Association of XRCC1 gene polymorphisms and pancreatic cancer risk in a Chinese population

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ABSTRACT. We conducted a case-control study to assess the role of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp gene polymorphisms in pancreatic cancer susceptibility in a Chinese population. A total of 152 patients diagnosed with pancreatic cancer and 264 control subjects were enrolled in this study between March 2012 and October 2014. XRCC1 Arg399Gln, Arg280His, and Arg194Trp were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method. As determined by the chi-squared test, a statistically significant difference was observed between pancreatic cancer patients and control subjects in regard to the genetic distribution of XRCC1 Arg399Gln (χ^2 = 16.13, P < 0.001). Using an unconditional regression analysis, we found that the TT genotype of Arg399Gln was associated with a significantly increased risk of pancreatic cancer (OR = 2.33, 95%CI = 1.20-4.51), and that the CT+TT genotype also significantly increased pancreatic cancer risk (OR = 1.58, 95%CI = 1.04-2.41), compared to the wild-type genotype. In conclusion, we found that XRCC1 Arg399Gln genetic

variations are associated with pancreatic cancer development, whereas the XRCC1 Arg280His and Arg194Trp polymorphisms did not affect pancreatic cancer risk.

Key words: XRCC1; Arg399Gln; Arg280His; Arg194Trp; Polymorphism; Pancreatic cancer

INTRODUCTION

Pancreatic cancer is the seventh leading cause of cancer-related death among both men and women worldwide (Torre et al., 2016). The development of pancreatic cancer occurs over a long period of time, involves multifactorial processes, and has many associated risk factors, including high fat and protein diets, tobacco smoking, diabetes, chronic pancreatitis, and a family history of cancer (Genkinger et al., 2015; Wada et al., 2015). Additionally, genetic factors contribute to the development of pancreatic cancer, and previous studies have reported that many genes are associated with this cancer, such as XRCC4, H63D, C282Y, UGT2B4, CYP2C19, and DNA repair genes (Ding and Li, 2015; Zhang et al., 2015; Shen et al., 2015; Che et al., 2015; Kattel et al., 2015).

The X-ray repair cross-complementing group 1 gene (XRCC1) is located on chromosome 19q13.2-13.3 (Siciliano et al., 1986; Stucki et al., 1998; Mohrenweiser et al., 2002). The main function of the XRCC1 protein is to participate in DNA repair after damage caused by ionizing radiation and chemical mutagenesis via base excision repair and single-break repair (Stucki et al., 1998). There are a variety of single nucleotide polymorphisms in XRCC1, of which those in the tenth, ninth, and sixth exons are common and cause Arg399Gln, Arg280His, and Arg194Trp amino acid substitutions, respectively. To date, several studies have reported on the association between XRCC1 gene polymorphisms and the risk of pancreatic cancer development, but the results have been inconsistent. Here, we conducted a case-control study to assess the role of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp gene polymorphisms in the susceptibility to pancreatic cancer in a Chinese population.

MATERIAL AND METHODS

Study population

The current study included 152 patients diagnosed with pancreatic cancer from Yantai Affiliated Hospital of Binzhou Medical University between March 2012 and October 2014. All patients were newly diagnosed with pancreatic cancer, and diagnoses were confirmed by histopathologic examination by two independent pathologists. Patients were excluded if they had a history of other malignant tumors, had recurrent and/or metastatic tumors, and/or had serious kidney or liver diseases. A total of 264 health controls were enrolled from the Gastrointestinal Surgery Department Clinics of Yantai Affiliated Hospital of Binzhou Medical University. All control subjects had no history of malignant tumors, had no serious infectious diseases, and had no serious gastrointestinal, kidney, or liver diseases. The general characteristics of the study participants were collected from medical records, and included data on gender, age, alcohol consumption, tobacco use, body mass index, diabetes mellitus, hypertension, and on the family history of pancreatic cancer. Written informed consent was

obtained from each subject prior to his or her participation in our study. This study was approved by the Ethics Committee of Yantai Affiliated Hospital of Binzhou Medical University.

DNA extraction and genotyping

Peripheral venous blood samples (5 mL) were obtained from each study participant. Genomic DNA was extracted from the peripheral venous blood using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). The XRCC1 Arg399Gln, Arg280His, and Arg194Trp polymorphisms were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers for XRCC1 Arg399Gln, Arg280His, and Arg194Trp were designed with the Primer Premier v5.0 software (Premier Biosoft International, Palo Alto, CA, USA), and are shown in Table 1. The digested PCR products were separated by 3% agarose gel electrophoresis, and the ethidium bromide-stained products were visualized and confirmed under ultraviolet light. For quality control, 10% of the samples were re-analyzed to verify repeatability, and 100% consistency was achieved.

Table 1. Primers and restriction enzymes for XRCC1 Arg399Gln, Arg280His, and Arg194Trp.

