RESEARCH COMMUNICATION

Polymorphism of DNA Repair Gene XRCC1 and Hepatocellular Carcinoma Risk in Chinese Population

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Abstract

Aim: We conducted a case-control study in China to clarify the association between the XRCC1-Arg399Gln polymorphism and HCC risk. Methods: A total of 202 cases and 236 controls were selected from the the Affiliated Hospital of Qingdao University from May 2008 to May 2010. Assessment of the XRCC1-Arg399Gln polymorphism was based upon duplex polymerase-chain-reactions with the confronting-two-pair primer (PCR-CTPP) method. All analyses were performed using the STATA statistical package. Results: A significant increase in risk was associated with the Arg/Gln genotype (adjusted OR 1.55, 95%CI=1.03-2.57) compared with Arg/Arg. However, the Gln/Gln genotype had non-significant increased risk of HCC with adjusted OR (95%CI) of 1.34(0.67-2.38). There was also a significant increasewith the Arg/Gln genotype among HCC patients above 50 years old (OR=1.95, 95% CI=1.14-3.57). Additionally, the risk of HCC was moderately increased in drinkers with Arg/Gln genotype compared with never drinkers, and the adjusted OR (95% CI) was 1.89 (1.13-3.45). Conclusion: This study demonstrated that a polymorphism in a DNA repair gene may influence the risk of HCC. The XRCC1 codon Arg/Gln was thuis associated with an increased risk of HCC, especiallyin patients above 50 years old and/or with a drinking habit.

Keywords: Polymorphism - DNA repair gene XRCC1 - hepatocellular carcinoma - China

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Introduction

Liver cancer is the fifth most common cancer in men (523 000 cases, 7.9% of the total) and the seventh in women (226 000 cases, 6.5% of the total), and most of the burden is in developing countries, where almost 85% of the cases occur, and particularly in men: the overall sex ratio male: female is 2.4. The regions of high incidence are Eastern and South-Eastern Asia, Middle and Western Africa, but also Melanesia and Micronesia/Polynesia (particularly in men). Low rates are estimated in developed regions, with the exception of Southern Europe where the incidence in men (ASR 10.5 per 100,000) is significantly higher than in other developed regions (International Agency for Research on Cancer, 2011). The wide geographic variation at an international levels of EC in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer.

As we know, chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is the most well-established environmental risk factor for HCC worldwide. However, only a fraction of HBsAg carriers eventually develop HCC and only 2.5% of HCV infected individuals develop HCC later in life. Therefore, the real reason of hepatocarcinogenesis is still incompletely understood, and the risk factors for HCC still need to be further elucidated (Bowen and Walker, 2005).

In recent years, it has been shown that variability in DNA repair capacity plays a role as a modifier of cancer risk. The XRCC1 gene (located at chromosome 19q13.2) produces an enzyme involved in the base excision repair (BER) pathway, amending small lesions such as singlestrand breaks (SSBs), non-bulky adducts, oxidative damage, alkylation, and methylation. Recently, the XRCC1 complex has also been described as part of an alternative route of DNA double-strand break (DSB) nonhomologous end-rejoining, i.e., PARP1–dependent end-joining of DSBs (Audebert et al., 2004). The XRCC1 protein is essential for mammalian viability and XRCC1-deficient cells are genetically unstable and sensitive to DNA damaging agents. Three common SNPs (variant allele frequency[0.05) lead to amino acid substitutions in XRCC1 at codons 194 (exon 6, C→T, Arg→Trp), 280 (exon 9, G→A, Arg→His), and 399 (exon 10, G A, Arg→Gln) (Shen et al., 1998). These mutations could alter XRCC1 function, diminish repair kinetics, influence susceptibility to adverse health effect, such as cancer. Previous studies reported the association between XRCC1-Arg399Gln polymorphism and HCC risk, but the results are conflicting. The aim of our study is to conduct a case-control study in China to clarify the association between XRCC1-Arg399Gln polymorphism and HCC risk.

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Materials and Methods

HCC patients were consecutively collected from the the Affiliated Hospital of Qingdao University from May 2008 to May 2010. All HCC patients with newly diagnosed primary HCC in the hospital were invited for face-to-face interviews within one months after diagnosis. All cases recruited in our study were histologically confirmed. Among a total of 210 eligible cases, 202 were interviewed with a participation rate of 96%. 236 controls were randomly selected from people who requested general health examinations in the same hospital during the same period and were confirmed to have no malignancy, digestive diseases, chronic diseases and also no prior history of malignancy. The controls were matched with cases by ages within five years age.

