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Inflammation-Related Gene Variants as Risk Factors for Pancreatic Cancer

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Abstract

BACKGROUND—Recent reports support an association between chronic inflammation and progression to pancreatic cancer (PC).

METHODS—This case-control, candidate gene association study evaluated 1,354 Caucasian patients with pancreatic ductal adenocarcinoma and 1,189 healthy Caucasian controls. We genotyped 1,538 SNPs in 102 genes from inflammatory pathways involving *NF-κB*. Primary tests of association assumed a multiplicative (log-additive) genotype effect; secondary analyses examined dominant, additive and recessive SNP effects.

RESULTS—After adjusting for known risk factors for PC, single SNP analysis revealed an association between four SNPs in *NOS1* and one in the *CD101* gene with PC risk. These results however were not replicated in a PC case-control and cohort population.

CONCLUSION—*NOS1* and *CD101* may be associated with a risk of PC; however, these findings did not replicate in other pancreatic cancer populations. Future research is needed into the possible role of *NOS1* and *CD101* for PC.

IMPACT STATEMENT—This research demonstrates a lack of association between genetic variation in 102 inflammation-related genes and pancreatic cancer. Future research is needed into the possible role of other inflammation-related genes and pancreatic cancer risk.

Keywords

Nitric oxide synthase; inflammation; pancreatic cancer; single nucleotide polymorphisms; gene

Introduction

Chronic inflammation has been recognized as a contributing factor in the development of a subset of highly lethal pancreatic adenocarcinomas (PC) (1). Studies linking chronic

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inflammation to PC report an upregulation of cyclooxygenase (*COX*) (2) and nitric oxide (*NO*) gene expression (3), important mediators of inflammation in PC tissue specimens. The trigger for upregulation of *COX* and *NO* is the transcription factor, nuclear factor kappa-light-chain-enhancer of activated B (*NF-κB*). NF-κB activates these genes and others that are involved in inflammation and apoptosis and appears to promote pancreatic cell growth by inhibition of apoptosis (4). We hypothesized that genetic polymorphisms in inflammation-related genes involving *NF-κB*-related inflammatory pathways are associated with risk of PC.

Methods

PC patients were recruited prospectively to a PC research registry using processes approved by the Mayo Clinic Institutional Review Board. Consenting patients completed a comprehensive questionnaire, donated a blood sample, and provided medical release for study of archival tissue.

Subjects

Cases were patients with histologically proven pancreatic ductal adenocarcinoma evaluated at Mayo Clinic from 2000 to 2008. Controls were healthy, clinic-based patients without a personal history of cancer (except non-melanoma skin cancer) and were frequency matched to cases for age (± 5 years), sex, race, and geographic region of residence(5).

Candidate Gene and Single Nucleotide Polymorphism Selection

Candidate genes were selected using a combination of literature review and the bioinformatics tools Ingenuity® Systems (Redwood, CA), and MetaCore™ from GeneGo, Inc. to identify genes involved in the pathogenesis of PC that are in the *NF-κB*-related inflammatory pathways. Genotyping and Quality Control measures were followed as reported previously (5). Call rates for SNPs were 99.2% and 97.3%. Eighty-four of 1,538 SNPs failed to amplify.

Statistical Analysis

Unconditional logistic regression analysis was used to estimate odds ratio (OR) and 95% confidence intervals (CI) for the risk of PC with each SNP. The regression model included covariates of: age, sex, smoking status, body mass index (kg/m^2), family history of PC, and preexisting diabetes (>2 years). Primary tests of association assumed a multiplicative (log-additive) genotype effect, equivalent to the Armitage test for trend. To account for multiple testing, SNPs were deemed significantly associated with PC if $P < 0.001$.

Validation Studies

Genotyping data from PanScan, the Pancreatic Cancer Cohort Consortium, and Pancreatic Cancer Case-Control Consortium (6, 7) were obtained from dbGap (8). Imputation in non-Mayo samples was accomplished with MACH software (9) using HapMap. The imputed allele dosage was used to validate SNPs of interest. All analyses were unadjusted.

Results

Demographics for all subjects are reported in Table 1. Single SNP analysis revealed an association between four SNPs in *NOS1* one SNP in the *CD101* gene and the risk of PC (Table 2). An additional 14 and four imputed SNPs with p values = $5E^{-4}$-$8E^{-4}$ were observed in CD101 and NOS1 respectively (data not shown). The significant SNPs failed confirmation in the validation set using both PanScan data sets.

Discussion

We report the results of a case-control study evaluating the risk of inflammation-related gene variants with PC. To date, this is the largest evaluation of risk for PC that focuses primarily on genes in the inflammation pathways involving *NF-κB*. The 102 genes code for proinflammatory mediators, inhibitors, or activators of *NF-κB*. Polymorphisms of *NOS1* and *CD101* demonstrated increased (*NOS1*) and decreased risk (*CD101*) risk association for PC. The *NOS1* SNPs were in high linkage disequilibrium (LD) and located across a region of two LD blocks on chromosome 12. Imputed and genotyped SNPs from *CD101* were located in a single LD block. Attempts to validate these data utilizing the PanScan cohort and PanScan case-control studies of PC were unsuccessful. Potential reasons for the lack of validation include the differences in study designs and accrual methods of the three data sets and the inability to adjust the PanScan data sets.

Conclusion

Of the 102 genes evaluated, *NOS1* and *CD101* may be associated with an increased risk and decreased risk of PC respectively. However, these findings did not replicate in a follow-up study of two PC populations. Future research is needed to determine the role, if any, of *NOS1* and *CD101* for risk of PC.

