

Research Article

Correlation Analysis of Cytochrome P450 SNPs in Hepatitis B-Caused Cirrhosis Patients

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Received 19 March 2022; Revised 31 March 2022; Accepted 8 June 2022; Published 5 July 2022

Academic Editor: Hafiz Ishfaq Ahmad

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Aim. The aim of the present research was to find the correlation of single nucleotide polymorphisms (SNPs) of cytochrome P450 (CYP450) and hepatitis B-caused cirrhosis. **Methods.** Collection of specimens was done from 297 volunteers with confirmed hepatitis B-caused cirrhosis as well as 120 healthy volunteers in China. Individuals were categorized into three classes, i.e., A, B, and C, on the basis of the Child-Pugh-Turcotte (CPT) value of diseased people, while the Child-Pugh-Turcotte score was determined by rating the below mentioned 5 parameters, i.e., serum volume of bilirubin as well as albumin, prothrombin time, ascites, and encephalopathy. Twenty-four SNPs in the CYP450 superfamily including *CYP1A2*, *CYP2A6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, and *CY3A4* were detected using the SNaPshot assay. **Results.** *CYP1A2*-G2964A, *CYP1A2*-C733A, and *CYP1A2*-T5347C in all 24 SNP loci attained significance. AA genotype at *CYP1A2*-G2964A ($P = 0.048$) and CC genotype at *CYP1A2*-T5347C ($P = 0.049$) were significantly correlated with hepatitis B-caused cirrhosis. Moreover, an allele at *CYP1A2*-G2964A ($P = 0.032$) and C allele at *CYP1A2*-T5347C ($P = 0.016$) were associated with hepatitis B-caused cirrhosis. Furthermore, AC plus AA genotype at *CYP1A2*-C733A correlated with a TCM syndrome, “damp abundance due to spleen asthenia syndrome” ($P = 0.039$) in individuals suffering from hepatitis B-resulted cirrhosis. **Conclusion.** The findings of the current study suggested that AA genotype at *CYP1A2*-G2964A and CC genotype at *CYP1A2*-T5347C may be higher risk in the occurrence of hepatitis B-caused cirrhosis. Moreover, patients with AC plus AA genotype at *CYP1A2*-C733A may be susceptible to appear having “damp abundance due to spleen asthenia syndrome.”

1. Introduction

Hepatitis B virus is considered as a crucial disease in China, as well as regarded as an important reason for virus-based hepatocellular disorders, i.e., liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1, 2]. Throughout the world, chronic hepatitis B virus disease is a key health risk in subsequent developing of hepatocellular carcinoma [3]. 350 million hepatitis B virus-affected individuals are present having 15-25% chances of death due to these virus-based hepatocellular diseases [4]. In the development of Hepatitis B virus disease, hepatotropic deoxyribonucleic acid virus is involved in damaging the hepatic system. Due to which, a wide range of physical indications appeared, from no symptoms/carrier formed to persistent hepatitis B or hepatitis B-caused cirrhosis, which can also cause HCC [5, 6]. Five-

year survival rate has been observed in individuals suffering from persistent hepatitis B, persistent hepatitis B virus disease that can also lead to pulmonary cirrhosis, persistent HBV disease, and chronic hepatitis B.

Already done researches have reported the association among genetic vulnerability of gene and hepatic disease, i.e., aldehyde dehydrogenase 2 (ALDH2) and hepatocellular carcinoma [7], interleukin-2, IFN- γ , interleukin-10, and hepatitis B and C virus or their coinfection [8] and CYP2E1 gene as well as hepatocellular carcinoma [9]. But this type of association is not fully explored yet.

The cytochrome P450 (CYP450) is a vast superfamily (46000 known members [10]) of haem-based monooxygenases. Compounds of this pervasive superfamily perform a significant function in the metabolism and production of exogenous medicine at large scale [11]. However,

the correlation between CYP450 gene and hepatitis B-caused cirrhosis remains poorly understood.

