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Association between rs9904341 G<C gene polymorphism and susceptibility to pancreatic cancer in a Chinese population

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ABSTRACT. We conducted a case-control study to investigate the association between the survivin rs9904341 G<C polymorphism and risk of pancreatic cancer. A total of 261 pancreatic cancer patients who were pathologically confirmed to have pancreatic cancer were included in the study. Polymorphisms of rs9904341 G<C were assessed by polymerase chain reaction-restriction fragment length polymorphism. Pancreatic cancer patients were more likely to be smokers, have a family history of cancer, and have diabetes. Using multivariate logistic regression analysis, the CC genotype was found to be associated with a significantly increased risk of pancreatic cancer risk compared with the GG genotype (odds ratio = 1.85, 95% confidence interval = 1.12-3.01). Moreover, those carrying the C allele had a higher risk of pancreatic cancer compared to those with the G allele (odds ratio = 1.38, 95% confidence interval = 1.08-1.77). In conclusion, we found that the survivin rs9904341 polymorphism was associated with an increased risk of acute pancreatitis.

Key words: rs9904341; Pancreatic cancer; Susceptibility

INTRODUCTION

Pancreatic cancer is one of the most fatal malignant tumors in humans and has a very poor prognosis. The 5-year survival rate is less than 5%, even in patients who received surgical and chemotherapy intervention (Jemal et al., 2007; Tanaka et al., 2011; Nakao et al., 2012; Yan et al., 2014). The development of pancreatic cancer is a multifactorial process that includes both environmental and genetic factors (Hansel et al., 2003; Vaccaro et al., 2012). It is well known that smoking status, advanced age, alcohol consumption, body mass index, diabetes mellitus, and family history of pancreatic cancer, as well as genetic factors, play key roles in the development of pancreatic cancer (Hansel et al., 2003; Lowenfels and Maisonneuve, 2006; Luo et al., 2007; Nakao et al., 2012; Vaccaro et al., 2012).

Apoptosis, which is programmed cell death, plays a crucial role in the development and maintenance of tissue homeostasis in multicellular organisms (Thompson, 1995; Raff, 1998). Apoptosis functions mainly through the death receptor and mitochondrial pathways, causing activation of cascade enzymes known as caspases (Hengartner, 2000; Danial and Korsmeyer, 2004). Moreover, defects in apoptosis can induce numerous human disorders such as intestinal disease and tumor development (Shivapurkar et al, 2003; Hajra and Liu, 2004; Dias et al., 2014).

Survivin is in the inhibitor of apoptosis protein family (Li et al., 1998). Recent studies have shown that high survivin expression is important in the development of several types of cancer, including gastric, colorectal, head and neck, urothelial, and other cancers (Kawasaki et al., 1998; Karam et al., 2007; Ulukus et al., 2007; Zhu et al., 2013; Qin et al., 2014). Therefore, the survivin gene may play a critical role in tumorigenesis and can be used for biological, prognostic, and therapeutic applications.

The human survivin gene baculoviral inhibitor of apoptosis repeat-containing 5 (*BIRC5*) is located on chromosome 17q25, is 14.7 kb in length, and contains 4 exons and 3 introns (Altieri, 2001). Various genetic polymorphisms have been reported to be located in the regulatory regions of *BIRC*, which are associated with survivin overexpression (Liu et al., 2013). There were approximately 10 single-nucleotide polymorphisms in the promoter region of survivin. The rs9904341 G<C variant is located in the cell cycle-dependent elements and cell cycle homology region repressor-binding site of the survivin promoter (Borbély et al., 2007; Mityaev et al., 2008). An increasing number of studies have reported that the rs9904341 G<C variant is associated with the risk of several types of cancers. However, few studies examined the association between survivin polymorphisms and pancreatic cancer. Therefore, we conducted a case-control study to investigate the association between the rs9904341 G<C polymorphism and the risk of pancreatic cancer.

MATERIAL AND METHODS

Subjects

This study included 261 pancreatic cancer patients who were pathologically confirmed to have pancreatic cancer. All patients involved in the study were recruited from Zhongda Hospital, School of Medicine, Southeast University between January 2010 and December 2013.

A random sample of 224 healthy individuals was selected from the health examination center of Zhongda Hospital between January 2010 and December 2013. The controls were

matched with pancreatic cancer patients in terms of age and gender.

