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BRCA1, BRCA2, PALB2, and CDKN2A Mutations in Familial Pancreatic Cancer (FPC): A PACGENE Study

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Supplemental Data

Supplemental Data include three tables (S1–S3).

Conflict of Interest: Ralph H. Hruban, Michael G. Goggins, and Alison P. Klein receive royalty payments from Myriad Genetics for the *PALB2* invention under an agreement through Johns Hopkins University. L.A. Cannon-Albright collaborated with Myriad on gene discovery and receives royalties for *BRCA1*, *BRCA2*, and *CDKN2A* clinical testing under an agreement with the University of Utah.

Abstract

Purpose—Familial Pancreatic Cancer (FPC) kindreds contain at least two affected first-degree relatives (FDR). Comprehensive data are needed to assist clinical risk assessment and genetic testing.

Methods—Germline DNA samples from 727 unrelated probands with positive family history (521 met criteria for FPC) were CLIA-tested for mutations in *BRCA1* and *BRCA2* (including analysis of deletions and rearrangements), *PALB2*, and *CDKN2A*. We compared prevalence of deleterious mutations between FPC probands and non-FPC probands (kindreds containing at least two affected biologic relatives, but not FDR). We also examined the impact of family history of breast and ovarian cancer and melanoma.

Results—Prevalence of deleterious mutations (excluding variants of unknown significance) among FPC probands was: *BRCA1*, 1.2%; *BRCA2*, 3.7%; *PALB2*, 0.6%; *CDKN2A*, 2.5%. Four novel deleterious mutations were detected. FPC probands carry more mutations in the four genes (8.0%) than non-FPC probands (3.5%) (odds ratio=2.40, 95% CI=(1.06, 5.44), p=0.03). The probability of testing positive for deleterious mutations in any of the four genes ranges up to 10.4%, depending upon family history of cancers. *BRCA2* and *CDKN2A* account for the majority of mutations in FPC.

Conclusion—Genetic testing of multiple relevant genes in probands with a positive family history is warranted, particularly for FPC.

Keywords

Familial pancreatic cancer (FPC); *BRCA1*; *BRCA2*; *PALB2*; *CDKN2A*

Introduction

Pancreatic adenocarcinoma is currently the fourth leading cause of cancer death in the United States¹ and is anticipated to be the second leading cause by the year 2020.² Despite medical advances, overall five-year survival rates have not significantly changed, with the vast majority of diagnoses made at advanced stages of disease.¹ The relatively lower incidence of pancreatic cancer compared to other malignancies makes it challenging to conduct the large-scale studies that are needed to determine appropriate early screening measures.³ It is critical to identify populations at high risk who may potentially benefit from earlier detection, with concomitant implications for intervention or therapy.⁴

Family history studies suggest that approximately 5–10% of pancreatic adenocarcinoma has a strong hereditary basis, and familial pancreatic cancer (FPC) is thought to be genetically heterogeneous. FPC, defined as a kindred with at least two affected first degree relatives (FDR), describes an established entity of inherited pancreatic cancer.⁵ Our knowledge of the genetic basis of FPC largely arises from observed increased pancreatic cancer risk in those with hereditary malignant syndromes. A number of candidate susceptibility genes have been proposed to date, and four genes, *BRCA1*, *BRCA2*, *PALB2*, and *CKDN2A*, appear to account for the majority of known genetic causes of FPC.⁵

