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Association Between Inherited Germline Mutations in Cancer Predisposition Genes and Risk of Pancreatic Cancer

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IMPORTANCE Individuals genetically predisposed to pancreatic cancer may benefit from early detection. Genes that predispose to pancreatic cancer and the risks of pancreatic cancer associated with mutations in these genes are not well defined.

OBJECTIVE To determine whether inherited germline mutations in cancer predisposition genes are associated with increased risks of pancreatic cancer.

DESIGN, SETTING, AND PARTICIPANTS Case-control analysis to identify pancreatic cancer predisposition genes; longitudinal analysis of patients with pancreatic cancer for prognosis. The study included 3030 adults diagnosed as having pancreatic cancer and enrolled in a Mayo Clinic registry between October 12, 2000, and March 31, 2016, with last follow-up on June 22, 2017. Reference controls were 123 136 individuals with exome sequence data in the public Genome Aggregation Database and 53 105 in the Exome Aggregation Consortium database.

EXPOSURES Individuals were classified based on carrying a deleterious mutation in cancer predisposition genes and having a personal or family history of cancer.

MAIN OUTCOMES AND MEASURES Germline mutations in coding regions of 21 cancer predisposition genes were identified by sequencing of products from a custom multiplex polymerase chain reaction–based panel; associations of genes with pancreatic cancer were assessed by comparing frequency of mutations in genes of pancreatic cancer patients with those of reference controls.

RESULTS Comparing 3030 case patients with pancreatic cancer (43.2% female; 95.6% non-Hispanic white; mean age at diagnosis, 65.3 [SD, 10.7] years) with reference controls, significant associations were observed between pancreatic cancer and mutations in *CDKN2A* (0.3% of cases and 0.02% of controls; odds ratio [OR], 12.33; 95% CI, 5.43-25.61); *TP53* (0.2% of cases and 0.02% of controls; OR, 6.70; 95% CI, 2.52-14.95); *MLH1* (0.13% of cases and 0.02% of controls; OR, 6.66; 95% CI, 1.94-17.53); *BRCA2* (1.9% of cases and 0.3% of controls; OR, 6.20; 95% CI, 4.62-8.17); *ATM* (2.3% of cases and 0.37% of controls; OR, 5.71; 95% CI, 4.38-7.33); and *BRCA1* (0.6% of cases and 0.2% of controls; OR, 2.58; 95% CI, 1.54-4.05).

CONCLUSIONS AND RELEVANCE In this case-control study, mutations in 6 genes associated with pancreatic cancer were found in 5.5% of all pancreatic cancer patients, including 7.9% of patients with a family history of pancreatic cancer and 5.2% of patients without a family history of pancreatic cancer. Further research is needed for replication in other populations.

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Cancer predisposition gene testing is useful for identifying individuals who may benefit from screening, prevention, and early detection of breast, ovarian, and colorectal cancer[1,2] and may be beneficial for individuals at risk of pancreatic cancer.[3,4] Family members of those with germline predisposition gene mutations may also benefit from enhanced cancer screening and prevention strategies. For instance, screening of *CDKN2A* (RefSeq NM_000077.4) mutation carriers has been associated with early detection of resectable pancreatic tumors.[3]

Epidemiologic studies have shown that 10% to 20% of pancreatic cancers are associated with an inherited component.[5] Deleterious mutations in *BRCA2* (RefSeq NM_000059.3), *PALB2* (RefSeq NM_024675.3), and *CDKN2A* cancer predisposition genes have been detected in families of patients with pancreatic cancer.[6-8] Germline mutations in *BRCA1* (RefSeq NM_007294.3) have also been associated with an increased risk of pancreatic cancer in families (relative risk, 2.26; 95% CI, 1.26-4.06),[9] similar to mutations in mismatch repair genes in families of patients with Lynch syndrome (hazard ratio of cumulative increased risk, 8.6; 95% CI, 4.7-15.7).[10] Germline mutations have also been observed in 7% of patients unselected for family history of pancreatic cancer[11] and in 3.9% of 854 patients with pancreatic adenocarcinoma.[12]

This study used a custom cancer predisposition gene panel developed for hereditary cancer genetic testing to assess the prevalence of deleterious germline mutations among patients with pancreatic cancer in 21 predisposition genes implicated in susceptibility to solid tumors (eTable 1 in the Supplement).[1,13,14] DNA for panel testing was obtained from a series of 3030 patients with pancreatic cancer from a Mayo Clinic pancreas cancer registry, and DNA sequence data for the same predisposition genes were obtained from publicly available Genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC) reference control groups.[15,16] Associations between mutations in each gene and pancreatic cancer were evaluated to establish a defined subset of genes that confer susceptibility to pancreatic cancer.

Key Points

Question Are there germline mutations in cancer predisposition that are associated with pancreatic cancer?

Findings In a case-control study that included 3030 patients with pancreatic cancer and 123 136 reference controls, 6 genes were independently associated with pancreatic cancer, with odds ratios between 2.58 and 12.33 after correction for multiple comparisons. In aggregate, these genes were observed in 5.5% of patients with pancreatic cancer.

