Null Results in Brief

Polymorphic Variants in Hereditary Pancreatic Cancer Genes Are Not Associated with Pancreatic Cancer Risk

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Abstract

Background: **Inherited risk of pancreatic cancer has been associated with mutations in several genes, including *BRCA2*, *CDKN2A* (p16), *PRSS1*, and *PALB2*. We hypothesized that common variants in these genes, single nucleotide polymorphisms (SNP), may also influence risk for pancreatic cancer development.**
Methods: **A clinic-based case-control study in non-Hispanic white persons compared 1,143 patients with pancreatic adenocarcinoma with 1,097 healthy controls. Twenty-eight genes directly and indirectly involved in the Fanconi/BRCA pathway (includes *BRCA1*, *BRCA2*, and *PALB2*) were identified and 248 tag SNPs were selected. In addition, 11 SNPs in *CDKN2A*, *PRSS1*, and *PRSS2* were selected. Association studies were done at the gene level by principal components analysis, whereas recursive partitioning analysis was used to investigate pathway effects. At the individual SNP level, adjusted additive, dominant, and recessive models were investigated, and gene-environment interactions were also assessed.**
Results: **Gene level analyses showed no significant association of any genes with altered pancreatic cancer risk. Multiple single SNP analyses showed associations, which will require replication. Exploratory pathway analyses by recursive partitioning showed no association between SNPs and risk for pancreatic cancer.**
Conclusion: **In a candidate gene and pathway SNP association study analysis, common variations in the Fanconi/BRCA pathway and other candidate familial pancreatic cancer genes are not associated with risk for pancreatic cancer.** (Cancer Epidemiol Biomarkers Prev 2009;18(9):2549-52)

Introduction

The double-stranded break repair pathway is a unique pathway of response to DNA cross-linking and subsequent repair; the exact mechanism of which is as yet undetermined (1). High-penetrance mutations in double-stranded break repair genes such as *BRCA1* and *BRCA2* increase susceptibility to cancer, most notably breast and ovarian cancers (2, 3), but also have been reported in familial pancreatic cancer kindreds (4-6). Truncating mutations in *FANCC* and *FANCG* have been reported in a few cases of sporadic young-onset pancreatic cancer, although their contribution to pancreatic cancer risk is unclear (7-9), whereas truncating mutations in *PALB2* have also recently been documented in familial pancreatic cancer kindreds (10). Other genes involved in hereditary susceptibility to pancreatic cancer include *CDKN2A* (familial melanoma) and *PRSS1* (hereditary pancreatitis).

We hypothesized that low-penetrance polymorphisms could confer a modest increase in risk for pancreatic cancer. Unlike highly penetrant truncating mutations or large deletions, these polymorphic variants may be associated with alterations in gene function or expression to a more limited extent.

Materials and Methods

Written informed consent was obtained from each subject for participation in this study and provision of biospecimens. This study was approved by the Mayo Clinic Institutional Review Board.

Cases. From October 2000 to March 2007, patients with clinically documented pancreatic adenocarcinoma were recruited to a prospective registry during their visit to Mayo Clinic (Rochester, Minnesota or Jacksonville, Florida), as previously described (11).

Controls. From May 2004 to February 2007, healthy controls were recruited from the General Internal Medicine clinics at Mayo Clinic (Rochester). Controls were frequency matched to cases on sex, location of residence, age at time of recruitment (in 5-y increments), and race/ethnicity, as previously described (11).

Single Nucleotide Polymorphism Selection. A linkage disequilibrium–based tag single nucleotide polymorphism

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(SNP) strategy was used (12). Known genes directly and indirectly involved in double-stranded break repair were identified ($n = 28$; Table 1), as well as *PRSS1*, *PRSS2*, and *CDKN2A*. Genotype data were compiled from HapMap, SeattleSNPs, and National Institute of Environmental Health Sciences SNPs. We used LdSelect software (version 1.0; ref. 13) for SNP selection from each gene, including 5 kb upstream/downstream, using criteria of $r^2 \geq 0.9$ and minor allele frequency > 0.05. A total of 259 SNPs in 31 genes were selected.