Classification of this infection is significant in examination of this infection. In liver cirrhosis, different genetic makeup has been applied for the categorization, i.e., CPT categorization, compensation/decompensation stage, pulmonary activities, and TCM syndrome. TCM disease, also known as “ZHENG,” is regarded as key basics of the concept of theory of this syndrome. Each examination and treatment technique in TCM is established on the basis of differentiation of “ZHENG” [12]. This also evaluated the TCM identification of outline of signs of the ailment rather than a normal collection of signs of syndrome.

In the current research, we evaluated the relationship between CYP450 single nucleotide polymorphism and hepatitis B-caused cirrhosis and the relationship between these polymorphisms and the phenotype of hepatitis B-caused cirrhosis.

2. Materials and Methods

2.1. Patients and Healthy Controls. A total of 297 individuals having cirrhosis caused by hepatitis B and 120 healthy controls were included in the current research. These individuals belonged to Longhua, Shuguang, Yueyang, and Putuo Hospitals located in Shanghai, the First Allied Hospital of Henan University of Traditional Chinese Medicine, and Ruikang Hospital in Guangxi, China, and had been chosen on the basis of age (from 18 to 65 years), gender, and ethnicity (Table 1). The healthy control subjects were volunteers from the Medical Examination Center. All patients and controls were Chinese yellow race. Blood sampling was done in all individuals, by providing informed consent and ethical review board acceptance according to the principles of the Declaration of Helsinki. 3 ml blood sample was obtained from every participant and then stored at -80°C prior to nucleic acid extraction.

2.2. Classification of Child-Pugh, Phase, and TCM Syndrome. Individuals that participated in the study were categorized in classes A, B, and C on the basis of the Child-Pugh-Turcotte (CPT) value of patients; the Child-Pugh-Turcotte value was computed with the help of 5 frameworks, i.e., serum volume of bilirubin as well as albumin, prothrombin time, ascites, and encephalopathy [13, 14]. When signs of patient were identified, compensation as well as decompensation phase was regarded as 2 categories of LC.

According to “diagnosis, syndrome differentiation of TCM and evaluate the curative effect of liver cirrhosis” [15], six of TCM syndrome types in individuals suffering from hepatitis B-caused cirrhosis were defined and classified. They are “liver-qi stagnation syndrome,” “damp abundance due to spleen asthenia syndrome,” “damp-heat syndrome,” “liver-kidney yin deficiency syndrome,” “blood stasis syndrome,” and “yang deficiency of spleen and kidney syndrome.”

2.3. Selection of SNPs in CYP450 Genes. In this study, the International Haplotype Mapping (<http://www.hapmap.org>), NCBI database (<http://www.ncbi.nlm.nih.gov/snp>), and FastSNP (<http://fastsnp.ibms.sinica.edu.tw>) were used

TABLE 1: Clinical data of patients with hepatitis B-caused cirrhosis.

<i>Gender</i>	
Male (%)	218 (73.40)
Female (%)	79 (26.60)
Mean age (y)	49.15 \pm 10.28
<i>Child-Pugh-Turcotte score (%)</i>	
A	218 (75.43)
B	57 (19.72)
C	14 (4.84)
<i>Phase (%)</i>	
Compensation phase	151 (50.84)
Decompensation phase	146 (49.16)
<i>Area</i>	
Shanghai	180 (60.61)
Guangxi	69 (23.23)
Henan	48 (16.16)

for single nucleotide polymorphism selection. Twenty-four SNP loci in 7 genes of the CYP450 superfamily were selected including 4 of the *CYP1A2* gene, 6 of the *CYP2A6* gene, 4 of the *CYP2C9* gene, 4 of the *CYP2C19* gene, 2 of the *CYP2D6* gene, 2 of the *CYP2E1* gene, and 2 of the *CYP3A4* gene (Table 2).

2.4. DNA Extraction. Blood specimens of every participant were taken in potassium EDTA (K_2EDTA) vials. Chromosomal DNA was from 1 ml peripheral blood from single specimen, with the help of TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) chromosomal DNA from 1 ml peripheral blood of each specimen. Afterwards, DNA was kept at -80°C for further processing.