Meaning Six genes were identified that were associated with pancreatic cancer; further research is needed for replication in other populations.

Methods

Study Sample

The participants in this study were recruited into the Mayo Clinic Biospecimen Resource for Pancreas Research, a prospective patient registry focused on pancreatic cancer.[17] Patients were identified and invited to participate at the time of diagnosis. Detailed information about the process of recruitment, biospecimen collection, and maintenance of the registry is provided in eAppendix 1 in the Supplement. All participants diagnosed as having pancreatic ductal adenocarcinoma, who were recruited from October 12, 2000, through March 31, 2016, with available genomic DNA extracted from peripheral blood lymphocyte samples were included in the study. Patients completed questionnaires on demographic and clinical characteristics and family history of cancer. Race was self identified as American Indian/Alaskan Native, Asian/Asian American, black/African American, Native Hawaiian/other Pacific Islander, white, and multiracial. Ethnicity was self identified as Hispanic/Latino or non-Hispanic/non-Latino. The study was approved by the Mayo Clinic Institutional Review Board. All patients provided written informed consent for research genetic testing. Results have not been systematically disclosed to participants.

Reference control data were obtained from the public gnomAD (http://gnomad.broadinstitute.org/),[15] which contains exome sequencing data from 123 136 unrelated individuals sequenced as part of various disease-specific and population genetic studies (eAppendix 2 in the Supplement). The gnomAD data set was generated using multiple exome capture methods and sequencing chemistries and was subset to racial and ethnic groups including African/African American, Hispanic, Asian, and non-Finnish European for this study. A second reference control data set of 53 105 germline exomes from ExAC (http://exac.broadinstitute.org),[15,16] excluding samples from cancer cases from The Cancer Genome Atlas (TCGA) project (ExAC non-TCGA) was used to assess consistency in results (eAppendix 2). The ExAC non-TCGA data set was generated using multiple exome sequencing methods and was also subset to racial and ethnic groups including African/African American, Hispanic, Asian, and non-Finnish European for this study. All reference control groups may have included a small number of pancreatic cancer cases because individuals with cancer were not excluded.

DNA Sequencing

Genomic DNA samples were subjected to multiplex polymerase chain reaction using a QIAseq (Qiagen Inc)[18] custom panel of target regions covering all coding regions and consensus splice sites from 21 cancer predisposition genes: *ATM* (RefSeq NM_000051.3), *BARD1* (RefSeq NM_000465.3), *BRCA1, BRCA2, BRIP1* (RefSeq NM_032043.2), *CDH1* (RefSeq NM_004360.4), *CDKN2A, CHEK2* (RefSeq NM_007194.3), *FANCC* (RefSeq NM_000136.2), *MLH1* (RefSeq NM_000249.3), *MRE11A* (RefSeq NM_005591.3), *MSH2* (RefSeq NM_000251.2), *MSH6* (RefSeq NM_000179.2), *NBN* (RefSeq NM_002485.4), *NF1* (RefSeq NM_001042492.2), *PALB2, PMS2* (RefSeq NM_000535.6), *PTEN* (RefSeq NM_000314.6), *RAD51C* (RefSeq NM_058216.2), *RAD51D* (RefSeq NM_001142571), and *TP53* (RefSeq NM_000546.5) (eAppendix 3 in the Supplement). Libraries derived from each DNA sample were individually bar coded by dual indexing. Sequencing was performed on a HiSeq 4000 with 150-bp paired-end reads of 768 pooled libraries per

lane. Median sequence read depth was 200×. These genes were selected based on inclusion in commercial hereditary cancer genetic testing panels as known or candidate predisposition genes for several solid tumors including breast, ovarian, endometrial, colorectal, or pancreatic cancers.[1,13,14,19] Results from 19 genes are presented because no mutations were identified in *RAD51D* or *PTEN*.

Bioinformatics Analysis

FASTQ files of DNA sequences were generated for each sample based on unique dual indexes. Reads were trimmed with Cutadapt version 1.10[20] and aligned with BWA-MEM version 0.7.10.[21] Sequence realignment, recalibration, haplotype calling, and depth of coverage were conducted using Genome Analysis Toolkit version 3.4-46 (University of Birmingham). A minimum quality threshold[22] of Q20 was applied to identify cases eligible for analyses. Annotation of variants from cases with pancreatic cancer and from gnomAD and ExAC non-TCGA reference controls[16,23] (eAppendix 4 in the Supplement) was provided through the Biological Reference Repository tool kit,[24] leveraging dbNSFP version 3.0,[25] ClinVar,[26] and CAVA.[27] Variants were viewed and filtered with VCF-Miner.[28] All loss-of-function variants (nonsense, frameshift, consensus splice sites [±1 or 2]) and any intronic or missense variants defined as pathogenic or likely pathogenic in ClinVar in patients with pancreatic cancer were validated by Sanger sequencing (eAppendix 3 in the Supplement). Variants in pancreatic cancer cases and in both gnomAD and ExAC non-TCGA reference controls were filtered using established approaches (eAppendix 5 in the Supplement).[1]

Study End Points

The primary outcome was case-control status, where case status was assigned to all individuals with pancreatic cancer in the Mayo Clinic registry. All individuals in the gnomAD and ExAC data sets were controls. A secondary outcome was overall survival after diagnosis of pancreatic cancer. Vital status was ascertained by using personal/family correspondence, a study follow-up questionnaire, medical records, or an external service (LexisNexis Accurint). Duration of overall survival was calculated from the date of diagnosis at a Mayo Clinic location until date of death, date last known alive, or date of censorship of June 22, 2017.

