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Original Article

Association of single nucleotide polymorphisms of DNA repair gene and susceptibility to pancreatic cancer

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Abstract: We conducted a case-control study to assess the XRCC4 genes polymorphism and development of pancreatic cancer. A case-control study including 248 cases and 496 controls was conducted in a Chinese population. Genotypes of XRCC4 rs2075685, rs10040363, rs963248 and rs1805377 were determined using Polymerase Chain Reaction combined with a restriction fragment length polymorphism (PCR-RFLP) assay (Applied Biosystems, Foster City, CA, USA). Pancreatic cancer cases were more likely to have a history of diabetes, a higher BMI, family history of cancer and a habit of alcohol drinking when compared with control. Conditional logistic regression analysis showed that individuals carrying TT genotype of XRCC4 rs2075685 was associated with increased risk of pancreatic cancer when compared with GG genotype, and the OR (95% CI) was 1.62 (1.04-2.52). Individuals with GT+TT genotype of XRCC4 rs2075685 were significantly associated with increased risk of pancreatic cancer in those with ever tobacco smoking habit, and the OR (95% CI) was 1.77 (1.07-2.98). In conclusion, our results suggest that XRCC4 rs2075685 polymorphism plays an important role in the risk of pancreatic cancer in a Chinese population, especially in tobacco smokers.

Keywords: XRCC4, polymorphism, pancreatic cancer

Introduction

Pancreatic cancer is the fourth leading cause of cancer death for both males and females in the United States of America, and ranks as the sixth-leading cause of cancer in China [1]. It is well known that cancer is strongly influenced by environmental and genetic factors, of which gene polymorphism is a critical cause for the difference of individual genetic susceptibility to cancer [2]. Therefore, understanding the genetic etiology of gliomas may help to reveal the mechanism of gliomas and provide new insight for the diagnosis and treatment.

Single strand breaks (SSBs) and double-strand breaks (DSBs) are important DNA damages. DSBs may lead to chromosomal breakage or rearrangement and are the most detrimental form. Moreover, defects in DSBs repair can induce disastrous consequences including genomic instability, cell death and carcinogenesis [3, 4]. Homologous recombination (HR) and non-homologous end-joining (NHEJ) play an important role in the DSBs repair pathway [5]. In the NHEJ process, XRCC4 play an important a role in NHEJ [6]. Several previous studies have reported the association between XRCC4 polymorphisms and risk of several cancers, including non-small-cell lung cancer, hepatocellular carcinoma, glioma and breast cancer [7-10]. However, no study reported the effects of XRCC4 gene polymorphisms on the glioma risk. Therefore, we conducted a case-control study to assess the XRCC4 genes polymorphism and development of pancreatic cancer.

Materials and methods

Study population

A case-control study including 248 cases and 496 controls was conducted in a Chinese population. 248 patients histopathologically diagnosed with pancreatic cancer were selected from the Henan Provincial People's Hospital between May 2011 and December 2013. Demographic and clinical data of included cases and controls were collected from medical records and a self-designed questionnaire.

Table 1. Distribution of included pancreatic cases and controls

Characteristics	Cases	%	Controls	%	χ^2	*P* value
Age, years						
< 60	105	42.34	202	40.73		
≥ 60	143	57.66	294	59.27	0.18	0.67
Gender						
Female	94	37.90	188	37.90		
Male	154	62.10	308	62.10	0.00	1.00
Body mass index						
< 25 kg/m^2	138	55.65	328	66.13		
≥ 25 kg/m^2	110	44.35	168	33.87	7.76	0.01
History of diabetes						
No	192	77.42	425	85.69		
Yes	56	22.58	71	14.31	7.98	0.01
Alcohol drinking						
Never	130	52.42	285	57.46		
Ever	118	47.58	211	42.54	1.71	0.19
Tobacco smoking						
Never	105	42.34	249	50.20		
Ever	143	57.66	247	49.80	4.1	0.04
Family history of Cancer						
No	224	90.32	413	83.27		
Yes	24	9.68	83	16.73	6.69	0.01

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

SNPs of XRCC1	Minor allele frequency in NCBI	Minor allele frequency in controls	*P* value for Hardy-Weinberg equilibrium
rs2075685	0.449	0.439	0.10
rs10040363	0.460	0.451	< 0.05
rs963248	0.465	0.438	< 0.05
rs1805377	0.375	0.377	0.11

496 cancer-free controls were randomly recruited from a pool of individuals who came to receive a health check-up in the health check-up center of the same hospitals, and the control subjects are free from any cancer, and two health control subjects were matched to one case by sex and age.

All the cases and control subjects signed an informed consent before participating into this study, and the protocol of this study was approved by the institutional ethnics committee of the Henan Provincial People's Hospital.

Data collection

We collected data regarding demographic and clinical characteristics from a self-designed questionnaire or medical records, including sex, age, tobacco smoking, alcohol drinking, history of diabetes and family history of cancer.