2.5. SNP Genotyping. By using ABI PRISM[®] SNaPshot[™] Multiplex Kit (Applied Biosystems, USA) and ABI 3730 XL DNA Analyzer (Applied Biosystems, USA), genotyping of single nucleotide polymorphisms was done. All procedures were done as earlier [16–18]. Primer sequences (Table 2) were chosen to amplify the DNA. Firstly, multiplex PCR was performed. Samples processed by multiplex polymerase chain reaction were examined/analyzed using two percent agarose-TBE gels for qualitative analysis and to check the yield. Secondly, other polymerase chain reaction products were processed with 5 U and 2 U shrimp alkaline phosphatase (rSAP) as well as exonuclease I, respectively, in order to exclude extra deoxyribonucleotide triphosphates. Thirdly, SNaPshot was done with the help of ABI PRISM[®] SNaPshot[™] Multiplex Kit. Experiment was done with volume of 10 μl consisting of 5 μl SNaPshot Multiplex Ready Reaction Mix, 3 μl rSAP/exonuclease-treated multiplex polymerase chain reaction samples, and 1 μl of probe mix (Table 3). Extension processes were done in a PCR machine consist of 45 rounds for 20 s denaturing at 96°C , 5 s annealing at 50°C , and then 30 s elongation at 60°C . 10 μl samples were processed with rSAP (1 U/sample) for 60 min at 37°C and then 15 min inactivation at 75°C . 0.5 μl extension product that was diluted was added to 8.6 μl of HiDi[™]

TABLE 2: Gene position, polymorphism, and primer sequences of CYP450 SNPs.

Gene position	RS number	Polymorphism	Primer sequence 5'-3'	Gene frequencies (%)
CYP1A2-C558A		C/A	F: 5'-CAGAATGCCCTCAACACCTT-3' R: 5'-CACTGACACCACCACCTGAT-3'	Low
CYP1A2-C733A	rs762551	C/A	F: 5'-CTACTCCAGCCCCAGAAGTG-3' R: 5'-CTGATGCGTGTCTGTGCTT-3'	62.06
CYP1A2-G2964A	rs2069514	G/A	F: 5'-AACACAACGGGACTTCTTGG-3' R: 5'-GGCATGACAATTGCTTGAAT-3'	41.77
CYP1A2-5347T>C	rs2470890	T/C	F: 5'-ATCTACGGGCTGACCATGAA-3' R: 5'-CTTGGCCTCCTAAAATGCTG-3'	14.29
CYP2A6-383G>A	rs4986891	G/A	F: 5'-CCCACCCTACTCCCTCTCTC-3' R: 5'-GTCCCCTGCTCACCGCCA-3'	Low
CYP2A6-5065G>A	rs28399454	A/G	F: 5'-TTCCTGCTCTGAGACCCCT-3' R: 5'-GAAACTTGGTGTCCCTTTTGACT-3'	Low
CYP2A6-6558T>C	rs5031016	C/T	F: 5'-GAACTTCCGCCTCAAGTCCT-3' R: 5'-GTCTTGGCCCTGCCCTTT-3'	Low
CYP2A6-1436G>T	rs5031017	G/T	F: 5'-GAACTTCCGCCTCAAGTCCT-3' R: 5'-GTCTTGGCCCTGCCCTTT-3'	Low
CYP2A6-6600G>T	rs28399468	G/T	F: 5'-GAACTTCCGCCTCAAGTCCT-3' R: 5'-GTCTTGGCCCTGCCCTTT-3'	Low
CYP2A6-479T>A	rs1801272	A/T	F: 5'-GAACTTCCGCCTCAAGTCCT-3' R: 5'-GTCTTGGCCCTGCCCTTT-3'	Low
CYP2C9-1003C>T	rs28371685	C/T	F: 5'-GCCATTTTCTCCTTTTCCA-3' R: 5'-GATACTATGAATTTGGGGACTTCG-3'	Low
CYP2C9-A1075C	rs1057910	A/C	F: 5'-GCCATTTTCTCCTTTTCCA-3' R: 5'-GATACTATGAATTTGGGGACTTCG-3'	4.61
CYP2C9-449G>A	rs7900194	A/G	F: 5'-GGGAGGATGGAAAACAGAGA-3' R: 5'-TAAGGTCAAGTATGATATGGAGTAGGG-3'	Low
CYP2C9-3276T>C		C/T	F: 5'-ATTTTGGCCTGAAACCCATA-3' R: 5'-GCACATGCACACCTACCAAA-3'	Low
CYP2C19-G681A	rs4244285	A/G	F: 5'-CAACCAGAGCTTGGCATATTG-3' R: 5'-TAAAGTCCCAGGGTTGTTG-3'	41.37
CYP2C19-G636A	rs4986893	A/G	F: 5'-AAATTGTTTCCAATCATTTAGCT-3' R: 5'-ACTTCAGGGCTTGGTCAATA-3'	4.55
CYP2C19-C1297T	rs56337013	C/T	F: 5'-ACTCATCCCTCCTATGATTCACC-3' R: 5'-TGTCAAGGTCCTTTGGGTCA-3'	Low
CYP2C19-A991G	rs3758581	A/G	F: 5'-ATGATGTTTGGATACCTTCATCAT-3' R: 5'-GAGGAATAAAAGAACATGGAGTTG-3'	5.10
CYP2D6-C188T	rs1065852	C/T	F: 5'-CCATTTGGTAGTGAGGCAGGT-3' R: 5'-CCTGGTCAAGCAGTATGGT-3'	85.14
CYP2D6-G4268C	rs1135840	C/G	F: 5'-AGCTTCTCGGTGCCCACT-3' R: 5'-CTGAGGAGGATGATCCCAAC-3'	40.90
CYP2E1-G1168A		A/G	F: 5'-ACTTCTAGCCACGGGTCTCC-3' R: 5'-GACTCACCCCTGTCCCTGT-3'	Low
CYP2E1-G10059A	rs55897648	A/G	F: 5'-CCAGATGAAAGCCCACATTT-3' R: 5'-CTGCTCCTCAAGGGAAGGTA-3'	Low
CYP3A4-T878C	rs28371759	C/T	F: 5'-TGAAACCACCCCAAGTGTAC-3' R: 5'-CCCTCCTTCTCCATGTACCA-3'	Low

