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Association study of c.910A>G and c.1686C>G polymorphisms in *XRCC1* gene with risk of hepatocellular carcinoma in the Chinese population

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ABSTRACT. *XRCC1* (human X-ray repair complementing defective repair in Chinese hamster cell 1) gene is considered a potentially important gene influencing the risk of hepatocellular carcinoma (HCC). Our analyses detected two allelic variants of *XRCC1*, c.910A>G and c.1686C>G. We aimed to investigate whether these polymorphisms influence the risk of HCC. The association between the *XRCC1* polymorphisms and the risk of HCC was analyzed in 719 patients and 662 controls by polymerase chain reaction-restriction fragment length polymorphism. Our data suggested that the genotypes and alleles of c.910A>G and c.1686C>G polymorphisms were statistically associated with the risk of HCC. For c.910A>G, the GG genotype was associated with increased risk of developing HCC compared with the AA wild

genotype (OR = 1.95, 95%CI = 1.40-2.70, P < 0.0001). For c.1686C>G, the risk of HCC was significantly higher for the GG genotype compared with the CC wild genotype (OR = 1.89, 95%CI = 1.375-2.599, P < 0.0001). Significant differences in the risk of HCC were also found with other genetic models for these two SNPs. The G allele of both c.910A>G and c.1686C>G may contribute to the risk of HCC (G versus A: OR = 1.40, 95%CI = 1.20-1.64, P < 0.0001 and G versus C: OR = 1.38, 95%CI = 1.19-1.61, P < 0.0001, respectively). Our findings suggest that the c.910A>G and c.1686C>G polymorphisms of *XRCC1* are associated with the risk of HCC in the Chinese population.

Key words: Hepatocellular carcinoma; *XRCC1*; Molecular marker; Single nucleotide polymorphisms; Risk factors

INTRODUCTION

Hepatocellular carcinoma (HCC) is a global health problem. It is the fifth most common solid cancer and the third leading cause of cancer-related deaths globally (Llovet et al., 2003; Parkin et al., 2005; Parikh and Hyman, 2007). HCC shows great geographical variation, more than 600,000 people die from HCC each year, >75% of the cases occur in the Asia-Pacific region (But et al., 2008; Yuen et al., 2009). In China, HCC has been the second leading cause of cancer deaths since the 1990s (Chen et al., 2010). China has a very high HCC incidence, with approximately 55% of the world's new cases annually (Parkin, 2001; Parkin et al., 2005; Schutte et al., 2009). Currently, the exact mechanism of HCC is poorly understood. Previous studies indicated that the human X-ray repair complementing defective repair in Chinese hamster cells 1 gene (*XRCC1*) was potentially an important gene influencing the risk of HCC (Rossit et al., 2002; Yu et al., 2003; Chen et al., 2005; Kirk et al., 2005; Borentain et al., 2007; Long et al., 2006, 2008; Kiran et al., 2009a,b; Liu et al., 2011; Pan et al., 2011; Han et al., 2012; Li et al., 2012). A number of single nucleotide polymorphisms (SNPs) have been identified in *XRCC1*, such as arg inine(Arg)194tryptophan(Trp), Arg280histidine(His), and Arg399glutamine(Gln) (Rossit et al., 2002; Yu et al., 2003; Long et al., 2008; Kiran et al., 2009a,b; Pan et al., 2011; Han et al., 2012; Li et al., 2012). Furthermore, several studies proved that *XRCC1* polymorphisms were associated with HCC (Rossit et al., 2002; Yu et al., 2003; Long et al., 2008; Kiran et al., 2009a,b; Pan et al., 2011; Han et al., 2012; Li et al., 2012). To date, however, there are no similar reports on the relationship between the c.910A>G and c.1686C>G polymorphisms in *XRCC1* and the risk of HCC. The aim of this study was therefore to evaluate whether human *XRCC1* c.910A>G and c.1686C>G polymorphisms influence the risk of HCC in the Chinese population.

