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Correlations between polymorphisms in the uridine diphosphate-glucuronosyltransferase 1A and C-C motif chemokine receptor 5 genes and infection with the hepatitis B virus in three ethnic groups in China

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Abstract

Objective: To determine whether genetic polymorphisms in the uridine diphosphate-glucuronosyltransferase 1A (*UGT1A*) and the C-C motif chemokine receptor 5 (*CCR5*) genes are associated with hepatitis B virus (HBV) infection in Yi, Yao and Han ethnic groups in the Guizhou Province of China.

Methods: The study enrolled subjects with and without HBV infection. Whole blood was used for DNA genotyping using standard techniques. The study determined the frequencies of several polymorphic alleles (*UGT1A6* [rs2070959], *UGT1A1* [rs8175347], *CCR5-59029* [rs1799987] and *CCR5Δ32* [rs333]) and then characterized their relationship with HBV infection.

Results: A total of 404 subjects were enrolled in the study: 138 from the Yao group, 101 from the Yi group and 165 from the Han group. There was a significant difference in the frequency of *UGT1A1* rs8175347 polymorphisms among the three groups. The rates of 7TA carriers of

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UGT1A1 rs8175347 in all three groups were significantly higher than the other genotypes. Individuals with genotype AA of *UGT1A6* rs2070959 in the Yi group had a higher risk for HBV infection than in the Yao and Han groups. The frequency of genotype GG in *CCR5-59029* in the Yao group was significantly higher than in the Yi group. The genotypes of *CCR5Δ32* were not associated with HBV infection.

Conclusion: These findings provide genetic and epidemiological evidence for an association of *UGT1A* and *CCR5-59029* polymorphisms with HBV infection in Chinese Yi and Yao populations.

Keywords

C-C motif chemokine receptor 5 (CCR5), ethnic minorities, gene polymorphisms, hepatitis B virus infection, uridine diphosphate-glucuronosyltransferase 1A (UGT-1A)

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Introduction

Approximately one-third of all human beings are infected with hepatitis B virus (HBV), the most serious hepatitis virus that is potentially life-threatening;[1] with 6% being chronic carriers and more than 600 000 dying each year from acute or chronic sequelae such as liver cirrhosis and hepatocellular carcinoma.[2,3] There is considerable geographic variation in the distribution of HBV and the areas with a high rate of infection are present, especially in Asia and Africa, where the most common routes of infection are still vertical transmission from mother to child and horizontal transmission between children.[4–6]

It has become clear that replication of HBV is the primary promoter of immune-mediated liver injury and disease progression.[7] This virus does not damage the liver directly, but rather an antibody-mediated immune reaction and non-specific inflammation leading to cytokine-mediated hepatocyte injury are triggered by the viral antigens.[8] Most Asian carriers of HBV are infected either during the perinatal period or in early childhood.[9] While most infected adults recover and develop protective antibodies, approximately 10–15% remain chronically infected and are thus at risk for developing severe hepatic pathology.[10]

Although not yet fully understood, the mechanisms of persistent HBV infection appear to involve both immunological and genetic components.[11,12] Host (e.g. age at the time of infection, sex, ethnicity, immune status), viral and external factors are all of importance in this connection.[13,14] Notably, genetic variation exerts an impact on susceptibility to persistent HBV infection and the associated development of hepatocellular carcinoma.[5,6,15,16]

The superfamily of uridine diphosphate-glucuronosyltransferases (UGTs) consists of cytosolic glucuronosyltransferases that catalyse the transfer of the glucuronic acid component of uridine diphosphate (UDP)-glucuronic acid to a small hydrophobic molecule.[17] A variety of genetic polymorphisms in these UGTs have been described.[18] UGT-1A6 protein is produced in a number of tissues, including the liver, bile ducts, colon, stomach and brain, and catalyses the glucuronidation of small, planar phenols and primary amines, including acetaminophen, β-blockers and salicylates.[19,20] Heterozygous expression of the *UGT1A6*2* gene enhances enzyme activity in both human liver tissue and cultured lever cells.[21] Among Caucasians, approximately 10% are heterozygous and 10% homozygous with respect to the UGT-1A6*2 forms of this protein.[18,22]

Moreover, among Taiwanese subjects, there is a relationship between the detoxifying activity of UGT-1A7 and hepatocellular carcinoma.[23] However, other host factors that may influence the outcome of persistent HBV infection remain elusive.