Individuals carrying germline mutations in *BRCA1* and *BRCA2* demonstrate increased risk for developing other malignancies, including pancreatic cancer.⁶⁻⁸ Although germline mutations in *BRCA1* and *BRCA2* are associated with Hereditary and Ovarian Breast Cancer (HBOC) syndrome, this increased risk of pancreatic malignancy can also manifest in families who do not meet criteria for HBOC.^{9,10} In the initial studies by the Breast Cancer Linkage Consortium, the relative risk of developing pancreatic cancer was increased by a mean of 2.26-fold for *BRCA1*⁶ and 3.5-fold for *BRCA2*⁷ carriers. However, risk ascertainment was obtained in hereditary breast cancer families rather than families ascertained through pancreatic cancer, thus likely underestimating the actual risk for pancreatic cancer in *BRCA1* and *BRCA2* mutation carriers. While initial investigations cite the presence of pancreatic cancer in HBOC families with deleterious *BRCA1* mutations^{8,11,12}, no germline *BRCA1* mutations were identified in a series of pancreatic cancer families.¹³ Thus, available evidence indicates that when individuals are ascertained through FPC kindreds, the risk of pancreatic cancer in *BRCA1* mutation carriers is less than it is for *BRCA2* mutation carriers. Murphy et al. reported 17% prevalence of *BRCA2* mutations among affected individuals from 26 European FPC kindreds containing three or more affected members with pancreatic cancer.¹⁴ Subsequent studies of individuals with pancreatic cancer from families meeting FPC criteria (two or more affected first-degree relatives) estimated *BRCA2* prevalence ranging between 6–10%.¹⁵ Furthermore, the ethnic variation of the population influences mutation prevalence rates of *BRCA1* and *BRCA2* and should be recognized when interpreting the literature. For example, among Ashkenazi Jews, similar mutation prevalences were observed for both *BRCA1* and *BRCA2*.^{16,17} The role of *BRCA1* and *BRCA2* mutations in larger samples of FPC kindreds remains to be elucidated. Determination of *BRCA* mutation status has potential therapeutic implications, as those carrying such mutations have been shown to benefit from therapies that inhibit poly[ADP ribose]polymerase (PARP inhibitors).^{18,19}

PALB2, a co-localizer and partner gene to *BRCA2*, is also proposed to be involved in FPC. *PALB2* was originally identified as a novel protein that complexes with *BRCA2*, leading to its stability and facilitating DNA repair.²⁰ Bi-allelic germline mutations in *PALB2* lead to the development of Fanconi anemia,²¹ while mono-allelic mutations increase breast cancer susceptibility. While searching for candidate pancreatic cancer susceptibility genes, Jones et al. discovered an inherited deleterious *PALB2* mutation coupled with a second inactivating hit in a patient with pancreatic cancer.²² Further *PALB2* sequencing in a cohort of 96 FPC patients showed that 3–4% carried deleterious mutations. With the exception of one European study,²³ subsequent studies have reported a lower prevalence of *PALB2* mutations in FPC.^{24,25} Studies with large sample sizes and unbiased selection criteria are needed to provide a more complete understanding of the role of *BRCA1*, *BRCA2*, and *PALB2* in pancreatic cancer susceptibility.

The *CDKN2A* gene located on chromosome 9p21 encodes the p16 protein, an important cell cycle regulator that inhibits cyclins, thus preventing premature transition from G1 to the S phase and serving as an important tumor suppressor. Germline mutations in *CDKN2A* are responsible for early-onset melanomas often associated with the development of Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome. Increased risk for pancreatic cancer development was observed in cases of *CDKN2A*-associated familial melanoma.²⁶

Examining *CDKN2A* in German FPC patients, Bartsch et al. found that mutations were rare, unless patients had concurrent melanoma.²⁷ Studies performed in other regions of Europe ultimately demonstrated the occurrence of *CDKN2A* mutations in FPC kindreds without melanoma, with prevalences ranging from 20–30%.²⁸ Such elevated rates, however, were likely influenced by specific founder mutations; one study also included patients of other familial cancer syndromes. In a large study in the United States of *CDKN2A* germline mutations among 1,537 unselected mostly sporadic pancreatic cancer cases, McWilliams et al.²⁹ found a much lower overall prevalence of *CDKN2A* mutations (0.6%), with higher rates in the subset of cases with affected first degree relatives (FDR); the limited family history data in this study left open the question of germline *CDKN2A* mutations in patients with FPC, particularly families without evidence of FAMMM.

In order to better inform genetic counseling of patients and families through more precise prevalence estimates, we comprehensively analyzed *BRCA1*, *BRCA2*, *PALB2*, and *CDKN2A* in a large cohort of FPC kindreds ascertained via the multicenter Pancreatic Cancer Genetic Epidemiology (PACGENE) Consortium.

