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Research Paper

Association between the C.1161G>A and C.1779C>G Genetic Variants of *XRCC1* Gene and Hepatocellular Carcinoma Risk in Chinese Population

Xin Deng, Jian Liang✉, Majun Jiang, Xiaoxiao Zhou and Hongdan Liu

Department of Oncology, Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, Nanning, Guangxi Zhuang Autonomous Region, People's Republic of China.

✉ Corresponding author: Jian Liang, Department of Oncology, Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, NO. 10 Huadong Road, Nanning 530011, Guangxi Zhuang Autonomous Region, People's Republic of China. Tel: +86-771-2238026; Fax: +86-771-2238026 E-mail: jianliang_12@sina.com.

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Abstract

The human X-ray repair complementing group 1 gene (*XRCC1*) is an important candidate gene influencing hepatocellular carcinoma (HCC) susceptibility. The objective of this study was to detect the association between c.1161G>A and c.1779C>G variants of *XRCC1* gene and HCC risk. This study was conducted in Chinese population consisting of 623 HCC cases and 639 controls. These two genetic variants could be genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The association of *XRCC1* gene variants with the risk of HCC was investigated under different genetic models. Our findings suggested that the genotypes/alleles from c.1161G>A and c.1779C>G genetic variants were statistically associated with HCC risk. As for the c.1161G>A, the AA genotype was statistically associated with the increased risk of HCC compared to GG wild genotype (OR = 2.36, 95% CI 1.63-3.40, P < 0.001). As for the c.1779C>G, the risk of HCC was significantly higher for GG genotype compared to CC wild genotype (OR = 2.17, 95% CI 1.51-3.12, P < 0.001). Furthermore, significant differences in the risk of HCC were also detected in other genetic models for these two variants. The allele-A of c.1161G>A and allele-G of c.1779C>G variants may contribute to the susceptibility of HCC (A versus G: OR = 1.48, 95% CI 1.26-1.75, P < 0.001 and G versus C: OR = 1.51, 95% CI 1.28-1.78, P < 0.001). Our data indicated that these two variants of *XRCC1* gene were statistically associated with HCC risk in Chinese population.

Key words: Hepatocellular carcinoma; *XRCC1* gene; genetic variants; molecular markers; susceptibility; risk factors.

Introduction

Hepatocellular carcinoma (HCC) is the fifth common cancer and the third leading cause of cancer-related death globally. It is a global health problem and a common liver malignancy in the world [1-3]. Several studies suggest that HCC shows great geographical variation, and more than 600,000 people die from HCC every year, and about > 75% of these HCC cases occur in the Asia-Pacific region [4,5]. China has a very high HCC incidence, with approximately 55% of annual new cases of HCC in the world [1,6,7]. Since the 1990s, HCC has been the second leading cause of cancer deaths in China [8]. Up to date, the exact mechanism of HCC remains poorly understood. There were several studies suggested that the human X-ray repair complementing group 1 gene (*XRCC1*) was an important candidate gene

which influencing HCC risk [9-21]. Many single nucleotide polymorphisms (SNPs) in *XRCC1* gene, such as Arginine (Arg)194 Tryptophan (Trp), Arg280 Histidine (His) and Arg399 Glutanine (Gln), have been analyzed to evaluate the possible association between the genetic variants of *XRCC1* gene and the risk of HCC [9-16]. Fortunately, several studies have proved that the genetic variants of *XRCC1* gene were associated with HCC [9-16]. However, there were no similar studies on the association between c.1161G>A and c.1779C>G genetic variants and the risk of HCC. Therefore, the purpose of this study was to assess whether these two genetic variants influence the risk of HCC.

Materials and methods

Study population

A total of 623 HCC patients and 639 cancer-free controls were enrolled from Ruikang Hospital (Guangxi University of Chinese Medicine, Nanning, Guangxi Zhuang Autonomous Region, China) between January 2009 to December 2011 in this case-control study. All HCC patients and cancer-free controls were unrelated Han Chinese. HCC patients were diagnosed by doctors based on the standards established by Chinese Society of Liver Cancer (CSLC). Cancer-free controls were matched with HCC patients with regard to gender and age, excluding those with a history of HCC and other medical diseases. The clinical characteristics were collected in Table 1, including gender, age, alcohol drinking, tobacco smoking, hypertension, diabetes mellitus, family history of HCC, hepatitis B virus (HBV) serological markers, and serum alpha-fetoprotein (a-FP) levels. This study was approved by the ethics committee of Ruikang Hospital (Guangxi University of Chinese Medicine, Nanning, Guangxi Zhuang Autonomous Region, China). Informed consent was obtained from all the subjects enrolled in the study.