MATERIAL AND METHODS

Study population

This case-control study consisted of 719 patients with HCC from Tongji Hospital (Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei, China) and 662 non-cancer controls. All HCC patients and healthy controls were unrelated

Han Chinese and were enrolled consecutively from January 2009 to December 2011. HCC patients were diagnosed by doctors using standards established by the Chinese Society of Liver Cancer (CSLC). Healthy controls were matched with the patients in terms of age and gender, excluding those with a history of cancer and other diseases. Clinical characteristics, including gender, age, tobacco smoking, alcohol drinking, diabetes mellitus, hypertension, family history of HCC, serum alpha-fetoprotein levels, and hepatites B virus (HBV) serological markers, were collected (Table 1). The present study protocol was approved by the independent Ethics Committee of Tongji Hospital, and all individuals gave informed consent.

Table 1. Clinical characteristics of the subjects.

Characteristics	Cases [N (%)]	Controls [N (%)]	P value
Number	719 (51.31)	662 (48.69)	
Gender			0.1290[a]
Male	547 (76.08)	480 (72.51)	
Female	172 (23.92)	182 (27.49)	
Age (years; mean ± SD)	56.66 ± 14.68	55.26 ± 14.29	0.5268[b]
<50	406 (56.47)	382 (57.70)	
≥50	313 (43.53)	280 (42.30)	
Tobacco smoking			0.0896[a]
Yes	430 (59.81)	366 (55.29)	
No	289 (40.19)	296 (44.71)	
Alcohol drinking			0.1238[a]
Yes	399 (55.49)	340 (51.36)	
No	320 (44.51)	322 (48.64)	
Diabetes mellitus			0.6054[a]
Yes	178 (24.76)	156 (23.56)	
No	541 (75.24)	506 (76.44)	
Hypertension			0.9200[a]
Yes	131 (18.22)	122 (18.43)	
No	588 (81.78)	540 (81.57)	
Family history of HCC			
Yes	67 (9.32)	-	
No	652 (90.68)	-	
Serum alpha-fetoprotein levels			
<400 ng/mL	232 (32.27)	-	
>400 ng/mL	487 (67.73)	-	
Hepatites B virus serological markers			
HBs Ag(+)	195 (27.12)	-	
HBs Ag(-)	524 (72.88)		

[a]Chi-square test; [b]Mann-Whitney test.

DNA extraction and *XRCC1* polymorphism genotyping

Genomic DNA was extracted from peripheral venous blood of all subjects using the standard phenol/chloroform extraction method. Specific PCR primers were designed using the Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA), based on the available DNA (GenBank ID: NC_000019.9) and mRNA sequences (GenBank ID: NM_006297.2) of human *XRCC1*. Primers, annealing temperature, region, fragment sizes, and selected restriction enzymes (MBI Fermentas, St. Leon-Rot, Germany) are shown in Table 2. Genotype was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Samples were randomly selected (50 from cases, 50 from controls) and verified by DNA sequencing (ABI3730xl DNA Analyzer; Applied Biosystems, Foster City,

CA, USA). PCRs were carried out in a total volume of 20 µL containing 50 ng template DNA, 1X buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl), 0.25 µM primers, 2.0 mM $MgCl_2$, 0.25 mM dNTPs, and 0.5 U *Taq* DNA polymerase. All solutions were supplied by Bioteke Corporation, Beijing, China, except for *Taq* polymerase from Promega, Madison, WI, USA. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, at the corresponding temperature (presented in Table 2) for 30 s, at 72°C for 30 s, and a final extension at 72°C for 5 min. After confirmation of successful amplification by 1.5% agarose gel electrophoresis, each PCR amplified product was digested with 5 U restriction enzyme (shown in Table 2) at 37°C for 10 h, following manufacturer protocols, electrophoresed on a 3% agarose gel containing 0.5 µg/mL ethidium bromide, and visualized under UV illumination.

