Review

Re-evaluation of ABO gene polymorphisms detected in a genome-wide association study and risk of pancreatic ductal adenocarcinoma in a Chinese population

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Abstract
    Pancreatic cancer is a fatal malignancy with an increasing incidence in Shanghai, China. A genome-wide association study (GWAS) and other work have shown that ABO alleles are associated with pancreatic cancer risk. We conducted a population-based case-control study involving 256 patients with pathologically confirmed pancreatic ductal adenocarcinoma (PDAC) and 548 healthy controls in Shanghai, China, to assess the relationships between GWAS-identified ABO alleles and risk of PDAC. Carriers of the C allele of rs505922 had an increased cancer risk [adjusted odds ratio (OR) = 1.42, 95% confidence interval (CI): 1.02–1.98] compared to TT carriers. The T alleles of rs495828 and rs657152 were also significantly associated with an elevated cancer risk (adjusted OR = 1.58, 95% CI: 1.17–2.14; adjusted OR = 1.51, 95% CI: 1.09–2.10). The rs630014 variant was not associated with risk. We did not find any significant gene-environment interaction with cancer risk using a multifactor dimensionality reduction (MDR) method. Haplotype analysis also showed that the haplotype CTTC was associated with an increased risk of PDAC (adjusted OR = 1.46, 95% CI: 1.12–1.91) compared with haplotype TGGT. GWAS-identified ABO variants are thus also associated with risk of PDAC in the Chinese population.

Key words   Pancreatic ductal adenocarcinoma, ABO gene, genome-wide association study, genetic variation, haplotype

Pancreatic cancer is a lethal digestive malignancy, with an overall age-standardized incidence of 3.9/100,000 and a mortality of 3.7/100,000 worldwide (Globocan 2008)[1]. Data from the Shanghai Cancer Registry between 1973 and 2007 show that the incidence of pancreatic cancer has increased significantly in urban Shanghai, with age-standardized incidence of 3.38/100,000 in 1973 and 6.29/100,000 in 2007 and an annual percent change (APC) of 1.8%[2]. Because pancreatic cancer is difficult to diagnose in its early stages, the prognosis of this disease is extremely poor. Pancreatic cancer kills more than 213,000 people worldwide each year and has the lowest 1- and 5-year survival rates among all cancers, with a 5-year survival rate of <5% and a median survival of less than 6 months[3,4].

    The etiology of pancreatic cancer is not well understood. However, the literature suggests that environmental and lifestyle factors, such as smoking, alcohol drinking, *Helicobacter pylori* infection, diabetes, obesity, family history, chronic pancreatitis, and occupational hazards, may play roles in pancreatic carcinogenesis[5]. Molecular epidemiologic studies have found associations between polymorphisms in several genes and pathways and the risk of pancreatic cancer[6]. Additional evidence suggests that pancreatic carcinogenesis involves complex interactions between genetic mutations, epigenetic alterations, and environmental risk factors[7].

    Early studies have shown that ABO blood type is associated

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with gastrointestinal cancers, including gastric, pancreatic, and esophageal cancers[8,9]. Recently, a genome-wide association study (GWAS) identified several pancreatic cancer susceptibility loci, including single nucleotide polymorphisms (SNPs) in the genic regions of ABO, sonic hedgehog (SHH), telomerase reverse transcriptase (TERT), nuclear receptor subfamily 5, group A, member 2 (NR5A2), and in putative genic regions of chromosomes 13q22.1 and 15q14[10]. Therefore, the ABO loci are biologically plausible candidate factors in pancreatic carcinogenesis.

This study aimed to confirm the association between pancreatic ductal adenocarcinoma (PDAC) and the previously identified ABO mutations (rs505922, rs495828, rs657152, and rs630014) in a large-scale, population-based case-control study in urban Shanghai.

Materials and Methods

Study subjects

Between December 2006 and January 2011, subjects were enrolled in a population-based case-control study of pancreatic cancer in urban Shanghai, China as described in a previous report[11]. All participants were Shanghai residents between 35 and 79 years of age. Potential cases were identified using an "instant case reporting system" established by the Shanghai Cancer Institute and 37 collaborating hospitals; the majority of individuals with pancreatic cancer in this region are diagnosed and treated in these institutions. During the same period, healthy control individuals were randomly selected from the Shanghai Resident Registry with frequency matching by age and gender. All study participants provided written, informed consent before participating in the study and were interviewed in person by trained medical professionals using a structured questionnaire. The study was approved by the institutional review boards of both the Shanghai Cancer Institute and Yale University.

