RESEARCH ARTICLE

# Association of XPD and XRCC1 Genetic Polymorphisms with Hepatocellular Carcinoma Risk

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## Abstract

**Aim: XRCC1 and XPD are two major repair genes involved in nucleotide excision repair (NER), which is reported to be associated with risk of several cancers. We explored the association of XRCC1 and XPD polymorphisms with the risk of HCC. Methods: A total of 410 cases with HCC and 410 health controls were collected. XRCC1 Arg194Trp, XRCC1 Arg399Gln, XPD Lys751Gln and XPD Asp312Asn genotyping was performed by duplex polymerase-chain-reaction with the confronting-two-pair primer (PCR-CTPP) method. Results: XRCC1 194Trp/Trp was strongly significantly associated with an increased risk of HCC cancer when compared with the wide-type genotype (OR=2.26, 95% CI=(1.23-5.38). Individuals carrying the XRCC1 399Gln/Gln showed increased risk of HCC (OR=1.74, 95%CI=1.06-2.74). The XPD 751Gln/Gln and Gln allele genotype were associated with strong elevated susceptibility to HCC (OR=3.51 and 1.42, respectively). Conclusion: These results suggest that polymorphisms in XRCC1 and XPD may have functional significance in risk of HCC.**

**Keywords:** XRCC1 - XPD - hepatocellular carcinoma - susceptibility

*Asian Pacific J Cancer Prev,* **13 (9)**, 4423-4426

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh in women and most of the burden is in developing countries. The regions of incidence of HCC showed wide geographic variation at an international level, high in Eastern and South-Eastern Asia, and low in developed regions (IARC, 2008). The difference in terms of incidence of HCC suggests the role of genetic and environmental factors in the development of HCC.

It is well known that chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) are the main risk factors for the pathogenesis of HCC. It is estimated 30% to 50% of the HBV related deaths are attributable to HCC, however, only less than 10% of HBV and HCV infected individuals developed HCC in their later life (Lavanchy, 2004; Bowen and Walker, 2005). It could be hypothesis that other factors might play a role in the development of HCC, such as environment and genetic factors. It is reported that individuals with HCC presented with DNA damaged by hepatitis virus, and this is a major underlying risk factors of HCC (Bowen and Walker, 2005). There are several DNA repair systems involved in the base excision (BER), nucleotide excision repair (NER), mismatch repair (MMR), double strand break repair (DSBR) and homologous recombination repair (HRR). The NER is the main DNA repair system, and constitutes the main defense against lesions generated by ionizing radiation and strong alkylating agents as well as lesions formed by endogenous DNA-damaging agents like viruses (Smith et al., 2003). The NER is reported to association with the development of several cancers (Bradbury et al., 2009; Yin et al., 2011; Mandal et al., 2012; Slyskovae et al., 2012).

The XRCC1 and XPD are two major repair genes involved in NER. Mutations and polymorphisms in DNA repair genes are associated with variations in the repair efficiency of DNA damage, and this repair deficit may increase the risk of cancer, birth defects and a reduced life span (Ronen and Glickman, 2001). There are two most common polymorphisms of XRCC1 identified in Arg194Trp and Arg399Gln, and two polymorphisms of XPD in Asp312Asn and Lys751Gln. These variations in the evolutionarily conserved amino acid residues in the protein-protein interface could alter the function of protein and increase the cancer risk (Chacko et al., 2005). However, there is few study on the association between these gene polymorphisms and HCC. Therefore, we conducted a case-control and case-cohort study to explore the association of XRCC1 and XPD polymorphisms with HCC.

## Materials and Methods

*Subjects*

A total of 410 cases with HCC were histological confirmed between Jan. 2008 and Dec. 2011. Case with secondary or recurrent tumors was excluded. We reviewed