TABLE 2: Continued.

Gene position	RS number	Polymorphism	Primer sequence 5' -3'	Gene frequencies (%)
CYP3A4-A13989G	rs55951658	C/T	F: 5'-CAGTGGACTACCCCTTGGAA-3' R: 5'-GCATCTAGCATAGGGCCCAT-3'	Low

TABLE 3: SNaPshot probes for CYP450 detection.

Gene position	Probe sequence (5'→3')	Size (bp)	Probes of type
CYP1A2-C558A	TTTTTTTTTTTTTTTTTTCACCACCTGATTGTAAGGGTC	37	G/T
CYP1A2-C733A	TTTTTTTTTTTTTTTTTAGGGTGAGCTCTGTGGGC	33	A/C
CYP1A2-G2964A	CGCAACCTCCGCCTCTC	17	A/G
CYP1A2-5347T>C	TTTCAGAATGGTGGTGTCTTCTTCA	25	A/G
CYP2A6-383G>A	TGGCGATGGAGAAGCGC	17	C/T
CYP2A6-5065G>A	CGAGATCCAAAGATTGGAGAC	22	A/G
CYP2A6-6558T>C	TTTTTTTTCCTCCAGTCACCTAAGGACA	28	C/T
CYP2A6-1436G>T	TTTTTTTTTTTTTTTTTTGTCCCCAAACACGTGG	36	G/T
CYP2A6-6600G>T	TTTTTTTTTTTTTTTTTTTCAGGAAGCTCATGGTGTAGTTT	40	A/C
CYP2A6-479T>A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTTCTCATCGACGCC	46	A/T
CYP2C9-1003C>T	AACGTGTGATTGGCAGAAAC	20	C/T
CYP2C9-A1075C	TTTTTTTTTTTTTTTTTTTTTTTGCACGAGGTCCAGAGATAC	41	A/C
CYP2C9-449G>A	CGTGTTCAGAGGAAGCCC	19	A/G
CYP2C9-3276T>C	AAGGAAGCCCTGATTGATCT	20	C/T
CYP2C19-G681A	CCCACTATCATTGATTATTTCCC	23	A/G
CYP2C19-G636A	TTTTTTTTTAAACTTGGCCTTACCTGGAT	30	C/T
CYP2C19-C1297T	TTTTTTTTTTTTTTTTTCCCTCTCCACACAAATCC	34	A/G
CYP2C19-A991G	TTTTTTTTTTTTTTTTTTTTTTTGGCTCCGGTTCTTGCCAA	38	C/T
CYP2D6-C188T	GCTGGGCTGCACGCTAC	17	C/T
CYP2D6-G4268C	TTTTTTTTTTTTTTTTTTTTTTGTCTTTGCTTTCCTGGTGA	40	C/G
CYP2E1-G1168A	GTACGTGGGCTCGCAGC	17	A/G
CYP2E1-G10059A	TTGTTTCTCCTAGGGCACAGTC	22	A/G
CYP3A4-T878C	TTTTTTTTTTCCTTTCAGCTCTGTCCGATC	30	C/T
CYP3A4-A13989G	TTTTTTTTTTTTTTGTGGGATTTATGAAAAGTGCC	34	A/G