Individuals that develop a broad immunological response to HBV involving T cells are more likely to recover.[24] Chemokines attract and recruit specific populations of immune cells to the sites of injury or infection.[25] The C-C motif chemokine receptor 5 (CCR5), which is produced by granulocytes, macrophages, immature dendritic cells, CD8+ lymphocytes and type 1 helper T (Th_1) cells, influences the migration and activation of these same cells.[26] In mice, CCR5 deficiency is associated with a more potent T cell response to several infectious agents.[27,28] Moreover, *CCR5Δ32*, a promoter polymorphism, might play a role in eliminating HBV infection.[27,29]

In Guizhou Province in China, there are 49 minority ethnic groups. Among them, 17 typical ethnic groups live in mountainous areas of the province, and have little contact with each other or the outside world due to transportation difficulties and the customs of their own cultures, languages and marriages, which results in relatively isolated genetic populations.[30] This current study determined the frequencies of several polymorphic alleles (*UGT1A6* [rs2070959], *UGT1A1* [rs8175347], *CCR5-59029* [rs1799987] and *CCR5Δ32* [rs333]); and then characterized their relationship with HBV infection in subjects from two ethnic minority groups (Yi and Yao groups) compared with a Han group (the majority of Chinese) from Guizhou Province.

Subjects and methods

Study subjects

This study enrolled subjects from: (i) the minority Yao group living in Yaoshan village of Libo county; (ii) the minority Yi group living in Longjie village of Weining county; and (iii) the Han majority ethnic group living in Changchunpu village of Bijie county. The study was undertaken in the Key Laboratory of Endemic and Ethnic Diseases of the Ministry of Education of PR China (Guizhou Medical University), Guiyang, Guizhou Province, China between May 2012 and September 2014. All three counties are in Guizhou Province, China and are characterized by poor healthcare, where the populations have not been vaccinated against the HBV. These subjects were identified as infected or non-infected by determining serum levels of hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs) and hepatitis B core antibody (anti-HBc) by enzyme-linked immunosorbent assays (China Bio-Technology, Shanghai, China). Infected individuals exhibited repeated seropositivity for one or more of these over a period of 6 months. None of the subjects were infected with other types of viral hepatitis or HIV.

All study subjects provided written informed consent to participate and ethical permission for this study was granted by the Ethical Committee of Guizhou Medical University, Guiyang, Guizhou Province, China (no. 2012-12038).

DNA extraction and genotyping

Genomic DNA was isolated from whole blood drawn from a vein in the elbow region (3 ml) using a kit based on extraction with phenol-chloroform and then redissolved in 10 mM tris-ethylenediaminetetraacetic acid buffer (pH 8.0) as described previously (Shanghai Shenbo Chemical, Shanghai, China).[31] On the same day, the blood samples were extracted for DNA isolation at room temperature and the DNA was stored at –20°C. Before isolation, the blood samples were kept at 4°C.

The sequences of the primers (Shanghai Shenbo Chemical) employed for polymerase chain reaction (PCR) genotyping are documented in Table 1.

In the case of *UGT1A6* rs2070959 A and G, two alleles of a single nucleotide polymorphism (SNP), were characterized by using confronting two-pair primers (PCR-CTPP), specific for each allele. In primer pair 1, the base at the 3′-end of the reverse primer was T and, therefore, only the A allele was amplified; while the forward primer was upstream. With primer pair 2, the base at the 3′-end of the forward primer was C and, therefore, only the G allele was amplified; and the reverse primer was downstream. All four of these primers were added to each tube, and the AA genotype resulted in products of 145 and 300 base pairs (bp) in length; and the GG genotype gave 200 and 300 bp products; and heterozygotes showed all three of these products. The cycling programme involved preliminary denaturation at 95°C for 10 min, followed by 14 cycles of denaturation at 95°C for 30 s, annealing at 66°C for 45 s, and elongation at 72°C for 30 s for each cycle; and then followed by 18 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 45 s, and elongation at 72°C for 30 s; and a final extension step at 72°C for 7 min. The resulting PCR products were separated by electrophoresis on a 1.5% agarose gel.