Blood samples and genotyping

After participating into this study, cases and control subjects were asked to provide 5 mL blood sample. 0.5 mg/ml EDTA was used for anticoagulant of blood and stored in -70°C until use. Genomic DNA was isolated from peripheral blood with TIANamp Blood DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) and DNA dissolved in water according to the manufacturer's instructions. Genotypes of XRCC4 rs2075685, rs10040363, rs963248 and rs1805377 were determined using Polymerase Chain Reaction combined with a restriction fragment length polymorphism (PCR-RFLP) assay (Applied Biosystems, Foster City, CA, USA). Probes and primers of XRCC4 rs2075685, rs10040363, rs963248 and rs1805377 were designed using Sequenom Assay Design 3.1 software (Sequenom®) according to the manufacturer instructions. Briefly PCR was carried out in a final volume of 25 μL containing 50 ng genomic DNA template, 1X PCR buffer with 2 mM $MgCl_2$, 0.5 μM of each primer, 50 μM dNTPs and 0.5 U DNA polymerase. For PCR amplification, the standard program was used as follows: one initial denaturation step at 94°C for 7 min, followed by 35 denaturation cycles of 1 min at 94°C, 1 min of annealing at 60°C, and 1 min of extension at 72°C, followed by a final elongation cycle at 72°C for 10 min. For quality control, 5% of the cases and controls were randomly selected to repeat genotyping, and the results were 100% concordance with previous.

Statistical analysis

Continuous variables were shown as the mean ± SD and analyzed by student t test. Categorical variables were expressed as n (%) of study subjects and analyzed by χ^2-test. The χ^2 test was used to compare the differences in demo-

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	65	26.21	166	33.47	Ref.	
GT	117	47.18	226	45.56	1.32 (0.91-1.94)	0.13
TT	66	26.61	104	20.97	1.62 (1.04-2.52)	0.02
rs10040363						
AA	82	33.06	174	35.08	Ref.	
AG	100	40.32	197	39.72	1.08 (0.74-1.56)	0.68
GG	66	26.61	125	25.20	1.12 (0.74-1.70)	0.57
rs963248						
AA	76	30.65	184	37.10	Ref.	
AG	98	39.52	189	38.10	1.26 (0.86-1.83)	0.22
GG	74	29.84	123	24.80	1.46 (0.96-2.20)	0.06
rs1805377						
AA	92	37.10	201	40.52	Ref.	
AG	112	45.16	216	43.55	1.13 (0.80-1.61)	0.47
GG	44	17.74	79	15.93	1.22 (0.76-1.94)	0.39

[1]Adjusted for sex, age, BMI, history of diabetes, alcohol drinking, tobacco smoking and family history of cancer.

graphic characteristics and genotypes of XRCC4 genes. The Hardy-Weinberg equilibrium (HWE) was tested by Fisher's exact test for each SNP in controls. The association between gene polymorphisms of XRCC4 and development of pancreatic cancer was assessed by conditional logistic regression models adjusted for potential confounding factors, and the results was expressed by OR and 95% confidence interval (CI) were calculated. The SPSS software (SPSS, Chicago, IL) was used for statistical analyses. All *P*-values were two sided, and a *P*-value was regarded as statistically significant when it less than 0.05.

Results

The demographic and clinical characteristics of 248 pancreatic cancer cases and 496 health controls were shown in **Table 1**. The mean ± standard deviation ages of cases and controls were 65.1 ± 10.5 and 61.5 ± 9.6 years old, respectively. Cases and controls were matched on age and sex, and no significant difference between them ($P = 0.67$ and 1.0, respectively). We found that pancreatic cancer cases were more likely to have a history of diabetes, a higher BMI, family history of cancer and a habit of alcohol drinking when compared with controls ($P < 0.05$).

The allele and genotype distributions of XRCC4 rs2075685 and rs1805377 were found to be in Hardy-Weinberg equilibrium in the control group, but the XRCC4 rs10040363 and rs963248 was not (**Table 2**). We found the Minor allele frequencies in controls were similar to them in NCBI.

Conditional logistic regression analysis showed that individuals carrying TT genotype of XRCC4 rs2075685 was associated with increased risk of pancreatic cancer when compared with GG genotype, and the OR (95% CI) was 1.62 (1.04-2.52) (**Table 3**). Moreover, we found that GG genotype of XRCC4 rs963248 was associated with marginally significantly increased risk of pancreatic cancer, and the OR (95% CI) was 1.46 (0.96-2.20). However, no association was found between XRCC4 rs10040363 and rs1805377 polymorphisms and development of pancreatic cancer.

We further analyzed the association of XRCC4 rs2075685 polymorphism with risk of pancreatic cancer risk stratified by sex, age, BMI, history of diabetes, alcohol drinking, tobacco smoking and family history of cancer (**Table 4**). Individuals with GT + TT genotype of XRCC4 rs2075685 were significantly associated with increased risk of pancreatic cancer in those with ever tobacco smoking habit, and the OR (95% CI) was 1.77 (1.07-2.98). However, we did not find significant association between XRCC4 rs2075685 polymorphism and sex, age, BMI, history of diabetes, alcohol drinking and family history of cancer in pancreatic cancer risk.

