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RESEARCH ARTICLE

Association between CLPTM1L-TERT rs401681 polymorphism and pancreatic cancer risk among Chinese Han population

Chengli Liu · Yingjie Wang · Hui Huang · Cheng Wang · Hui Zhang · Yalin Kong · Hongyi Zhang

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Abstract Pancreatic cancer is one of the human cancers with the highest fatality rates; however, the etiology still remains largely unknown. Recently, one genome-wide association study (GWAS), conducted exclusively among women of European ancestry, has discovered that cleft lip and palate transmembrane 1-like telomerase reverse transcriptase (CLPTM1L-TERT) rs401681 polymorphism was significantly associated with pancreatic cancer risk. Few studies have been conducted to evaluate whether this finding could be generalized to Chinese people. In the current study, we explored the association between rs401681 polymorphism and risk of pancreatic cancer in a case–control study of 1,587 Chinese people (including 766 pancreatic cancer cases and 821 healthy controls). Under the log-additive model, each additional copy of minor allele T was associated with a 1.24-fold increased risk of pancreatic cancer (odds ratio (OR)=1.24, 95 % confidence interval (CI) 1.06–1.44, $P=5.61\times10^{-3}$). While compared with individuals with the CC genotype, the OR for developing pancreatic cancer was 1.09 (95 % CI 0.88–1.34) among those with the CT genotype and 1.66 (95 % CI 1.20–2.29) among those with the TT genotype. Additional adjustments for the confounding factors did not change the results materially. Our data suggests that the T allele of rs401681 in CLPTM1L-TERT locus predisposes its carriers to pancreatic cancer, and further research into the function of CLPTM1L-TERT locus and its potential biological mechanism association may be warranted.

Keywords Pancreatic cancer · CLPTM1L-TERT · SNP · Genetic susceptibility

C. Liu (✉) · C. Wang · H. Zhang · Y. Kong · H. Zhang
Department of Hepatobiliary Surgery, Air Force General Hospital of PLA, 30 Fucheng Road, 100142 Beijing, China
e-mail: liuchengli_beijing@163.com

Y. Wang
Department of Radiation Oncology, Air Force General Hospital of PLA, 100142 Beijing, China

H. Huang
Department of Hepatobiliary Surgery, 309 Hospital of PLA, 100091 Beijing, China

Introduction

Pancreatic cancer (PC) is one kind of the human cancers with the highest fatality rates and is the fourth-highest cancer killer among both men and women worldwide [1, 2]. Globally, as of 2010, pancreatic cancer resulted in 310,000 deaths up from 200,000 in 1990 [3]. Furthermore, the incidence of and number of deaths caused by pancreatic tumors have been gradually rising, and only about 4 % of patients will live 5 years after diagnosis, despite developments in detection and management of pancreatic cancer [1]. There are no effective markers for its screening and early diagnosis [1]. Extensive research efforts have been implemented, which revealed some potential risk factors for PC including gender, age, smoking status, alcohol consumption, overweight, body mass index (BMI), diabetes mellitus, and family history of pancreatic cancer [4]; however, the etiology of pancreatic cancer remains poorly understood.

Genome-wide association study (GWAS), which examines many common genetic variants in different individuals to see if any variant is associated with a trait, has been identified to be a useful and efficient method to detect susceptibility gene for traits like major diseases [5]. GWAS have identified multiple genetic loci for pancreatic cancer; however, most of these GWAS were exclusively conducted in European-ancestry populations [6–9]. Given the considerable differences in genetic architecture including allele frequencies, linkage disequilibrium (LD) structure, and genetic diversity across ethnic

groups, it is important to investigate whether GWAS-identified variants are associated with pancreatic cancer in non-European populations [10].

Petersen et al. [7] found that cleft lip and palate transmembrane 1-like telomerase reverse transcriptase (CLPTM1L-TERT) rs401681 polymorphism was significantly associated with pancreatic cancer risk among European subjects. However, only one study has been conducted to evaluate whether this finding could be generalized to Chinese people [11]. The CPTM1L-TERT region on chromosome 5p15.33 has been implicated in a disease spectrum that also includes urothelial cancer, bladder cancer, lung cancer, brain tumors, acute myelogenous leukemia, bone marrow failure syndromes, and pulmonary fibrosis [12–16]. Given the mounting evidence showing a positive association between CPTM1L-TERT region and cancer risk, we therefore investigated the association between CLPTM1L-TERT rs401681 polymorphism and pancreatic cancer risk in a case–control study in China.