The PCR reaction mix consisted of 25 mmol/l $MgCl_2$, 2.5 mmol/l dNTPs for each, 10.0 μmol/l upstream or downstream primer for each (Table 1), 2.5 U/μl *Taq* DNA Polymerase and 100 ng DNA in a total volume of 25 μl diluted with 1X PCR buffer (Shanghai Shenbo Chemical). This reaction mixture was employed for all PCRs. The PCR reaction was performed using an Applied Biosystems® Veriti® 96-Well Thermal Cycler (Thermo Fisher Scientific, Rockford, IL, USA). PCR products were separated on 2.5% agarose gels

Table 1. Sequences of the primers used for genotyping in this study that investigated the relationship between polymorphisms in the C-C motif chemokine receptor 5 (*CCR5*) and uridine diphosphate- glucuronosyltransferase 1A (*UGT1A*) genes and hepatitis B virus infection in two ethnic minority Chinese groups compared with a Han Chinese group.

Gene and loci	Upstream primer	Downstream primer	Product length, base pairs
UGTA1 rs8175347	5′-CTACATAGTCGTCCTTCTTC-3′	5′-ACAGTATCTTCCCAGCAT-3′	433
UGT1A6 rs2070959	a:5′-TAAGGAGAGCAAGTTTGATGCTC-3	5′-GTCTGGGCTTCTGCTGATTGT-3′	145
	b:5′-GGTTTTCCGTGTTCCCTGGAGGATG-3	5′-CTCTTGAGGACAGCTGATGC-3′	200
	c:5′-TAAGGAGAGCAAGTTTGATGCTC-3′	5′-CTCTTGAGGACAGCTGATGC-3′	300
CCR5-59029 rs1799987	5′-GAAGAATCCTGCCACCTAT-3′	5′-CTCTGCTCATCCCCACTACA-3′	559
CCR5△*32* rs333	5′-AGGTCTTCATTACACCTGCAGC-3′	5′-CTTCTCATTTCGACACCGAAGC-3′	169 (wild-type)
			137 (mutant)

and visualized using ethidium bromide staining and UV light.

The *UGT1A1* rs8175347 polymorphism (Table 1), a micro-satellite containing a thymine-adenine (TA) repeat, was detected by short tandem repeats PCR on a sequencing instrument (ABI PRISM® 310 Genetic Analyzer; Thermo Fisher Scientific). The upstream 5′-end was labelled with a 6-carboxyfluorescein fluorescent tag (Shanghai GeneCore BioTechnologies, Shanghai, China). The cycling programme involved preliminary denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 61.3°C for 30 s, and elongation at 72°C for 30 s, followed by a final elongation step at 72°C for 7 min.

The *CCR5-59029* rs1799987 polymorphism (Table 1) was identified using an improved protocol for PCR-restriction fragment length polymorphism, including an internal control for incomplete digestion by restriction enzymes following preincubation at 95°C for 5 min. Hot start PCR was run for 30 cycles of denaturation at 95°C for 15 s, annealing at 63.3°C for 30 s, and elongation at 72°C for 30 s. Digestion of the 559-bp product with Bsp1286 I (Promega, Madison, WI, USA) resulted in two (405 and 154 bp) and three (278, 154 and 127 bp) fragments from the A and G alleles, respectively, which were resolved by electrophoresis on a 2.5% agarose gel.

For *CCR5Δ32* (rs333) polymorphism genotyping (Table 1), PCR amplification of genomic DNA was used.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Each SNP/deletion was assessed for association by using χ^2-test with one or two degrees of freedom in different minority groups. All tests for significance were two-sided and a P-value < 0.05 considered statistically significant. Hardy–Weinberg equilibrium was performed for each locus.

Results

A total of 404 subjects living in Guizhou Province, China were enrolled in the study: 138 subjects from the minority Yao group living in Libo county; 101 from the minority Yi group living in Weining county; and 165 from the Han majority group living in Bijie county. The demographic and clinical characteristics of the three ethnic groups are presented in Table 2.

The distributions of the *UGT1A1*, *UGT1A6* and *CCR5-59029* genotypes in the three groups conformed to Hardy–Weinberg equilibrium. The frequency of the *UGT1A1* rs8175347 genotypes in the three groups are shown in Table 3. There was a significantly different frequency of *UGT1A1* rs8175347 among the Yi, Yao and Han groups ($P < 0.01$). Specifically, there was a significant difference in the frequency of 6TA among the three ethnic groups ($P < 0.05$), in which the highest frequency of 6TA was in the Yi group; and there was a significant difference in the frequency of 8TA between the Yi and Han groups ($P < 0.05$), in which the Han group had the highest frequency.

As shown in Table 4, the frequency of 7TA carriers of *UGT1A1* rs8175347 were significantly higher than those of other genotypes in both subjects with and without HBV infection from the Yi, Yao and Han groups ($P < 0.01$). The Yi carriers of 8TA exhibited a significantly lower risk of being HBV-infected than those with this same genotype in the other two groups ($P < 0.01$).

