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RESEARCH ARTICLE

# Individuals having variant genotypes of cytochrome P450 2C19 are at increased risk of developing primary liver cancer in Han populations, without infection with the hepatitis virus

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**Abstract** Recently, many researchers have reported that the genetic polymorphisms of CYP2C19 may account for the interpatient variability of the clinical course in cancers including primary liver cancer (PLC). Besides the genetic polymorphisms of CYP2C19, hepatitis viruses (HV, including HAV, HBV, HCV, HDV, HEV, especially HBV and/or HCV) also account for the interpatient variability of the clinical course in PLC. This research covered the above two factors and divided the patients with PLC into two groups (one group with HBV infection and another without any HV infection) to find out whether the genetic polymorphisms of CYP2C19 have different effects in the progressing of PLC in different groups of patients. Eight hundred sixty-four cancer-free Han people (controls, named group 1), 207 Han PLC patients with HBV infection (group 2), and 55 Han PLC patients without any HV infection (group 3) were involved in this study. A wild-type allele (CYP2C19*1) and two mutated alleles (CYP2C19*2 and CYP2C19*3) were identified. The frequencies of the mutant alleles and genotypes were then compared with each other. The frequencies of the homozygous and heterozygous variant genotypes (*2/*2, *2/*3, *3/*3) in group 3 (25.5 %) were significantly higher than those in other groups (11.9 % in group 1 and 13.5 % in group 2, $P$=0.014, 95 % confidence interval (CI)). The differences were statistically significant between group 1 and group 3 ($P$=0.004, 95 % CI), but they were not statistically significant between group 1 and group 2 ($P$=0.527, 95 % CI). Thus, we conclude that people which were not infected with HV but with the homozygous or heterozygous variant genotypes (*2/*2, *2/*3, *3/*3) of CYP2C19 may have higher possibilities of getting PLC than people with other allelic genotypes (*1/*1, *1/*2, *1/*3) (odds ratio (OR)=2.523, 95 % CI=1.329~4.788). However, in patients with HBV infection, the genetic polymorphisms of CYP2C19 did not seem to be an important factor in the risk of developing PLC (OR=1.156, 95 % CI=0.738~1.810).

**Keywords** CYP2C19 polymorphisms · Primary liver cancer · Hepatitis virus

## Introduction

Being exposed to endogenous or environmental substances may modulate the carcinogenic process. Polymorphic enzymes of cytochrome P450 (CYP) play an important role in detoxication/toxication of such substances. As an important member of the CYP family of enzymes, CYP2C19 is also involved in the metabolism and elimination of such substances. Two common polymorphisms of CYP2C19 (CYP2C19*2 and CYP2C19*3) that are responsible for cancer susceptibility have been investigated extensively [1–6]. Many researchers had reported the relationship between the liver cancer and genetic polymorphisms of CYP2C19 [7–11]. But, among the various etiological factors being implicated in the cause of primary liver cancer (PLC), the most important cause, however, is infection with HBV and/or HCV [12, 13].

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**Table 1** Clinical characteristics of the study participants

| General characteristics | Groups | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 | Group 2 | *P* | Group 1 | Group 3 | *P* |
| Alcohol drinker[a] (*n*, %) | 99 (11.5 %) | 34 (16.4 %) | 0.052 | 99 (11.5 %) | 7 (12.7 %) | 0.775 |
| Smoking[b] (*n*, %) | 205 (23.7 %) | 62 (29.9 %) | 0.063 | 205 (23.7 %) | 12 (21.8 %) | 0.747 |
| Family history of PLC (*n*, %) | 7 (0.8 %) | 21 (10.1 %) | 0.000 | 7 (0.8 %) | 1 (1.8 %) | 0.435 |
| HV status | No HV | HBV | | No HV | No HV | |

[a] Persons using tobacco every day in the previous 6 months

[b] Persons drinking alcohol almost every day in the previous 6 months

So, researching the association between genetic polymorphisms of CYP2C19 and PLC without precluding the infection situation of the patients is not thoughtful [7, 10, 11]. The distribution of the CYP2C19 genotypes in PLC patients with HCV infection had been researched by some scientists [8, 9], but researches have not been carried out on the distribution of the CYP2C19 genotypes, neither in PLC patients with HBV infection nor in PLC patients without hepatitis virus (HV) infection. Based on this, the PLC patients in this study were divided into two groups; one group was composed of PLC patients infected with HBV, and the other group was composed of PLC patients infected without HV. The control group was cancer-free people without HV infection. In this way, we can distinguish the effects of genetic polymorphisms of CYP2C19 and HBV on liver cancer in different groups of patients.

