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An evaluation study of reported pancreatic adenocarcinoma risk-associated SNPs from genome-wide association studies in Chinese population

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ABSTRACT

Background: Previous genome-wide association studies (GWAS), and a pathway study of pancreatic ductal adenocarcinoma (PDAC) identified 14 significantly associated single nucleotide polymorphisms (SNPs) along with another 7 promising loci in European, Japanese, and Chinese descents. In this study, we aimed to evaluate the potential association of these SNPs with PDAC risk in the Chinese population.
Methods: In this Chinese population-based case-control study with 254 cases and 1200 controls, we tested 20 PDAC risk associated SNPs from previous GWAS and one SNP from a pathway-based study.
Results: All 21 SNPs were polymorphic in the Chinese population. Twenty SNPs were included in the final analysis after the quality check (QC). Among these SNPs, three were significantly associated with PDAC risk after Bonferroni correction ($P < 2.5E-03$) including rs7779540 (at 7q36.2, $P = 3.89E-06$, $OR = 2.59$, 95%CI: 1.73–3.87), rs10919791 (at 1q32.1, $P = 6.07E-05$, $OR = 1.52$, 95%CI: 1.24–1.86) and rs401681 (at 5p15.33, $P = 5.15E-04$, $OR = 1.42$, 95%CI: 1.17–1.73). Rs2255280 (at 5p13.1, $P = 8.16E-03$, $OR = 1.31$, 95% CI: 1.07–1.6) showed significant association at the $p < 0.05$ level. The directions of effect of these SNPs were consistent with previous studies.
Conclusion: Four PDAC risk-associated SNPs identified in GWAS of various populations are associated with PDAC risk in the Chinese population. Information on PDAC risk-associated SNPs and their ORs may facilitate risk assessment of PDAC risk in the Chinese population.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal cancers worldwide. The mortality and morbidity of PDAC are roughly equal. PDAC is the fourth leading cause of cancer-related deaths across the United States (US) and other developed countries, independent of race or sex [1–4]. From 1998 to 2007, the incidence rates of PDAC for men and women in Chinese rural areas increased by 7.54% and 7.83% [5]. The overall 5-year survival rate is below 5% [3,4]. The etiology of PDAC remains largely unknown and probably multifactorial. Previous epidemiological studies have revealed smoking [6,7], diabetes [8], and chronic pancreatitis [9] to be risk factors. In addition, familial aggregation of PDAC has been associated with the possible involvement of genetic factors [6].

From 2008 to 2014, 20 genomic regions that harbor inherited genetic variants associated with PDAC risk have been identified from multiple genome-wide association studies (GWAS) in European, Japanese, and Chinese populations [10–14]. In addition, Li et al. [15] found a new locus on 9q34.2 to be potentially associated with PDAC using an adaptive rank truncated product method in a pathway-based analysis of GWAS data from PanScan 1 study. The loci from different ethnic groups scatter on chromosomes 1q32,

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5p15, 7q36, 9q34, 16q23, 21 and 22. More importantly, loci reported from different ethnic groups are located on different regions on the genome. Overlapping of these loci among different ethnic groups has not been observed. This might be due to the different genetic mechanisms of PDAC amongst different ethnic groups. The SNPs from European and Japanese descent have not been systemically evaluated in the Chinese population. In this study, we aimed to evaluate the reported SNPs with PDAC risk in the Chinese population and estimate their strength of association.

Materials and methods

Study subjects

With approval of the Institutional Review Board at Shanghai Huashan Hospital (HIRB), our department (the department of pancreatic surgery, Shanghai Huashan Hospital) consecutively recruited 254 pancreatic cancer patients during March 2013 to August 2014. Patients who had been diagnosed prior to the approval date were recruited during their chemotherapy or outpatient follow-up sessions. Two copies of the consent forms were signed by participant and researcher. One copy was given to the patient; the other was kept with the patient's medical record. HIRB approved this consent procedure. Participants were de-identified after data collection. As a national referral hospital, our patients came from all over the country. Inclusion criteria were as follows: pathologically diagnosed with PDAC, Han Chinese ethnicity, and provided informed consent to participate in the study. Blood samples were taken during routine examinations. The recruitment of controls was described in a previous publication [16]. Age and other factors such as smoking status were not matched for PDAC patients. Family history was not required for enrollment.