Genotyping

Genomic DNA was extracted from buffy coats using a Genomic DNA Extraction Kit (Promega, Madison, WI, USA) or from oral epithelial cells (collected using the oragene method) using a GTpure Buccal Cell DNA Extraction Kit (Gene Tech, Shanghai, China). Genotyping was performed via TaqMan assay using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) in 384-well format, with Aequorea victoria green fluorescent protein (VIC) and carboxyfluorescein (FAM) dual-fluorescence reporter probes. The genotyping failure rate among all samples was <1.0%. The quality and potential misclassification of the genotyping results were assessed by evaluating 5% of duplicate DNA samples that were randomly placed within the same reaction plates used for the study subjects.

Statistical analysis

We used SAS software (version 9.1; SAS Institute, Inc.) for statistical analyses. Differences in the distribution of selected characteristics, including age, gender, education, alcohol drinking, tea drinking, cigarette smoking, and body mass index (BMI, weight/height^2, kg/m^2), were evaluated using the χ^2 test. Associations between SNPs and risk of PDAC were measured by odds ratios (ORs) and their 95% confidence intervals (CIs), as estimated by unconditional logistic regression models. A multifactor dimensionality reduction (MDR) approach was used to analyze the interactions between SNPs and environmental factors that might contribute to cancer risk[12]. The linkage disequilibrium between loci in the ABO gene and the deviation of allele frequencies from Hardy-Weinberg equilibrium were assessed in HaploView version 4.0[13]. We used HAPSTAT software to evaluate associations between haplotypes and cancer risk[14].

Results

Population characteristics

In total, 1,241 patients with newly diagnosed pancreatic cancer were referred to the Shanghai Cancer Institute. Of these patients, 149 could not be contacted or refused to participate; the remaining 1,092 patients were recruited into the study (88% participation rate). All relevant hospital records, pathologic reports, pathologic slides, and/or imaging results [computer tomography (CT), positron emission tomography (PET-CT), and/or magnetic resonance imaging (MRI)] were collected for case eligibility review by a panel of expert pathologists and clinicians. Among the 1,092 patients, 261 were confirmed histopathologically with PDAC, of which 256 provided blood or buccal cell samples. For the control group, 1,653 individuals were contacted, among whom 462 refused to participate, 94 were diagnosed with malignancies or other severe diseases, and 30 died before the interview date. The remaining 1,067 subjects were recruited as controls (65% participation rate), among which 548 controls were frequency-matched to the 256 cases by age (within 5 years) and gender. **Table 1** presents selected characteristics of the cases and controls. Subjects were comparable with respect to age and gender. The cases included more smokers (49.6% vs. 43.8%) and exhibited a higher education level than the controls, although these differences were not statistically significant. The cases tended to have slightly higher BMI than the controls.

Single marker associations

Buffy coats were collected from 458 controls and 230 patients and oral epithelial cells were collected from 90 controls and 26 patients for genomic DNA extraction. DNA genotyping was re-examined on 42 samples to evaluate the quality and accuracy of the genotyping results. The replicates were 100% concordant.

Associations of the ABO gene variants with the risk of PDAC are presented in **Table 2**. The genotype distributions of these variants in the controls did not deviate from the expected Hardy-Weinberg equilibrium ($P > 0.05$), except for rs657152 ($P = 0.03$). Both the C allele of rs505922 and the T allele of rs495828 were associated

Table 1. Comparison of selected characteristics of pancreatic cancer patients and healthy controls recruited from Shanghai