formamide, 0.9 μ l Genescan-120 LIZ size. Denaturation was done at 95°C for 5 minutes prior to cooling for 4 min; after that, separation was done by ABI PRISM 3730 XL Genetic Analyzer. The GeneMapper 4.0 tool (Applied Biosystems, USA) was applied for evaluation.

2.6. Statistical Analysis. From the dataset, it was evaluated that occurrence of genetic makeup followed the Hardy-Weinberg equilibrium between calculated and already predicted genotype scores through SNaPshot analysis. Genotypic and allelic frequency between groups and comparison between genotypes and phenotypes in terms of correlation were done through the X^2 test. $P < 0.05$ was regarded statistically significant for all analysis.

3. Results

3.1. Features of Study Population. Frequencies of 24 CYP 450 gene loci were assessed in 297 individuals that suffered from

hepatitis B-caused cirrhosis and 120 nondiseased volunteers as controls in China. HWE analysis evaluated nonsignificant difference for distribution of analyzed genotypes when compared with predicted distribution ($P > 0.05$). Age range was 18-65 years (mean \pm SD, 49.15 \pm 10.28, Table 1) of the research participants. Furthermore, age and gender of gene polymorphisms were not significantly different in our study population ($P > 0.05$).

3.2. Genotype and Allele of CYP450 in Patients and Healthy Controls. The SNaPshot method showed that 9 in 24 SNP loci with detectable frequencies were available for statistical analysis in volunteers that suffered from HBV infection-caused cirrhosis and controls (Table 4).

CYP1A2-G2964A and CYP1A2-T5347C showed some evidence of relevance between cases with hepatitis B-caused cirrhosis and controls (Table 4). It was showed that the AA homozygous genotype ($P = 0.048$, 95% CI: 0.160-1.016, OR = 0.403) and AG heterozygous genotype

TABLE 4: Frequency of *CYP1A2*-G2964A, *CYP1A2*-C733A, and *CYP1A2*-T5347C genotypes between patients with hepatitis B-caused cirrhosis and healthy controls.

Gene/genotype	Cases (%) (n = 297)	Control (%) (n = 120)	OR (95% CI)	P
<i>CYP1A2</i> -G2964A				
GG	133 (45.08)	66 (55.00)	1.0 (reference)	
AG	132 (44.75)	48 (40.00)	0.733 (0.471-1.141)	0.168
AA	30 (10.17)	6 (5.00)	0.403 (0.160-1.016)	0.048
<i>CYP1A2</i> -C733A				
CC	29 (9.80)	16 (13.33)	1.0 (reference)	
AC	149 (50.34)	60 (50.00)	0.730 (0.370-1.441)	0.363
AA	118 (39.86)	44 (36.67)	0.676 (0.335-1.363)	0.272
<i>CYP1A2</i> -T5347C				
TT	6 (2.02)	5 (4.39)	1.0 (reference)	
CT	49 (16.50)	46 (40.35)	1.127 (0.322-3.944)	0.852
CC	242 (81.48)	63 (55.26)	0.312 (0.092-1.057)	0.049

