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Null Results in Brief

Lack of Replication of Seven Pancreatic Cancer Susceptibility Loci Identified in Two Asian Populations

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Abstract

Background: Two recent genome-wide association studies (GWAS) of pancreatic ductal adenocarcinoma (PDAC), conducted, respectively, in a Japanese and in a Chinese population, identified eight novel loci affecting PDAC risk.

Methods: We attempted to replicate the novel loci in a series of PDACs and healthy controls of European ancestry in the context of the newly formed PANcreatic Disease ReseArch (PANDoRA) consortium. We genotyped seven single-nucleotide polymorphisms (SNP): rs12413624, rs1547374, rs372883, rs5768709, rs6464375, rs708224, rs9502893 (one SNP identified in the Chinese GWAS is not polymorphic in Caucasians) in 1,299 PDAC cases and 2,884 controls. We also attempted stratified analysis considering the different stages of the disease and addressed the possible involvement of the selected SNPs on the survival of patients.

Results: None of the SNPs were significantly associated with PDAC risk if considering the overall population of the consortium. When stratifying for country of origin, we found that in the Polish subgroup, the G allele of rs372883 was statistically significantly associated with increased risk [OR, 6.40; 95% confidence interval (CI), 2.28–17.91]. However, the sample size of the subgroups was rather small; therefore, this result can be due to chance. None of the SNPs was associated with disease progression or survival.

Conclusions: None of the SNPs associated with PDAC risk in two Asian populations were convincingly associated with PDAC risk in individuals of European descent.

Impact: This study illustrates the importance of evaluation of PDAC risk markers across ethnic groups. *Cancer Epidemiol Biomarkers Prev; 22(2); 320–3. ©2012 AACR.*

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Introduction

Pancreatic cancer mortality rates approach incidence rates (1). Due to the lack of sufficient risk factors, finding genetic variants associated with disease risk is of utmost importance. A genome-wide association study (GWAS) performed in a Caucasian population identified 4 loci associated with pancreatic cancer risk; 2 more showed a strong association only in samples from prospective cohorts but not in retrospective case–control series (2, 3). Two recent GWAS of pancreatic ductal adenocarcinoma (PDAC), conducted in Japan (4) and China (5), identified 8 novel loci affecting PDAC risk. We attempted to replicate these loci in a series of 1,299 PDAC and 2,884 healthy controls of European ancestry in the context of the PANcreatic Disease ReseArch (PANDoRA) consortium. We also attempted stratified analysis considering the different stages of the disease and addressed the possible involvement of the single-nucleotide polymorphisms (SNP) on the survival of patients.

Materials and Methods

Characteristics of the study population were described in detail elsewhere (6). A total of 1,299 PDAC cases and 2,884 controls were used in this study. We genotyped 4 SNPs identified by Wu (5) and 3 identified by Low (4). Genotyping was conducted using the KASPar SNP genotyping system (KBiosciences; ref. 7). Genotyping for British controls has been conducted in the context of a GWAS as described before (7).

Risk analysis was conducted by logistic regression for multivariate analyses to assess the main effects of the genetic polymorphism on pancreatic cancer risk using the same inheritance model reported by Wu and Low. The most common allele in the controls was assigned as the reference category. Survival analysis was conducted using HRs and 95% confidence intervals (CI) in Cox proportional hazard models. All analyses were adjusted for age, gender, tumor–node–metastasis (TNM) stage (for survival only), and nationality. We also conducted stratified analysis for risk and survival considering the various nationalities and the different stages as different strata. All analyses were conducted with STATA software (StataCorp).