Materials and methods

Subjects

This study consisted of 766 patients with pathologically confirmed pancreatic cancer cases and 821 geographically matched healthy controls. All individuals involved in the study were of Chinese Han ethnicity and were recruited between April 2008 and September 2011. The controls were genetically unrelated and were frequency matched with the PC patients in terms of age and sex, excluding those with a history of cancer and other medical diseases. Each subject was interviewed face-to-face by trained personnel using a formatted questionnaire to obtain demographic data and overall health characteristics, including gender, age, smoking status, alcohol consumption, body mass index, diabetes mellitus, and family history of pancreatic cancer. After the interview, each subject provided 3–5 mL of venous blood. The present study was approved by the institutional review board and informed written consent was obtained from each subject.

DNA sampling and genotyping

Genomic DNA was extracted from peripheral lymphocytes using the Axygen DNA Isolation Kit (Axygen, CA, USA) and stored at −30 °C. TaqMan® assays were used for genotyping the polymorphisms in 96-well plates on ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The primers and probes of the TaqMan® assays were designed using Primer Express Oligo Design software v2.0 (ABI PRISM) and available upon request as TaqMan® predesigned single-nucleotide polymorphism (SNP) genotyping assays. Individual genotype identification was performed by SDS software 2.0 (ABI). Each plate contained blank samples as negative controls for the genotyping quality confirmation. There was 100 % consistency in a 5 % sample of duplicate testing.

Statistical analyses

All statistical analyses were performed using the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software programs. A two-side P value less than 0.05 was regarded as statistically significant. The chi-squared (χ^2) test was performed to assess the Hardy–Weinberg equilibrium in genotype frequencies in control subjects and the differences in characteristics such as gender, age, smoking status, alcohol consumption, body mass index, diabetes mellitus, and family history of pancreatic cancer between cases and controls. The odds ratios (ORs) with their 95 % confidence intervals (CIs) for pancreatic cancer risk in relation to selected SNP were estimated using an unconditional logistic regression analysis. We evaluated the cumulative risk and predictive value for pancreatic cancer using the area under the receiver operating characteristic (ROC) curve.

Results

Characteristics of the study population are shown in Table 1. A total of 766 patients with pathologically confirmed pancreatic cancer and 821 healthy controls were included in this study. The pancreatic cancer patients and the healthy controls were comparable in regard to the distribution of gender, age, smoking status, alcohol consumption, and body mass index, while significant distribution differences for diabetes mellitus and family history of pancreatic cancer were detected between cases and controls. Generally speaking, cases were slightly elder; have higher education; more likely to be male, smoker, drinker, diabetes patients; have higher BMI; and have a family history of pancreatic cancer.

Table 2 shows the association between CLPTM1L-TERT rs401681 and pancreatic cancer risk. The distribution of genotypes among control subjects did not deviate from the Hardy–Weinberg equilibrium (P=0.263). Compared with individuals with the CC genotype, the age- and gender-adjusted OR for developing pancreatic cancer was 1.09 (95 % CI 0.88–1.34) among those with the CT genotype and 1.66 (95 % CI 1.20–2.29) among those with the TT genotype. Under the log-additive model, each additional copy of minor allele T was associated with a 1.24-fold increased risk of pancreatic cancer (OR=1.24, 95 % CI 1.06–1.44, $P=5.61\times10^{-3}$). After adjusting for additional potentially confounding factors such as smoking status, alcohol consumption, body mass index, diabetes mellitus, and family history of pancreatic cancer, the results did not

Table 1 Characteristics of the study population

Characteristics	Cases		Controls		*P* value
	N	Percent	*N*	Percent	
Total number	766		821		
Gender					
Male	452	59.0	472	57.5	0.540
Female	314	41.0	349	42.5	
Age					
<50	365	47.7	374	45.5	0.403
≥50	401	52.3	447	54.5	
Smoking					
Never	435	56.8	503	61.3	0.698
Ever	331	43.2	318	38.7	
Drinking					
Never	526	68.7	579	70.5	0.436
Ever	240	31.3	242	29.5	
BMI					
<25	493	64.4	536	65.3	0.707
≥25	273	35.6	285	34.7	
Diabetes					
Yes	117	15.3	88	10.7	*<0.001*
No	649	84.7	733	89.3	
PC history					
Yes	179	23.4	111	13.5	*<0.001*
No	587	76.6	710	86.5	

Categorical variables: numbers, percentages, and *P* values from χ^2 test

PC history family history of pancreatic cancer

P value <0.05 means statistically significant for italic entries

change materially. When including the three significant risk factors, diabetes, family history of pancreatic cancer, and rs401681 in multivariate models, the ROC was 0.695 (Fig. 1).