Selection and genotyping of SNP

The PDAC risk-associated SNP were defined according to the five GWAS reports published from the year 2008 to the year 2014 [10–14], and an additional pathway study [15]. Among the 20 loci selected for verification of previous GWAS (with P value lower than 10E-5), three were from a Japanese population, six were from a Chinese population, and the remaining eleven were from a population with European ancestry. The final locus (rs2073828, at 9q34.2) was selected from a promising pathway-based study by Li et al. [15]. At the time of genotyping, the PanSCAN 4 study was not published; Therefore, the new locus discovered by PanSCAN 4 was not included in this study. Information regarding selected SNPs is presented in Tables 2 and 3

Genotyping was performed by the iPLEX of Sequenom platform (Sequenom, Inc., San Diego, CA). Polymerase chain reaction (PCR) and extension primers were designed using MassARRAY Assay Design 3.1 software (Sequenom, Inc., San Diego, CA). Genotyping procedures were performed according to the manufacturer's iPLEX Application Guide (Sequenom Inc. SanDiego, CA). 384 well plates were used for all genotyping reactions. Each plate included duplicates for three or four subjects selected at random as well as six to nine negative controls in which water was substituted for DNA. The average concordance rate was 99%. Due to unsuccessful genotyping, genotype data of two SNPs (rs4885093 and rs10919791) were unavailable. Thus, additional Tagman PCR was used for the genotyping of these two SNPs. Laboratory technicians were blinded to case–control status.

Statistical analysis

Genotype frequencies of all SNPs in the control subjects were analyzed by Pearson's Chi-squared tests for Hardy-Weinberg equilibrium (HWE). Rs2736098 was excluded from the final analysis due to the high missing rate in the control group (26.33%). Twenty SNPs were included in the final association analysis. Logistic regression under the additive genetic model was conducted using PLINK program version 1.9 (URL: http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml; PLINK 1.9) [17]. The odds ratio (OR) and 95% confidence interval (CI) were calculated for each SNP. To ensure a Type I error of 5%, Bonferroni correction was used for multiple comparison correction. A p value lower than 2.5E-03 (0.05/20) was considered statistically significant. SNPs with p value less than 0.05 were also considered of interest. Since the control group was significantly younger ($p < 0.05$, Table 1), age adjusted p values and OR were also calculated.

Results

This study consecutively enrolled 254 PDAC patients, 156 males and 98 females. The mean age of study subjects was 63.31 years with a standard deviation of 10.01. All participants were pathologically confirmed with surgery or endoscopic ultrasonic-fine needle aspiration (EUS-FNA). For patients who underwent surgery, the TNM stage was defined according to their surgical exploration and pathologic results. Two patients who did not undergo surgery were diagnosed through EUS-FNA. The staging of these two patients was defined by all results of clinical exams (abdominal enhanced CT, chest x-ray, EUS-FNA and PET/CT). Detailed information of patients and control subjects is displayed in Table 1.

Rs2736098 was excluded because of its high missing rate in the control group (26.33%). The call rates for all 20 SNPs included in the final analysis were over 97%. According to data from the "1000 Genomes Project" (http://www.1000genomes.org) along with our genotyping data in cases and controls, all 20 SNPs appear to be polymorphic in Chinese population (Table 2). Among the control subjects, none of the SNPs significantly deviated from HWE at the $p < 0.05$ levels (Table 3).

The association between all 20 previously reported SNPs and PDAC risk in our study subjects are summarized in Table 3. Among

Table 1
Characteristics of pancreatic cancer cases and controls.

Characteristics	Cases N (%)	Controls N (%)
Number of subjects	254	1200
Sex[++]		
male	156 (61.41%)	748 (62.33%)
female	98 (38.59%)	452 (37.67%)
Age, years (Mean, SD)[$]	63.31, 10.01	48.80, 15.49
Stage[&]		
0 (TisN0M0)	1 (0.39%)	/
IA (T1N0M0)	11 (4.33%)	/
IB (T2N0M0)	39 (15.35%)	/
IIA (T3N0M0)	36 (14.17%)	/
IIB (T1-3N1M0)	65 (25.59%)	/
III (T4NxM0)[+]	30 (11.81%)	/
IV (TxNxM1)[*]	70 (27.56%)	/
NA	2 (0.80%)	/

$: $p < 0.05$ & TNM staging of pancreatic tumor: T (primary tumor) staging: T0 No evidence of primary tumor; Tis Carcinoma in situ; T1 Tumor < 2 cm; T2 Tumor >2 cm; T3 Tumor extends beyond the pancreas without involvement of celiac axis or mesenteric artery; T4 Tumor involves the celiac axis or SMA.
N (regional lymph nodes) staging:
+A T3 tumor without metastasis is counted as stage III tumor, regardless of lymph node metastasis status.
*Tumor with distant metastasis is counted as stage IV tumor, regardless of T and N staging.

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