Association between single nucleotide polymorphisms of X-ray repair cross-complementing protein 4 gene and development of pancreatic cancer

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ABSTRACT. We performed a study to evaluate X-ray repair cross-complementing protein 4 (XRCC4) gene polymorphisms and the development of pancreatic cancer. A case-control study including 206 patients with newly diagnosed primary pancreatic cancer and 412 controls was performed between January 2011 and October 2013 in a Chinese population. Genotypes of *XRCC4* rs1805377, rs2075685, rs2075686 and rs1056503 were determined using polymerase chain reaction combined with a restriction fragment length polymorphism assay. Compared with controls, pancreatic cancer patients were more likely to have a higher body mass index, family history of cancer, and a habit of alcohol drinking compared with controls (P < 0.05). Logistic regression analysis showed that individuals carrying the TT genotype of *XRCC4* rs2075685 had an increased risk of pancreatic cancer compared to those with the GG genotype, with an odds ratio (95% confidence interval) of 1.88 (1.15-3.08). Our results suggest that the

XRCC4 rs2075685 polymorphism could influence the susceptibility to pancreatic cancer in a Chinese population.

Key words: X-ray repair cross-complementing protein 4; Pancreatic cancer; Polymorphism

INTRODUCTION

Pancreatic cancer is the 4th leading cause of cancer deaths among human in the US and ranks as the 6th leading cause of cancer in China (International Agency for Research on Cancer, 2012). The five-year survival rate of pancreatic cancer is less than 5% even though these patients receive surgical and chemotherapy intervention (Tanaka et al., 2011; Nakao et al., 2012; Yan et al., 2014).

It is well-known that the development of pancreatic cancer is involved in complex and multifactorial processes. Previous studies reported that environmental factors are strong contributors to the development of pancreatic cancer, including smoking status, advanced age, alcohol consumption, body mass index and diabetes mellitus (Larsson et al., 2007; Luo et al., 2007; Nakao et al., 2012). Moreover, it has been reported that genetic variations have an important role in the development of pancreatic cancer (Avan et al., 2014; Wolpin et al., 2014). Therefore, understanding the genetic etiology of pancreatic cancer may contribute to clarify the mechanism of pancreatic cancer.

Previous studies have reported that DNA repair gene polymorphisms contribute to the development of pancreatic cancer (Li et al., 2009; Dong et al., 2011, 2012). There are several types of DNA damge, such as double-strand breaks (DSBs). Defects in DSBs repair can cause disastrous consequences, including genomic instability and carcinogenesis (Morgan et al., 1998; Yin et al., 2012). Non-homologous end-joining (NHEJ) plays important roles in DSB repair pathways (van Gent et al., 2001). In the NHEJ process, X-ray repair cross-complementing protein 4 (*XRCC4*) is located on the chromosomal 5q14.2 and restores DNA DSB repair (Li et al., 1995). Since XRCC4 repair gene alterations could cause a reduction in DNA repair capacity, we hypothesis that polymorphisms in *XRCC4* gene contribute to the development of pancreatic cancer. Currently, no studies have examined the role of *XRCC4* gene polymorphisms in glioma risk. Therefore, we performed a study to evaluate the relationship between *XRCC4* (rs1805377, rs2075685, rs2075686 and rs1056503) polymorphisms and the risk of pancreatic cancer.

MATERIAL AND METHODS

Study population

In this study, we recruited 206 patients with newly diagnosed primary pancreatic cancer from the Daqing Oil Field General Hospital between January 2011 and October 2013. Second primary and recurrent pancreatic cancer patients were excluded from this study.

Four hundred and twelve controls were randomly selected from individuals who underwent a routine physical examination during the same period. Two controls were matched to 1 case by age (± 3 years) and gender. Individuals who were direct relatives to the cases or

had a previous history of cancer were excluded. All epidemiological data of pancreatic cancer patients and control subjects were obtained from medical records and a self-designed questionnaire through in-person interviews.

All case and control subjects agreed to the study and signed a consent form. Our study was approved by the institutional ethnics committee of the Daqing Oil Field General Hospital.

The epidemiological characteristics were collected using a standardized questionnaire, such as gender, age, dietary status, tobacco smoking, alcohol drinking and family history of pancreatic cancer.