Case-Control Statistical Analysis

Analyses were based on patients (**Table 1** and eTable 2 in the Supplement) with good-quality sequence data. Frequencies of mutations in individual genes were calculated overall and by patient characteristics (personal history of other cancer; family history of breast, colorectal, ovarian, gynecologic, and pancreatic cancer). Associations between mutations in each gene and pancreatic cancer were assessed by logistic regression, comparing combined mutation frequencies by gene in patients with pancreatic cancer with frequencies in gnomAD reference controls after weighting for the relative frequency of racial and ethnic populations. Association analysis included patients with pancreatic cancer after exclusion of patients with missing race information or other race (multiracial, American Indian/Alaskan Native, and Native Hawaiian/other Pacific Islander) (Table 1). Confidence

Table 1. Characteristics of Case Patients

Characteristics	No. (%)[a] All Case Patients (n = 3030)	Mutation Carriers (n = 249)[b]
Sex		
Female	1308 (43.2)	101 (40.6)
Male	1722 (56.8)	148 (59.4)
Race/ethnicity		
African American	50 (1.6)	4 (1.6)
Hispanic	42 (1.4)	3 (1.2)
Asian	11 (0.4)	1 (0.4)
Non-Hispanic white	2896 (95.6)	236 (94.8)
Other[c]	19 (0.6)	2 (0.8)
Missing	12 (0.4)	3 (1.2)
Age at diagnosis of pancreatic cancer, y		
<50	242 (8.0)	22 (8.8)
50-59	639 (21.1)	75 (30.1)
60-69	1023 (33.7)	81 (32.5)
≥70	1125 (37.2)	71 (28.5)
Missing	1 (<0.1)	0
Overall mean (SD)	65.3 (10.7)	63.1 (10.6)
Overall range	20-92	34-90
Body mass index		
Overall mean (SD)	28.5 (5.6)	29.2 (5.6)
Overall range	15.3-59.0	17.8-49.9
Missing data	341 (11.3)	22 (08.83)
Diabetes		
No	2263 (74.7)	184 (73.9)
Yes	767 (25.3)	65 (26.1)
Smoking status		
Missing	99 (3.3)	9 (3.6)
No	1246 (41.1)	106 (42.6)
Yes	1685 (55.6)	134 (53.8)
Family history of cancer (first- or second-degree relative)		
Pancreatic	343 (11.3)	43 (17.3)
Breast	675 (22.3)	75 (30.1)
Ovarian	152 (5)	21 (8.4)
Colorectal	513 (16.9)	45 (18.1)
Gynecologic, nonovarian	162 (5.3)	17 (6.8)
Personal history of other cancers		
Breast	82 (2.7)	11 (4.4)
Ovarian	10 (0.3)	0
Colorectal	65 (2.1)	12 (4.8)
Gynecologic (nonovarian)	11 (0.4)	2 (0.8)
Disease staging		
Resectable	850 (28.1)	76 (30.5)
Locally advanced	1115 (36.8)	72 (28.9)
Metastatic	1056 (34.9)	99 (39.8)
Missing	9 (3.0)	2 (0.8)

[a] Data are No. (%) of case patients unless otherwise noted.

[b] Panel of cancer predisposition genes evaluated for mutations: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDKN2A*, *CHEK2*, *FANCC*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *NBN*, *NF1*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*.

[c] Including multiracial, American Indian/Alaskan Native, and Native Hawaiian/other Pacific Islander.

intervals were estimated by the profile likelihood method. Sensitivity analyses using ExAC non-TCGA reference controls, selected for race/ethnicity, as with gnomAD controls, were undertaken to assess consistency in results (eAppendix 6 in the Supplement). Sensitivity analyses were conducted to account for the influence of personal and family history of pancreatic, breast, ovarian, colorectal, and gynecologic cancer on the associations between each gene and pancreatic cancer and to evaluate associations between genes and pancreatic cancer using non-Hispanic white cases and gnomAD non-Finnish European and ExAC non-Finnish European non-TCGA reference controls. For comparisons within individual populations, odds ratios (ORs) and corresponding 95% confidence intervals were estimated by inverting Fisher exact test.[29] Significance of associations was adjusted for multiple testing by Bonferroni correction. Associations between mutation status in each predisposition gene and age at diagnosis were tested using the Kolmogorov-Smirnov test,[30] and associations with patient characteristics were evaluated using logistic regression adjusted for age at diagnosis (eAppendix 6). All analyses were performed with R software version 3.4.2 (R Project for Statistical Computing). All statistical tests were 2-sided, and an adjusted $P<.05$ was considered statistically significant.