($P = 0.168$, 95% CI: 0.471-1.141, OR = 0.733) at *CYP1A2*-G2964A were significant differences compared to GG wild-type genotype, respectively. CT heterozygous genotype ($P = 0.852$, 95% CI: 0.322-3.944, OR = 1.127) and CC homozygous genotype ($P = 0.049$, 95% CI: 0.092-1.057, OR = 0.312) at *CYP1A2*-T5347C were compared to TT wild-type genotype, respectively. But there was no significant correlation among genotypes of *CYP1A2*-C733A and hepatitis B-caused cirrhosis and controls.

Moreover, the allele of *CYP1A2*-G2964A and *CYP1A2*-T5347C was analyzed in cases with hepatitis B-caused cirrhosis and controls. G allele at *CYP1A2*-G2964A has significant differences compared to that of A allele ($P = 0.032$, 95% CI: 1.031-2.032, OR = 1.447). T allele at *CYP1A2*-T5347C has significant differences compared to that of C allele ($P = 0.016$, 95% CI: 0.404-0.912, OR = 0.607). However, there was no significant correlation between A allele at *CYP1A2*-G2964A and C allele at *CYP1A2*-T5347C and hepatitis B-caused cirrhosis and controls, respectively (Table 5).

3.3. Correlation between the Genotypes of *CYP1A2* and Child-Pugh Classification and Compensation or Decompensation Phase in Hepatitis B-Caused Cirrhosis. Nonsignificant correlation among genotypes of *CYP1A2* and Child-Pugh classification for hepatitis B-caused cirrhosis was observed. This was evaluated; P values were 0.181 and 0.198 between genotypes of *CYP1A2*-G2964A and *CYP1A2*-C733A and Child-Pugh classification (classes A and B+C), respectively (Table 6). P value was 0.605 between genotypes of *CYP1A2*-T5347C and Child-Pugh classification (class A, B+C).

No statistically significant correlation was observed among the genetic makeup of *CYP1A2* and hepatitis B-caused cirrhosis phase. The P values were 0.496 and 0.290 between genotypes of *CYP1A2*-G2964A and *CYP1A2*-C733A and phase (compensation, decompensation phase), respectively. The P value was 0.291 between genotypes of *CYP1A2*-T5347C and phase (compensation, decompensation phase).

3.4. Correlation between the Genotypes of *CYP1A2* and TCM Syndromes in Hepatitis B-Caused Cirrhosis. The correlation between *CYP1A2*-G2964A, *CYP1A2*-C733A, and *CYP1A2*-T5347C and traditional Chinese medicine syndromes was analyzed in volunteers having hepatitis B-caused cirrhosis. It was showed that AC heterozygous and AA homozygous genotypes at *CYP1A2*-C733A were significant differences compared to CC wild-type genotype ($P = 0.039$) between the “damp abundance due to spleen asthenia syndrome” and other traditional Chinese medicine syndromes. But no significance was observed in the correlation among the genetic makeup of *CYP1A2*-G2964A, *CYP1A2*-T5347C, and TCM syndromes (Table 7).

4. Discussion

The CYP450 is a superfamily consisting of catalytic molecules which can speed up metabolism of xenobiotic chemicals or medicines, compounds present in the environment and endogenous compounds. The role of CYP450 enzymes in cancer was studied over the last decade [19], and the relationships between genetic polymorphism and many types of cancer are the major research, such as colorectal cancer [20, 21] and breast cancer [22, 23]. However, the correlation between CYP450 polymorphism and hepatitis B-caused cirrhosis is less studied.

Single nucleotide polymorphisms are mutations in one base pair of genomic DNA that can be stably inherited in most human populations and has no difference in the various tissues of human. Hence, we can obtain the polymorphism information from expediently acquired materials such as peripheral blood, saliva, and hair for clinical diagnosis. In this study, peripheral blood specimens were obtained from 297 individuals suffering from hepatitis B-caused cirrhosis as well as 120 healthy controls.