## Materials and methods

### Subjects

A total of 1,126 unrelated adults from Henan Provincial People's Hospital, Henan Province, China, were recruited for the study. Eight hundred sixty-four adults (600 male, 264 female, 61±17 years old) were selected into the control group (group 1) after the diagnosis of no HV infection and cancer-free. Two hundred seven patients infected with HBV and diagnosed with primary liver cancer (160 male, 47 female, 54±10 years old) were selected into one experimental group (group 2), and 55 patients who had not been infected with HV but diagnosed with primary liver cancer (32 male, 23 female, 55±14 years old) were selected into another experimental group (group 3). Clinical data about the subjects were collected from 2012 to 2013. General characteristics of the study population which are considered to be risk factors for PLC are presented in Table 1. Both the patients and the controls are of Chinese Han ethnic origin. The study was approved by the ethical committees of Henan Provincial People's Hospital. Informed consents were obtained from all patients.

### Laboratory determinations

Venous blood samples were drawn and collected in ethylenediaminetetraacetic acid (EDTA) tubes, and DNA was extracted by using a whole blood DNA extraction and purification kit (BaiO, Shanghai, China) according to the manufacturer's instructions. Samples were coded to allow blinding of the investigators who carried out the genotyping. The CYP2C19 genotype was determined by PCR-genotyping microarray analysis of the two important allelic variants (CYP2C19*2 and CYP2C19*3) by using the BaiO gene detection kit (genotyping microarray method, BaiO, Shanghai, China). Cases which were homozygous or heterozygous for either the CYP2C19*2 or/and CYP2C19*3 mutation (*2/*2, *3/*3, *2/*3) were categorized as poor metabolizer phenotypes (PMs). Cases that were homozygous for the wild type (WT) (*1/*1) or heterozygous for the WT and mutation (*1/*2, *1/*3) were categorized as extensive metabolizer phenotypes (EMs) [14].

### Statistical methods

The genotype frequencies of different genes were tested for the Hardy-Weinberg equilibrium for both patient groups and control groups using the chi-square test. Logistic regression analysis was used to estimate the odds ratio (OR) and its 95 % confidence interval (CI) as a measure of the association between polymorphism of CYP2C19 and risk of PLC. The

**Table 2** Distribution of CYP2C19 of the controls

| Entries | Frequency of CYP2C19 genotypes (*n*, %) | | | | | |
|---|---|---|---|---|---|---|
| | *1/*1 | *1/*2 | *1/*3 | *2/*2 | *2/*3 | *3/*3 |
| Entry 1 (group 1) | 352 (40.7) | 357 (41.3) | 52 (6.0) | 76 (8.8) | 25 (2.9) | 2 (0.2) |
| Entry 2 [15] | 68 (32.4) | 94 (44.8) | 17 (8.1) | 22 (10.5) | 8 (3.8) | 1 (0.5) |

**Table 3** CYP2C19 polymorphism of the subjects

| CYP2C19 genotypes | Group 1 (*n*, %) | Group 2 (*n*, %) | Group 3 (*n*, %) |
|---|---|---|---|
| *1/*1 | 352 | 85 | 18 |
| *1/*2 | 357 | 81 | 18 |
| *1/*3 | 52 | 13 | 5 |
| EMs | 761 (88.1 %) | 179 (86.5 %) | 41 (74.5 %) |
| *2/*2 | 76 | 20 | 9 |
| *2/*3 | 25 | 7 | 4 |
| *3/*3 | 2 | 1 | 1 |
| PMs | 103 (11.9 %) | 28 (13.5 %) | 14 (25.5 %) |

differences were considered significant if the *P* value did not exceed 0.05.

## Results

### General characteristics of the participants

The general characteristics of the study population are presented in Table 1. There were no significant differences ($P>0.05$) between the PLC patients (group 2 and group 3) and the control subjects (group 1) in tobacco smoking and alcohol drinking which are considered to be risk factors for PLC. However, there was significant difference in family history of PLC between the HBV-infected patients and the other two groups ($P<0.05$).

### Distribution of the CYP2C19 genotypes

Firstly, the genetic data of group 1 were summarized in Table 2 (entry 1). Entry 2 of Table 2 was the result of a previous research which studied the genetic polymorphisms of drug-metabolizing enzymes CYP2C19 in a Han Chinese population from the Henan area [15]. In Table 2, it is shown that the distribution of CYP2C19 was approximate between the two groups ($P=0.309$, 95 % CI). So, the distribution of CYP2C19 in our control subjects (group 1) can represent the distribution of CYP2C19 in the healthy Han Chinese population from the Henan area.