Survival Analysis

The patient population for survival analysis was restricted to the subset of 2698 adenocarcinoma cases diagnosed at a Mayo Clinic location within 3 months (≤92 days) of an initial diagnosis. This date of diagnosis was defined as either (1) the date of tissue-based diagnosis for those with pathology-proven disease (97%) or (2) the date of first clinical diagnosis for patients without pathology information (3%) and was used to avoid immortal time bias.[31] The association between mutations in pancreatic cancer predisposition genes and overall survival was evaluated using Cox regression models adjusted for age at diagnosis, sex, and disease staging (resectable, locally advanced, or metastatic). The significance of associations with survival was estimated by likelihood ratio test (eAppendix 6 in the Supplement). The proportional hazards assumption was tested using the residuals from the Cox model.[32] All analyses were performed with R software version 3.4.2.

Results

Characteristics of the Pancreatic Cancer Case Series

The participation rate in the Mayo Clinic Biospecimen Resource for Pancreas Research was 65.6%. High-quality sequencing data were obtained for 3030 of 3046 patients with pancreatic cancer in this case series (eTable 2 in the Supplement). Among the 3030 participants, 2591 (85.5%) consented to registry participation within 30 days of diagnosis. The sample was 95.6% non-Hispanic white and 43.2% female, with 37.2% of patients receiving diagnoses at age 70 years or older. The mean age at diagnosis was 65.3 (SD, 10.7) years, and approximately 5.5% of cases had an additional personal history of breast, ovarian, colorectal, or nonovarian gynecologic cancers (Table 1). While 11.3% of patients had a family history (among first- and second-degree relatives) of pancreatic cancer, 22.3% reported family histories of breast cancer, 16.9% colorectal cancer, and 5.0% ovarian cancer.

In 19 of 21 candidate pancreatic cancer predisposition genes, 253 deleterious germline mutations were identified in 249 patients (8.2%; 95% CI, 7.26%-9.25%) (Table 1 and **Table 2**; eTables 2 and 3 in the Supplement). While *ATM* had the highest prevalence of mutations (n = 69) (2.28%; 95% CI, 1.78%-2.87%), mutations were also frequently observed in *BRCA2* (1.95%; 95% CI, 1.49%-2.50%), *CHEK2* (1.09%; 95% CI, 0.75%-1.53%; excluding the low-risk p.Ile157Thr missense variant), *BRCA1* (0.59%; 95% CI, 0.35%-0.94%), *PALB2* (0.40%; 95% CI, 0.20%-0.69%), and *CDKN2A* (0.33%; 95% CI, 0.16%-0.61%). Among the 59 patients with *BRCA2* mutations, only 3 carried the Ashkenazi Jewish c.5946delT (6174delT) founder mutation. Smaller numbers of mutations were observed in candidate pancreatic cancer predisposition genes, including *FANCC* (0.26%; 95% CI, 0.11%-0.52%) and *TP53* (0.20%; 95% CI, 0.07%-0.43%). Germline mutations in the *MLH1*, *MSH2*, *PMS2*, and *MSH6* mismatch repair genes were detected in aggregate in 0.50% (95% CI, 0.28%-0.82%) of study participants (Table 2).

Patients with pancreatic cancer and mutations were more likely to have personal and family histories of other cancers (Table 1 and eTable 4 in the Supplement). In terms of personal history of cancer, 65 of 513 patients (12.3%; 95% CI, 9.9%-15.9%) with at least 1 other cancer in addition to pancreatic cancer had mutations in the panel genes. Additionally, mutations were detected in 43 of the 343 patients (12.9%; 95% CI, 9.2%-16.5%) with at least 1 first- or second-degree relative with pancreatic cancer. Mutations were also identified in 75 of 675 patients (11.3%; 95% CI, 8.8%-13.7%) with a family history of breast cancer, 21 of 152 patients (13.8%; 95% CI, 8.8%-20.3%) with a family history of ovarian cancer, and 45 of 513 patients (8.9%; 95% CI, 6.5%-11.6%) with a family history of colorectal cancer (Table 1). Overall, 25.7% (95% CI, 20.4%-31.5%) of all mutations (65/253) were associated with multiple primary cancers, 17.8% (95% CI, 13.3%-23.1%) were associated with a family history of pancreatic cancer, and 30.0% (95% CI, 23.5%-36.1%) were associated with a family history of breast cancer (eTable 4). In addition, 124 of 253 mutations (49%; 95% CI, 42.7%-55.3%) were identified in patients with a family history of at least 1 common epithelial cancer (pancreatic, breast, ovarian, gynecologic, or colorectal) (eTable 4).