According to the literature and SNP-related database, 24 loci of 7 genes were screened. It showed that 15 loci of them are low frequencies. Finally, 3 SNP loci of *CYP1A2* gene (*CYP1A2*-G2964A, *CYP1A2*-C733A, and *CYP1A2*-T5347C)

TABLE 5: Frequency of *CYP1A2*-G2964A, *CYP1A2*-C733A, and *CYP1A2*-T5347C alleles between patients with hepatitis B-caused cirrhosis and healthy controls.

Gene/allele	Cases (%) (n = 297)	Control (%) (n = 120)	OR (95% CI)	P
<i>CYP1A2</i> -G2964A				
A	192 (32.54)	60 (25.00)	1.447 (1.031-2.032)	0.032
G	398 (67.46)	180 (75.00)		
<i>CYP1A2</i> -C733A				
A	385 (65.03)	148 (61.67)	1.156 (0.848-1.577)	0.359
C	207 (34.97)	92 (38.33)		
<i>CYP1A2</i> -T5347C				
C	514 (87.71)	195 (81.25)	0.607 (0.404-0.912)	0.016
T	72 (12.29)	45 (18.75)		

TABLE 6: Correlation between *CYP1A2* and Child-Pugh classification and phase in patients with hepatitis B-caused cirrhosis.

Gene/genotype	Child classification			P	Phase		P
	Class A (%) (n = 218)	Class B (%) (n = 57)	Class C (%) (n = 14)		Compensation (n = 151)	Decompensation (n = 146)	
<i>CYP1A2</i> -G2964A							
GG	101 (46.76)	21 (36.84)	8 (57.14)	0.181	71 (47.02)	62 (42.47)	0.496
AG	93 (43.06)	31 (54.39)	3 (21.43)		67 (44.37)	65 (44.52)	
AA	22 (10.19)	5 (8.77)	3 (21.43)		13 (8.61)	17 (11.64)	
<i>CYP1A2</i> -C733A							
CC	19 (8.76)	6 (10.53)	4 (28.57)	0.198	11 (7.28)	18 (12.33)	0.290
AC	110 (50.69)	30 (52.63)	5 (35.71)		76 (50.33)	73 (50.00)	
AA	88 (40.55)	21 (36.84)	5 (35.71)		64 (42.38)	54 (36.99)	
<i>CYP1A2</i> -T5347C							
TT	4 (1.83)	2 (3.51)	0 (0)	0.605*	5 (3.31)	1 (0.68)	0.291 [▲]
CT	37 (16.97)	6 (10.53)	3 (21.43)		26 (17.22)	23 (15.75)	
CC	177 (81.19)	49 (85.96)	11 (78.57)		120 (82.19)	122 (83.56)	

*Fisher's exact test between class A and class B+C. [▲]Fisher's exact test.

TABLE 7: Correlation between *CYP1A2*-G2964A, *CYP1A2*-C733A, and *CYP1A2*-T5347C and TCM syndromes in patients with hepatitis B-caused cirrhosis.

TCM syndrome type	<i>CYP1A2</i> -G2964A		P	<i>CYP1A2</i> -C733A		P	<i>CYP1A2</i> -T5347C		P
	GG	AG+AA		CC	AC+AA		TT	CT+CC	
Liver-qi stagnation syndrome	29	32	0.759	5	61	0.566	2	59	0.630*
Damp abundance due to spleen asthenia syndrome	21	21	0.575	8	30	0.039	0	46	
Damp-heat syndrome	29	40	0.613	7	62	0.944	1	69	1.000*
Liver-kidney yin deficiency syndrome	17	24	0.636	2	39	0.399*	0	42	
Blood stasis syndrome	31	37	0.977	6	62	0.794	3	60	0.200*
Yang deficiency of spleen and kidney syndrome	6	6	0.753	1	11	1.000*	0	12	
Total	133	160		29	265		6	288	

*Fisher's exact test.