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**Table 1. Comparison of the Selected Characteristics of HCC Cases and Controls**

| Characteristics | Case N=410 | % | Control N=410 | % | P-value |
|---|---|---|---|---|---|
| Age (mean±SD), years | 51.5±7.5 | | 51.4±7.9 | | 0.68 |
| Gender | | | | | |
| Male | 269 | 65.6 | 269 | 65.6 | 1.0 |
| Female | 141 | 34.4 | 141 | 34.4 | |
| Smoking status, % | | | | | |
| Smokers | 150 | 36.5 | 93 | 22.8 | <0.05 |
| Non-smokers | 260 | 63.5 | 317 | 77.2 | |
| Drinking status, % | | | | | |
| Drinkers | 169 | 41.3 | 131 | 31.9 | 0.07 |
| Non-drinkers | 241 | 58.7 | 279 | 68.1 | |
| Family history of cancer, % | | | | | |
| Yes | 44 | 10.7 | 7 | 1.8 | <0.05 |
| No | 366 | 89.3 | 403 | 98.2 | |
| HBsAg | | | | | |
| + | 150 | 36.5 | 35 | 8.6 | <0.05 |
| - | 260 | 63.5 | 375 | 91.4 | |
| Anti-HCV | | | | | |
| + | 21 | 5.1 | 4 | 0.9 | 0.14 |
| - | 389 | 94.9 | 406 | 99.1 | |

clinicopathological features such as tumor differentiation, tumor size, metastasis, cirrhosis, child-pugh class, chemotherapy and surgery from medical records.

The control group consisted of participants in the health examination center from Jan. 2008 and Dec. 2011, and they were matched with the cases by age and sex. The controls with a history of cancer and digestive system disease were excluded. All the cases and controls signed the formed consent and then provided their blood in our study.

*DNA collection and genotyping*

All participants provided 5 ml blood, and the blood was stored at -20°C. DNA was extracted from the buffy-coat fractions with TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). SNP genotyping was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction (PCR) amplification and single base extension (SBE) assays were designed by Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions. For quality control, genotyping was performed without the knowledge of the case/control status of the subjects, and a 5% random sample of cases and controls was genotyped twice by different researchers. The reproducibility was 100%.

All patients were investigated with a uniformed questionnaires including demographic information (sex and age), smoking and drinking status, and clinical characteristics (HBV and HCV infection).

The XRCC1 Arg194Trp, XRCC1 Arg399Gln, XPD Lys751Gln and XPD Asp312Asn genotyping were performed by duplex polymerase-chain-reaction with the confronting-two-pair primer (PCR-CTPP) method. The sequences of primers used for polymorphism of XRCC1 were amplified by primers described previous (Kiran et al., 2009; Long et al., 2009). The PCR conditions for XRCC1 Arg194Trp, XRCC1 Arg399Gln and XPD Lys751Gln included initial denaturation at 95°C for 2 min followed by 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s. Final extension was done at 72°C for 7 min.

**Table 2. Clinical Features of HCC Patients**

| Parameter | Cases N=410 | % |
|---|---|---|
| Tumor differentiation | | |
| Well | 146 | 35.7 |
| Moderate | 137 | 33.4 |
| Poor | 98 | 23.8 |
| Unknown | 29 | 7.1 |
| Tumor size (%) of liver | | |
| ≤50 | 215 | 52.4 |
| >50 | 195 | 47.6 |
| Distant metastasis | | |
| Yes | 96 | 23.4 |
| No | 314 | 76.6 |
| Cirrhosis | | |
| Yes | 211 | 51.4 |
| No | 199 | 48.6 |
| Child-Pugh class | | |
| A | 244 | 59.6 |
| B | 119 | 29.1 |
| C | 46 | 11.3 |
| Chemotherapy | | |
| Yes | 259 | 63.2 |
| No | 151 | 36.8 |
| Surgery | | |
| Yes | 188 | 45.9 |
| No | 222 | 54.1 |

*Statistical analysis*

Stata 8.0 (StataCorp, College Station, USA) was used to perform statistical analyses. Continuous variables were expressed as mean±standard deviation (SD) while categorical variables were shown as frequencies and percentages. Demographic characteristics were compared between cases and controls by means of chi-square test and Student's t test. We compared differences in genotype distributions of XRCC1 Arg194Trp, XRCC1 Arg399Gln, XPD Lys751Gln and XPD Asp312Asn among cases and controls, as well as tests for Hardy-Weinberg equilibrium in controls. Adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) of the association between genotype and HCC were calculated by using conditional logistic regression models with adjustments for potential confounding factors, such as sex, age, smoking, and drinking status. P were considered statistically significant which was less than or equal to 0.05.