Material and Methods

Subjects

Institutional review board approval was obtained at all participating sites, and written consent was obtained from all probands in order to be included in the study. PACGENE Consortium sites had assembled 2,853 unrelated kindreds containing at least two family members affected with pancreatic cancer from which subjects for this study sample were drawn (i.e., not all probands had available biospecimens). Ascertainment and recruitment methods were previously described.³⁰ Probands were biopsy-proven or clinically documented to have a diagnosis of pancreatic adenocarcinoma. We identified 727 unrelated kindreds that contained at least two biologically related family members affected with pancreatic cancer and for which a proband DNA sample was available. PACGENE sites include Mayo Clinic (Rochester, Minnesota) (n=341), Johns Hopkins University (Baltimore, Maryland) (n=107), Barbara Ann Karmanos Cancer Institute (Detroit, Michigan) (n=45), University of Toronto (Ontario, Canada) (n=131), and Dana Farber Cancer Institute (Boston, Massachusetts) (n=58). Subjects from kindreds similar to the PACGENE sites were contributed from the University of Texas MD Anderson Cancer Center (Houston, TX) (n=38) and University of Utah (Salt Lake City, UT) (n=7). In general, probands were unselected for hereditary cancer syndrome patterns or whether genetic mutation status in one of the four tested genes may have been previously known. Some potential probands with known mutations in one of the genes being tested may have been excluded by some sites, but this was not systematic. DNA was extracted at each contributing site from peripheral blood or buccal cell samples. Baseline demographic and family history information were available, typically self-reported.

Of the 727 kindreds in this study, a subset of 521 met criteria for FPC (having two FDRs with pancreatic cancer), and the remaining 206 were familial non-FPC cases (these kindreds contained at least two affected biologic relatives, but not FDR). A small proportion (1.2%) of the total sample also had a personal history of melanoma; among females, personal

history of breast and ovarian cancers occurred in 6.4% and 0.6%, respectively. All subjects were assigned a unique identifier, and all samples were de-identified during analysis by Myriad Genetic Laboratories, Inc.

Mutation Analysis

Re-sequencing analysis for germline mutations in *BRCA1*, *BRCA2*, *PALB2*, and *CDKN2A* and large rearrangement analysis for *BRCA1* and *BRCA2* was conducted by Myriad Genetic Laboratories, Inc. Full-sequence DNA analysis of these four genes and breakpoint analysis for five large genomic rearrangements in *BRCA1* (exon13del3835bp, exon13ins6kb, exon14-20del26kb, exon22del510bp, and exon8-9del7.1kb) were performed using previously described methods.^{31,32} All testing adhered to Clinical Laboratory Improvement Amendments (CLIA) requirements.

Briefly, for each of the four genes, full-gene sequencing was performed in both forward and reverse directions. The non-coding intronic regions of each gene that are analyzed do not extend more than 20 base pairs proximal to the 5' end and ten base pairs distal to the 3' end of each exon. Aliquots of subjects' DNA are each subjected to polymerase chain reaction (PCR) amplification to generate exon-specific amplicons that can be directly sequenced. The amplified products are each sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers. Electropherogram tracings of each amplicon are analyzed by a proprietary computer-based review system followed by visual inspection and confirmation of all clinically significant variants. Genetic variants are detected by comparison with a consensus wild-type sequence constructed for each gene. All potential clinically significant variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above. In addition, large-rearrangement analysis of *BRCA1* and *BRCA2* was performed for each sample using the BRCAAnalysis Rearrangement Test (BART), a quantitative multiplex endpoint PCR assay that detects all large deletions and duplications across the coding regions and promoters of *BRCA1* and *BRCA2* using a quantitative endpoint multiplex PCR assay. BART uses a set of 12 reactions comprising 11 multiplex PCR reactions containing 9 to 14 amplicons per multiplex, and one contamination detection reaction. These amplicons cover coding exons, promoters, and flanking regions for *BRCA1* and *BRCA2*.³³

