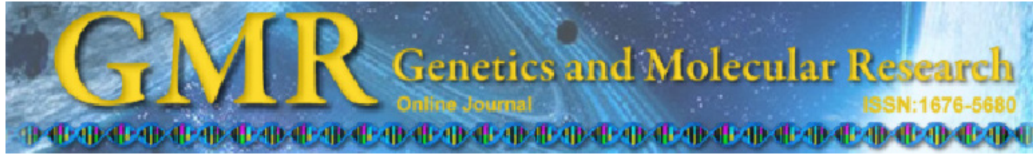


# Association between a single nucleotide polymorphism of the *XRCC1* gene and hepatocellular carcinoma susceptibility in the Chinese Han population

**X.F. Li, Y.X. Chen, W.W. Ye, X.F. Tao, J.H. Zhu, S. Wu and L.Q. Lou**

Department of Infectious Diseases, the Yiwu Central Hospital,
Yiwu, Zhejiang Province, China

Corresponding author: L.Q. Lou
E-mail: lianqinglou@sina.com

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**ABSTRACT.** The human X-ray repair cross-complementing protein 1 (*XRCC1*) gene is a potentially gene determining hepatocellular carcinoma (HCC) susceptibility. The purpose of this study was to evaluate the association between *XRCC1* and susceptibility to HCC. The association of *XRCC1* polymorphisms with HCC susceptibility was investigated in 460 HCC patients and 463 controls using the created restriction site-polymerase chain reaction method. Our results indicate that the c.1471G>A variant could be detected and that the allele and genotype frequencies were statistically different between cases and controls. The AA genotype was strongly associated with increased HCC susceptibility as compared with the GG wild genotype (OR = 2.214, 95%CI = 1.493-3.283, $\chi^2$ = 15.97, P < 0.0001). In addition, significantly increased HCC susceptibility was also found in a dominant and recessive model (P < 0.01). The allele A could contribute to HCC susceptibility compared with the G allele (OR = 1.480, 95%CI = 1.224-1.789, $\chi^2$ = 16.44, P = 0.0001). Results from this study indicate

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that the *XRCC1* c.1471G>A polymorphism is associated with HCC susceptibility in the Chinese Han population. Future studies on larger populations are essential to confirm this association.

**Key words:** Hepatocellular carcinoma; *XRCC1* gene; Single nucleotide polymorphism; Molecular marker; Susceptibility

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths globally (Llovet et al., 2003; Parkin et al., 2005; Parikh and Hyman, 2007). More than 600,000 people die from HCC each year, and >75% of these cases occur in the Asia-Pacific region (But et al., 2008; Yuen et al., 2009). China has a very high HCC incidence, where it is the second leading cause of cancer deaths since the 1990s (Chen et al., 2010), and it accounts for approximately 55% of new HCC cases worldwide (Parkin, 2001; Parkin et al., 2005; Schutte et al., 2009). To date, the mechanism of hepatocarcinogenesis remains poorly understood. There are indications in the literature that the human X-ray repair cross-complementing protein 1 (*XRCC1*) gene may influence 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). Several studies proved that *XRCC1* polymorphisms, such as Arg194Trp, Arg280His and Arg399Gln, 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). However, the association between the c.1471G>A polymorphism in *XRCC1* and HCC susceptibility has not been investigated. The purpose of this study was to evaluate whether the *XRCC1* c.1471G>A polymorphism influences the susceptibility to HCC in the Chinese population.

## MATERIAL AND METHODS

### Subjects

Patients with HCC (460) and non-cancer controls (463) were recruited from January 2010 to December 2011 in the Yiwu Central Hospital. All subjects were unrelated Han Chinese. Clinical characteristics, including gender, age, alcohol drinking, tobacco smoking, hypertension, diabetes mellitus, family history of HCC, HBV serological markers, and serum a-FP levels are summarized in Table 1. The protocol for this study was approved by the local Ethics Committee, and all subjects signed an informed consent form.

### *XRCC1* polymorphism genotyping

Genomic DNA was extracted from peripheral venous blood using the standard phenol/chloroform extraction method. Primers were designed based on the DNA and mRNA sequences of human *XRCC1* (GenBank IDs: NC_000019.9 and NM_006297.2) using the Primer Premier 5.0 software. Primers, annealing temperature, region, fragment sizes, and selected

restriction enzymes (MBI Fermentas, St. Leon-Rot, Germany) are shown in Table 2. Genotyping was accomplished by the created restriction site-polymerase chain reaction (CRS-PCR) method, with one of the primers containing a nucleotide mismatch. This enabled the use of restriction enzymes for discriminating sequence variations (Yuan et al., 2012, 2013a,b). PCRs were performed 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 (Promega, Madison, WI, USA). PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 64°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR amplified products were digested with 2 U restriction enzyme (shown in Table 2) at 37°C for 10 h, electrophoresed on a 3% agarose gel, and visualized under UV light.

## Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, Windows version, release 15.0; SPSS Inc., Chicago, IL, USA). The chi-squared ($\chi^2$) test was used to evaluate Hardy-Weinberg equilibrium, allele and genotype frequencies, and general characteristics of healthy controls and HCC patients. Associations between allele and genotype frequencies and HCC susceptibility were estimated using the odds ratios (ORs) with their 95% confidence intervals (CIs). A P value <0.05 was defined as statistically significant.

# RESULTS

## General characteristics of the subjects

In this case-control study, 923 Chinese subjects were enrolled, including 460 HCC cases and 463 healthy controls. The general characteristics of the subjects are shown in Table 1. There was no significant difference between cases and controls with regard to gender and age (P = 0.4426 and P = 0.3363, respectively). Additionally, no significant differences were found in alcohol drinking, tobacco smoking, hypertension, or diabetes mellitus between case and control groups (P = 0.3700, P = 0.4816, P = 0.4252, and P = 0.2605, respectively).

## Identification and genotyping of the *XRCC1* polymorphism

A novel allelic variant (c.1471G>A) was identified by CRS-PCR and DNA-sequencing methods. Sequence analysis revealed a G to A mutation in exon 13 of the *XRCC1* gene, resulting in a glutanine (Glu) to lysine (Lys) amino acid substitution (p.Glu491Lys, reference sequences GenBank IDs: NC_000019.9, NM_006297.2 and NP_006288.2). PCR products were digested with *Alw*NI restriction enzyme and divided into three genotypes, based on restriction fragment pattern: GG (194 and 21 bp), GA (215, 194 and 21 bp) and AA (215 bp). Results are shown in Table 2. The allele and genotype frequency distributions in both HCC patients and healthy controls are summarized in Table 3. The allele frequencies of HCC patients (G = 58.15%; A = 41.85%) were significantly different from healthy controls (G = 67.28%; A = 32.72%; $\chi^2$ = 16.4413, P < 0.0001; Table 3). In addition, significant differences

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in genotype frequencies between cases and controls were found ($\chi^2$ = 16.0887; P = 0.0003; Table 3). The genotype distributions in the studied subjects did not significantly deviate from Hardy-Weinberg equilibrium (for case group: $\chi^2$ = 4.8066, P = 0.0904; for control group: $\chi^2$ = 0.5234, P = 0.7697). Results are presented in Table 3.

**Table 1.** Clinical characteristics of the hepatocellular carcinoma (HCC) cases and controls.

| Characteristics | Cases (N) | % | Controls (N) | % | $\chi^2$ value | P value |
|---|---|---|---|---|---|---|
| Number | 460 | | 463 | | | |
| Gender | | | | | 0.5895 | 0.4426 |
| Male | 312 | 67.83 | 303 | 65.44 | | |
| Female | 148 | 32.17 | 160 | 34.56 | | |
| Age (years) | | | | | 0.9244 | 0.3363 |
| mean ± SD | 57.23 ± 12.55 | | 56.43 ± 13.68 | | | |
| <55 | 282 | 61.30 | 298 | 64.36 | | |
| ≥55 | 178 | 38.70 | 165 | 35.64 | | |
| Alcohol drinking | | | | | 0.8038 | 0.3700 |
| Yes | 251 | 54.57 | 239 | 51.62 | | |
| No | 209 | 45.43 | 224 | 48.38 | | |
| Tobacco smoking | | | | | 0.4951 | 0.4816 |
| Yes | 257 | 55.87 | 248 | 53.56 | | |
| No | 203 | 44.13 | 215 | 46.44 | | |
| Hypertension | | | | | 0.6359 | 0.4252 |
| Yes | 63 | 13.70 | 72 | 15.55 | | |
| No | 397 | 86.30 | 391 | 84.45 | | |
| Diabetes mellitus | | | | | 1.2661 | 0.2605 |
| Yes | 79 | 17.17 | 67 | 14.47 | | |
| No | 381 | 82.83 | 396 | 85.53 | | |
| Family history of HCC | | | | | | |
| Yes | 23 | 5.00 | - | | | |
| No | 437 | 95.00 | - | | | |
| HBV serological markers | | | | | | |
| HBs Ag(+) | 126 | 27.39 | - | | | |
| HBs Ag(-) | 334 | 72.61 | - | | | |
| Serum a-FP levels | | | | | | |
| <400 ng/mL | 156 | 33.91 | - | | | |
| >400 ng/mL | 304 | 66.09 | - | | | |

**Table 2.** Primer and CRS-PCR analysis used for detecting the *XRCC1* gene c.1471G>A polymorphism.

| Primer sequences | Annealing temperature (°C) | Amplification fragment (bp) | Region | Restriction enzyme | Genotype (bp) |
|---|---|---|---|---|---|
| 5'-AAGATTCTGGGGACACAGAGGCT-3'<br>5'-GTGTTCTCATCCGTGGAGCCTG-3' | 64.0 | 215 | Exon 13 | *Alw*NI | GG: 194,21<br>GA: 215,194,21<br>AA: 215 |

CRS-PCR = created restriction site-polymerase chain reaction.