XRCC1 variants genotyping

Genomic DNA was isolated from venous blood of all the subjects using the standard protocol [22]. PCR primers were designed using Primer Premier 5.0 software. Primers, region, fragment size, and annealing temperature were summarized in Table 2. *XRCC1* genetic variants were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and almost 10% of the samples showing genetic polymorphisms for *XRCC1* gene were verified by DNA sequencing (ABI3730xl DNA Analyzer, Applied Biosystems, Foster City, CA) to ensure the concordance of the PCR-RFLP results. The PCR reactions were performed in a total volume of 20 μL solution containing 50ng 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 (Bioteke Corporation, Beijing, China), and 0.5U Taq DNA polymerase (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 (given in Table 2) for 30 s, at 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR amplified products were digested with 5 units corresponding restriction enzyme (MBI Fermentas, St. Leon-Rot, Germany, Table 2) at 37°C for 10 h following the supplier's manual and electrophoresed on 2.5% agarose gel containing ethidium-bromide.

Statistical analysis

The chi-squared (χ^2) test was performed to assess the Hardy–Weinberg equilibrium in genotype/allele frequencies, and compare the differences of the clinical characteristics between HCC patients and cancer-free controls. The odds ratios (ORs) with its 95% confidence intervals (95% CIs) of the association between allele/genotype frequencies and the risk of HCC were evaluated by multivariate logistic regression models. A P value < 0.05 was suggested as statistically significant. The statistical analyses were analyzed using the Statistical Package for Social Sciences software (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA).

Results

Subjects and general characteristics

In total, 1262 subjects were recruited in this case-control study, including 623 HCC cases and 639 cancer-free controls. The clinical characteristics of subjects were performed in Table 1. There was no statistically significant difference between HCC patients and cancer-free controls in terms of age and gender (P = 0.3851 and P = 0.4423, respectively). Furthermore, no statistically significant differences were obtained in other clinical characteristics, such as alcohol drinking, tobacco smoking, hypertension, and diabetes mellitus between HCC cases and cancer-free controls (P = 0.3115, P = 0.6512, P = 0.8074 and P = 0.1363, respectively).

Identification and genotyping of *XRCC1* variants

Two allelic variants (c.1161G>A and c.1779C>G) were found by PCR-RFLP and DNA sequencing methods in this study. Based on the reference se-

quences GenBank ID: NC_000019.9, NM_006297.2 and NP_006288.2, the sequence analysis suggested that c.1161G>A allelic variant was a synonymous mutation, which caused by G to A mutations in exon10 of *XRCC1* gene [p. Leucine (Leu) 387Leu]. As for the c.1779C>G variant, sequence analysis proved that this variant was caused by C to G mutations in exon16 of *XRCC1* gene, which leading to the Serine (Ser) to Arginine amino acid (Arg) replacement (p.Ser593Arg). The PCR amplified products of c.1161G>A variant was digested with *Mae*I restriction enzyme and divided into three genotypes, GG (213 bp), GA (213, 183 and 30 bp) and AA (183 and 30 bp, Table 2). As for the c.1779C>G variant, it was digested with *Alu*I restriction enzyme and divided into three genotypes, CC (177 and 58 bp), CG (235, 177 and 58 bp) and GG (235 bp, Table 2). The genotype/allele frequencies of these two genetic variants in both HCC patients and cancer-free controls were performed in Table 3. As shown in Table 3, the genotype distributions of these two genetic variants in the subjects didn't significantly deviate from Hardy-Weinberg equilibrium (all P values > 0.05). Allele-G and allele-C were predominant alleles in the studied group for c.1161G>A and c.1779C>G variants, respectively. As for the c.1161G>A variant, the allele frequencies of HCC patients (G, 61.16%; A, 38.84%) were statistically significantly different from cancer-free controls (G, 70.03%; A, 29.97%; $\chi^2 = 22.0390$, P < 0.001). Genotype frequencies in HCC patients were not consistent with cancer-free controls, the differences being statistically significant ($\chi^2 = 22.4559$, P < 0.001). As for the c.1779C>G variant, the allele frequencies of HCC patients (C, 61.08%; G, 38.92%) were statistically significantly different from cancer-free controls (C, 70.34%; G, 29.66%, $\chi^2 = 24.075$, P < 0.001). The genotype distributions in HCC patients were statistically significantly different from cancer-free controls ($\chi^2 = 23.4151$, P < 0.001, Table 3).