Deleterious (including suspected deleterious) mutations, variants of uncertain significance (VUS), and single nucleotide polymorphisms (SNPs) in the four genes were detected and distinguished for the data analysis. Deleterious (including suspected deleterious) mutations, VUS, and SNPs were defined as those established in the current published literature as well as those previously catalogued in Myriad's established genetic mutation database for these genes. Novel, previously unreported mutations discovered in this study were defined as those not present in the Myriad gene mutation database. All variants were classified in accordance with the recommendations of the American College of Medical Genetics and Genomics (ACMG) for standards in the interpretation and reporting of sequence variations.³⁴

Data Analysis

Prevalence of deleterious mutations and VUS of the four genes studied were compared between individuals of FPC and familial non-FPC kindreds. We focused on the sub-analysis of the probands who had complete results on all four genes to better gain insight for genetic counseling for *BRCA1*, *BRCA2*, *PALB2*, and *CDKN2A*. Descriptive statistics and mutation rates were calculated. Comparisons of the mutation prevalence between groups were measured using either chi-square or Fisher's exact tests, depending on sample sizes. All statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC).

Results

Baseline demographic characteristics of the 727 probands included in this study are shown in Table 1. Among these, 521 met criteria for FPC while the remaining 206 were classified as familial non-FPC. A slight majority of probands were males (50.9%), and median age of diagnosis was 65 years (range 20–95 years). The sample was largely White/Caucasian (87.3%), and 43 (8.0%) were of Ashkenazi Jewish descent among the 538 who self-reported this information. A majority of kindreds (70.4%) contained two affected members with pancreatic cancer, 19.3% reported 3 affected individuals, and 10.3% contained 4 or more affected individuals.

Table 2 summarizes the deleterious mutations, VUS, and SNPs in the four genes for this sample, highlighting those which were not previously established in the current literature. The four novel deleterious mutations detected were: *BRCA2* 6224insT, *PALB2* E837X (2509G>T), *PALB2* W1038X (3113G>A), and *CDKN2A* 286delG. Novel VUS detected were: *BRCA1* H1860Q (5699C>A); *BRCA2* S538N (1841G>A), T1586I (4985C>T), and dup exon 1; *PALB2* E1018D (3054G>T), E892K (2674G>A), I887S (2660T>G), P1009S (3025C>T), P65L (194C>T), P806L (2417C>T), S578G (1732A>G), Y334D (1000T>G); and *CDKN2A* G101R (301G>A), L65P (194T>C), and T18P (52A>C). These novel VUS, particularly in *PALB2*, have been classified as incidental findings and not likely related to the pathogenesis of FPC at this time.³⁵

Table 3 summarizes germline mutation prevalences in the subset of 716 probands who had results for all four genes tested (Tables of all results in the full sample and by gene are provided in Supplemental Data: Table S1). Results in Table 3 are stratified by deleterious mutations and VUS among probands from FPC kindreds and probands from kindreds that did not strictly meet the FPC criterion of containing at least two affected FDR (familial non-FPC). Gene for gene, probands from FPC kindreds carry more deleterious mutations than those from familial non-FPC kindreds. The probability that a proband carried a deleterious mutation in any of these genes was 8.0% and 3.5% in FPC versus familial non-FPC probands, respectively (odds ratio=2.40, 95% confidence interval (1.06, 5.44), p=0.03). The aggregate prevalence is 48/716 (6.7%) for all cases with any positive family history. Overall, deleterious mutations in *BRCA2* and *CDKN2A* were more prevalent compared to either *BRCA1* or *PALB2*.

