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Research Paper

Association Study of Single Nucleotide Polymorphisms in *XRCC1* Gene with the Risk of Gastric Cancer in Chinese Population

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Abstract

Gastric cancer is one of highly cancer-related deaths in the world. Previous evidence suggests that the X-ray repair cross-complementing group 1 gene (*XRCC1*) is one of the most important candidate genes for influencing gastric cancer risk. The objective of this study was to detect the potential association of genetic variants in *XRCC1* gene with gastric cancer risk in Chinese Han population. In total, we enrolled 395 gastric cancer patients and 398 cancer-free controls in this study. The genotyping of c.910A>G and c.1804C>A genetic variants in *XRCC1* gene were investigate by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and created restriction site-PCR (CRS-PCR) methods, respectively. We found the genotypes/alleles from these two genetic variants were statistically associated with the increased risk of gastric cancer (for c.910A>G**,** GG versus (vs.) AA: OR = 2.00, 95% CI 1.21-3.31; AG vs. AA: OR = 1.50, 95% CI 1.12-2.02; GG/AG vs. AA: OR = 1.59, 95% CI 1.20-2.10; GG vs. AG/AA: OR = 1.68, 95% CI 1.03-2.73; G vs. A: OR = 1.47, 95% CI 1.18-1.83; for c.1804C>A**,** AA vs. CC: OR = 2.68, 95% CI 1.46-4.94; AA vs. CA/CC: OR = 2.62, 95% CI 1.44-4.76; A vs. C: OR = 1.33, 95% CI 1.06-1.66). The allele-G of c.910A>G and allele-A of c.1804C>A genetic variants may contribute to gastric cancer susceptibility. These preliminary results indicate that these *XRCC1* genetic variants are potentially related to gastric cancer susceptibility in Chinese Han population, and might be used as molecular markers.

Key words: Gastric cancer; *XRCC1* gene; Single nucleotide polymorphisms; Molecular markers; Risk factors.

Introduction

Gastric cancer is a common solid tumor, and causes global health problem in the world. It is causing highly cancer-related deaths worldwide [1-5]. In the last few decades, a gradual decrease in incidence and mortality rate in gastric cancer has been observed [6-8]. However, in China, gastric cancer still remains one of the leading cause of cancer death [8-11]. Many factors could affect the development of gastric carcinogenesis. It has been reported that genetic factors may play key roles in the pathogenesis of gastric cancer [6,12,13]. Previous evidence suggests that the X-ray repair cross-complementing group 1 gene (*XRCC1*) is one of the most important candidate genes for influencing gastric cancer risk [5-10,12-15]. Evidence from published studies indicated that the single nucleotide polymorphisms (SNPs) in *XRCC1* gene may alter the efficiency of DNA repair and influence gastric cancer susceptibility [5,8-10,12-19]. Three common genetic polymorphisms, more often found in the *XRCC1* conserved sites, lead to a C to T substitu-

tion at codon 194 in exon 6, to a G to A substitution at codon 280 in exon 9, and to a G to A substitution at codon 399 in exon 10 of *XRCC1* gene, leading to the amino acid alterations arginine (Arg) to tryptophan (Trp), Arg to histidine (His), and Arg to glutamine (Gln), respectively. These changes in conserved protein sites may alter the base excision repair (BER) capacity, increasing the chances of DNA damage [10,13,19-21]. Both of Arg194Trp, Arg280His and Arg399Gln genetic variants have potential affection on the susceptibility to gastric cancer [5,8-10,12-18]. However, the exact mechanism of the pathogenesis of gastric cancer still remains poorly understood. Up to now, there are no similar studies which reported the potential affection of c.910A>G and c.1804C>A genetic variants in *XRCC1* on gastric cancer susceptibility. Thus, in this case-control study, the objective of this study aimed to assess whether these genetic variants influencing on gastric cancer susceptibility in Chinese Han population.