Table 2. Primers and PCR-RFLP analysis used for detecting the *XRCC1* gene polymorphisms.

SNPs	Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
c.910A>G	5'-CTGGACTGCTGGGTCTGAGGG-3' 5'-TGGAAGCCACTCAGCACCACTAC-3'	64.0	251	Exon 9	*Hha*I	AA: 251 AG: 251, 169, 82 GG: 169, 82
c.1686C>G	5'-CACATGTGGACAGCCCTCTCCT-3' 5'-CACCCCAGTCCCTGGAGACTC-3'	63.6	224	Exon 15	*Mbo*I	CC: 224 CG: 224, 174, 50 GG: 174, 50

PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; SNPs = single nucleotide polymorphisms.

Statistical analysis

The Mann-Whitney test was used to analyze age variables between different groups. The chi-square (χ^2) test was performed to evaluate the Hardy-Weinberg equilibrium in allele and genotype frequencies, and compare the differences between other characteristics of HCC patients and healthy controls. The odds ratios (ORs) with their 95% confidence intervals (CIs) of the association between allele and genotype frequencies and HCC risk were estimated by multivariate logistic regression models. $P < 0.05$ was defined as statistically significant. All statistical analyses were used by the Statistical Package for Social Sciences software (SPSS, Windows version, release 15.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Subjects and general characteristics

Subjects were enrolled in the current study, including 719 HCC cases and 662 healthy controls. The general characteristics of the subjects are presented in Table 1. No significant differences were found between HCC patients and healthy subjects with regard to gender and age ($P = 0.1290$ and $P = 0.5268$, respectively). In addition, there were no significant differences in tobacco smoking, alcohol drinking, diabetes mellitus, or hypertension between the cases and controls ($P = 0.0896$, $P = 0.1238$, $P = 0.6054$ and $P = 0.9200$, by the χ^2 test).

Identification and genotyping of *XRCC1* SNPs

In this study, we detected two allelic variants (c.910A>G and c.1686C>G) by PCR-RFLP and DNA sequencing methods. Sequence analysis showed that the c.910A>G variant is caused by an A to G mutation in exon 9 of the human *XRCC1* gene. This DNA sequence change also causes a threonine (Thr) to alanine (Ala) amino acid substitution (p.Thr304Ala) (GenBank reference sequence IDs: NC_000019.9, NM_006297.2, and NP_006288.2). As for the c.1686C>G variant, sequence analysis showed that this allelic variant is a synonymous mutation, caused by a C to G mutation in exon 15 of the human *XRCC1* gene (p.leucine(Leu)562Leu). The PCR products of the c.910A>G variants were digested with *Hha*I restriction enzyme and divided into three genotypes on the basis of the restriction fragments produced: AA (251 bp), AG (251, 169 and 82 bp) and GG (169 and 82 bp; Table 2). The c.1686C>G variants were digested with *Mbo*I restriction enzyme and divided into three genotypes: CC (224 bp), CG (224, 174 and 50 bp) and GG (174 and 50 bp; Table 2). The genotype and allele frequencies of the c.910A>G and c.1686C>G polymorphisms in both HCC patients and healthy controls are presented in Table 3. The genotype distributions of the two variants in the studied subjects did not significantly deviate from that expected for Hardy-Weinberg equilibrium (all $P > 0.05$). As presented in Table 3, allele A and allele C predominated in the study subjects over c.910A>G and c.1686C>G, respectively. For the c.910A>G polymorphism, the allele frequencies of HCC patients (A, 59.94%; G, 40.06%) were significantly different from those of healthy controls (A, 67.67%; G, 32.33%; $\chi^2 = 17.80$, $P < 0.0001$). In addition, genotype frequencies in HCC patients were different from those in healthy controls, the differences being statistically significant ($\chi^2 = 16.95$, $P = 0.0002$). For the c.1686C>G polymorphism, the allele frequencies of HCC patients (C, 56.05%; G, 43.95%) were significantly different from those of healthy controls (C, 63.82%; G, 36.18%, $\chi^2 = 17.32$, $P < 0.0001$). The genotype frequencies in HCC patients were also different from those in healthy controls, the differences being statistically significant ($\chi^2 = 16.99$, $P = 0.0002$; Table 3).