The vast majority of probands who did test positive for a mutation carried a mutation in only one of the four genes. Only two individuals whose only personal cancer history was that of

pancreatic cancer had multiple mutations: one proband carried two novel mutations in *PALB2*: E837X (2509G>T) (classified as deleterious) and P806L (2417C>T) (classified as VUS); another proband from a familial non-FPC kindred carried a mutation in both *BRCA1* (187delAG) and *BRCA2* (6174delT). Conversely, two probands who had malignancies in addition to pancreatic cancer tested positive for one mutation each: one proband had breast and ovarian in addition to pancreatic cancer and was found to carry 816delGT in *BRCA1*; another proband had breast cancer and melanoma in addition to pancreatic cancer and was found to carry V932M (2794G>A) in *PALB2*. The number of individuals affected with pancreatic cancer in a kindred did not correlate with the prevalence of deleterious mutations in FPC kindreds (ANOVA $p=.97$) (Shown in Supplemental Data: Table S2).

Table 4 displays germline mutation prevalences in the subset of 716 probands who had results for all four genes tested, stratified by deleterious mutations and VUS and by whether they also had family history of breast cancer, ovarian cancer, or melanoma. As expected, probands with a family history of breast cancer were more likely to test positive for deleterious mutations in *BRCA1* (1.9%) or *BRCA2* (4.2%), probands with a family history of ovarian cancer were more likely to test positive for deleterious mutations in *BRCA1* (5.2%) and *BRCA2* (5.2%), and probands with a family history of melanoma were more likely to test positive for deleterious mutations in *CDKN2A* (7.8%). Overall, the probability that a proband with a family history of any of these three cancers would test positive for a deleterious mutation in any of the four genes is 8.7%. Similar results for probands when family history is restricted to FDR are shown in Supplemental Data (Table S3): those data show that a proband with a family history in a FDR of any of these three cancers has an overall 9.5% probability of testing positive for a deleterious mutation in any of the four genes. To facilitate genetic counseling, we have aggregated a summary of our data showing the distributions of the probabilities of deleterious mutations by various cancer family histories in Figure 1. As can be seen, *BRCA2* and *CDKN2A* constitute the majority of deleterious mutations across cancer family histories. Proband with pancreatic cancer who have a family member with ovarian cancer have 10.4% probability of testing positive for a deleterious mutation in *BRCA1* or *BRCA2*. Proband with melanoma in their family history have a 10.4% probability of testing positive for *CDKN2A* or *BRCA2* mutations. Interestingly, 7/14 (50%) of patients who carried *CDKN2A* mutations did not have a personal or family history of melanoma. Of the six *BRCA1* mutation carriers, 1 (16.7%) had no personal or family history of breast cancer, and 2/6 (33.3%) had no personal or family history of ovarian cancer. Similarly, the numbers among the 25 *BRCA2* mutation carriers were 14 (56%) and 21 (84%), respectively. For *PALB2*, one of four (25%) and none of the mutation carriers had a personal or family history of breast or ovarian cancer, respectively.

We also examined age at onset differences by mutation carrier status among 710 probands who had all four gene tests and available age data. Forty-five carried deleterious mutations and were younger than the others ($p=0.03$); median ages were 60 (range 42–93) and 65 (20–95), respectively.

Discussion

In this large study, we provide a comprehensive analysis of germline mutations occurring in the four genes, *BRCA1*, *BRCA2*, *PALB2*, and *CDKN2A*, among familial pancreatic cancer probands. With the exception of one FPC proband and one familial non-FPC proband, the vast majority of tested individuals carry only one germline mutation in these four genes. We found that 8% of probands who have a FDR with pancreatic cancer (and therefore meet the definition of FPC) harbor a deleterious mutation in one of these four genes and that even probands who have a biological relative other than FDR with pancreatic cancer may carry a deleterious mutation, although with significantly less probability. We demonstrated that these genes together account in total for approximately 5–10% of deleterious mutations in FPC. Overall, any proband with a positive family history of pancreatic cancer has a 6.7% probability of carrying a deleterious mutation in one of the genes. Mutations in *BRCA2* and *CDKN2A* were detected more often than those in *BRCA1* and *PALB2*, consistent with the published literature. We also found a younger age of onset among probands who carried a mutation in one of the four genes. Our study confirms and highlights the genetic heterogeneity of FPC. Thus, when genetic testing of probands is considered, multiple genes will need to be evaluated.