Genotyping of XRCC1

Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pairprimer (PCR-CTPP) method. Briefly, the sequences of primers used for XRCC1 polymorphisms are CTAACTggCATCTTCACTTCTg and CATTgCCCAgCACAggATAAg. Each 30 μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg2+, 2.4μL dNTPs, 8 primers, 15 pmol of each primer and 5-8 μL template. 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 62°C for 30 s, at 72°C for 30 s, and a final extension at 72°C for 5 min. After transient centrifugation, agarose electrophoresis was conducted. The PCR products included 198 bp fragments of Arg/Arg, 145 bp fragments of Gln/Gln, 198 bp fragments of Arg/Gln.

Statistical analysis

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX). Hardy-Weinberg equilibrium of alleles at controls was assessed by using exact tests. Categorical variables were compared with use of the chi-square test or Fisher's exact test (when one expected value was <5). Unconditional logistic regression was undertaken to estimate odds ratios (ORs) and their 95% confidence intervals (95% CIs) after controlling for potential confounding factors, including age, sex, cigarette smoking (yes or no), alcohol consumption (yes or no), first degree family history of HCC. All statistical tests were two sided.

Results

The mean age at enrollment of this case-control study was 50.5±9.7years for cases and 50.2±9.5 years for controls. There were no significant differences for sex, age, HBsAg and anti-HCV status (P>0.05, table 1).

Table 1. Demographic Characteristics of the Study Population

Parameter	Cases N=202(%)	Controls N=236(%)	Test statistics	P
Sex				
Male	136(67.33)	151(63.98)	$\chi2$=0.54	0.46
Female	66(32.67)	85(36.02)		
Age (mean±SD)	50.5±9.7	50.2±9.5	t=0.33	0.63
<50 years old	86(42.57)	101(42.80)	$\chi2$=0.002	0.96
≥50 years old	116(57.43)	135(57.20)		
Smoking	89(44.06)	71(30.08)	$\chi2$=9.17	0.002
Drinking	127(62.87)	78(33.05)	$\chi2$=38.87	<0.001
HBsAg(+)	120(52.48)	108(48.73)	$\chi2$=0.611	0.43
Anti-HCV(+)	25(11.88)	18(9.32)	$\chi2$=0.76	0.38

Table 3. Estimates of Odds Ratio for HCC Associated with XRCC1-Arg399Cln Polymorphism in Different Age Groups

Ages	XRCC1-Arg 399Cln	Cases(%)	Controls(%)	OR[1]
<50	Arg/Arg	21(10.40)	26(11.02)	1.0(reference)
years	Arg/Gln	44(21.78)	50(21.19)	1.32(0.62-2.57)
old	Gln/Gln	21(10.40)	25(10.59)	1.14(0.51-2.69)
≥50	Arg/Arg	24(11.88)	42(17.80)	1.0(reference)
years	Arg/Gln	61(30.20)	62(26.27)	1.95(1.14-3.57)
old	Gln/Gln	31(15.35)	31(13.14)	1.53(0.77-3.61)

[1]Adjusted for sex, smoking and drinking status

The HCC patients had significant higher consumption of smoking and drinking than controls (P<0.05, Table 1). The genotype distribution of XRCC1-Arg399Cln for both HCC cases and controls were shown in Table 2. The genotype frequency distribution in controls fit well to Hardy-Weinberg equilibrium (p=0.46). The frequencies of Arg/Arg, Arg/Gln and Gln/Gln in cases were 22.28%, 51.98% and 25.74%, respectively, which was significant difference with those in controls (28.81%, 47.46% and 23.73%, respectively). A significant increased risk was associated with the Arg/Gln genotype (adjusted OR 1.55, 95%CI=1.03-2.57) compared with genotype Arg/Arg. But Gln/Gln genotype had non-significant increased risk of HCC with adjusted OR (95%CI) of 1.34(0.67-2.38).

The XRCC1-Arg399Cln genotypes and allelic frequencies in patients and controls, and the estimates of relative risks of HCC associated with XRCC1-Arg399Cln polymorphisms among the study population in different age groups are presented in Table 3. There was a significant increased risk for Arg/Gln genotype among HCC patients above 50 years old (OR=1.95, 95% CI=1.14-3.57). In contrast, no significant increased risk was found among patients below 50 years old.