Associations Between Germline Mutations and Pancreatic Cancer

Six genes were significantly associated with pancreatic cancer compared with gnomAD controls. These included *CDKN2A*, with mutations in 0.30% of cases and 0.02% of controls (OR, 12.33; 95% CI, 5.43-25.61); *TP53*, with mutations in 0.20% of cases and 0.02% of controls (OR, 6.70; 95% CI, 2.52-14.95); *MLH1*, with mutations in 0.13% of cases and 0.02% of controls (OR, 6.66; 95% CI, 1.94-17.53); *BRCA2*, with mutations in 1.90% of cases and 0.30% of controls (OR, 6.20; 95% CI, 4.62-8.17); *ATM*, with mutations in 2.30% of cases and 0.37% of controls (OR, 5.71; 95% CI, 4.38-7.33); and *BRCA1*, with mutations in 0.60% of cases and 0.20% of controls (OR, 2.58; 95% CI, 1.54-4.05) (**Table 3**). Similar results were obtained using the ExAC non-TCGA reference controls for *CDKN2A*, *ATM*, *MLH1*,

Table 2. Frequency of Mutations Among Pancreatic Cancer Cases by Category of Personal and Family History of Cancer

Genes	No. (%) of Case Patients[a]				
			Family History		
	Overall (n = 3030)	Personal History, Other Cancers (n = 513)	Pancreatic Ductal Adenocarcinoma (n = 343)	Breast Cancer (n = 675)	Colorectal Cancer (n = 513)
ATM	69 (2.28)	14 (2.73)	11 (3.29)	18 (2.72)	10 (1.98)
BARD1	4 (0.13)	0	1 (0.30)	0	0
BRCA1	18 (0.59)	6 (1.17)	2 (0.60)	4 (0.60)	2 (0.40)
BRCA2	59 (1.95)	14 (2.73)	7 (2.10)	21 (3.17)	10 (1.98)
BRIP1	5 (0.17)	0	1 (0.30)	0	0
CDH1	1 (0.03)	0	0	0	0
CDKN2A	10 (0.33)	2 (0.39)	5 (1.50)	2 (0.30)	2 (0.40)
CHEK2	33 (1.09)	9 (1.75)	8 (2.40)	11 (1.66)	5 (0.99)
FANCC	8 (0.26)	2 (0.39)	1 (0.30)	1 (0.15)	1 (0.20)
MLH1	5 (0.17)	3 (0.58)	0	1 (0.15)	3 (0.59)
MRE11A	2 (0.07)	0	0	1 (0.15)	0
MSH2	1 (0.03)	0	1 (0.30)	1 (0.15)	1 (0.20)
MSH6	7 (0.23)	3 (0.58)	1 (0.30)	3 (0.45)	4 (0.79)
NBN	4 (0.13)	1 (0.19)	1 (0.30)	1 (0.15)	1 (0.20)
NF1	4 (0.13)	3 (0.58)	0	1 (0.15)	1 (0.20)
PALB2	12 (0.40)	3 (0.58)	2 (0.60)	5 (0.76)	3 (0.59)
PMS2	2 (0.07)	2 (0.08)	0	1 (0.15)	2 (0.40)
RAD51C	3 (0.10)	0	0	0	0
TP53	6 (0.20)	3 (0.58)	2 (0.60)	4 (0.60)	0
All genes	253 (8.36)	65 (12.33)	43 (12.89)	75 (11.31)	45 (8.92)

[a] Number of cases in each category with a mutation in the specified gene.

BRCA2, and *BRCA1* (eTables 5 and 6 in the Supplement), while *TP53* exhibited a statistically significant but attenuated association (OR, 3.03; 95% CI, 1.14-6.74).

NBN and *BRIP1* were not significantly associated with pancreatic cancer, but the numbers of mutations in these genes were too low to allow for definitive evaluation of associations with pancreatic cancer. In contrast, *CHEK2* was associated with little or no risk of pancreatic cancer (Table 3 and eTable 6 in the Supplement). Similar frequencies of mutations in each gene by phenotypic category and similar associations between 5 of the predisposition genes (other than *MLH1*) and pancreatic cancer were observed for non-Hispanic white cases (n = 2896), which account for the majority of the study population (eTables 7, 8, and 9 in the Supplement). Sensitivity analyses yielded similar OR estimates for pancreatic cancer for the 6 predisposition genes other than *MLH1* when restricting to patients with pancreatic cancer as the first cancer diagnosis (eTable 10 in the Supplement). Similarly, no substantial changes in associations between predisposition gene mutations and pancreatic cancer were observed when restricting analyses to patients with a family history of common epithelial cancers (pancreatic, breast, ovarian, colorectal, and endometrial) (eTable 11 in the Supplement) or to patients without a family history of these cancers (eTables 12, 13, 14, 15, 16, and 17 in the Supplement), except for reduced risk for *MLH1* following exclusion of patients with a family history of colorectal cancer (eTables 12 and 16). Similarly, exclusion of patients and reference controls with Ashkenazi Jewish founder mutations in *BRCA1*, *BRCA2*, and *CHEK2* had little influence on results. Thus, 6 genes significantly associated with pancreatic cancer were designated as pancreatic cancer predisposition genes.