**Table 3.** Genotype and allele frequencies of the *XRCC1* c.1471G>A polymorphism in cases and controls.

| Groups | Genotype frequencies | | | | | | Allele frequencies | | | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GG | % | GA | % | AA | % | G | % | A | % | | |
| Cases (N = 460) | 167 | 36.30 | 201 | 43.70 | 92 | 20.00 | 535 | 58.15 | 385 | 41.85 | 4.8066 | 0.0904 |
| Controls (N = 463) | 213 | 46.00 | 197 | 42.55 | 53 | 11.45 | 623 | 67.28 | 303 | 32.72 | 0.5234 | 0.7697 |
| Total (N = 923) | 380 | 41.17 | 398 | 43.12 | 145 | 15.71 | 1158 | 62.73 | 688 | 37.27 | 5.5890 | 0.0611 |
| | $\chi^2$ = 16.0887, P = 0.0003 | | | | | | $\chi^2$ = 16.4413, P < 0.0001 | | | | | |

## *XRCC1* polymorphism and HCC susceptibility

As shown in Table 4, alleles and genotypes of the c.1471G>A polymorphism were significantly associated with HCC susceptibility. There was significantly increased susceptibility to HCC in the homozygote comparison (AA *vs* GG: OR = 2.214, 95%CI = 1.493-3.283, $\chi^2$ = 15.97, P = 0.0001), dominant model (AA+GA *vs* GG: OR = 1.495, 95%CI = 1.148-1.946, $\chi^2$ = 12.75, P = 0.0004), recessive model (AA *vs* GA+GG: OR = 1.934, 95%CI = 1.341-2.789, $\chi^2$ = 8.96, P = 0.0028), and allele contrast (A *vs* G: OR = 1.480, 95%CI = 1.224-1.789, $\chi^2$ = 16.44, P = 0.0001), while a marginal significance was found in heterozygote comparison (GA *vs* GG: OR = 1.301, 95%CI = 0.981-1.726, $\chi^2$ = 3.35, P = 0.0672; Table 4).

**Table 4.** Association between the risk of hepatocellular carcinoma (HCC) and the *XRCC1* c.1471G>A polymorphism.

| Comparisons | OR (95%CI) | $\chi^2$ value | P value |
|---|---|---|---|
| Homozygote comparison (AA *vs* GG) | 2.214 (1.493-3.283) | 15.97 | 0.0001 |
| Heterozygote comparison (GA *vs* GG) | 1.301 (0.981-1.726) | 3.35 | 0.0672 |
| Dominant model (AA/GA *vs* GG) | 1.495 (1.148-1.946) | 12.75 | 0.0004 |
| Recessive model (AA *vs* GA/GG) | 1.934 (1.341-2.789) | 8.96 | 0.0028 |
| Allele contrast (A *vs* G) | 1.480 (1.224-1.789) | 16.44 | 0.0001 |

SNPs = single nucleotide polymorphisms; OR = odds ratio; 95%CI = 95% confidence interval.

# DISCUSSION

HCC is a common malignant tumor resulting from complex interactions between multiple environmental and genetic factors (Marrero et al., 2005; Farazi and DePinho, 2006; El-Serag and Rudolph, 2007; Amarapurkar et al., 2008). Previous studies suggested that environmental risk factors, including cigarette smoking, alcohol consumption, chronic hepatitis B or hepatitis C viral infections, and exposure to dietary aflatoxin B1 (AFB1), are associated with HCC (Bosch et al., 2004; Suriawinata and Xu, 2004; Farazi and DePinho, 2006; Gomaa et al., 2008). Furthermore, 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). Several studies have evaluated how Arg194Trp, Arg280His and Arg399Gln polymorphisms in *XRCC1* affect 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), but with conflicting rather than conclusive results. In this study, we firstly evaluated the association between the *XRCC1* c.1471G>A polymorphism and susceptibility to HCC in the Chinese Han population. Our results indicate that the AA genotype was strongly associated with increased susceptibility to developing HCC compared with the GG genotype and GA/GG carriers (P = 0.0001 and P = 0.0028). Using GG as the reference genotype, the AA/GA carriers had increased susceptibility to HCC (P = 0.0004). The A allele may contribute to the susceptibility to HCC (P = 0.0001; Table 4). Our data indicate that the *XRCC1* c.1471G>A polymorphism is associated with increased susceptibility to HCC in the Chinese population.

In conclusion, to the best of our knowledge, this is the first investigation of the association between the *XRCC1* c.1471G>A polymorphism and HCC susceptibility. Results from this study provide additional evidence of the role of *XRCC1* in HCC susceptibility. The

c.1471G>A polymorphism in *XRCC1* could be a useful molecular marker for evaluating susceptibility to HCC. Further investigations on larger populations are needed to confirm these results and to explain the role of the c.1471G>A polymorphism and other *XRCC1* polymorphisms in susceptibility to HCC.

## Conflicts of interest

The authors declare no conflict of interest.

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