When family history of breast or ovarian cancer, or melanoma is considered, there are varying ranges of probabilities; it is of interest that a proband with a family history of pancreatic cancer and any of the three other cancers has an 8.7% probability of carrying a mutation. As familial pancreatic cancer probands are increasingly referred for genetic risk assessment, we aggregated in Figure 1 selected family history scenarios from our data that will help inform the probability of genetic test outcomes.

With respect to genetic testing of probands, our data can inform the strategy to identify particular FPC individuals as candidates for genetic testing and whose families could potentially benefit from genetic risk assessment. We found that, gene for gene, significantly more deleterious mutations were found in FPC kindreds than in those of familial non-FPC kindreds. As such, the yield of identifying a mutation would be greatest among those whose kindreds meet the criteria for FPC. Interestingly, the number of family members affected with pancreatic cancer in a kindred did not correlate with the probability of detecting deleterious mutations (Supplemental Data: Table S2). We could not confidently explore this relationship in the familial non-FPC cases due to the smaller number of mutations detected.

Previous studies have emphasized the importance of family history in pancreatic cancer risk^{12,36} and increased incidence of early pancreatic lesions detected via early screening measures.³⁷ While our findings could lend promise towards use of genetic testing in early pancreatic cancer screening,³⁸ many questions remain on how to appropriately translate this into the clinical setting for genetically high risk individuals.³

In addition to informing genetic counseling, this report provides perhaps the most comprehensive mutation analysis of *PALB2* and *CDKN2A* in the familial setting. We utilized conventional methods and available databases from Myriad Genetic Laboratories as well as the research community at large to determine the classification of deleterious

have the data to adequately interrogate cancer risk in *BRCA1* or *BRCA2* mutation carriers of Ashkenazi Jewish heritage. Third, we did not test for mutations in mismatch repair genes associated with Lynch Syndrome. Although mutations in these genes confer increased risk for pancreatic cancer, the risk is more moderate compared to the four genes we did report⁵. Fourth, some of the sites may have excluded potential probands with already-known gene mutations. The estimated prevalences we present here are therefore underestimates. At this time, we cannot firmly fix the degree of underestimation because the exclusions were not systematic. Future studies should further identify other subject characteristics or risk factors that may assist in selecting appropriate affected individuals for genetic testing with the ultimate hope that this will enhance ongoing efforts toward an effective clinical strategy for screening high risk individuals for pancreatic cancer.⁴⁰

In this comprehensive study of germline mutations in *BRCA1*, *BRCA2*, *PALB2*, and *CDKN2A* in a sample of probands with familial pancreatic cancer, we have confirmed genetic heterogeneity and that a greater proportion of mutations occur in *BRCA2* and *CDKN2A*. Our data suggest there is a role for genetic testing in high risk FPC families, especially those containing at least two FDRs, supporting proposals made in previous pancreatic cancer screening guidelines.³ Further studies will elucidate the functional relevance of FPC genes as well as their potential interplay with complex intracellular pathways in the pathogenesis of pancreatic cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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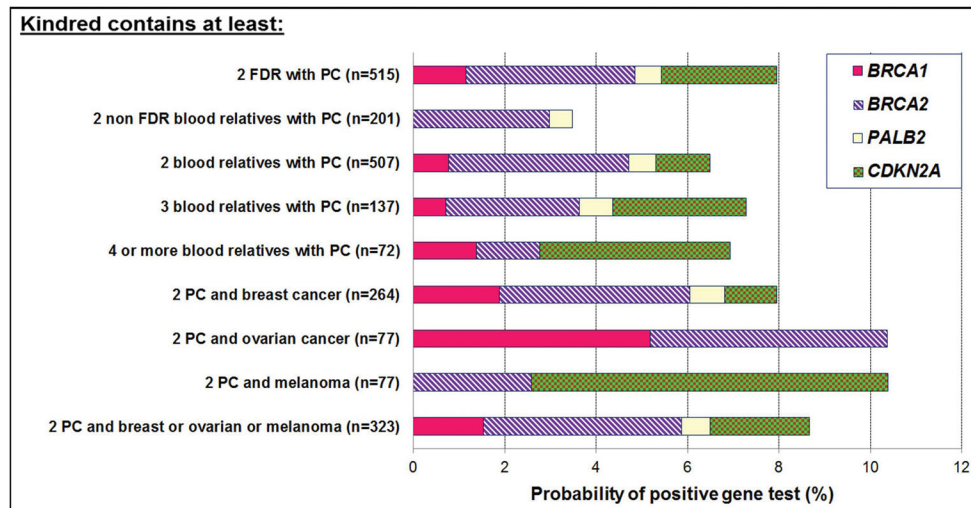