Selected characteristic	Patients [cases (%)]	Controls [cases (%)]	P
Age (years)			0.12
< 60	91 (35.5)	177 (32.3)	
60–69	86 (33.6)	161 (29.4)	
⩾ 70	79 (30.9)	210 (38.3)	
Gender			0.19
Male	162 (63.3)	320 (58.4)	
Female	94 (36.7)	228 (41.6)	
Education			0.09
Elementary or below	33 (12.9)	97 (17.7)	
Middle school	83 (32.4)	169 (30.8)	
High school	72 (28.1)	172 (31.4)	
College and above	68 (26.6)	110 (20.1)	
Regularly drank alcohol			0.27
No	135 (52.7)	266 (48.5)	
Yes	121 (47.3)	282 (51.5)	
Regularly drank tea			0.33
No	106 (41.4)	247 (45.1)	
Yes	150 (58.6)	301 (54.9)	
Regularly smoke			0.12
No	129 (50.4)	308 (56.2)	
Yes	127 (49.6)	240 (43.8)	
BMI (kg/m^2)			0.03
< 18.5	8 (3.1)	22 (4.0)	
18.5–22.9	95 (37.1)	245 (44.7)	
23.0–24.9	79 (30.9)	118 (21.5)	
⩾ 25.0	74 (28.9)	163 (29.8)	

with a higher risk of PDAC. Carriers of the T allele of rs657152 were also significantly associated with an increased risk. Significant associations with rs630014 were not observed.

Interactions between SNPs and environmental factors

MDR analysis was applied to explore the gene-environment interactions. The best predictive models for up to 4 orders of interaction, along with their cross-validation consistency (CVC) and testing balanced accuracy (TBA), are summarized in **Table 3**. The best 1-locus model was found for rs495828, with a CVC of 10/10 and a TBA of 0.557,5. Among the 2-locus interactions, the combination of rs6310014 and education was most significant, with a CVC of 5/10 and a TBA of 0.499,2. The best 3-locus model consisted of rs505922, education, and BMI, with a CVC of 7/10 and a TBA of 0.493,8. The 4-locus interaction model comprised rs505922, age, education, and BMI, with a maximum CVC of 10/10 and a TBA of 0.557,4. However, the P values for TBA were not significant (data not shown).

Associations between haplotypes and risk of PDAC

The studied genetic variants in the ABO gene fall into a major haplotype block and were in close linkage disequilibrium (D' > 0.98). **Table 4** shows the associations between ABO haplotype and risk of PDAC. Carriers of the ABO CTTC haplotype (rs505922-rs495828-rs657152-rs630014) had an excess risk of cancer relative to carriers of the TGGT haplotype. This result was consistent with the individual SNP results.

Discussion

The present study re-evaluated the associations between pancreatic cancer risk and four ABO variants (rs505922, rs495828, rs657152, and rs630014) identified in a genome-wide screen among a population from urban Shanghai, China. We largely replicated the GWAS results. All 4 SNPs had the same directions of risk as reported in the previous GWAS study[10] although the rs630014 polymorphism was not statistically significant, possibly due to insufficient statistical

Table 2. Associations of polymorphisms in the ABO gene and pancreatic cancer risk in a population from Shanghai, China

SNP	Genotypes	Patients [cases (%)]	Controls [cases (%)]	Crude OR (95% CI)[a]	Adjusted OR (95% CI)[b]	P
rs505922	TT	69 (27.0)	186 (34.0)	1.00	1.00	
	TC	124 (48.4)	246 (45.0)	1.35 (0.95–1.92)	1.40 (0.98–2.00)	0.08
	CC	63 (24.6)	115 (21.0)	1.46 (0.96–2.20)	1.47 (0.97–2.23)	0.08
	TC+CC	187 (73.0)	361 (66.0)	1.38 (1.00–1.92)	1.42 (1.02–1.98)	0.05
rs495828	GG	133 (52.0)	344 (62.9)	1.00	1.00	
	GT	101 (39.5)	173 (31.6)	1.51 (1.10–2.08)	1.53 (1.11–2.10)	0.04
	TT	22 (8.5)	30 (5.5)	1.92 (1.07–3.46)	1.88 (1.04–3.40)	0.01
	GT+TT	123 (48.0)	203 (37.1)	1.57 (1.16–2.13)	1.58 (1.17–2.14)	0.004
rs657152	GG	71 (27.7)	199 (36.4)	1.00	1.00	
	GT	124 (48.5)	240 (43.9)	1.43 (1.01–2.03)	1.48 (1.04–2.10)	0.04
	TT	61 (23.8)	108 (19.7)	1.56 (1.03–2.37)	1.58 (1.04–2.40)	0.03
	GT+TT	185 (72.3)	348 (63.6)	1.47 (1.07–2.04)	1.51 (1.09–2.10)	0.02
rs630014	CC	114 (44.5)	213 (38.9)	1.00	1.00	
	CT	108 (42.2)	250 (45.7)	0.81 (0.59–1.12)	0.83 (0.60–1.15)	0.21
	TT	34 (13.3)	84 (15.4)	0.76 (0.48–1.20)	0.73 (0.46–1.16)	0.24
	CT+TT	142 (55.5)	334 (61.1)	0.80 (0.59–1.08)	0.81 (0.59–1.09)	0.16