Results

The 7 SNPs were genotyped in all cases and healthy controls. Relevant characteristics of the study population are given in Table 1. The average call rate was 95.86% (range, 93.93%–97.83%). Approximately 10% of the samples were analyzed in duplicate; the concordance rate of the genotypes was above 99%. The genotype distributions at all loci were in Hardy–Weinberg equilibrium in controls, with nonsignificant χ^2 values (data not shown). The frequencies and distribution of the genotypes, the ORs and 95% CIs for the association with PDAC are shown in Table 2. Heterozygous AG carriers of rs1547374 were associated with increased risk (OR, 1.16; 95% CI, 1.00–1.35; $P = 0.04$), whereas we observed a trend for rs5768709 and decreased risk ($P_{trend} = 0.04$) and in heterozygous AG carriers of rs9502893 (OR, 0.84; 95% CI, 0.71–0.98; $P = 0.03$). After Bonferroni correction, none of the above variants remained significant. Stratifying by nationality, we found that in the Polish subgroup, the G allele of rs372883 was significantly associated with increased risk (OR, 6.40; 95% CI, 2.28–17.91; $P = 0.0004$). The sample size of the subgroup was rather small; there were 7 and 21 cases with AA or AG + GG genotypes, respectively, and 43 and 80 Polish controls with either AA or AG + GG genotypes. Therefore, this result has to be taken with caution. None of the other SNPs were significantly associated with PDAC risk, even when stratifying for tumor stage, or with patient survival, considering the correction for multiple testing. Finally, we observed no statistically significant association between the SNPs and survival when stratifying for nationality or tumor stage (Table 2).

Table 1. Characteristics of patients and healthy controls included in this study

	Czech		German		Italian		English		Polish		Greek	
Nationality	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
N	228	305	672	1,579	159	462	98	280	54	163	88	95
Type of controls		Blood donors and hospitalized individuals		Blood donors		Blood donors and hospitalized individuals		General population		Blood donors		Hospitalized individuals
Gender												
Female	82	132	297	775	66	196	36	112	35	87	40	46
Male	146	173	375	804	91	263	61	168	19	75	48	49
Missing	0	0	0	0	2	3	1	0	0	1	0	0
Age at diagnosis/recruitment												
Mean (SD)	62.13 (10.46)	59.6 (12.25)	64.08 (9.83)	56.56 (9.7)	69.72 (9.94)	55.15 (10.96)	64.5 (10.15)	66.81 (9.23)	60.5 (9.27)	66.83 (10.34)	64.11 (10.11)	47.51 (15.66)
Median (25%–75%)	63 (57–70)	59.5 (57–68)	65 (58–71)	59 (54–63)	71 (63–77)	58 (47–63)	64 (58–72)	67 (60–74)	61 (53–67)	68 (62–73)	66 (58–72)	45 (35–53)
Survival days												
Mean (SD)	289 (243)		490 (406)		355 (227)		410 (442)		327 (176)		358 (275)	
Median (25%–75%)	252.5 (100–305)		353 (207–667)		319 (180–461)		294 (160–487)		382 (206–458)		300 (180–420)	
Deaths	150		474		97		85		21		78	