Figure 1. Probability (%) that probands affected with pancreatic cancer (PC) will test positive for a deleterious mutation in *BRCA1*, *BRCA2*, *PALB2*, or *CDKN2A*, if from kindreds with various cancer family histories. Number of PC includes proband. Sizes of sample subsets from which probabilities were estimated are shown in parentheses.

Table 1

Baseline characteristics of pancreatic cancer probands in the study

Characteristic	Familial Pancreatic Cancer (FPC)	Familial, not meeting FPC definition	Total
N	521	206	727
Median age at diagnosis of pancreatic cancer (range)	66 (37–95)	62 (20–88)	65 (20–95)
Sex, N (%)			
Male	259 (49.7)	111 (53.9)	370 (50.9)
Female	262 (50.3)	95 (46.1)	357 (49.1)
Race, N (%)			
White/Caucasian	401 (85.7)	163 (91.6)	564 (87.3)
Black/African-American	14 (3.0)	4 (2.2)	18 (2.8)
Asian/Asian-American	4 (0.9)	4 (2.2)	8 (1.2)
American Indian/Alaskan	0 (0.0)	1 (0.6)	1 (0.2)
Native	42 (9.0)	4 (2.2)	46 (7.1)
Multiracial	7 (1.5)	2 (1.1)	9 (1.4)
Other	53	28	81
Ashkenazi Jewish origin, N (%)			
No	346 (91.8)	149 (92.6)	495 (92.0)
Yes	31 (8.2)	12 (7.4)	43 (8.0)
Unknown/Unreported	144	45	189
Number in pedigree with pancreatic cancer, N (%)			
Two	327 (62.8)	185 (89.8)	512 (70.4)
Three	121 (23.2)	19 (9.2)	140 (19.3)
Four or more	73 (14.0)	2 (0.1)	75 (10.3)

Table 2

Germline mutations and counts in 727 sequenced pancreatic cancer probands with positive family history. Deleterious mutations include suspected deleterious mutations. Novel variants are in **bold**. Two individuals had multiple mutations: a) *PALB2* E837X (2509G>T) and *PALB2* P806L (2417C>T); and b) *BRCA1* 187delAG and *BRCA2* 6174delT. Variants were present in one proband unless otherwise noted by n in brackets.

Gene	Deleterious mutations	Variants of uncertain significance	Single Nucleotide Polymorphisms
<i>BRCA1</i>	187delAG [n=3]	C328R (1101T>C)	S1217P (3768T>C)
	4507ins7	H1860Q (5699C>A)	
	5385insC	R496S (1605C>A)	
	816delGT		
<i>BRCA2</i>	10095delT	S538N (1841G>A)	K1434I (4529A>T)
	2041insA [n=2]	T1586I (4985C>T)	R2341C (7249C>T)
	3635ins>100bp	dup exon 1	T544I (1859C>T)
	3972del4		V2652V (8184G>A)
	4075delGT		
	4206ins4		
	5175delAA [n=2]		
	5950delCT		
	6174delT [n=5]		
	6224insT		
	6601insA		
	8765delAG [n=2]		
	9663delGT		
	E1953X (6085G>T) [n=2]		
	K1323X (4195A>T)		
	Q321X (1189C>T)		
	Y1655X (5193C>G)		
<i>PALB2</i>	E837X (2509G>T)	A712V (2135C>T)	IVS6+10A>G
	R1086X (3256C>T) [n=2]	E1018D (3054G>T)	IVS7-18C>T [n=2]
	W1038X (3113G>A)	E892K (2674G>A)	P864S (2590C>T) [n=5]
		G881S (2641G>A)	S1165S (3495G>A) [n=3]
		I887S (2660T>G)	V932M(2794G>A) [n=4]
		L939W (2816T>G) [n=6]	
		N241D (721A>G)	
		P1009S (3025C>T)	
		P65L (194C>T)	
		P806L (2417C>T)	
		S578G (1732A>G)	
		Y334D (1000T>G)	
	<i>CDKN2A</i>	131insAA	5'UTR-25C>T
225del19		5'UTR-33G>C [n=5]	