Characteristics of Patients With Mutations in Pancreatic Cancer Predisposition Genes

Overall, 167 of 3030 patients (5.5%; 95% CI, 4.7%-6.4%) with pancreatic cancer had deleterious mutations in 1 of the 6 predisposition genes: *CDKN2A*, *TP53*, *MLH1*, *BRCA2*, *ATM*, and *BRCA1* (**Table 4** and eTable 18 in the Supplement). Among all tested patients, 27 of 343 patients (7.9%; 95% CI, 5.3%-11.2%) with a family history of pancreatic cancer and 140 of 2687 patients (5.2%; 95% CI, 4.4%-6.1%) with no family history of pancreatic cancer had a mutation in 1 of the 6 predisposition genes (P = .06) (Table 4; eTable 18). Thus, family history of pancreatic cancer did not inform on the presence of 83.8% of mutations. In addition, 40 of 495 patients (8.1%; 95% CI, 5.8%-10.8%) with another primary cancer diagnosis prior to pancreatic cancer had mutations in these genes (Table 4). Although prior primary cancer was significantly associated with mutation status (OR, 1.67; 95% CI, 1.17-2.48; P = .009), 76% of patients with mutations (127/167) did not exhibit this phenotype. Overall, significant associations were observed between mutations in the 6 predisposition genes combined and advanced stage of disease (resectable: 48/850; locally advanced: 50/1115; and metastatic: 67/1056; P = .04), personal history of other cancers (OR, 1.67; 95% CI, 1.17-2.48; P = .009), family history of breast cancer (OR, 1.58; 95% CI, 1.11-2.23; P = .01), or family history of common epithelial cancers (OR, 1.40; 95% CI, 1.01-1.92; P = .04) (Table 4). Patients with

Table 3. Comparisons of Mutation Carriers by Panel Gene Between Pancreatic Cancer Cases and gnomAD Controls

Genes	Cases			gnomAD Controls			Cancer Risk[a]	
	Cases With Mutations, No.	Individuals Tested, No.[b]	Carrier Frequency, %	Controls With Mutations, No.	Individuals Tested, No.	Carrier Frequency, %	Odds Ratio (95% CI)	Adjusted *P* Value[c]
Genes Significantly Associated With Pancreatic Cancer								
CDKN2A	9	2999	0.30	15	99 493	0.02	12.33 (5.43-25.61)	<.001
TP53	6	2999	0.20	25	104 162	0.02	6.70 (2.52-14.95)	<.001
MLH1	4	2999	0.13	25	103 526	0.02	6.66 (1.94-17.53)	.01
BRCA2	57	2999	1.90	313	102 739	0.30	6.20 (4.62-8.17)	<.001
ATM	69	2999	2.30	386	104 016	0.37	5.71 (4.38-7.33)	<.001
BRCA1	18	2999	0.60	208	104 122	0.20	2.58 (1.54-4.05)	.002
Genes Not Significantly Associated With Pancreatic Cancer								
NF1	4	2999	0.13	31	103 812	0.03	3.70 (1.11-9.22)	.25
PALB2	12	2999	0.40	153	104 169	0.15	2.33 (1.23-4.01)	.09
CDH1	1	2999	0.03	15	102 110	0.01	2.30 (0.13-11.39)	>.99
MSH6	6	2999	0.20	101	102 802	0.10	1.98 (0.77-4.14)	>.99
FANCC	8	2999	0.27	129	104 042	0.12	1.69 (0.76-3.21)	>.99
MSH2	1	2999	0.03	16	103 327	0.02	1.58 (0.09-7.54)	>.99
BARD1	4	2999	0.13	86	102 189	0.08	1.32 (0.40-3.15)	>.99
CHEK2	33	2999	1.10	572	102 856	0.56	1.31 (0.91-1.83)	>.99
RAD51C	3	2999	0.10	94	104 128	0.09	1.11 (0.27-2.97)	>.99
NBN	4	2999	0.13	125	103 912	0.12	0.86 (0.27-2.04)	>.99
BRIP1	4	2999	0.13	194	104 071	0.19	0.78 (0.28-1.71)	>.99
MRE11A	2	2999	0.07	96	104 071	0.09	0.71 (0.12-2.23)	>.99
PMS2	2	2999	0.07	86	101 976	0.08	0.70 (0.12-2.22)	>.99

Abbreviation: gnomAD, Genome Aggregation Database.

[a] Logistic regression analysis weighted by race and ethnicity.

[b] Analyses do not include cases with race/ethnicity reported as other (n = 19) or cases with missing race/ethnicity information (n = 12), for a total denominator of 2999.

[c] Adjusted by Bonferroni correction for 19 genes with mutations from 21 tested genes.

mutations in these 6 genes also had a significantly earlier mean age of diagnosis (62.5 vs 65.5 years; $P < .001$) (Table 4). In particular, mutations in *BRCA2* alone were significantly associated with an earlier age at diagnosis of pancreatic cancer (mean age, 60.5 years vs 63.3 years for noncarriers; $P = .01$) (eTable 19 in the Supplement).

