

**A.** A high prevalence of AJ *BRCA1/2* founder mutations was demonstrated in the entire AJ surgical cohort (19%, p<.001), AJ PDAC cohort (18.9%, p<.001), and AJ IPMN cohort (28.6%, p=.01).  **B.** A high prevalence of individual *BRCA1* and *BRCA2* mutations was demonstrated in the AJ PDAC cohort and AJ 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><em>BRCA1</em> 185delAG</td>
<td>IPMN</td>
<td>81</td>
<td>M</td>
<td>Former</td>
<td>Mild</td>
<td>-</td>
<td>Alive</td>
<td>1526</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><em>BRCA1</em> 185delAG</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><em>BRCA1</em> 185delAG</td>
<td>PDAC</td>
<td>53</td>
<td>F</td>
<td>Unknown</td>
<td>Unknown</td>
<td>IB</td>
<td>Deceased</td>
<td>1883</td>
<td>Complete</td>
<td>Negative</td>
</tr>
<tr>
<td><em>BRCA1</em> 185delAG</td>
<td>PDAC</td>
<td>85</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>IIA</td>
<td>Alive</td>
<td>1635</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td><em>BRCA1</em> 5382insC</td>
<td>PDAC</td>
<td>53</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>IIB</td>
<td>Alive</td>
<td>1005</td>
<td>Wild Type</td>
<td>Negative</td>
</tr>
<tr>
<td><em>BRCA2</em> 6174delT</td>
<td>PDAC</td>
<td>61</td>
<td>M</td>
<td>Former</td>
<td>Mild</td>
<td>IIB</td>
<td>Alive</td>
<td>1744</td>
<td>Complete</td>
<td>Negative</td>
</tr>
<tr>
<td><em>BRCA2</em> 6174delT</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><em>BRCA2</em> 6174delT</td>
<td>PDAC</td>
<td>57</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>IIA</td>
<td>Deceased</td>
<td>1002</td>
<td>Partial</td>
<td>Focal Nuclear</td>
</tr>
<tr>
<td><em>BRCA2</em> 6174delT</td>
<td>PDAC</td>
<td>59</td>
<td>F</td>
<td>No</td>
<td>Heavy</td>
<td>IIB</td>
<td>Alive</td>
<td>1061</td>
<td>Complete</td>
<td>Positive</td>
</tr>
<tr>
<td><em>BRCA2</em> 6174delT</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>
Figure 1. BRCA1 185delAG Loss of Heterozygosity.

Panel A. Chromatogram demonstrating 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. Panel B. Chromatogram demonstrating sequence in germline BRCA1 185delAG carrier. This sequence was generated from normal pancreatic DNA. Arrow indicates mutation site. Panel C. Chromatogram demonstrating LOH at BRCA1 185delAG. DNA was obtained from microdissected PDAC tumor tissue from the same patient in Panel B. Arrow indicates mutation site. Panel D. Chromatogram demonstrating wild-type sequence of BRCA1 gene at region of 5382insC mutation. This patient does not carry a germline BRCA1 5382insC mutation. Panel E. Representative chromatogram demonstrating sequence in the PDAC patient with germline BRCA1 5382insC mutation. Arrow indicates mutation site. DNA from multiple normal pancreas tissue blocks demonstrated the same sequence. Panel F. Chromatogram demonstrating wild-type sequence at BRCA1 5382insC site in the same patient. Arrow indicates mutation site. Microdissected DNA from multiple tumor blocks demonstrated the same sequence. DNA profiling confirmed the samples in Panel D and Panel E belong to the same subject.

Figure 2. Representative immunohistochemistry studies from a 59 year-old man with PDAC and a BRCA2 6174delT mutation with LOH.

Panel A is a representative hematoxylin and eosin (H&E) stain of tumor region and Panel B demonstrates extensive staining for p53 in same tumor region, counterstained with hematoxylin.
only. Panel C demonstrated H&E stain of different tumor section, and Panel D demonstrates greater heterogeneity in p53 staining.
Figure 1.
Figure 2.
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.

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