XRCC1 polymorphism	SNP	Primers (5'-3')	Restriction enzyme	Products length (bp)
Arg399Gln	rs25487	GCCCGCTCTGGATTATACG CTATCATCTCCTGGCCCCC	*Pvu*II	485
Arg280His	rs25489	TTGTGCTTTCTCTGTGTCCA TCCTCCAGCCTTTTCTGATA	*Rsa*I	615
Arg194Trp	rs1799782	CCAGTGGTGCTAACCTAATC CACTCAGCACCACTACCACA	*Msp*I	201

Statistical analysis

All statistical analyses were conducted using the Statistical Package for the Social Science (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA). Data regarding gender, age, alcohol consumption, tobacco use, body mass index, diabetes mellitus, hypertension, and pancreatic cancer family history are reported as frequencies (N) or percentages (%). The differences in these general characteristics between patients and controls were analyzed using chi-squared (χ^2) tests. The goodness-of-fit chi-squared test was used to evaluate whether the genotype frequencies of XRCC1 Arg399Gln, Arg280His, and Arg194Trp were in line with Hardy-Weinberg equilibrium. Moreover, the minor allele frequencies were compared with those in the National Center for Biotechnology Information (NCBI) SNP database (http://www.ncbi.nlm.nih.gov/snp/) to determine whether they were similar. An unconditional logistic analysis was performed to evaluate the role of XRCC1 Arg399Gln, Arg280His, and Arg194Trp in the development of pancreatic cancer. P values less than 0.05 were regarded as statistically significant.

RESULTS

The general characteristics of the study participants are summarized in Table 2. Of the 152 pancreatic cancer patients, there were 55 (36.18%) females and 97 (63.82%) males. Of the 264 control subjects, there were 111 (42.05%) females and 153 (57.95%) males. As determined by chi-squared tests, there were no significant differences between pancreatic

cancer patients and control subjects regarding age ($\chi^2 = 0.35$, P = 0.56), gender ($\chi^2 = 1.38$, P = 0.24), alcohol consumption ($\chi^2 = 2.02$, P = 0.16), tobacco use ($\chi^2 = 2.41$, P = 0.12), body mass indexes ($\chi^2 = 0.26$, P = 0.61), diabetes mellitus ($\chi^2 = 0.65$, P = 0.42), or hypertension ($\chi^2 = 0.69$, P = 0.41). We found that there was a difference in cancer family history between the pancreatic cancer patients and the control subjects ($\chi^2 = 12.37$, P < 0.05).

Table 2. General characteristics of pancreatic cancer patients and control subjects.

Variables	Patients	%	Controls	%	χ^2 test	P value
Age (years)						
<55	69	45.39	112	42.42		
≥55	83	54.61	152	57.58	0.35	0.56
Gender						
Female	55	36.18	111	42.05		
Male	97	63.82	153	57.95	1.38	0.24
Alcohol consumption						
Never	86	56.58	168	63.64		
Ever	66	43.42	96	36.36	2.02	0.16
Tobacco use						
Never	85	55.92	168	63.64		
Ever	67	44.08	96	36.36	2.41	0.12
Body mass index						
<23	68	44.74	125	47.35		
≥23	84	55.26	139	52.65	0.26	0.61
Diabetes mellitus						
No	130	85.53	233	88.26		
Yes	22	14.47	31	11.74	0.65	0.42
Hypertension						
No	115	75.66	209	79.17		
Yes	37	24.34	55	20.83	0.69	0.41
Family history of pancreatic cancer						
No	145	95.39	264	100		
Yes	7	4.61	0	0.00	12.37	<0.001

The distributions of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp gene polymorphisms are summarized in Table 3. As determined by the chi-squared test, a statistically significant difference was observed between pancreatic cancer patients and control subjects in regards to the genetic distribution of XRCC1 Arg399Gln ($\chi^2 = 16.13$, P < 0.001). However, no significant differences were observed in the genetic distributions of XRCC1 Arg280His ($\chi^2 = 3.37$, P = 0.19) and Arg194Trp ($\chi^2 = 1.85$, P = 0.40) between the two groups. According to the goodness-of-fit chi-squared test, the genetic distributions of XRCC1 Arg399Gln (P = 0.03), Arg280His (P < 0.001), and Arg194Trp (P = 0.01) were not in Hardy-Weinberg equilibrium in the control group. The minor allele frequencies of XRCC1 Arg399Gln, Arg280His, and Arg194Trp in controls were similar to those reported in the NCBI SNP database. Using an unconditional regression analysis, we found that the TT genotype of Arg399Gln was associated with a significant increase in pancreatic cancer risk (OR = 2.33, 95%CI = 1.20-4.51), and that the CT+TT genotype significantly increased pancreatic cancer risk (OR = 1.58, 95%CI = 1.04-2.41), compared to the wild-type genotype.

Table 3. Genotype frequencies of XRCC1 Arg399Gln, Arg280His, and Arg194Trp and their association with pancreatic cancer risk.