Control subjects with chronic diseases, brain, severe endocrinological, metabolic, and nutritional diseases as well as a history of cancer were excluded from our study. A total of 224 patients met the requirements and agreed to participate in the study (85.8%).

Each subject was interviewed in-person by trained nurses or doctors using a self-designed questionnaire to determine demographic characteristics, including gender, age, smoking and drinking status, family history of cancer, body mass index, and diabetes.

DNA isolation

To determine the presence of the rs9904341 G<C polymorphism, genomic DNA was purified from peripheral blood. All study participants provided 5 mL venous blood, which were stored at -20°C with 0.5 mg/mL ethylenediaminetetraacetic acid used as the anticoagulant until use. Genomic DNA was isolated from peripheral blood leukocytes using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to manufacturer instructions. Genomic DNA was stored at -20°C until use.

Determination of rs9904341 G<C polymorphism

Polymorphisms at rs9904341 G<C were assessed by polymerase chain reaction-restriction fragment length of polymorphism (PCR-RFLP). Primers for rs9904341 G<C were designed using the Sequenom Assay Design 3.1 software (Sequenom, Inc., San Diego, CA, USA). The forward primer sequence was 5'-CGTGCGCTCCCGACAT-3' and the reverse primer sequence was 5'-GATGCGGTGGTCCTTGAGAA-3'.

The PCR was performed in a 50-μL reaction solution containing 25 mM $MgCl_2$, 2 mM 4X dNTPs, 20 μM primers, and 5 U/μL *Taq* DNA polymerase. The PCR was performed using the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 62°C for 60 s, and extension at 72°C for 60 s, and final extension at 72°C for 10 min. PCR products were visualized by 1.0% agarose gel electrophoresis and stained using ethidium bromide staining with ultraviolet light. For quality control, we selected 5% of the cases and control subjects for repeated genotyping; the results were 100% concordant.

Statistical analysis

Continuous variables are reported as means ± standard deviation, while categorical variables are expressed as N (%) of study participants. The Student *t*-test was used to compare continuous variables between patients and control subjects, while the χ^2 test was used to compare categorical variables between patients and control subjects. Hardy-Weinberg equilibrium among controls was compared using the χ^2 test. Unconditional logistic regression was conducted to assess the association between the rs9904341 G<C polymorphism and risk of acute pancreatitis; the results are presented as odds ratios (ORs) and corresponding 95% confidence intervals (CIs). Homozygotes of the rs9904341 G<C polymorphism were considered to be the reference group. $P < 0.05$ was regarded as statistically significant, and all of statistical tests were 2-sided. All statistical analyses were performed using SPSS statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS

The demographic characteristics of pancreatic cancer patients and controls are shown in Table 1. There was no significant difference between the cases and controls in terms of gender and age. We compared pancreatic cancer patients and healthy controls in terms of demographic characteristics and found that pancreatic cancer patients were more likely to be smokers (P = 0.003), have a family history of cancer (P = 0.005), and have diabetes.

Table 1. Demographic characteristics of pancreatic cancer patients and controls.

Variables	Cases	%	Controls	%	*t*- or χ^2-test	P value
Age (years) (mean ± SD)	63.4 ± 11.5		62.6 ± 10.7			
≤ 60	104	46.43	101	45.09		
> 60	120	53.57	123	54.91	0.08	0.78
Gender						
Male	137	61.16	137	61.16		
Female	87	38.84	87	38.84	0.000	1.00
Smoking status						
Never	93	41.52	124	55.36		
Ever	131	58.48	100	44.64	8.59	0.003
Drinking status						
Never	149	66.52	162	72.32		
Ever	75	33.48	62	27.68	1.78	0.18
Body mass index						
≤25	148	66.07	153	68.30		
>25	76	33.93	71	31.70	0.25	0.61
Diabetes						
No	43	19.20	27	12.05		
Yes	181	80.80	197	87.95	4.33	0.04
Family history of pancreatic cancer						
No	39	17.41	19	8.48		
Yes	185	82.59	205	91.52	7.92	0.005

The genotype distributions of survivin rs9904341 G<C between pancreatic cancer cases and controls are shown in Table 2. The observed genotype frequencies of survivin rs9904341 G<C in controls were in Hardy-Weinberg equilibrium (P > 0.05). The frequencies of rs9904341 G<C were significantly different between cases and controls, with P values of 0.03 and 0.009 for genotype and allele, respectively. According to multivariate logistic regression analysis, subjects carrying the CC genotype had a significantly increased risk of pancreatic cancer when compared with those carrying the GG genotype (OR = 1.85, 95%CI = 1.12-3.01). Moreover, those carrying the C allele had a higher risk of pancreatic cancer compared with the G allele (OR = 1.38, 95%CI = 1.08-1.77).