were used for analysis. It was illustrated that *CYP1A2*-G2964A and *CYP1A2*-T5347C are correlated to varying degrees of the risk levels of hepatitis B-caused cirrhosis. The result also showed that the *CYP1A2*-T5347C and *CYP1A2*-G2964A have

stronger correlation with this disease (Table 4). Both the homozygous mutants in these 2 loci (AA for *CYP1A2*-G2964A and CC for *CYP1A2*-T5347C) have significant difference between cases and controls (both $P < 0.05$), whereas their

heterozygous mutants (AG for *CYP1A2*-G2964A and CT for *CYP1A2*-T5347C) between cases and controls have no significant difference, indicating that the homozygous mutants may play a more important role than heterozygous mutants in these loci in the process of hepatitis B-caused cirrhosis.

Single nucleotide polymorphism constitutes almost 90% human DNA polymorphism [24]. Less rate of mutation as well as significant arbitrary nature of variations in the bp constitutes single nucleotide polymorphism alleles well stable [25] and most (>80%) of them are usual in the entire human populations; however, allele frequencies vary [26]. In this study, the allele A frequency of *CYP1A2*-G2964A and the allele C frequency of *CYP1A2*-T5347C are both significantly correlated with hepatitis B-caused cirrhosis (both $P < 0.05$), whereas allele A frequency of *CYP1A2*-C733A has no correlation with this disease (Table 5). These results supported the above finding that there is significant correlation between the *CYP1A2*-G2964A genotype and hepatitis B-caused cirrhosis.

CYP1A2 is considered as the main member of the P450 superfamily [27]; it contributes 13% of total CYP protein of the liver [28]. *CYP1A2* action might be applied for monitoring the changes in hepatic activities in clinical work [29]. Moreover, this also performs function of metabolism of various clinical medicines, environmental toxic materials, and endogenous substrates [30]. Previous studies showed that the abundance of the *CYP1A2* *1F variant was higher in Caucasians, after its comparison with Japanese subjects [31]. On the other hand, people of Egypt had more incidence of *CYP1A2* *1F variant (0.68) than people of Japan (0.61). We analyzed the genotype distribution and allele frequency of 120 healthy people in our study, the presence of *CYP1A2* G2964A allele showed similarity when compared with individuals of Japan (G: 0.75 vs. 0.77; A: 0.25 vs. 0.23) [32], and the incidence of *CYP1A2* C2964A allele was the same when comparison was done with Germans (C: 0.62 vs. 0.68; A: 0.38 vs. 0.32) [33]. The frequency of A allele was similar compared with Qidong and Changsha in China (0.25 vs. 0.25 vs. 0.22) [34].

The TCM syndrome type is a consequence of disease classification. According to TCM theory, the patients of the same disease can be classified into different syndrome types. There is significant correlation between *CYP1A2*-C733A mutation genotypes and the TCM syndrome “damp abundance due to spleen asthenia syndrome.” It can be perceived for the classification of traditional Chinese medicine syndrome type in hepatitis B-resulted cirrhosis and may be helpful in the clinical diagnosis of TCM.

5. Conclusion

The current study evaluated the relationship between CYP450 SNPs and hepatitis B-caused cirrhosis. The results suggested that there is a correlation between AA genotype at *CYP1A2*-G2964A and CC genotype at *CYP1A2*-T5347C and hepatitis B-caused cirrhosis. Moreover, there is a correlation between AC plus AA genotype at *CYP1A2*-C733A and the “damp abundance due to spleen asthenia syndrome” of TCM.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qing-Ya Li collected samples and clinical data and performed the research and drafted the manuscript; Xiaona Yang and Zhi-Zhong Guo revised the manuscript.

Acknowledgments

This paper is a phased research result of the project of “Henan Provincial Scientific Research Project of TCM (2013ZY02061).” We thank Longhua Hospital, Shuguang Hospital, Putuo Hospital, and Yueyang Hospital of Shanghai University of TCM, Ruikang Hospital and the First Affiliated Hospital of Guangxi University of TCM, and the First Affiliated Hospital of Henan University of TCM, respectively.

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