Table 2 Association between the CLPTM1L-TERT rs401681 and pancreatic cancer risk

rs401681	Cases	Controls	Age- and sex-adjusted OR	Multivariable-adjusted OR
CC	360	422	1.00 (reference)	1.00 (reference)
CT	300	324	1.09 (0.88–1.34)	1.08 (0.87–1.36)
TT	106	75	1.66 (1.20–2.29)	1.64 (1.19–2.28)
T vs C	766	821	1.24 (1.06–1.44)	1.20 (1.04–1.38)
P trend			5.61×10^{-3}	9.89×10^{-3}

Multivariable-adjusted OR: adjusted for age, sex, smoking status, alcohol consumption, body mass index, diabetes mellitus, and family history of pancreatic cancer

P value <0.05 means statistically significant for italic entries

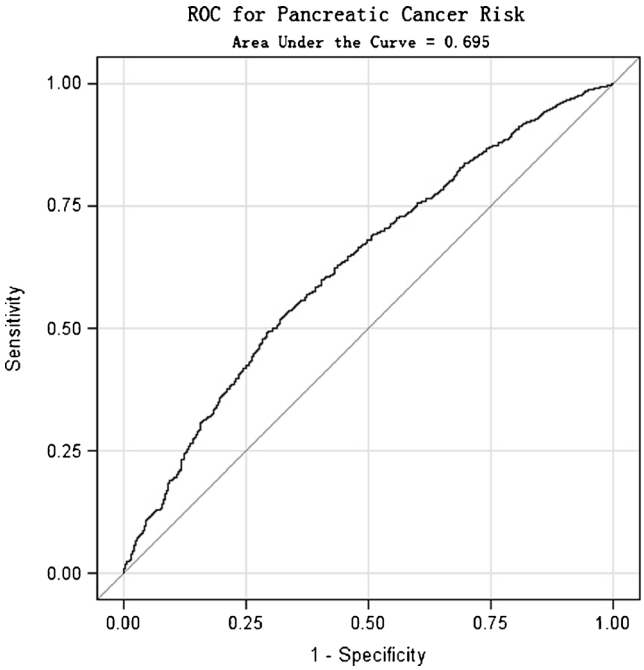

Fig. 1 ROC curve for the pancreatic cancer risk. The three significant risk factors, diabetes, family history of pancreatic cancer, and rs401681 were included in the multivariate models

Discussion

In this hospital-based, case–control study, we observed a significant association between CLPTM1L-TERT rs401681 polymorphism and risk of pancreatic cancer. The association was similar when additionally adjusted by age, education, body mass index, hormone replacement therapy, smoking status, and histological subtypes. This provides evidence to implicate CLPTM1L-TERT rs401681 polymorphism as a novel susceptibility factor for pancreatic cancer risk. Further exploration of the functional explanation of this locus is warranted to understand the mechanism for this association.

The rs401681 polymorphism resides in intron 13 of the CLPTM1L gene (also known as cisplatin resistance-related protein 9), which is mapped to chromosome 5p15.33 and consists of 16 exons and 15 introns [17]. CLPTM1L gene was part of the CLPTM1L-TERT locus that includes the TERT gene, only 23 kb away [17, 18]. Both genes have been implicated in carcinogenesis: the CLPTM1L gene was first identified as an upregulated transcript in a cisplatin-resistant ovarian tumor cell line, although no implication of mechanism and the effect of overexpression of CLPTM1L in cisplatin sensitivity was conflicting in different ovarian tumor cell lines, depending on their preexisting level of resistance [19]. The TERT gene is a catalytic subunit of the enzyme telomerase, which, together with the telomerase RNA component (TERC), comprises the most important unit of the telomerase complex [20]. The enzyme complex acts through the addition of telomeric repeats to the ends of chromosomal DNA, and this generates immortal

cancer cells [21]. There is a strong correlation between telomerase activity and malignant tumors or cancerous cell lines.

To date, many studies have indicated that CLPTM1L and TERT genes were candidate susceptible genes for many diseases, in which SNPs and CNV were reported to be associated with risk of multiple diseases, including urothelial cancer, bladder cancer, lung cancer, brain tumors, acute myelogenous leukemia, bone marrow failure syndromes, and pulmonary fibrosis [12–16, 22–32]. In the current study, we replicated the association between CLPTM1L-TERT rs401681 polymorphism and pancreatic cancer risk in Asians and identified rs401681 polymorphism as a novel susceptibility factor for pancreatic risk. We have a study power of 81 % (α=0.05) to detect a per-copy-deletion OR of 1.35 (which occurred at a frequency of 14.86 % in the controls). The limitations of hospital-based, case–control study should also be addressed in this study. The selection bias is unavoidable; since this study was restricted to a Chinese Han population, it is uncertain whether our findings can be replicated to other ethnic groups.

In conclusion, this study found that CLPTM1L-TERT rs401681 polymorphism was associated with an increased pancreatic cancer risk in Chinese. The results suggest that the rs401681 may be a new biomarker for pancreatic cancer susceptibility. Validations with larger population-based studies in different ethnic groups and further research into the function of CLPTM1L-TERT locus and its potential biological mechanism association may be warranted.

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Conflicts of interest None

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