Table 2. Association between rs9904341 G<C polymorphism and pancreatic cancer risk.

Polymorphisms	Cases	%	Controls	%	χ^2-test	P value	Adjusted OR (95%CI)[1]	P value
rs9904341 G<C								
GG	45	20.3	66	29.5			1.0 (Ref.)	-
CG	106	47.3	101	45.1			1.52 (0.97-2.36)	0.05
CC	73	32.4	57	25.4	6.92	0.03	1.85 (1.12-3.01)	0.01
Allele								
G	196	65.63	233	52.01			1.0 (Ref.)	-
C	252	83.48	215	47.99	6.88	0.009	1.38 (1.08-1.77)	0.009

[1]Adjusted for gender, age, smoking status, diabetes status, and family history of cancer.

We conducted stratification analyses between the rs9904341 G<C polymorphism and smoking status, diabetes, and family history of cancer. However, we found no interaction between the rs9904341 G<C polymorphism and smoking status, diabetes, and family history of cancer on the risk of pancreatic cancer.

DISCUSSION

In the present study, we found that the rs9904341 G<C polymorphism in the promoter region of survivin is associated with the susceptibility to pancreatic cancer in a Chinese population. This is the first study to evaluate the role of the rs9904341 G<C polymorphism in the survivin gene in the susceptibility to pancreatic cancer.

Several clinical and experimental studies showed that alterations in survivin gene expression can contribute to the susceptibility to common cancers (Kawasaki et al., 1998; Karam et al., 2007; Ulukus et al., 2007; Zhu et al., 2013; Qin et al., 2014). The rs9904341 G<C polymorphism was reported to be associated with altering gene expression in cancer cell lines (Xu et al., 2004). The rs9904341 G<C polymorphism is located in the cell cycle-dependent repressor elements and depressed aberrant cell-cycle-dependent transcription, and thus causes overexpression of survivin. In our study, we found that subjects carrying the CC genotype had a significantly increased risk of pancreatic cancer compared with subjects carrying the GG genotype. This CC genotype of rs9904341 indicates a functional modification in *BIRC5* expression and is associated with higher survivin concentrations, which promotes apoptosis inhibition, decreases the ability to eliminate damaged cells, and promotes disease development (Gazouli et al., 2009). Several previous studies reported that the rs9904341 G<C polymorphism likely contributed to increased susceptibility to gastric, colorectal, bladder, and prostate cancers (Chen et al., 2013; Liu et al., 2013; Zhu et al., 2013). Chen et al. (2013) conducted a case-control study to investigate the association between the rs9904341 G<C polymorphism and the development of prostate cancer, and they found that the CC genotype increased the risk of prostate cancer compared with the GG genotype (OR = 1.57, 95%CI = 1.17-2.13). Liu et al. (2013) conducted a meta-analysis by pooling 9 case-control studies and found that the survivin rs9904341 polymorphism may increase the risk of gastric and colorectal cancers. The results of our study agree with those of previous studies.

There were several limitations to our study. First, this study was conducted in a single hospital, and thus the participants may not have been representative of other areas in China. Second, the sample size of cases was relatively small. This may have decreased the statistical power for identifying the role of genetic variation in the survivin gene on the risk of pancreatic cancer. Third, other genetic polymorphisms may have a large influence the risk of pancreatitis cancer in addition to the survivin rs9904341 polymorphism. Therefore, further large sample studies including different ethnicities should be conducted to investigate the association between survivin gene polymorphisms and pancreatitis cancer risk.

In conclusion, we found that the survivin rs9904341 polymorphism is associated with an increased risk of acute pancreatitis. Because of the limitations of our study, further large sample studies including different ethnicities are needed.

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