XRCC1 polymorphisms and risk of HCC

The genotypes and alleles from the c.910A>G and c.1686C>G polymorphisms were statistically associated with the risk of HCC (Table 4). For the c.910A>G polymorphisms, significantly increased risk of liver cancer was found in the homozygote comparison (GG versus AA: OR = 1.95, 95%CI = 1.40-2.70, $\chi^2 = 16.05$, $P < 0.0001$), heterozygote comparison (AG versus AA: OR = 1.31, 95%CI = 1.04-1.65, $\chi^2 = 5.43$, $P = 0.0200$), dominant model (GG+AG versus AA: OR = 1.45, 95%CI = 1.17-1.79, $\chi^2 = 11.42$, $P = 0.0007$), recessive model (GG versus AG+AA: OR = 1.70, 95%CI = 1.25-2.30, $\chi^2 = 11.52$, $P = 0.0007$) and allele contrast (G versus A: OR = 1.40, 95%CI = 1.20-1.64, $\chi^2 = 17.80$, $P < 0.0001$) (Table 4). Similarly, there were significant differences in the c.1686C>G polymorphisms for the homozygote comparison (GG versus CC: OR = 1.89, 95%CI = 1.375-2.599, $\chi^2 = 15.54$, $P < 0.0001$), heterozygote comparison (CG versus CC: OR = 1.39, 95%CI = 1.10-1.75, $\chi^2 = 7.54$, $P = 0.0060$), dominant model (GG+GC versus CC: OR = 1.50, 95%CI = 1.21-1.87, $\chi^2 = 13.12$, $P = 0.0003$), recessive model (GG versus GC+CC: OR = 1.57, 95%CI = 1.18-2.10, $\chi^2 = 9.44$, $P = 0.0021$) and allele contrast (G versus C: OR = 1.38, 95%CI = 1.19-1.61, $\chi^2 = 17.32$, $P < 0.0001$) (Table 4).

Table 3. Genotypic and allelic frequencies of the c.910A>G and c.1686C>G polymorphisms in HCC patients and healthy subjects.

Groups	c.910A>G										c.1686C>G									
	Genotype frequencies					Allele frequencies					Genotype frequencies					Allele frequencies				
	AA	%	AG	%	GG	%	A	%	G	%	CC	%	CG	%	GG	%	C	%	G	%
HCC patients (N = 719)	271	37.69	320	44.51	128	17.80	862	59.94	576	40.06	228	31.71	350	48.68	141	19.61	806	56.05	632	43.95
Healthy subjects (N = 662)	309	46.68	278	41.99	75	11.33	896	67.67	428	32.33	272	41.09	301	45.47	89	13.44	845	63.82	479	36.18
			$\chi^2 = 16.95$					$\chi^2 = 17.80$					$\chi^2 = 16.99$					$\chi^2 = 17.32$		
			P = 0.0002*					P < 0.0001*					P = 0.0002*					P < 0.0001*		

*P value for χ^2 test.

Table 4. Association between *XRCC1* polymorphisms and hepatocellular carcinoma (HCC) risk.