XRCC1 variants and the risk of HCC

We found that the genotypes/alleles from the c.1161G>A and c.1779C>G variants were statistically associated with the risk of HCC (Table 4). As for the c.1161G>A variant, statistically significantly increased risk of HCC were detected in the homozygote comparison (AA versus (vs.) GG: OR = 2.36, 95% CI 1.63-3.40, $\chi^2 = 21.63$, P < 0.001), heterozygote comparison (GA vs. GG: OR = 1.34, 95% CI 1.06-1.70, $\chi^2 = 5.79$, P = 0.016), dominant model (AA/GA vs. GG: OR = 1.51, 95% CI 1.21-1.89, $\chi^2 = 13.17$, P < 0.001), recessive model (AA vs. GA/GG: OR = 2.04, 95% CI 1.44-2.88, $\chi^2 = 16.66$, P = < 0.001) and allele contrast (A vs. G: OR = 1.48, 95% CI 1.26-1.75, $\chi^2 = 22.03$, P < 0.001, Table 4). Similarly, as for the c.1779C>G variant, we also found that there were statistically significant differences in the homozygote comparison (GG vs. CC: OR = 2.17, 95% CI 1.51-3.12, $\chi^2 = 18.07$, P < 0.001), heterozygote comparison (CG vs. CC: OR = 1.55, 95% CI 1.22-1.96, $\chi^2 = 12.98$, P < 0.001), dominant model (GG/GC vs. CC: OR = 1.67, 95% CI 1.33-2.09, $\chi^2 = 20.03$, P < 0.001), recessive model (GG vs. GC/CC: OR = 1.75, 95% CI 1.24-2.46, $\chi^2 = 10.44$, P = 0.001) and allele contrast (G vs. C: OR = 1.51, 95% CI 1.28-1.78, $\chi^2 = 24.07$, P < 0.001, Table 4).

Table 1. The clinical characteristics of hepatocellular carcinoma (HCC) cases and controls.

Characteristics	Cases (n)	%	Controls (n)	%	χ^2-value	P-value
Number	623		639			
Gender (n)					0.5902	0.4423
Male	369	59.23	392	61.35		
Female	254	40.77	247	38.65		
Age (years)						
Mean ± SD	58.25±13.28		59.22±14.37		0.7544	0.3851
< 55	387	62.12	412	64.48		
≥ 55	236	37.88	227	35.52		
Tobacco smoking					0.2043	0.6512
Yes	348	55.86	365	57.12		
No	275	44.14	274	42.88		
Alcohol drinking					1.0243	0.3115
Yes	351	56.34	378	59.15		
No	272	43.66	261	40.85		
Hypertension (n)					0.0594	0.8074
Yes	105	16.85	111	17.37		

No	518	83.15	528	82.63		
Diabetes mellitus(n)					2.2197	0.1363
Yes	121	19.42	146	22.85		
No	502	80.58	493	77.15		
Family history of HCC (n)						
Yes	42	6.74	-			
No	581	93.26	-			
HBV serological markers(n)						
HBs Ag (+)	158	25.36	-			
HBs Ag (-)	465	74.64	-			
Serum a-FP levels						
< 400 ng/ml	210	33.71	-			
> 400 ng/ml	413	66.29	-			

Table 2. Primers and PCR-RFLP analysis for genotyping *XRCC1* gene polymorphisms.

SNPs	Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
c.1161G>A	5′-GAGGCCGCATCGTGCGTAAG-3′	66.4	213	Exon10	*Mae*I	GG:213
	5′-TGCCCCGCTCCTCTCAGTAGTCT-3′					GA:213,183,30
						AA:183,30
c.1779C>G	5′-AGGACAATATGAGTGACCGGGT-3′	59.9	235	Exon16	*Alu*I	CC:177,58
	5′-CATTGCAACTGTAGATCCATCG-3′					CG:235,177,58
						GG:235

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

Table 3. The genotypic and allelic frequencies of *XRCC1* polymorphisms in case and groups.