SNP, single nucleotide polymorphism; OR, odd ratio; CI, confidence interval. [a]Adjusted for age and gender. [b]Adjusted for age, gender, education, cigarette smoking, and body mass index (BMI).

Table 3. Summary of gene–environment interaction results

Model	Training balanced accuracy	Testing balanced accuracy	Cross validation consistency
rs495828	0.558,4	0.557,5	10/10
rs630014, education	0.579,6	0.499,2	5/10
rs505922, education, BMI	0.621,3	0.493,8	7/10
rs505922, age, education, BMI	0.673,2	0.557,4	10/10

Table 4. Association of major haplotypes in the ABO gene with pancreatic cancer risk in a population from Shanghai, China

Haplotype[a]	Patients (%)	Controls (%)	Crude OR (95% CI)[b]	Adjusted OR (95% CI)[c]
TGGT	34.4	38.0	1.00	1.00
CTTC	28.1	21.2	1.45 (1.11–1.90)	1.46 (1.12–1.91)
CGTC	19.5	20.4	1.03 (0.77–1.38)	1.04 (0.78–1.40)
TGGC	16.8	18.4	1.01 (0.74–1.39)	1.02 (0.75–1.40)

[a] rs505922, rs495828, rs657152, rs630014. [b]Adjusted for age and gender. [c]Adjusted for age, gender, education, cigarette smoking, and BMI.

power. Further, one haplotype that contained risk alleles of the four variants was associated with increased cancer risk.

The relationship between ABO blood group and pancreatic cancer has been thoroughly examined. Carriers with blood type

O have a lower risk of pancreatic cancer than those with blood type A, and blood types B and AB appear to be associated with an increased pancreatic cancer risk in western populations but not in Asian populations[15,16]. A GWAS has also identified loci in the ABO gene that conferred increased pancreatic cancer risk[10]. However, a GWAS performed in a Chinese population did not find an association between the ABO rs505922 allele and risk of pancreatic cancer[17].

Although the precise mechanism linking ABO blood type to pancreatic cancer risk has not been determined, several hypotheses have been suggested. The 2-SNP haplotype (rs8176746 and rs8176719) determines ABO blood type for all but certain rare groups[18]. A study in Japan has also shown that the rs8176719 and rs8176746 alleles in the ABO gene determine ABO blood group. The rs505922 allele is in high linkage disequilibrium with rs8176719 and is strongly correlated with the O allele (r^2 = 0.96)[19].

Variants in the ABO gene have been suggested to be linked to serum inflammation markers, such as tumor necrosis factor-α (TNF-α)[20], soluble intercellular adhesion molecule 1 (sICAM-1), and liver-derived alkaline phosphatase (ALP)[21], suggesting that ABO blood type may influence the systemic inflammatory response. An increased serum TNF-α level has been associated with enhancement of the invasive properties of PDAC cell lines and tumor growth in animals[22].

The strengths of our study include the population-based design, which minimized selection bias; the detailed review of cancer diagnoses (all cases in this study were histopathologically confirmed), which minimized disease misclassification; and the use of high-quality extracted DNA, which minimized inaccurate genotyping results. The limitations of this study include its relatively small sample size, which limited the statistical power to detect an effect and the ability to evaluate specific effects in subgroups, and the lack of clear biological mechanisms to explain the findings.

Conclusion

The GWAS-identified ABO variants (rs505922, rs495828, rs657152, and rs630014) were found to be associated with risk of PDAC in a Chinese population in our study.

Acknowledgments

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