Genotyping. DNA samples were analyzed in the Mayo Clinic Genotyping Shared Resource using an Illumina Golden Gate Custom 768-plex OPA panel using the standard protocol. BeadStudio II software was used to analyze the data and prepare reports. DNA samples from cases and controls were randomly placed on plates.

Quality Control. Positive and negative controls were run in parallel to assess the quality of genotyping. All genotype clusters were manually inspected by a molecular geneticist (J.M.C.). Call rates were high for SNPs overall, at 99.6% rate for samples, and 95.1% for loci. Forty-seven pairs were used for duplicate concordance, with a 99.9% concordance rate. Eighteen SNPs failed to amplify and 91 samples had a call rate of 0.

Statistical Methods. Risk factor questionnaires were completed by 100% of controls and 71% of cases. For cases missing risk factor questionnaires, clinical data were extracted from available medical records, with a high intermethod reliability as previously reported (12). Hardy-Weinberg equilibrium was confirmed in controls for each SNP by χ^2 test. Those failing were excluded from the analysis ($n = 2$). A principal components analysis (14) approach was used to test for an overall association between disease and the multiple SNPs genotyped within each gene. The necessary number of principal components needed for each gene was determined using a 90% explained variance criteria. Once the necessary principal components were determined, multivariable logistic regression models were constructed to assess the significance of each gene. We had 88% power to detect an odds ratio of 1.35 with a minor allele frequency of 0.10 and 90% power to detect an odds ratio of 1.25 with a minor allele frequency of 0.25.

Allele associations were assessed using the Pearson χ^2 or Fisher's exact test (when sample sizes were small), and genotype associations were assessed using the Cochran-Armitage trend test. Multivariate analysis compared genotype frequencies in cases and controls adjusted for age, sex, ever/never smoking status, family history of pancreatic cancer (first degree), and body mass index (BMI).

Results

Demographic characteristics of cases and controls are shown in Table 1. There were differences in BMI, sex, percent of ever smokers, percent reporting a first-degree relative with pancreatic cancer, and diabetes. Adjusted principal components analyses for each gene (Table 2) showed no association for any gene with pancreatic cancer risk. Logistic regression analyses at the single SNP level for each gene were also done using multivariable additive, dominant, and recessive models. Statistically significant associations are shown in Supplementary Table S1, although no associations would

Table 1. Demographic and clinical characteristics of cases and controls

Variable	Cases (n = 1,143)	Controls (n = 1,097)	P*
Age at diagnosis (cases) or study entry (controls; +SD)	65.5 ±10.7	65.6 ±10.8	0.79
Age (<60 y)	329 (29%)	297 (27%)	0.37
Male sex	668 (58%)	557 (51%)	<0.001
Non-Hispanic whites†	1,143 (100%)	1,097 (100%)	
Ever smoker	682 (60%)	505 (46%)	<0.001
Smoking status			<0.001
Never smoker‡	455 (40%)	592 (54%)	
Former smoker	527 (47%)	458 (42%)	
Current smoker	148 (13%)	41 (4%)	
Missing	13	6	
Years smoked (+SD)	22.4 ±16.9	18.2 ±14.0	<0.001
Pack-years smoked (+SD)	17.0 ±23.0	9.3 ±17.2	<0.001
BMI (+SD)	27.8 ±5.5	27.2 ±4.7	0.010
Region			<0.001
Minnesota, Iowa, or Wisconsin (Tristate)	579 (51%)	748 (68%)	
North Dakota or South Dakota	94 (8%)	40 (4%)	
Other United States	448 (39%)	308 (28%)	
Other country	22 (2%)	1 (0%)	
Diabetes mellitus			<0.001
No	801 (70%)	1,008 (92%)	
Yes	342 (30%)	89 (8%)	
Diabetes mellitus (>2 y before pancreatic cancer diagnosis)	224	0	
Pancreas cancer stage at enrollment			
Resectable	328 (29%)	0 —	
Locally advanced	379 (33%)	0 —	
Metastatic	430 (38%)	0 —	
Not otherwise specified	6 (1%)	0 —	
Family history of pancreatic cancer (first degree)	79 (7%)	43 (4%)	0.002

*P value unadjusted.
†Only Non-Hispanic whites included in the analysis.
‡Defined as <100 cigarettes in lifetime.