Blood samples and genotyping

A 5-mL non-fasting blood sample were collected from each participant with a vacuum tube; 0.5 mg/mL EDTA was used as an anticoagulant of blood and the samples were stored at -70°C until use. Genomic DNA was extracted from peripheral blood using the DNA Extraction Kit (Bo Fu Rui Biotechnology Company, Beijing). The genotypes of *XRCC4* rs1805377, rs2075685, rs2075686 and rs1056503 were determined using polymerase chain reaction-restriction fragment length polymorphism (Applied Biosystems, Foster City, CA, USA). Primers of *XRCC4* rs1805377, rs2075685, rs2075686, and rs1056503 were designed using the MassARRAY Assay Design 3.1 software (Sequenom). The PCR reaction was carried out as follows: initial denaturation at 94°C for 5 min, and then at 94°C for 30 s, 58°C 30s and 72°C for 30 s. After 35-cycles of amplification, additional extensions were done at 72°C for 7 min.

Statistical analysis

The statistical difference between cases and controls was analyzed by Student *t*-test or the χ^2-test. The χ^2 test was used to compare differences in demographic characteristics and genotypes of *XRCC4* genes. Hardy-Weinberg equilibrium was tested using χ^2-test for *XRCC4* rs1805377, rs2075685, rs2075686, and rs1056503 in controls. The association between *XRCC4* rs1805377, rs2075685, rs2075686, and rs1056503 polymorphisms and the risk of pancreatic cancer was evaluated by logistic regression analysis, and the results was expressed as the OR and 95% confidence interval. The SPSS 19.0 statistical software (SPSS, Chicago, IL, USA) was used for statistical analyses. All P values were 2-sided, and P values less than 0.05 were regarded as statistically significant.

RESULTS

Baseline characteristics of 206 pancreatic cancer patients and 412 health controls were shown in Table 1. The mean age was 62.52 ± 9.85 years for pancreatic cancer patients, and was 61.95 ± 10.10 years for control subjects. As expected, no significant differences was found between pancreatic cancer patients and controls in terms of age and gender (P > 0.05). Compared with controls, pancreatic cancer patients were more likely to have a higher body mass index, family history of cancer, and a habit of alcohol drinking compared with controls (P < 0.05).

Table 1. Baseline characteristics of included pancreatic cancer patients and controls.

Characteristics	Patients (N = 206)	%	Controls (N = 412)	%	t or χ^2	P value
Mean age, years	62.52 ± 9.85		61.95 ± 10.10		0.67	0.25
Gender						
Female	74	35.92	148	35.92		
Male	132	64.08	264	64.08	0.00	1.00
Body mass index						
<23 kg/m^2	94	45.63	238	57.77		
≥23 kg/m^2	112	54.37	174	42.23	8.14	0.004
Alcohol drinking						
Never	116	56.31	267	64.81		
Ever	90	43.69	145	35.19	4.21	0.04
Tobacco smoking						
Never	135	65.53	307	74.51		
Ever	71	34.47	105	25.49	5.44	0.02
Diabetes mellitus						
Yes	77	37.38	142	34.47		
No	129	62.62	270	65.53	0.51	0.48
Family history of cancer						
No	181	87.86	377	91.50		
Yes	25	12.14	35	8.50	2.08	0.15

The genotype distributions of *XRCC4* rs2075685, rs2075686, rs1056503, and rs1805377 in pancreatic cancer patients and controls were shown in Table 2. Significant difference was found in the genotype distributions of rs2075685 between pancreatic cancer patients and controls. The allele and genotype distributions of *XRCC4* rs2075685, rs2075686, and rs181805377 were found to be in Hardy-Weinberg equilibrium in the control group, while *XRCC4* rs1056503 were not (Table 2). The minor allele frequencies in controls were similar to those listed in NCBI.

Table 2. Allele frequencies of *XRCC4* in pancreatic cancer and control subjects.

SNPs	Patients	Controls	χ^2	P value	Minor allele frequency in NCBI	Minor allele frequency in controls	P value for Hardy-Weinberg equilibrium
rs2075685							
GG	53	143					
GT	98	190					
TT	55	79	7.14	0.028	0.4449	0.4223	0.26
rs2075686							
CC	166	352					
CT	25	42					
TT	15	18	3.05	0.22	0.0733	0.0947	<0.001
rs1056503							
GG	76	166					
GT	98	186					
TT	32	60	0.77	0.68	0.3756	0.3714	0.50
rs1805377							
AA	74	159					
AG	95	184					
GG	37	69	0.44	0.80	0.3752	0.3908	0.21

Logistic regression analysis showed that individuals carrying the TT genotype of *XRCC4* rs2075685 was associated with an increased risk of pancreatic cancer compared with the GG genotype, and the odds ratio (95% confidence interval) was 1.88 (1.15-3.08) (Table 3). However, no association was found between the XRCC4 rs2075686, rs1056503, and rs1805377 polymorphisms and the development of pancreatic cancer.

