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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 (SNPs), 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 performed at the gene level by principal components analysis, while recursive partitioning analysis was utilized 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 demonstrated associations, which will require replication. Exploratory pathway analyses by recursive partitioning demonstrated 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.

Introduction

The double-stranded break repair pathway is a unique pathway of response to DNA crosslinking 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, though their contribution to pancreatic cancer risk is unclear,(7–9) while truncating mutations in *PALB2* have also recently been documented in familial pancreatic cancer

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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.

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 through 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-year increments), and race/ethnicity, as previously described.(11)

Single Nucleotide Polymorphism Selection

A linkage disequilibrium (LD) based tag-SNP strategy was employed. (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 NIEHS SNPs. We used LdSelect software (Version 1.0, Seattle, Washington) (13) for SNP selection from each gene including 5kb upstream/downstream using criteria of $r^2 \geq 0.9$ and minor allele frequency (MAF) ≥ 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 (JC). 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 (RFQs) were completed by 100% of controls and 71% of cases. For cases missing RFQs, clinical data were extracted from available medical records, with a high inter-method 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 utilized in order 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 OR of 1.35 with a MAF 0.10, and 90% power to detect an OR of 1.25 with a MAF of 0.25. Allele associations were assessed using the Pearson χ^2 or Fisher 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 (1^{st} 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 performed using multivariable additive, dominant, and recessive models. Statistically significant associations are shown in Supplemental Table s1, though no associations would 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 performed 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. (Supplemental Figure 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 appear 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 germline variation in DNA repair capacity does not appear 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Demographic and Clinical Characteristics of Cases and Controls

Variable	Cases (N=1143)		Controls (N=1097)		P ***
Age at diagnosis (Cases) or study entry (Controls) (±SD)	65.5	± 10.7	65.6	± 10.8	0.79
Age < 60	329	(29%)	297	(27%)	0.37
Male Sex	668	(58%)	557	(51%)	<.001
Non-Hispanic Whites*	1143	(100%)	1097	(100%)	
Ever-Smoker	682	(60%)	505	(46%)	<.001
Smoking status					<.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	<.001
Pack-years smoked (±SD)	17.0	± 23.0	9.3	± 17.2	<.001
Body Mass Index (±SD)	27.8	± 5.5	27.2	± 4.7	0.010
Region					<.001
Minnesota, Iowa, or Wisconsin (Tristate)	579	(51%)	748	(68%)	
North Dakota or South Dakota	94	(8%)	40	(4%)	
Other USA	448	(39%)	308	(28%)	
Other Country	22	(2%)	1	(0%)	
Diabetes Mellitus (DM)					<.001
No	801	(70%)	1008	(92%)	
Yes	342	(30%)	89	(8%)	
DM (> 2 yrs before Pancreatic Cancer dx)	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 (1st degree)	79	(7%)	43	(4%)	0.002

*Only Non-Hispanic whites included in the analysis

** Defined as less than 100 cigarettes in lifetime.

*** p-value unadjusted

Table 2

Gene-Level Principal Components Analysis of Pancreatic Cancer Risk Associations

Gene Name	# SNPs	Chromosome Location	Unadjusted p-value *	Adjusted p-value **	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

Gene Name	# SNPs	Chromosome Location	Unadjusted p-value *	Adjusted p-value **	Principal Components ***
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, body mass index, diabetes, 1st degree family history of pancreatic cancer

*** Number of Principal Components needed to meet 90% explained variance criteria