XRCC1	Patients	%	Controls	%	χ^2 test	P value	Hardy-Weinberg equilibrium in controls	Minor allele frequencies in controls	OR (95%CI)[1]	P value
Arg399Gln										
CC	70	46.05	151	57.20					1.0 (Ref.)	-
CT	56	36.84	88	33.33					1.37 (0.86-2.18)	0.16
TT	27	17.76	25	9.47	16.13	<0.001	0.03	0.2614	2.33 (1.20-4.51)	0.01
CT+TT	83	54.61	113	42.80					1.58 (1.04-2.41)	0.02
Arg280His										
AA	128	84.21	236	89.39					1.0 (Ref.)	-
AG	16	10.53	22	8.33					1.34 (0.63-2.78)	0.4
GG	8	5.26	6	2.27	3.37	0.19	<0.001	0.0644	2.46 (0.73-8.78)	0.09
AG+GG	24	15.79	28	10.61					1.58 (0.84-2.96)	0.12
Arg194Trp										
GG	109	71.71	200	75.76					1.0 (Ref.)	-
GA	33	21.71	54	20.45					1.12 (0.66-1.88)	0.65
AA	10	6.58	10	3.79	1.85	0.40	0.01	0.1402	1.83 (0.66-5.07)	0.18
GA+AA	43	28.29	64	24.24					1.23 (0.76-1.98)	0.36

[1]Adjusted for gender and age.

DISCUSSION

We conducted a case-control study herein to assess the role of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp gene polymorphisms in the development of pancreatic cancer, and found that the TT and CT+TT genotypes of XRCC1 Arg399Gln were associated with a significantly increased risk of pancreatic cancer.

Previous studies have assessed the role of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp polymorphisms in the development of several types of cancers, such as non-small cell lung cancer (NSCLC), prostate cancer, glioma, breast cancer, ovarian cancer, and colorectal cancer (Kang et al., 2015; Han et al., 2015; Zhu et al., 2015; Wang et al., 2015; Guo et al., 2015; Malisic et al., 2015; Nissar et al., 2015). For example, Kang et al. (2015) conducted a study on 210 NSCLC patients and 210 health control subjects in China to investigate the role of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp polymorphisms in the risk of NSCLC. They found that the XRCC1 Arg194Trp polymorphism increased the risk of NSCLC development. Zhu et al. (2015) examined the role of the XRCC1 Arg399Gln, Arg280His, and Arg194Trp polymorphisms in the risk of prostate cancer, and showed an increased risk for prostate cancer in individuals with the XRCC1 Arg194Trp polymorphism. Wang et al. (2015) conducted a study on 387 glioma patients and 400 cancer-free controls, and found that the XRCC1 Arg399Gln polymorphism was associated with glioma susceptibility. Guo et al. (2015) performed a meta-analysis on 13 published case-control studies, which revealed a lack of association between the XRCC1 Arg399Gln polymorphism and breast cancer risk. Malisic et al. (2015) and Han et al. (2015) suggested that the XRCC1 Arg399Gln polymorphism may be a predictive biomarker for ovarian cancer and NSCLC susceptibility. Lastly, Nissar et al. (2015) found that the XRCC1 Arg194Trp polymorphism was associated with a risk of developing colorectal cancer.

In regards to the role of XRCC1 polymorphisms in pancreatic cancer risk, several previous studies have reported conflicting results (Duell et al., 2002; Jiao et al., 2006; Wang et al., 2006; Nakao et al., 2012; Jiang et al., 2013; Yan et al., 2013; He et al., 2014; Hou et al., 2015). First, Duell et al. (2002) analyzed the role of the XRCC1 Arg399Gln polymorphism

in the risk of pancreatic cancer, and reported that the XRCC1 399Gln allele was an important biomarker for pancreatic cancer risk in an American population. An additional three studies conducted in Chinese populations and another in a Japanese population reported that the XRCC1 Arg399Gln polymorphism contributed to the development of pancreatic cancer (Wang et al., 2006; Nakao et al., 2012; Jiang et al., 2013; Yan et al., 2013). Furthermore, two studies found that the XRCC1 Arg194Trp polymorphism was a potential risk factor for pancreatic cancer (Jiao et al., 2006; Yan et al., 2013). However, another three studies did not find a significant association between the XRCC1 Arg194Trp polymorphism and pancreatic cancer development (Wang et al., 2006; He et al., 2014; Hou et al., 2015). In the current study, we found that only the XRCC1 399Gln allele was significantly associated with pancreatic cancer risk. The differences in these results may be caused by different populations, genotyping methods, and/or sample sizes. Therefore, additional studies with larger sample sizes are needed to validate our findings.

In conclusion, we found that XRCC1 Arg399Gln genetic variations are associated with the development of pancreatic cancer, whereas the XRCC1 Arg280His and Arg194Trp polymorphisms were not associated with pancreatic cancer risk. The mechanisms of how XRCC1 gene polymorphisms affect pancreatic cancer risk remain to be elucidated.

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