Materials and methods

Study population

A total of 793 subjects were enrolled from the First Affiliated Hospital, Medical School of Xi'an Jiaotong University in this case-control study, which consisting of 395 gastric cancer patients with a pathology-confirmed diagnosis and 398 healthy age-matched subjects who had no history of any gastric diseases as controls. All subjects were unrelated Chinese with Han nationality and lived in Xi'an city, Shaanxi province of China. Table 1 shows the demographic clinical characteristics, including sex, age, smoking, drinking, H.pyori infection, family history of gastric cancer. The protocol of this study was approved by the Ethics Committee of the First Affiliated Hospital, Medical School of Xi'an Jiaotong University. The informed consent for participation was obtained from all subjects.

PCR amplification

Genomic DNA was extracted from peripheral venous blood of each participant using the standard extraction method and then stored at -80°C [22]. Based on the human *XRCC1* gene mRNA sequences and DNA sequences (GenBank IDs: NM_006297.2 and NC_000019.9), the specific polymerase chain reaction (PCR) primers were designed by Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, CA). Table 2 shows the information of primers sequences, annealing temperature, fragment sizes and region. The PCR reaction was performed in a total volume of 20 μL solution containing 50 ng template DNA, 1×buffer (Tris-HCl 100 mmol/L, pH 8.3; KCl 500 mmol/L), 0.25 μmol/L primers, 2.0 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, and 0.5U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR amplification condition was carried out in an initial 94°C for 5 minutes, then followed by 32 cycles of 94°C for 32 seconds, annealing at the corresponding temperature (given in Table 2) for 32 seconds and 72°C for 32 seconds, and a final extension at 72°C for 5 minutes. The PCR amplified products were separated by 2.0% agarose gel electrophoresis and then observed under UV light.

Table 1. Clinical characteristics of gastric cancer cases and cancer-free controls.

Characteristics	Cases (n)	%	Controls (n)	%	χ^2-value	P-value
Number	395	49.81	398	50.19		
Age (years)					0.8001	0.3711
Mean ± SD	60.28 ± 10.66		61.67 ± 12.56			
< 60	181	45.82	195	48.99		
≥ 60	214	54.18	203	51.01		
Sex					0.4443	0.5050
Male	218	55.19	229	57.54		
Female	177	44.81	169	42.46		
Drinking					0.5502	0.4582
Yes	201	50.89	213	53.52		
No	194	49.11	185	46.48		
Smoking					2.1668	0.141015
Yes	226	57.22	207	52.01		
No	169	42.78	191	47.99		
Family history of gastric cancer (n)					1.5743	0.2096
Yes	158	40.00	142	35.68		
No	237	60.00	256	64.32		
H.pyori infection (n)					3.1951	0.0739
Positive	245	62.03	222	55.78		
Negative	150	37.97	176	44.22		

Table 2. The primers, PCR-RFLP and CRS-PCR analysis for *XRCC1* genetic polymorphisms

SNPs	Primer sequences	Amplification fragment (bp)	Region	Annealing temperature (°C)	Genotype method	Restriction enzyme	Genotype (bp)
c.910A>G	5'-GACTGCTGGGTCTGAGGGAGG-3'	238	Exon9	63.2	PCR-RFLP	*Hha*I	AA:238
	5'-TCAGCACCACTACCACACCCTG-3'						AG:238,166,72
							GG:166,72
c.1804C>A	5'-AGGACAATATGAGTGACCGGGTTC-3'	209	Exon17	64.0	CRS-PCR	*Mae*II	CC:209
	5'-CGAACGAATGCCAGGGACG-3'						CA:209,192,17
							AA:192,17

Note: SNPs, single nucleotide polymorphisms; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; CRS-PCR, created restriction site PCR; Underlined nucleotides mark nucleotide mismatches enabling the use of the selected restriction enzymes for discriminating sequence variations.