Groups	c.1161G>A							c.1779C>G						
	Genotype frequencies			Allele frequencies				Genotype frequencies			Allele frequencies			
	GG	GA	AA	G	A	χ^2	P	CC	CG	GG	C	G	χ^2	P
Cases (n = 623)	241(38.68)	280(44.94)	102(16.37)	762(61.16)	484(38.84)	1.8189	0.4027	235(37.72)	291(46.71)	97(15.57)	761(61.08)	485(38.92)	0.1932	0.9079
Controls (n = 639)	31 (48.83)	271 (42.41)	56 (8.76)	895 (70.03)	383 (29.97)	0.0687	0.9663	321 (50.23)	257 (40.22)	61 (9.55)	899 (70.34)	379 (29.66)	0.8294	0.6605
Total (n = 1262)	553 (43.82)	551 (43.66)	158 (12.52)	1657 (65.65)	867 (34.35)	1.2880	0.5252	556 (44.06)	548 (43.42)	158 (12.52)	1660 (65.77)	864 (34.23)	1.6012	0.4491
	χ^2 = 22.4559, P < 0.001			χ^2 = 22.0390, P < 0.001				χ^2 = 23.4151, P < 0.001			χ^2 = 24.075, P < 0.001			

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

SNPs	Comparisons	Test of association		
		OR (95% CI)	χ^2-value	P-value
c.1161G>A	Homozygote comparison			
	(AA vs. GG) Heterozygote comparison	2.36 (1.63-3.40)	21.63	< 0.001
	(GA vs. GG) Dominant model	1.34 (1.06-1.70)	5.79	0.016
	(AA/GA vs. GG) Recessive model	1.51 (1.21-1.89)	13.17	< 0.001
	(AA vs. GA/GG) Allele contrast	2.04 (1.44-2.88)	16.66	< 0.001
	(A vs. G)	1.48 (1.26-1.75)	22.03	< 0.001
c.1779C>G	Homozygote comparison			
	(GG vs. CC) Heterozygote comparison	2.17 (1.51-3.12)	18.07	< 0.001

SNPs	Comparisons	Test of association		
		OR (95% CI)	χ^2-value	P-value
	(CG vs. CC) Dominant model	1.55 (1.22-1.96)	12.98	< 0.001
	(GG/CG vs. CC) Recessive model	1.67 (1.33-2.09)	20.03	< 0.001
	(GG vs. CG/CC) Allele contrast	1.75 (1.24-2.46)	10.44	0.001
	(G vs. C)	1.51 (1.28-1.78)	24.07	< 0.001

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

Discussion

Emerging evidence suggests that HCC is a common and polygenic malignant solid cancers resulting from complex interactions between environmental and genetic factors [23-27]. It represents a major and constantly rising health burden worldwide. Previous studies have approved that genetic factors have the key roles in the pathogenesis of HCC [28-32]. In recent years, the *XRCC1* gene is regarded as an important candidate gene for HCC, and several association studies have been conducted to assess the role of Arg194Trp, Arg280His and Arg399Gln variants on the risk of HCC [9-16]. The findings from the previous association studies still remain conflicting rather than conclusive [9-16]. Kiran et al., indicated that Arg194Trp, Arg280His and Arg399Gln variants were significantly associated with the risk of hepatitis virus related HCC in Indian population [12]. Pan et al., suggested that the Arg399Cln Arg/Gln indicated an increased risk of HCC, especially for patients above 50 years old or with drinking habits in Chinese population [13]. However, Liu et al., demonstrated that the Arg399Gln is not statistically associated with altered HCC susceptibility according to the findings from meta-analysis method [17]. However, up to date, the c.1161G>A and c.1779C>G genetic variants have not been analyzed in the risk of HCC. In this case-control study, we evaluated the association between these two SNPs and the risk of HCC in Chinese Han population. Results from this study, statistical significant differences were shown in allele and genotype frequencies between HCC patients and cancer-free controls for these two genetic variants (Table 3). As for the c.1161G>A variant, the risk of HCC was significantly higher for AA genotype compared to GG genotype and GA/GG carriers. As for the c.1779C>G variant, the GG genotype was significantly associated with the increased risk of HCC compared to CC genotype and CG/CC carriers. The allele-A of c.1161G>A and allele-G of c.1779C>G variants could contribute to the risk of HCC (Table 4), and these two alleles might be associated with a protection from HCC. These genetic variants might affect the function of *XRCC1* protein in base excision DNA repair pathway, which is significantly associated with the risk of HCC. The results from the current study might provide more evidences to assess the role of *XRCC1* gene in the development of HCC. Our date indicated that the c.1161G>A and c.1779C>G variants of *XRCC1* gene were statistically associated with the increased risk of HCC in Chinese Han population.

In conclusion, to the best of our knowledge, this is the first investigation about the association between *XRCC1* gene c.1161G>A and c.1779C>G genetic variants and HCC risk. Our findings supported that the *XRCC1* genetic variants may be used as molecular markers for assessing the risk of HCC. Further studies will be needed to confirm these findings and explain the molecular mechanisms between *XRCC1* genetic variants and HCC risk on larger different populations.

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Competing Interests

The authors have declared that no competing interest exists.

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