Table 2. Associations between selected SNPs and PDAC risk and patients survival

		Risk analysis					Survival analysis				
SNP	Genotypes	Cases[a]	Controls[a]	OR (95% CI)[b]	P_{value}	P_{trend}	Test	Subjects[a]	Deaths[a]	HR (95% CI)[c]	P_{value}
rs12413624	TT	392	882			0.7528	Per allele	1,091	887	0.95 (0.86–1.06)	0.38
	AT	616	1393	1.02 (0.87–1.19)	0.832		TT vs. AT			0.99 (0.83–1.19)	0.91
	AA	269	585	1.05 (0.86–1.28)	0.626		TT vs. AA			0.90 (0.72–1.12)	0.35
	AT + AA			1.03 (0.88–1.19)	0.727		TT vs. (AT + AA)			0.96 (0.81–1.14)	0.63
rs1547374	AA	526	1,279			0.1864	Per allele	1,094	895	0.94 (0.83–1.06)	0.29
	AG	611	1,265	**1.16 (1.00–1.35)**	**0.043**		AA vs. AG			0.89 (0.75–1.05)	0.17
	GG	144	335	1.05 (0.83–1.32)	0.685		AA vs. GG			0.92 (0.71–1.21)	0.56
	AG + GG			1.14 (0.99–1.31)	0.067		AA vs. (AG + GG)			0.90 (0.76–1.05)	0.18
rs372883	AA	344	775			0.952	Per allele	1,059	860	0.90 (0.80–1.00)	0.06
	AG	613	1,446	0.97 (0.82–1.14)	0.7		AA vs. AG			0.90 (0.74–1.10)	0.31
	GG	288	650	0.98 (0.80–1.19)	0.83		AA vs. GG			0.81 (0.65–1.01)	0.06
	AG + GG			0.97 (0.83–1.14)	0.715		AA vs. (AG + GG)			0.87 (0.72–1.04)	0.13
rs5768709	AA	510	951			**0.0378**	Per allele	1,083	883	0.97 (0.86–1.09)	0.57
	AG	580	1,221	0.92 (0.79–1.07)	0.277		AA vs. AG			0.98 (0.82–1.16)	0.79
	GG	180	408	0.86 (0.69–1.07)	0.166		AA vs. GG			0.93 (0.73–1.19)	0.56
	AG + GG			0.90 (0.78–1.04)	0.17		AA vs. (AG + GG)			0.96 (0.82–1.14)	0.67
rs6464375	GG	1124	2,522			0.3589	Per allele	1,111	904	1.26 (1.00–1.58)	0.05
	AG	169	353	1.13 (0.92–1.38)	0.258		GG vs. AG			1.22 (0.96–1.55)	0.10
	AA	6	9	1.83 (0.62–5.36)	0.271		GG vs. AA			3.26 (0.80–13.21)	0.10
	AG + AA			1.14 (0.93–1.40)	0.199		GG vs. (AG + AA)			1.24 (0.98–1.57)	0.07
rs708224	GG	449	919			0.7476	Per allele	1,104	900	1.11 (0.99–1.23)	0.08
	AG	598	1,378	0.90 (0.77–1.05)	0.189		GG vs. AG			1.09 (0.91–1.30)	0.37
	AA	246	501	1.07 (0.87–1.30)	0.524		GG vs. AA			1.23 (0.98–1.53)	0.07
	AG + AA			0.94 (0.82–1.09)	0.441		GG vs. (AG + AA)			1.12 (0.95–1.33)	0.17
rs9502893	AA	452	899			0.5069	Per allele	1,084	880	1.00 (0.90–1.11)	0.98
	AG	555	1,338	**0.84 (0.71–0.98)**	**0.027**		AA vs. AG			0.98 (0.79–1.21)	0.85
	GG	265	536	0.98 (0.81–1.19)	0.861		AA vs. GG			1.07 (0.90–1.26)	0.44
	AG + GG			0.88 (0.76–1.02)	0.087		AA vs. (AG + GG)			1.13 (0.94–1.35)	0.20

[a]Numbers may not add up to 100% of subjects due to genotyping failure. All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to 2 additional rounds of genotyping. Data points that were still not filled after this procedure were left blank.

[b]All analyses were adjusted for age, gender, and nationality. Significant associations ($P < 0.05$) are reported in bold.

[c]All analyses were adjusted for age, gender, TNM stage, and nationality.

Discussion and Conclusion

We previously replicated the majority of loci identified by the first GWAS on pancreatic cancer in a subset of the cases and controls used in the present study (7). GWAS on PDAC risk in the Japanese (4) and Chinese population (5) yielded 3 new loci on chromosomes 6p25.3 (rs9502893, upstream of *FOXQ1*), 12p11.21 (rs708224, in the second intron of *BICD1*), and 7q36.2 (rs6464375, in the first intron of *DPP6*) and 5 novel susceptibility loci at chromosomes 21q21.3 (rs372883, in the *BACH1* gene), 5p13.1 (rs2255280, in the *DAB2* gene), 21q22.3 (rs1547374, upstream of *TFF1* gene), 22q13.32 (rs5768709), and 10q26.11 (rs12413624). The last 2 SNPs are not located in the immediate vicinity of any gene. We attempted to validate 7 of the novel hits in an independent cohort of different ethnicity. We did not analyze rs2255280 because it is monomorphic in Caucasians (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs = 2255280). Such validation is necessary because attempts to generalize genetic associations across ethnicities have had mixed results. Also, the incidence of pancreatic cancer is substantially different among populations of distinct ancestry (5), possibly reflecting differences in genetic susceptibility. We had more than 95% statistical power to detect the reported associations; nevertheless, we observed none. Our results highlight the genetic differences across human populations and illustrate the importance of evaluating PDAC risk markers across ethnic groups.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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