Genotyping

The genotyping for c.910A>G genetic variants were detected through the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The c.1804C>A genetic variants were genotyped by the created restriction site-PCR (CRS-PCR) method with one of the primers containing a nucleotide mismatch, which enables the use of restriction enzymes for discriminating sequence variations [23-27]. According to the supplier's manual, aliquots of 5 μL PCR products were digested with 2U selected restriction enzyme (MBI Fermentas, St. Leon-Rot, Germany, given in Table 2) at 37°C for 10 hours. The digested products were separated by electrophoresis in 2.5% agarose gel and observed under UV light for analyzing the genotyping of *XRCC1* genetic polymorphisms. In order to ensure the concordance of genotype results from CRS-PCR and PCR-RFLP methods, about 10% of random samples were re-analyzed by DNA sequencing method (ABI3730xl DNA Analyzer, Applied Biosystems, Foster City, CA).

Statistical analyses

The Hardy–Weinberg equilibrium (HWE) in the frequencies of allele and genotype, and general characteristics between gastric cancer patients and cancer-free controls were analyzed by chi-squared (χ^2) test. The odds ratios (ORs) and 95% confidence intervals (95% CIs) from unconditional logistic regression were utilized to evaluate the potential associations between genetic variants of *XRCC1* gene and the risk of gastric cancer. P value less than 0.05 was defined as statistically significant. All statistical analyses were evaluated by the Statistical Package for Social Sciences software (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA).

Results

General characteristics

In this case-control study, we enrolled 395 gastric cancer patients and 398 healthy age-matched controls in Chinese Han population. The chi-squared (χ^2) test indicated that there were no significant differences between gastric cancer patients and cancer-free controls in regarded to sex, age, drinking, smoking, family history of gastric cancer, and H. pyori infection (All P values > 0.05, Table 1). The demographic general characteristics of the subjects are performed in Table 1.

XRCC1 genetic variants identification

Through the PCR-RFLP and DNA sequencing methods, the c.910A>G genetic variants of *XRCC1* gene were detected. DNA sequence analyses indicate that this genetic variant is a non-synonymous mutation, which causes by A to G mutations in exon9 of human *XRCC1* gene and leading to the threonine (Thr) to alanine (Ala) amino acid replacement (p.Thr304Ala, Reference sequences GenBank IDs: NC_000019.9, NM_006297.2 and NP_006288.2). The PCR products were digested with *Hha*I restriction enzyme and divided into three genotypes, AA (238 bp), AG (238,166 and 72bp) and GG (166 and 72bp, Table 2). As for the c.1804C>A genetic variants, this genetic variants were genotyped by CRS-PCR and DNA sequencing methods. According to the DNA sequence analyses, this genetic polymorphism is a non-synonymous mutation, which causes by C to A mutations in exon17 of human *XRCC1* gene and leading to the proline (Pro) to threonine (Thr) amino acid replacement (p.Pro602Thr, Reference sequences GenBank IDs: NC_000019.9, NM_006297.2 and NP_006288.2). The PCR products were digested with *Mae*II restriction enzyme and divided into three genotypes, CC (209 bp), CA (209,192 and 17 bp) and AA (192 and 17 bp, Table 2).

Allelic and genotypic frequencies

The allelic and genotypic frequencies of c.910A>G and c.1804C>A genetic variants are summarized in Table 3. The allele-A frequencies of c.910A>G and allele-C of c.1804C>A genetic variants were maximums in gastric cancer patients and can-

cer-free controls. As for c.910A>G, the allele frequencies of gastric cancer patients (A, 66.96%; G, 33.04%) were statistically significantly different from cancer-free controls (A, 74.87%; G, 25.13%, χ^2 = 12.0393, P = 0.0005). The genotype frequencies in gastric cancer patients were not consistent with cancer-free controls, the differences being statistically significant (χ^2 = 11.6137, P = 0.0030, Table 3). As for c.1804C>A, significantly differences were detected between the allele frequencies of gastric cancer patients (C, 71.65%; A, 28.35%) and those of cancer-free controls (C, 77.01%; A, 22.99%, χ^2 = 5.9812, P = 0.0145), and the genotypic frequencies in gastric cancer patients were statistically significant different from those of cancer-free controls (χ^2 = 10.6912, P = 0.0048, Table 3). The genotypic frequencies of these two genetic variants sites were corresponded to HWE in gastric cancer patients and cancer-free controls (P > 0.05, Table 3).