Table 2. Gene level principal components analysis of pancreatic cancer risk associations

Gene name	No. SNPs	Chromosome location	Unadjusted P*	Adjusted P†	Principal components‡
ATM	11	11q22.3	0.7714	0.70308	5
ATR	10	3q22-24	0.7710	0.69677	5
BRCA1	7	17q21	0.7091	0.70542	3
BRCA2	22	13q12.3	0.8191	0.69003	7
BRIP1	16	17q22	0.2312	0.57038	6
CDKN2A	7	9p21	0.8854	0.80569	5
CHEK1	10	11q22-23	0.5427	0.43054	5
FANCA	12	16q24.3	0.8330	0.99897	4
FANCC	17	9q22.3	0.5472	0.18791	6
FANCD2	5	3p25.3	0.2406	0.19372	4
FANCE	4	6p21-22	0.6380	0.34821	2
FANCF	3	11p15	0.1601	0.16791	2
FANCG	1	9p13	0.7913	0.91465	1
FANCL (*PHF9*)	22	2p16.1	0.3318	0.45418	5
FANCM	7	14q21.3	0.2517	0.19150	4
PALB2	5	16p12	0.1992	0.38942	3
MCPH1	1	8p23	0.1268	0.20708	1
MDC1	2	6pter-p21.3	0.1056	0.12931	2
MRE11A	18	11q21	0.3097	0.54468	4
NBN (*NBS1*)	21	8q21	0.3246	0.52926	8
PRSS1	1	7q35	0.3033	0.40413	1
PRSS2	3	7q35	0.8843	0.87790	2
RAD50	4	5q31	0.9222	0.98552	3
RAD51	6	15q15.1	0.5609	0.88217	3
RAD51L3	4	17q11	0.3954	0.42135	3
RAD54	1	8q21.3-22	0.4097	0.17993	1
RBBP8	7	18q11.2	0.5038	0.69827	3
SHFM1	8	17q21	0.8274	0.72815	4
TOPBP1	12	3q22.1	0.3638	0.23532	4
TP53BP1	9	15q15-21	0.9888	0.98525	3
USP1	3	1p31-32	0.2560	0.27569	2
Total					
31	259				

*Likelihood ratio test.
†Likelihood ratio test adjusted for age, sex, ever/never smoking, BMI, diabetes, and first-degree family history of pancreatic cancer.
‡Number of principal components needed to meet 90% explained variance criteria.

remain significant after Bonferroni adjustment. The proportion of positive findings (4.0-4.2% for the three models) are within the range expected by chance ($\alpha = 0.05$). Recursive partitioning analysis was done as an exploratory method to assess SNP-SNP associations within the pathway and SNP-environment interactions. No partitions by SNPs reached statistical significance in these analyses, and no interaction was identified from this analysis (Supplementary Fig. S1).

Discussion

This large case-control study designed to assess common variants in genes associated with hereditary cancer or familial pancreatic cancer did not find associations of polymorphic variants with pancreatic cancer risk. Therefore, we conclude that functional variations of modest effect that might be associated with common polymorphisms in these genes do not seem to confer increased risk for pancreatic cancer. For instance, for the DNA repair genes, it is probable that only somatic loss of heterozygosity in the setting of a defective allele results in a neoplastic transformation, but minor germ-line variation in DNA repair capacity does not seem to meaningfully influence risk for pancreatic cancer. When high-throughput DNA sequencing is practically scaled to large numbers of subjects, it may be possible to identify high-penetrance mutations in key pathways that confer risk in "sporadic" pancreatic cancer patients as well as in the familial pancreatic cancer setting.

Conclusion

In a tag SNP analysis of genes associated with familial pancreatic cancer and genes associated with DNA double-stranded break repair, polymorphic variants were not associated with risk for pancreatic cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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