Table 3. Association between *XRCC4* gene polymorphisms and development of pancreatic cancer.

SNPs of XRCC4	Cases	%	Controls	%	OR (95%CI)[1]	P value
rs2075685						
GG	53	25.73	143	34.71	Ref.	
GT	98	47.57	190	46.12	1.39 (0.92-2.12)	0.10
TT	55	26.70	79	19.17	1.88 (1.15-3.08)	0.008
rs2075686						
CC	166	80.58	352	85.44	Ref.	
CT	25	12.14	42	10.19	1.26 (0.71-2.20)	0.39
TT	15	7.28	18	4.37	1.77 (0.81-3.81)	0.11
rs1056503						
GG	76	36.89	166	40.29	Ref.	
GT	98	47.57	186	45.15	1.15 (0.79-1.69)	0.45
TT	32	15.54	60	14.56	1.20 (0.70-2.04)	0.48
rs1805377						
AA	74	35.92	159	38.59	Ref.	
AG	95	46.12	184	44.66	1.11 (0.75-1.64)	0.58
GG	37	17.96	69	16.75	1.15 (0.69-1.92)	0.57

[1]Adjusted for age, gender, body mass index, alcohol drinking and tobacco smoking, diabetes mellitus, and family history of cancer.

DISCUSSION

In present study, we evaluated the association of rs1805377, rs2075685, rs2075686, and rs1056503 to the development of pancreatic cancer in a Chinese population. The findings in our study suggest that the TT genotype of *XRCC4* rs2075685 was associated with a significantly increased risk of pancreatic cancer compared with the GG genotype, but no significant association between the XRCC4 rs2075686, rs1056503, and rs1805377 polymorphisms and the risk of pancreatic cancer. These results implied that the genetic polymorphisms of *XRCC4* rs2075685 may be significantly correlated with a higher risk for developing pancreatic cancer.

Numerous epidemiologic studies have reported that *XRCC4* gene polymorphisms are associated with the risk of several types of cancers, such as colorectal cancer, acute lymphoblastic leukemia, breast cancer, glioma, hepatocellular carcinoma, non-small-cell lung cancer, esophageal cancer and prostate cancer (Bau et al., 2010; Chokkalingam et al., 2011; Zhou et al., 2012; He et al., 2013; Long et al., 2013; Zhao et al., 2013; Fan et al., 2013; Shao et al., 2014). Bau et al. (2010) investigated the association between *XRCC4* gene polymorphisms and the susceptibility to colorectal cancer, and suggested that *XRCC4* rs6869366 polymorphism may contribute to the development of esophageal cancer and may be useful for early detection of colorectal cancer. Zhou et al. (2013) conducted a meta-analysis that included five studies, and suggested that rs2075685 and rs6869366 polymorphisms increase the risk of breast cancer. He et al. (2013) investigated the association between the *XRCC4* gene polymorphisms and the risk of non-small-cell lung cancer and suggested that *XRCC4* rs6869366 polymorphism had a significantly increased NSCLC risk. Zhao et al. (2013) investigated the association between DSB gene polymorphisms and glioma risk in a Chinese population and found that the *XRCC4* rs1805377 polymorphism was associated with an increased risk of gliomas. Fan et al. (2013) reported that XRCC4 rs6869366 polymorphism may be involved in esophageal tumorigenesis. The inconsistent study results may be caused by discrepancies in cancer types, ethnicity, sample size, control selection, and study design.

However, no study reported the association between *XRCC4* polymorphisms has association with the risk of pancreatic cancer. In this study, we found that the *XRCC4* rs2075685

polymorphism may contribute to the risk of pancreatic carcinogenesis and may be useful for early detection of pancreatic cancer. Therefore, further large sample studies, including more ethnicities, are greatly needed to confirm our results.

Two limitations should be considered in our study. First, the study subjects were selected from one hospital and *XRCC4* rs1056503 were not in Hardy-Weinberg equilibrium in the control group. Our study sample may not be representative of the general populations. Second, the small sample size may have limited the statistical power for identifying associations between groups. This finding merits large sample size study to validate our results and screen more genetic polymorphisms.

In summary, our findings provided statistical evidence that the *XRCC4* rs2075685 polymorphism could influence the susceptibility to pancreatic cancer in a Chinese population. The result should prompt to perform a larger prospective study to test the role of *XRCC4* gene polymorphisms in pancreatic cancer patients.

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