Association between *XRCC1* genetic variants and gastric cancer risk

Table 4 presents the potential association between *XRCC1* genetic variants and the risk of gastric cancer. As for c.910A>G, we detected statistically increased risk of gastric cancer in the homozygote comparison (GG versus (vs.) AA: OR = 2.00, 95% CI 1.21-3.31, χ^2 = 7.43, P = 0.006), heterozygote model (AG vs. AA: OR = 1.50, 95% CI 1.12-2.02, χ^2 = 7.21, P = 0.007), dominant model (GG/AG vs. AA: OR = 1.59, 95% CI 1.20-2.10, χ^2 = 10.42, P = 0.001), recessive model (GG vs. AG/AA: OR = 1.68, 95% CI 1.03-2.73, χ^2 = 4.39, P = 0.036) and allele comparison (G vs. A: OR = 1.47, 95% CI 1.18-1.83, χ2 = 12.03, P = 0.001). As for c.1804C>A, statistically significantly increased risk of gastric cancer were found in the homozygote comparison (AA vs. CC: OR = 2.68, 95% CI 1.46-4.94, χ^2 = 10.59, P = 0.001), recessive model (AA vs. CA/CC: OR = 2.62, 95% CI 1.44-4.76, χ^2 = 10.51, P = 0.001) and allele comparison (A vs. C: OR = 1.33, 95% CI 1.06-1.66, χ^2 = 5.98, P = 0.014, Table 4).

Table 3. The genotype and allele frequencies of *XRCC1* gene polymorphisms in cases and controls

Groups	c.910A>G							c.1804C>A						
	Genotype frequencies (%)			Allele frequencies (%)				Genotype frequencies (%)			Allele frequencies (%)			
	AA	AG	GG	A	G	χ^2	P	CC	CA	AA	C	A	χ^2	P
Cases (n = 395)	180(45.57)	169(42.78)	46(11.65)	529(66.96)	261(33.04)	0.4307	0.8063	210(53.17)	146(36.96)	39(9.87)	566(71.65)	224(28.35)	3.2183	0.2001
Controls (n = 398)	227(57.03)	142(35.68)	29(7.29)	596(74.87)	200(25.13)	1.0657	0.5869	231(58.04)	151(37.94)	16(4.02)	613(77.01)	183(22.99)	2.0327	0.3619
Total (n = 793)	407(51.32)	311(39.22)	75(9.46)	1125(70.93)	461(29.07)	1.8990	0.3869	441(55.61)	297(37.45)	55(6.94)	1179(74.34)	407(25.66)	0.26737	0.8749
	χ^2 = 11.6137, P = 0.0030			χ^2 = 12.0393, P = 0.0005				χ^2 = 10.6912, P = 0.0048			χ^2 = 5.9812, P = 0.0145			

Table 4. The association between gastric cancer risk and *XRCC1* gene polymorphisms.

SNPs	Comparisons	Test of association		
		OR (95% CI)	χ^2-value	P-value
c.910A>G	Homozygote comparison (GG vs. AA)	2.00(1.21-3.31)	7.43	0.006
	Heterozygote comparison (AG vs. AA)	1.50(1.12-2.02)	7.21	0.007
	Dominant model (GG/AG vs. AA)	1.59(1.20-2.10)	10.42	0.001
	Recessive model (GG vs. AG/AA)	1.68(1.03-2.73)	4.39	0.036
	Allele contrast (G vs. A)	1.47(1.18-1.83)	12.03	0.001
c.1804C>A	Homozygote comparison (AA vs. CC)	2.68(1.46-4.94)	10.59	0.001
	Heterozygote comparison (CA vs. CC)	1.06(0.79-1.43)	0.17	0.682
	Dominant model (AA/CA vs. CC)	1.22(0.92-1.61)	1.91	0.167
	Recessive model (AA vs. CA/CC)	2.62(1.44-4.76)	10.51	0.001
	Allele contrast (A vs. C)	1.33(1.06-1.66)	5.98	0.014

SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; vs., versus.