Then, the CYP2C19 genotypes of all the individuals recruited were collected in Table 3. In Table 3, we can see that the frequencies of the CYP2C19 PM genotype were significantly higher in group 3 than in group 1 or in group 2 (25.5 % of group 3 versus 11.9 % of group 1 and 13.5 % of group 2, $P=0.014$). The genotype distribution of CYP2C19 showed a large difference between group 1 and group 3 ($P=0.004$), but there was no apparent difference between group 1 and group 2 ($P=0.527$). The CYP2C19 PM genotype in the whole PLC group (groups 2 and 3) were a little higher than that of the control group (group 1), but it was not statistically significant (29.0 % in the PLC group and 24.7 % in the control group, $P=0.272$).

### Association of CYP2C19 PM genotype with risk of PLC

Among participants who were not infected with HV, the PM genotype of CYP2C19 was associated with an increased risk of PLC (OR=2.523, 95 % CI=1.329~4.788), compared with EM genotype carriers. But, among participants infected with HV, the association between the CYP2C19 PM genotype and risk of PLC was not significant (OR=1.156, 95 % CI=0.738~1.810). The results are listed in Table 4.

## Discussion

In this study, on the basis of ruling out other factors (such as smoking, drinking, and family history) contributing to PLC, we found that the CYP2C19 PM genotype was related with PLC in the Chinese Han population. But, the relation was significant only in PLC patients without HV infection. Compared with cancer-free people, the distribution of CYP2C19 genotypes in PLC patients with HBV infection was free from significant difference. This can be attributed to a more important role of HBV than that of the CYP2C19 genotypes in the development of PLC in patients infected with HBV. Prior to this study, some researchers reported that the PM genotype caused by the mutation of the CYP2C19 gene in patients with HCV infection was related to some extent with a high risk for developing hepatocellular carcinoma [8, 9]. That does not conflict with our studies in that the patients in group 2 were

**Table 4** CYP2C19 genotype and PLC susceptibility

| Groups[a] | | *B* | S.E. | Wald | *P* | OR | 95 % CI |
|---|---|---|---|---|---|---|---|
| Group 2 | Intercept | −1.447 | 0.083 | 303.526 | 0.000 | | |
| | Genotype = PMs | 0.145 | 0.229 | 0.400 | 0.527 | 1.156 | 0.738~1.810 |
| | Genotype = EMs | 0[b] | | | | | |
| Group 3 | Intercept | −2.921 | 0.160 | 331.952 | 0.000 | | |
| | Genotype = PMs | 0.925 | 0.327 | 8.015 | 0.005 | 2.523 | 1.329~4.788 |
| | Genotype = EMs | 0[b] | | | | | |

[a] Reference was group 1

[b] Referent; this parameter was set to zero because it is redundant

infected with HBV. The reason may be that in patients with HCV infection, the risk of getting PLC may partly depend on the CYP2C19 PM genotype, but for patients with HBV infection, the risk of getting PLC mainly depends on the HBV infection. Because the HBV infection has higher possibility of family history, that may be the reason why the family history of PLC in group 2 is different from that of the other two groups.

Polymorphic enzymes of cytochrome P450 (CYP) are the main drug-metabolizing enzymes in the human body and participate in the metabolism of carcinogens or procarcinogens. As one of the most important cytochrome P450s, CYP2C19 is known as a key enzyme in the in vivo metabolism of a number of such substances and drugs. CYP2C19*2 shows a single-base mutation (G→A) in exon 5 which produces an aberrant splice site. CYP2C19*3 is a single-base-pair G636 A mutation in exon 4 of CYP2C19 which results in a premature stop codon [16]. Thus, people with the PM genotype of CYP2C19 may have a higher carcinogen level and potent cell toxicity because of the lower ability for detoxificating carcinogens. That may be the reason why the CYP2C19 PM genotype was related with PLC in the Chinese Han population.

## Conclusion

Even the worldwide incidence of PLC is mainly associated with chronic HBV and/or HCV infections [12, 13]. We found an association between the CYP2C19 PM genotype and PLC in the Chinese Han population. For individuals not infected with HV, the CYP2C19 PM genotype plays an important role in the development of PLC. But, in patients infected with HBV, the HBV infection plays an even more important role in the development of PLC, so no effects of the CYP2C19 PM genotype in such patients had been found.

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**Conflicts of interest** None

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