## Results

The demographic characteristics of subjects included and clinical features of HCC patients are shown in Table 1 and Table 2. The average age is 51.5±7.5 years in HCC cases, and is 51.4±7.9 years in controls. There was no significant difference for gender, age, drinking status and anti-HCV (P>0.05). Smoking was associated with a higher risk of HCC (P<0.05), and HCC patients with positive HBsAg have high risk of HCC (P<0.05). Moreover, first relatives have a history of HCC would increase the risk of HCC (P<0.05).

**Table 3. Genotype Characteristics of the Five SNPs**

| Single nucleotide polymorphism | Alleles | MAF[a] | | | HWE (P value)[b] | |
|---|---|---|---|---|---|---|
| | | Case | Control | From dbSNP | Case | Control |
| XRCC1 Arg194Trp (rs1799782) | Arg/Trp | 0.184 | 0.145 | 0.13 | 0.06 | 0.15 |
| XRCC1 Arg399Gln (rs25487) | Arg/Gln | 0.32 | 0.248 | 0.26 | 0.12 | 0.19 |
| XPD Lys751Gln (rs13181) | Lys/Gln | 0.284 | 0.245 | 0.24 | 0.43 | 0.21 |
| XPD Asp312Asn (rs1799793) | Asp/Asn | 0.224 | 0.187 | 0.19 | 0.11 | 0.08 |

[a]Minor Allele Frequency; [b]Hardy-Weinberg equilibrium

**Table 4. The Genotype Distributions and Association of Risk of HCC**

| Single nucleotide polymorphism | Cases N=410 | % | Controls N=410 | % | P value | OR (95% CI)[1] |
|---|---|---|---|---|---|---|
| XRCC1 Arg194Trp(rs1799782) | | | | | | |
| Arg/Arg | 264 | 64.4 | 292 | 71.2 | 0.22 | 1.0(Ref.) |
| Arg/Trp | 109 | 26.5 | 96 | 23.3 | | 1.17(0.83-1.55) |
| Trp/Trp | 37 | 9.1 | 23 | 5.5 | | 2.26(1.23-5.38) |
| Trp allele | 92 | 22.4 | 70 | 17.2 | | 1.42(0.91-2.48) |
| XRCC1 Arg399Gln(rs25487) | | | | | | |
| Arg/Arg | 203 | 49.6 | 227 | 55.3 | 0.17 | 1.0(Ref.) |
| Arg/Gln | 136 | 33.1 | 128 | 31.3 | | 1.16(0.86-1.62) |
| Gln/Gln | 71 | 17.3 | 55 | 13.4 | | 1.74(1.06-2.74) |
| Gln allele | 139 | 33.9 | 119 | 29.1 | | 1.50(0.94-2.04) |
| XPD Lys751Gln (rs13181) | | | | | | |
| Lys/Lys | 190 | 46.3 | 233 | 56.9 | <0.05 | 1.0(Ref.) |
| Lys/Gln | 183 | 44.6 | 159 | 38.7 | | 1.14(0.87-1.53) |
| Gln/Gln | 37 | 9.1 | 18 | 4.4 | | 3.51(1.50-6.31) |
| Gln allele | 129 | 31.4 | 97 | 23.8 | | 1.42(1.05-3.45) |
| XPD Asp312Asn (rs1799793) | | | | | | |
| Asp/Asp | 260 | 63.5 | 282 | 68.8 | 0.18 | 1.0(Ref.) |
| Asp/Asn | 107 | 26.1 | 96 | 23.5 | | 1.23(0.78-1.67) |
| Asn/ Asn | 43 | 10.4 | 32 | 7.7 | | 1.66(0.87-2.98) |
| Asn allele | 96 | 23.5 | 80 | 19.5 | | 1.37(0.90-2.34) |

[1]Adjusted for sex, age, smoking, drinking, family history of cancer and HBsAg status

Most of the HCC patients had well differentiation, and most of the tumor size was less than 50% of the liver. Almost 60% of the HCC patients were grade A of Child-Pugh class. Most of the HCC patients received chemotherapy and surgery treatment.