SNPs	Comparisons	OR (95%CI)	χ^2 value	P value*
c.910A>G				
	GG *vs* AA (Homozygote comparison)	1.95 (1.40-2.70)	16.05	<0.0001
	AG *vs* AA (Heterozygote comparison)	1.31 (1.04-1.65)	5.43	0.0200
	GG/AG *vs* AA (Dominant model)	1.45 (1.17-1.79)	11.42	0.0007
	GG *vs* AG/AA (Recessive model)	1.70 (1.25-2.30)	11.52	0.0007
	G *vs* A (Allele contrast)	1.40 (1.20-1.64)	17.80	<0.0001
c.1686C>G				
	GG *vs* CC (Homozygote comparison)	1.89 (1.38-2.60)	15.54	<0.0001
	CG *vs* CC (Heterozygote comparison)	1.39 (1.10-1.75)	7.54	0.0060
	GG/CG *vs* CC (Dominant model)	1.50 (1.21-1.87)	13.12	0.0003
	GG *vs* CG/CC (Recessive model)	1.57 (1.18-2.10)	9.44	0.0021
	G *vs* C (Allele contrast)	1.38 (1.19-1.61)	17.32	<0.0001

SNPs = single nucleotide polymorphisms; OR = odds ratio; 95%CI = 95% confidence interval. *P value for χ^2 test.

DISCUSSION

HCC is a common polygenic malignant cancer resulting from complex interactions between multiple genetic and environmental factors (Marrero et al., 2005; Farazi and DePinho, 2006; El-Serag and Rudolph, 2007; Amarapurkar et al., 2008), which presents a major and constantly rising health burden in the world. It is generally accepted that genetic factors play a key role in the pathogenesis of HCC (Thorgeirsson and Grisham, 2002; Nault and Zucman-Rossi, 2011). Because *XRCC1* is potentially one of the most important genes implicated in HCC, a large number of association studies have been conducted in recent years to evaluate the role of Arg194Trp, Arg280His and Arg399Gln polymorphisms in *XRCC1* on the risk of HCC (Rossit et al., 2002; Yu et al., 2003; Long et al., 2008; Kiran et al., 2009a,b; Pan et al., 2011; Han et al., 2012; Li et al., 2012). The results of these association studies are inconsistent. Kiran et al. (2009b) reported that these three polymorphisms were significantly associated with risk of hepatitis virus-related HCC in the Indian population. Pan et al. (2011) demonstrated that *XRCC1*-Arg399Gln Arg/Gln in the Chinese population showed an increased risk of HCC, especially for patients over 50 years old, or with excessive alcohol consumption. However, Liu et al. (2011) suggested that the *XRCC1* Arg399Gln polymorphism was not associated with altered susceptibility to HCC, based on the results from a meta-analysis. The current case-control study is the first to explore the association of c.910A>G and c.1686C>G polymorphisms in *XRCC1* with the risk of HCC in the Chinese population. Our data suggest that significant differences exist in the allele and genotype frequencies of c.910A>G and c.1686C>G polymorphisms between HCC patients and healthy controls (Table 3). The GG genotype of the c.910A>G polymorphism was associated with increased risk of developing HCC as compared with the AA and AG/GG genotypes. For the c.1686C>G polymorphism, the risk of HCC was significantly higher for the GG genotype compared with

the CC and CG/CC genotypes. The G allele of the c.910A>G and c.1686C>G polymorphisms may contribute to the risk of HCC (Table 4). Our findings suggest that the c.910A>G and c.1686C>G polymorphisms of *XRCC1* are associated with increased risk of HCC in the Chinese population. Although c.1686C>G is a synonymous coding polymorphism, it may be linked to other non-synonymous polymorphisms in *XRCC1*, such as Arg194Trp, Arg280His, and Arg399Gln. These are known to affect the function of *XRCC1* in the base excision DNA repair pathway, which is significantly associated with the risk of HCC. The results from the present study provide more evidence supporting the role of *XRCC1* in HCC. Nevertheless, further epidemiologic investigations of other larger populations are warranted to confirm these results and explain the molecular mechanisms underlying the links between *XRCC1* polymorphisms and the risk of HCC.

CONCLUSION

In conclusion, this is the first investigation of the correlation between the c.910A>G and c.1686C>G polymorphisms in *XRCC1* and the risk of HCC. Our findings show that *XRCC1* genetic polymorphism may be a useful molecular marker for evaluating the risk of HCC.

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Conflicts of interest

The authors declare no conflict of interest.

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