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Table 1

Patient Characteristics [a]

	Control (N=1171)	Case (N=1329)	Total (N=2500)	p value
Sex				0.0010
Female	574 (49%)	564 (42.4%)	1138 (45.5%)	
Male	597 (51%)	765 (57.6%)	1362 (54.5%)	
Race				
White/Caucasian	1171 (100%)	1329 (100%)	2500 (100%)	
Age at time of pancreatic cancer diagnosis				0.3356
N	1171	1329	2500	
Mean (SD)	66.1 (10.51)	65.7 (10.71)	65.9 (10.62)	
Median	67.0	67.0	67.0	
Q1, Q3	59.0, 74.0	58.0, 74.0	59.0, 74.0	
Range	(30.0–95.0)	(28.0–91.0)	(28.0–95.0)	
Ever Smoker				<0.0001
No	627 (53.5%)	532 (40%)	1159 (46.4%)	
Yes	544 (46.5%)	797 (60%)	1341 (53.6%)	
Pack Years				<0.0001
N	1146	1083	2229	
Mean (SD)	9.6 (17.61)	14.8 (22.16)	12.1 (20.12)	
Median	0.0	0.4	0.0	
Q1, Q3	0.0, 12.8	0.0, 25.0	0.0, 19.0	
Range	(0.0–125.0)	(0.0–140.0)	(0.0–140.0)	
Diabetes > 2 years prior to study entry				<0.0001
No	1106 (94.4%)	1156 (87%)	2262 (90.5%)	
Yes	65 (5.6%)	173 (13%)	238 (9.5%)	
Body Mass Index				<0.0001
N	1171	1329	2500	
Mean (SD)	27.2 (4.63)	28.4 (5.69)	27.8 (5.26)	
Median	26.6	27.6	27.1	
Q1, Q3	24.0, 29.5	24.4, 31.1	24.3, 30.3	
Range	(17.7–54.2)	(15.3–59.0)	(15.3–59.0)	
First Degree Relative with Pancreatic Cancer				0.0029
No	1127 (96.2%)	1244 (93.6%)	2371 (94.8%)	
Yes	44 (3.8%)	85 (6.4%)	129 (5.2%)	

[a] Only subjects included in final analysis

Table 2

Genotyping and Validation Results

		Primary Set				PanScan (Cohort)					PanScan (Case-Control)				
SNP	**Location**	**Number Cases**	**Number Controls**	**OR**	**P-value**	**Number Cases**	**Number Controls**	**Frequency of Coded Allele**	**OR**	**P-value**	**Number Cases**	**Number Controls**	**Frequency of Coded Allele**	**OR**	**P-value**
CD101, rs10923193	117338325	**1312**	**1169**	**0.8**	**0.000903**	**1408**	**1461**	**0.27**	**1.04**	**0.48**	**1090**	**1168**	**0.26**	**0.96**	**0.58**
G/G		755 (0.58)	613 (0.52)	1	1										
A/G		492 (0.42)	460 (0.35)	0.87	0.00126										
A/A		65 (0.05)	96 (0.08)	0.54	0.00126										
dominant				0.81	0.013										
recessive				0.58	0.000996										
NOS1, rs3782203	116204794	**1328**	**1171**	**1.24**	**0.0016**	**1408**	**1461**	**0.2**	**1**	**0.95**	**1090**	**1168**	**0.2**	**0.97**	**0.64**
G/G		780 (0.59)	748 (0.64)	1	1										
A/G		464 (0.4)	378 (0.28)	1.17	0.0733										
A/A		84 (0.06)	45 (0.04)	1.8	0.00222										
dominant				1.23	0.0109										
recessive				1.7	0.005										
NOS1, rs9658350	116208811	**1327**	**1170**	**1.24**	**0.00175**	**1408**	**1461**	**0.2**	**0.99**	**0.87**	**1090**	**1168**	**0.2**	**0.96**	**0.58**
A/A		774 (0.58)	742 (0.63)	1	1										
G/A		469 (0.4)	383 (0.29)	1.16	0.0779										
G/G		84 (0.06)	45 (0.04)	1.79	0.00225										
dominant				1.23	0.0124										
recessive				1.7	0.005										
NOS1, rs532967	116216722	**1329**	**1170**	**1.25**	**0.00159**	**1408**	**1461**	**0.18**	**0.98**	**0.76**	**1090**	**1168**	**0.18**	**0.92**	**0.31**
G/G		817 (0.61)	780 (0.67)	1	1										
A/G		447 (0.38)	357 (0.27)	1.19	0.0502										
A/A		65 (0.05)	33 (0.03)	1.89	0.00356										
dominant				1.25	0.00972										
recessive				1.79	0.00733										

		Primary Set				PanScan (Cohort)					PanScan (Case-Control)				
SNP	**Location**	**Number Cases**	**Number Controls**	**OR**	**P-value**	**Number Cases**	**Number Controls**	**Frequency of Coded Allele**	**OR**	**P-value**	**Number Cases**	**Number Controls**	**Frequency of Coded Allele**	**OR**	**P-value**
NOS1, rs547954	116238889	**1329**	**1171**	**1.27**	**0.00085**	**1408**	**1461**	**0.17**	**0.98**	**0.79**	**1090**	**1168**	**0.17**	**0.90**	**0.18**
G/G		829 (0.62)	796 (0.68)	1	1										
A/G		437 (0.37)	343 (0.26)	1.21	0.0273										
A/A		63 (0.05)	32 (0.03)	1.9	0.00373										
dominant				1.27	0.00433										
recessive				1.79	0.00882										