The allele and genotype distribution of polymorphisms in XRCC1 Arg194Trp, Arg399Gln XPD Lys751Gln and XPD Asp312Asn were showed in table 3. The minor allele frequencies among selected controls were consistent with the MAF from NCBI SNP databases. Moreover, all the SNPs were in line with the Hardy-Weinberg equilibrium among cases and controls (All the P value >0.05).

The genotype distributions of XRCC1 Arg194Trp, XRCC1 Arg399Gln, XPD Lys751Gln and XPD Asp312Asn were significantly different between cases and controls (Table 4). The association between the SNPs and the risk of HCC was studies by using conditional logistical regression analysis, with frequency matched by age and sex. XRCC1 194Trp/Trp was strongly significantly associated with an increased risk of HCC cancer when compared with the wide-type genotype, with the adjusted OR (95% CI) of 2.26 (1.23-5.38). Individuals carrying the XRCC1 399Gln/Gln showed increased risk of HCC (OR=1.74, 95%CI=1.06-2.74). The XPD 751Gln/Gln and Gln allele genotype were associated with strong elevated susceptibility to HCC (OR=3.51 and 1.42, respectively).

## Discussion

The results of the present study showed polymorphisms in XRCC1 Arg194Trp, XRCC1 Arg399Gln, and XPD Lys751Gln were related to HCC risk in Chinese population. These results suggest that polymorphisms in XRCC1 and XPD may have functional significance in HCC.

Various DNA damage may be induced by normal endogenous metabolic processes or by environmental carcinogens. Most of these alterations, if not repaired, may result in genetic instability, mutagenesis and cell death, Moreover, these DNA damages may destroy genome integrity and induce carcinogenesis. NER is the predominant DNA damage repair pathway for the processing of small base lesions, derived from oxidation and alkylation's damage. XRCC1 gene is regarded an important proteins in the multistep NER pathway, and it is the first mammalian gene isolated that affects cellular sensitivity to ionizing radiation (Thompson et al., 1990). Mutations of XRCC1 may increase the risk of cancers by impairing the interaction of XRCC1 with other enzymatic proteins and consequently altering DNA repair activity (Basso et al., 2007; Tudek, 2007), and subsequently induce the carcinogenesis of several malignant tumors (Yu et al., 2003; Han et al., 2004). HBV and HCV were the risk factors of HCC, and these two factors may cause chromosomal instability or insertional mutations, and thus to induce the carcinoma development risk. The polymorphism in XRCC1 codon 399 locates in the BRCT1 domain, which could altering the function of XRCC1 enzyme activity and DNA repair captivities, further leading to carcinoma development, including cervical cancer, lung cancer, colorectal cancer and breast cancer (Cui et al., 2012; Liu et al., 2012; Yin et al., 2012; Zhang et al., 2012). Our study showed the polymorphism in XRCC1 Arg399Cln could increase the risk of HCC, which was in line of a previous studies (Kiran et al., 2009; Pan et al., 2011; Li et al., 2012).

XPD protein, encoded by XPD gene, plays a role in NER pathway. During the NER, XPD participates in the opening of the DNA helix to allow the excision of the DNA fragment containing the damaged base (Benhamou et al., 2002; Manuguerra et al., 2006). 312 (Asp to Asn) and 751 (Lys to Gln) were the main two polymorphisms that induce amino acid changes in the proteins (Shen et al., 1998). Previous experimental and epidemiologic studies showed the XPD codon Lys751Gln and/or Asp312Asn could modify the DNA repair ability in the NER capacity,

and XPD 312Asn alleles and/or 751Gln alleles had lower NER capacity than the wide-type genotypes (Spitz et al., 2001; Rzeszowska-Wolny et al., 2005). In our study, we only found polymorphism in XPD Lys751Gln was related to the risk of HCC, which provide evidence that the XPD protein influences HCC risk through NER pathway.

In conclusion, our present data provide evidence to suggest that polymorphisms in XRCC1 Arg194Trp, XRCC1 Arg399Gln, and XPD Lys751Gln were related to HCC risk in Chinese population. Moreover, the genotype of XRCC1 399Gln/Gln and XPD 751Gln/Gln were associated with a reduction of death from HCC. Our finding were based on relative small numbers and limited by small number subjects. More large sample studies from Chinese population are still needed.

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