When comparing characteristics of mutation carriers and noncarriers by individual gene, only patients with deleterious mutations in *CDKN2A* were more likely to have a family history of pancreatic cancer (OR, 7.91; 95% CI, 2.19-28.57; adjusted $P = .005$). Similarly, patients with mutations in *BRCA2* (OR, 2.07; 95% CI, 1.19-3.50; adjusted $P = .04$) were more likely to have a family history of breast cancer (eTable 18 in the Supplement).

Associations Between Germline Mutations and Survival

The median overall survival for patients with mutations in the 6 genes associated with pancreatic cancer was 13.6 months (95% CI, 11.5-15.7 months), whereas overall survival for patients without mutations was 11.4 months (95% CI, 10.8-12.1 months). The association between mutation status in these genes and survival was not statistically significant (hazard ratio, 0.86; 95% CI, 0.72-1.02; $P = .09$) (eFigure in the Supplement). There was no evidence of deviation from proportional hazards for the mutation carrier status ($\chi^2 = 0.03$; $P = .87$).

Discussion

In this case-control study, mutations in 6 genes (*ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, *MLH1*, and *TP53*) were found to be associated with pancreatic cancer and were found in 5.5% of patients with pancreatic cancer. Mutations in *CDKN2A* yielded the highest risks of pancreatic cancer, although the frequency of mutations was low (0.33%). Mutations in *ATM*, a gene that encodes a multifunctional protein involved in regulating the cellular response to DNA damage,[33] were significantly associated with pancreatic cancer. Homozygous *ATM* mutations cause ataxia-telangiectasia,[34] and heterozygous *ATM* mutations have been associated with moderate risks of breast cancer[1] but not pancreatic cancer.[35] In the current study, no substantial change in *ATM* associations were observed when excluding individuals with a personal or family history of breast cancer, suggesting that the association with pancreatic cancer was independent of breast cancer effects. Whether missense mutations, such as in *ATM* c.7271T>G (p.Val2424Gly), which has been associated with substantially increased risk of breast cancer (OR, 8.0; 95% CI, 2.3-27.4),[36] have alternative effects on pancreatic cancer risk remains to be determined. Mutations in *TP53* were also significantly associated with pancreatic cancer, but it was not known if the patients carrying

Table 4. Associations Between Characteristics of Patients With Pancreatic Cancer by Mutation Carrier Status of 6 Pancreatic Cancer Predisposition Genes

Characteristics	No. (%) of Case Patients[a]		
	Patients With Mutations (n = 167)[b]	Patients Without Mutations (n = 2863)	*P* Value[c]
Age at diagnosis, y			
Mean (SD)	62.5 (10.5)	65.5 (10.7)	<.001
Range	39.0-90.0	20.0-90.0	
Sex			
Female	64 (38.3)	1244 (43.5)	.22
Male	103 (61.7)	1619 (56.5)	
Race/ethnicity			
African American	3 (1.8)	47 (1.6)	.10
Hispanic	2 (1.2)	40 (1.4)	
Asian	1 (0.6)	10 (0.4)	
Non-Hispanic white	157 (94.0)	2739 (95.7)	
Other[d]	1 (0.6)	18 (0.6)	
Missing	3 (1.8)	9 (0.3)	
Personal history of other cancers			
Yes	40 (24.0)	455 (15.9)	.009
No	127 (76.0)	2408 (84.1)	
Disease staging			
Resectable	48 (28.7)	802 (28.0)	.04
Locally advanced	50 (29.9)	1065 (37.2)	
Metastatic	67 (40.1)	989 (34.5)	
Missing	2 (1.2)	7 (0.2)	
Family history (first- or second-degree relative)			
Pancreatic cancer			
No	140 (83.8)	2547 (89.0)	.06
Yes	27 (16.2)	316 (11.0)	
Breast cancer			
No	116 (69.5)	2239 (78.2)	.01
Yes	51 (30.5)	624 (21.8)	
Ovarian cancer			
No	154 (92.2)	2724 (95.1)	.13
Yes	13 (7.8)	139 (4.9)	
Gynecologic (nonovarian)/endometrial cancer			
No	157 (94.0)	2711 (94.7)	.84
Yes	10 (6.0)	152 (5.3)	
Colorectal cancer			
No	140 (83.8)	2377 (83.0)	.87
Yes	27 (16.2)	486 (17.0)	
Pancreatic, breast, ovarian, gynecologic (nonovarian), or colorectal			
No	86 (51.5)	1711 (59.8)	.04
Yes	81 (48.5)	1152 (40.2)	

[a] Data are No. (%) of case patients unless otherwise noted.

[b] Mutations in 6 genes significantly associated with pancreatic cancer: *ATM*, *BRCA1*, *BRCA2*, *CDKN2A*, *MLH1*, and *TP53*.

[c] Wilcoxon test for age of diagnosis; χ^2 test for all others.