We also did stratified analysis regarding drinking status in Table 4. The risk of HCC was moderately increased in drinkers with Arg/Gln genotype compared with never

Table 2. The Gene Frequencies of XRCC1-Arg399Cln in Cases and Controls

XRCC1-Arg399Cln	Cases(%)	Controls(%)	OR[1]	OR[2]
Arg/Arg	45(22.28)	68(28.81)	1.0(reference)	1.0(reference)
Arg/Gln	105(51.98)	112(47.46)	1.42(0.87-2.31)	1.55(1.03-2.57)
Gln/Gln	52(25.74)	56(23.73)	1.40(0.79-2.48)	1.34(0.67-2.38)

Table 4. Estimates of Odds Ratio for HCC Associated with XRCC1-Arg399Cln Polymorphism in Different Dinking Status

Drinking status	XRCC1-Arg 399Cln	Cases(%)	Controls(%)	OR[1]
Non-drinkers	Arg/Arg	26(12.87)	19(8.05)	1.0(reference)
	Arg/Gln	72(35.64)	39(16.53)	1.39(0.70-2.95)
	Gln/Gln	29(14.36)	20(8.47)	1.12(0.42-2.67)
Drinkers	Arg/Arg	19(9.41)	49(20.76)	1.0(reference)
	Arg/Gln	41(20.30)	73(30.93)	1.89(1.13-3.45)
	Gln/Gln	15(7.43)	36(15.25)	1.09(0.46-2.67)

drinkers, and the adjusted OR (95% CI) was 1.89 (1.13-3.45). We did not found interaction between alcohol drinking and XRCC1-Arg399Cln genotypes on HCC risk (data not shown).

Discussion

A wide variety of 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. DNA repair mechanisms are important for maintaining genome integrity and preventing carcinogenesis. BER 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 BER 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 head and neck and cancer of lung, esophagus, breast and many other malignancies (Han, et al., 2004; Yu et al., 2004). As we known, HBV and HCV may promote chromosomal instability or insertional mutations, and thus to induce the carcinoma development risk. The XRCC1, DNA repair gene, play a protective way of genetic abnormalities, but the polymorphism of XRCC1 has been functional in altering the XRCC1 enzyme activity and DNA repair captivities, further leading to carcinoma development. There were three reported polymorphisms at codons 194, 280 and 399 of XRCC1, codon 194 and 280 do not locate in the important domain, but codon 399 locates in the BRCT1 domain. Previous experimental study showed the amino acid replacement of codon 399 could injury the DNA repair captivities and increase the susceptibility to ionizing radiation. Therefore, the polymorphism of XRCC1-Arg399Cln could increase the cancer risk of individuals. Our study also found the Arg/Gln genotype could increase the risk of HCC, which was in line of a previous study conducted in Taiwan (Yu et al., 2003).

Our study found the Arg/Gln and Gln/Gln genotypes could increased the risk of HCC among patients above 50 years old. However, it is contrary to the protective effect of Gln/Gln genotype reported by previous studies (Yu et al., 2003). The explanation might be the HBV infection in developing counties usually happened in children, and the exposure time was long. However, the HBV infection in developed counties usually in adults, and the mean age of onset is usually 10 late than developing countries. Therefore, the Gln/Gln genotype may play a protective way on HCC.

Our study also found the polymorphism of XRCC1-Arg399Cln may increase the risk among drinkers. Chronic alcohol consumption is associated with the production of free radical intermediates, such as hydroxyethyl free radicals and reactive oxygen species, which are produced during ethanol metabolism (Clot et al., 1994). Several reports suggest that free radicals and oxidative stress play an important role in the pathogenesis of alcoholic and toxic liver diseases (Nordmann et al., 1992; Ishii et al., 1997; Navasumrit et al., 2000). The XRCC1-Arg399Cln may have functional significance in the repair of alcohol induced genetic lesions. However, when the amino acid replacement of codon 399, the DNA repair capacity may be reduced. Our study showed the Arg/Gln genotype may moderate increase the risk of HCC, which support information on above assumption.

In conclusion, this study demonstrates that polymorphisms in DNA repair gens may influence the risk of HCC. XRCC1-Arg399Cln Arg/Gln showed an increased risk of HCC, especially for patients above 50 years old or with drinking habits. Our study presented here provide information of identifying genetic polymorphisms in DNA repair genes on the susceptibility of carcinogenesis.

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