Discussion

In the present study, we investigated the relationship between XRCC4 gene polymorphisms and development of pancreatic cancer. We found that TT genotype of XRCC4 rs2075685 significantly increased the risk of pancreatic cancer, but we found no significant association

Table 4. Association between XRCC4 rs2075685 and demographic characteristics in the risk of pancreatic cancer

Characteristics	XRCC4 rs2075685				OR (95% CI)[1]	*P* value
	GG		GT+TT			
	Cases	Controls	Cases	Controls		
Age, years						
< 60	29	68	76	134	1.33 (0.77-2.32)	0.28
≥ 60	36	98	107	196	1.49 (0.93-2.40)	0.08
Gender						
Female	25	66	69	122	1.49 (0.84-2.70)	0.15
Male	40	100	114	208	1.37 (0.87-2.17)	0.15
Body mass index						
< 25 kg/m^2	36	111	102	217	1.45 (0.91-2.33)	0.1
≥ 25 kg/m^2	29	55	81	113	1.36 (0.77-2.41)	0.26
History of diabetes						
No	49	138	143	287	1.40 (0.94-2.10)	0.08
Yes	16	28	40	43	1.63 (0.72-3.71)	0.2
Alcohol drinking						
Never	34	98	96	187	1.48 (0.91-2.43)	0.09
Ever	31	68	87	143	1.33 (0.79-2.29)	0.26
Tobacco smoking						
Never	35	87	70	162	1.07 (0.65-1.80)	0.77
Ever	30	79	113	168	1.77 (1.07-2.98)	0.02
Family history of cancer						
No	60	136	164	277	1.34 (0.92-1.96)	0.11
Yes	5	30	19	53	2.15 (0.68-8.08)	0.16

[1]Adjusted for sex, age, BMI, history of diabetes, alcohol drinking, tobacco smoking and family history of cancer.

between XRCC4 rs10040363, rs963248 and rs1805377 polymorphisms and development of pancreatic cancer.

Since there is increasing evidence that genetic variation leads to different DNA repair capacities in the human population, hence such common polymorphisms can play a role in an individual's genetic susceptibility to cancer [11]. Mutations in XRCC4 gene may lead to decrease or loss of its DNA repair capacity and confer the variation in susceptibility to diverse malignant tumors among individuals. Previous studies showed that NHEJ repair pathway had an important role in repairing DSBs in mammalian cells, and XRCC4 genes play an important role in performing the ending-joining reaction and promoting various cancer tumorigenesis [12].

Many studies have shown that XRCC4 polymorphisms are associated with risk of several kinds of cancers, such as esophageal cancer, non-small-cell lung cancer, hepatocellular carcinoma, glioma and breast cancer [7-10, 13]. Fan et al. investigated the association between XRCC4 gene polymorphisms and susceptibility to esophageal cancer, and found that XRCC4 rs6869366 polymorphism contributed to the development of esophageal cancer [13]. He et al. found that rs1056503 and rs9293337 polymorphisms are risk factors for developing NSCLC [7]. Long et al. reported that XRCC4 rs3734091 polymorphism may be a genetic modifier for the risk of hepatocellular carcinoma induced by AFB1 exposure [8]. Zhao et al. conducted a case-control study to investigate the association between DSBs gene polymorphisms and risk of gliomas, and they found that XRCC4 rs1805377 polymorphism increased the risk of gliomas [9]. A recent meta-analysis with 31 case-control studies found that rs28360071 polymorphism was significantly associated with cancer risk [14]. However, no study reported the association between XRCC4 polymorphisms and pancreatic cancer. In our study, we firstly reported that XRCC4 rs2075685 polymorphism significantly influences the risk of pancreatic cancer. Therefore, further large sample studies are with more ethnicities are greatly needed to confirm our results.

Moreover, our study found that XRCC4 rs2075685 polymorphism has association with cigarette smoking in pancreatic cancer risk. Cigarette smoking may induce various types of DNA damages including benzopyrene diol epoxide adduct, strand breaks, cross-links, and recombination, and these damages are repaired through different DNA repair pathways, including NHEJ [15]. Several previous studies

reported a significant gene-smoking association for XRCC4 polymorphisms in cancer risk [16, 17]. Therefore, cigarette smoking has a synergistic effect with XRCC4 polymorphisms in pancreatic cancer risk.

Two limitations should be considered in our study. First, cases and controls were selected from one hospital, and XRCC4 rs10040363 and rs963248 were not in Hardy-Weinberg equilibrium in the control group. The sample of our study did not be representative of other populations. However, the controls were a random sample from a pool of individuals who came to receive a health check-up, which may well represent the general population. Second, the small sample size could limit the statistical power to find the association between groups. Therefore, further studies with more subjects are greatly needed to confirm the association between XRCC4 genes polymorphisms and risk of pancreatic cancer.

In conclusion, our results suggest that XRCC4 rs2075685 polymorphism plays an important role in the risk of pancreatic cancer in a Chinese population, especially in tobacco smokers. Further multicenter studies involving various populations are greatly needed to confirm our results.

Disclosure of conflict of interest

None.

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