[d] Including multiracial, American Indian/Alaskan Native, and Native Hawaiian/other Pacific Islander.

these mutations exhibited Li-Fraumeni syndrome phenotypes or had a family history of Li-Fraumeni syndrome.

These results were consistent with a recent study of 854 patients with pancreatic adenocarcinoma that identified mutations in these 6 genes and *PALB2* in 3.5% of patients.[12] Although mutations in *PALB2* are thought to increase risk of pancreatic cancer,[7,8] the current study did not find a significant association after Bonferroni correction. *CHEK2* mutations were also not significantly associated with pancreatic cancer, even though mutations were frequently observed. It may be that mutations in *CHEK2* and other cancer predisposition genes can provide information about risk of other cancers in patients and their relatives.

Given the high frequency of predisposing mutations in this series of patients (>5%) and the absence of effective predictors of mutations, genetic testing of all patients with pancreatic

cancer by panel tests may be warranted. In addition, genetic testing and identification of germline mutations may have implications for the relatives of patients with pancreatic cancer because of risks of pancreatic and other cancers. Overall, genetic testing guidelines for patients with pancreatic cancer and for their unaffected relatives must be developed. Currently the National Comprehensive Cancer Network does not provide guidelines for selection of patients with pancreatic cancer for multigene panel testing,[37] instead focusing on patients with pancreatic cancer in the context of hereditary breast and ovarian cancer. The best predictors of mutations in patients with pancreatic cancer in the current study were a personal history of another primary cancer, a personal history of breast cancer, and a family history of 1 or more first- or second-degree relatives with epithelial cancers (pancreatic, breast, ovarian, endometrial, or colorectal). However, the specificity for mutations was too low for effective selection of patients for clinical genetic testing.

Although patients with *BRCA1* and *BRCA2* predisposing mutations may derive therapeutic benefit from testing because tumors may display sensitivity to platinum agents or poly adenosine diphosphate–ribose polymerase (PARP) inhibitors,[38,39] it remains to be determined whether patients with germline or somatic mutations in other predisposition genes will benefit from these and other targeted therapies. Benefits of panel testing may also extend to cancer screening and prevention. The International Cancer of the Pancreas Screening (CAPS) Consortium[4] and the American College of Gastroenterology (ACG) guidelines,[40] based on expert opinion, currently recommend imaging surveillance for individuals with greater than 5% lifetime risk of pancreatic cancer due to mutations in *STK11* (RefSeq NM_000455.4), *CDKN2A*, and hereditary pancreatitis genes; individuals with mutations in *BRCA1*, *BRCA2*, *ATM*, *PALB2*, or mismatch repair genes and a first- or second-degree relative with pancreatic cancer; and individuals with a first-degree relative with pancreatic cancer. Thus, the surveillance guidelines already include all of the predisposition genes identified in this study. In addition, the value of surveillance based on germline mutations, but not family history alone, has been empirically demonstrated,[3,4,41,42] supporting the potential importance of mutation testing. The genes included in the CAPS and ACG guidelines are consistent with results from the current study except that the moderate risks associated with *BRCA1* mutations may not be sufficient to warrant this level of intervention. Given the high case-fatality rate for pancreatic cancer, testing for inherited cancer susceptibility may identify candidates for participation in innovative approaches to screening and prevention.

Limitations

This study has several limitations. First, public reference controls were used to estimate the prevalence of each of the 21 cancer predisposition genes in race/ethnicity–matched general populations. However, extensive data cleaning and filtering were used in an effort to normalize the pancreatic cancer cases and the control data. These large reference control data sets were needed because study-matched control data sets are generally not of sufficient size for association studies because of the rarity of individual deleterious mutations in the general population. In this study, both the ExAC non-TCGA and gnomAD reference data sets resulted in very similar findings. Despite partial overlap in these data sets, this consistency strongly suggests that the pancreatic cancer predisposition genes identified in this study are drivers of pancreatic cancer risk in the general population. Second, the custom panel of 21 genes used in this study did not account for all possible cancer predisposition genes, and the possibility remains that other untested genes may contribute to risk of pancreatic cancer. Third, there are a number of variants of uncertain significance in genes with insufficient data for classification as deleterious or neutral. Fourth, because cases were identified from Mayo Clinic populations in Minnesota, Arizona, and Florida and were younger and less likely to be black or Hispanic than pancreatic cancer patients included in the Surveillance, Epidemiology, and End Results registry, study results may lack generalizability. Further analyses in more racially and ethnically diverse populations are necessary to identify other potential pancreatic cancer susceptibility genes. Fifth, the study did not have sufficient information to estimate lifetime probability of cancer (penetrance) in carriers of the predisposition gene mutations.

Conclusions

In this case-control study, mutations in 6 genes associated with pancreatic cancer were found in 5.5% of all pancreatic cancer patients, including 7.9% of patients with a family history of pancreatic cancer and 5.2% of patients without a family history of pancreatic cancer. Further research is needed for replication in other populations.

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