Discussion

Many emergence reports indicate that gastric cancer is a common solid cancer causing by complex interactions between environmental and genetic factors. The genetic factors of candidate genes might play key roles in the susceptibility to gastric cancer. The *XRCC1* gene is an important candidate gene for influencing gastric cancer susceptibility [5-10,12-15]. Earlier studies on the potential association between *XRCC1* genetic variants (such as Arg194Trp, Arg280His and Arg399Gln) and gastric cancer susceptibility revealed inconsistent results that might be attributed to environmental and genetic factors [5,8-10,12-18]. ENGIN et al., suggested that the *XRCC1* homozygous Gln allele at Arg399Gln was associated with 2.54 times higher risk of gastric cancer [17]. Li et al., indicated that subjects carrying the combined *XRCC1*-194Arg/Trp + Trp/Trp genotype had an elevated chance of regression of gastric lesions (adjusted OR = 1.44; 95% CI 1.06–1.96), whereas subjects carrying the *XRCC1*-399Arg/Gln + Gln/Gln genotype had a decreased chance of regression (OR = 0.68, 95% CI 0.49–0.92) in a Chinese population [15]. Yuan et al., reported that the *XRCC1* 194Trp allele significantly increased the risk of gastric cancer (OR = 2.72, 95% CI 1.04-7.24, P = 0.027), and also associated with risk of gastric cardia carcinoma and promoted distant metastasis of gastric cancer [12]. Duarte reported that there were no evidence of a relationship between the polymorphisms *XRCC1* Arg194Trp and Arg399Gln and the risk of gastric cancer in the Brazilian population [13]. Lee et al., indicated none of these three amino acid substitution polymorphisms (Arg194Trp, Arg280His, and Arg399Gln) were associated with increased risk of gastric cancer in the Korean population [5]. Furthermore, Lee and his colleagues found that the haplotype A (194Trp, 280Arg, and 399Arg) was associated with significant reduction in gastric cancer risk (adjusted OR = 0.65, 95% CI 0.43–0.99), whereas haplotype D (194Arg, 280Arg, and 399Arg alleles) was a risk type for gastric cancer (adjusted OR = 1.57, 95% CI 0.93–2.65) [5]. Ratnasinghe et al., observed no association between the variant genotype in *XRCC1* Arg194Trp and gastric cardia cancer, while found that the Arg399Gln was associated with reduced risk of gastric cardia cancer (relative risks (RR): 0.60, 95% CI 0.37–0.97). In the present study, the influencing of c.910A>G and c.1804C>A genetic variants in *XRCC1* on the susceptibility to gastric cancer was evaluated by association analysis in 395 gastric cancer patients and 398 cancer-free control subjects. We demonstrated that these two SNPs have statistically significant impacts on the susceptibility to gastric cancer in Chinese Han population (Table 4). Our data indicated that the significant differences were shown in the frequencies of allele and genotype between gastric cancer patients and cancer-free controls for these two SNPs (All $P < 0.05$, Table 3). As for c.910A>G, the GG genotype was statistically associated with the increased susceptibility of developing gastric cancer compared to AA genotype and AG/AA carriers ($P < 0.05$). As for the c.1804C>A genetic variants, the AA genotype was statistically associated with the increased susceptibility of developing gastric cancer compared to CC genotype and CA/CC carriers (all $P < 0.01$). Our data indicated that the allele-G of c.910A>G and allele-A of c.1804C>A genetic variants were increased risk factors for gastric cancer susceptibility (Table 4). To the best of our knowledge, this is the first report regarding the association between c.910A>G and c.1804C>A SNPs and the risk of gastric cancer. These findings could provide new evidence for further analysis of the biological function role of *XRCC1* gene variants on gastric cancer risk. These observations suggested that *XRCC1* genetic variants could be useful molecular biomarkers for assessing the susceptibility to gastric cancer. Future functional studies are necessary to verify these findings for use of c.910A>G and c.1804C>A SNPs of *XRCC1* gene as molecular biomarkers in gastric cancer.

Competing Interests

The authors have declared that no competing interest exists.

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