Gene	Deleterious mutations	Variants of uncertain significance	Single Nucleotide Polymorphisms
	286delG	G101R (301G>A)	
	32ins24 (in-frame ins)	L16R (47T>G) [n=3]	
	5'UTR-34G>T	L65P (194T>C)	
	D153Y (457G>T)	Q50R (149A>G)	
	G101W (301G>T)	T18P (52A>C)	
	M53I (159G>A)		
	M53I (159G>C)		
	Q50X (148C>T) [n=2]		
	R24P (71G>C)		
	V126D (377T>A) [n=2]		

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Table 3

Germline mutation prevalences stratified by deleterious mutations and variants of uncertain significance among probands from Familial Pancreatic Cancer (FPC) kindreds, and probands from kindreds that included at least two affected relatives, but not first degree (Non-FPC). Results shown for probands who were tested for all four genes (total n=716).

Gene	Deleterious Mutations n (%)			Variants of Uncertain Significance n (%)		
	FPC (n=515)	Non-FPC (n=201)	Total (n=716)	FPC (n=515)	Non-FPC (n=201)	Total (n=716)
<i>BRCA1</i>	6 (1.2)	0 (0.0)	6 (0.8)	3 (0.6)	0 (0.0)	3 (0.4)
<i>BRCA2</i>	19 (3.7)	6 (3.0)	25 (3.5)	2 (0.4)	1 (0.5)	3 (0.4)
<i>PALB2</i>	3 (0.6)	1 (0.5)	4 (0.6)	11 (2.1)	5 (2.5)	16 (2.2)
<i>CDKN2A</i>	13 (2.5)	0 (0.0)	13 (1.8)	10 (1.9)	3 (1.5)	13 (1.8)
Total	41 (8.0)	7 (3.5)	48 (6.7)	26 (5.0)	9 (4.5)	35 (4.9)

Table 4

Germline mutation prevalences among pancreatic cancer probands with any biological family history of breast cancer, ovarian cancer, or melanoma. Results shown for probands who were tested for all four genes (total n=716).

Gene	Deleterious Mutations n (%)				Variants of Uncertain Significance n (%)			
	Probands with family history of pancreatic cancer AND family history of:							
	Breast (n=264)	Ovarian (n=77)	Melanoma (n=77)	Any* (n=323)	Breast (n=264)	Ovarian (n=77)	Melanoma (n=77)	Any* (n=323)
<i>BRCA1</i>	5 (1.9)	4 (5.2)	0 (0.0)	5 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>BRCA2</i>	11 (4.2)	4 (5.2)	2 (2.6)	14 (4.3)	2 (0.8)	0 (0.0)	1 (1.3)	2 (0.6)
<i>PALB2</i>	2 (0.8)	0 (0.0)	0 (0.0)	2 (0.6)	4 (1.5)	3 (3.9)	2 (2.6)	7 (2.2)
<i>CDKN2A</i>	3 (1.1)	0 (0.0)	6 (7.8)	7 (2.2)	6 (2.3)	2 (2.6)	5 (6.5)	8 (2.5)
Total	21 (8.0)	8 (10.4)	8 (10.4)	28 (8.7)	12 (4.5)	5 (6.5)	8 (10.4)	17 (5.3)

* Any = Breast cancer, ovarian cancer, or melanoma