The individuals with genotype AA of the *UGT1A6* rs2070959 polymorphism in the Yi group had a higher risk for HBV infection than in the Yao and Han groups and

Table 2. Clinical and demographic characteristics of the three Chinese ethnic groups enrolled in this study that investigated the relationship between polymorphisms in the C-C motif chemokine receptor 5 (*CCR5*) and uridine diphosphate- glucuronosyltransferase 1A (*UGT1A*) genes and hepatitis B virus (HBV) infection.

Characteristic	Yi Group $n = 101$	Yao Group $n = 138$	Han Group $n = 165$
Age, years	46.39 ± 13.91	56.00 ± 13.20	44.72 ± 16.75
Sex			
Male	50 (49.5)	55 (39.9)	66 (40.0)
Female	51 (50.5)	83 (60.1)	99 (60.0)
Infected with HBV[a]	53 (52.5)	80 (58.0)	130 (78.8)
Not infected with HBV[b]	48 (47.5)	58 (42.0)	35 (21.2)

Data presented as mean $\pm$ SD of *n* of subjects (%).
[a]Repeated seropositivity for hepatitis B core antibody (anti-HBc), hepatitis B surface antibody (anti-HBs) and/or hepatitis B surface antigen (HBsAg) over a 6-month period.
[b]Seronegativity for anti-HBc, anti-HBs or HBsAg.

Table 3. The frequency of the *UGTA1* rs8175347 genotypes among the three Chinese ethnic groups who were enrolled in this study that investigated the relationship between polymorphisms in the C-C motif chemokine receptor 5 (*CCR5*) and uridine diphosphate- glucuronosyltransferase 1A (*UGT1A*) genes and hepatitis B virus infection.

Length of product (genotype)	Yi Group $n = 101$	Yao Group $n = 138$	Han Group $n = 165$	Statistical significance[a]
431 bp (6TA)[b]	33 (16.3)	28 (10.1)	26 (7.9)	$\chi^2 = 17.56$
433 bp (7TA)	160 (79.2)	231 (83.7)	267 (80.9)	$P = 0.002$
435 bp (8TA)[c]	9 (4.5)	17 (6.2)	37 (11.2)	

Data presented as *n* of genes (%).
[a]The statistical analysis ($\chi^2 = 17.56$ and $P = 0.002$) indicated a significant difference in the frequency of *UGTA1* rs8175347 among these three groups.
[b]$P < 0.05$, significant differences in the frequency of 6TA among the three ethnic groups; χ^2-test.
[c]$P < 0.05$, significant difference in the frequency of 8TA between the Yi and Han groups; χ^2-test.
bp, base pairs.

also constituted a relatively larger proportion with infection as compared with the uninfected subjects in the same group (Table 5). In the Yao and Han groups, the genotype AA of the *UGT1A6* rs2070959 polymorphism was overrepresented in the subjects with HBV infection as compared with the uninfected subjects.

As shown in Table 6, the frequency of genotype GG in the *CCR5-59029* polymorphism in the Yao group was significantly higher than in the Yi and Han groups ($P < 0.01$), but lower in the subjects infected with HBV as compared with uninfected subjects. The frequencies of the GA genotype in Han group were higher than the other two genotypes, and in the Yi and Yao groups the frequency of the GA genotype was higher in the infected subjects than the uninfected subjects. The frequency of the AA genotype in the subjects in the Yi and Yao groups with HBV infection were higher than those of the uninfected subjects in the same groups, but there was no significant difference between the infected and uninfected subjects.

Table 4. The frequency of the *UGTA1* rs8175347 genotypes among the three Chinese ethnic groups stratified according to hepatitis B virus (HBV) infection status.

Ethnic groups	Genotype	HBV infected	HBV uninfected	Statistical significance		Odds ratio (95% CI)
Yi*		$n = 53$	$n = 48$	$\chi^2 = 4.974$ [a]NS		
	431 bp (6TA)	21 (19.8)	12 (12.5)	$\chi^2 = 1.970$ [b]NS		1.585 (0.825, 3.046)
	433 bp (7TA)	83 (78.3)	77 (80.2)	$\chi^2 = 0.111$ [b]NS		
	435 bp (8TA)	2 (1.9)	7 (7.3)	$\chi^2 = 3.457$ [b]NS	$\chi^2 = 16.7$ [c]$P < 0.001$	
Yao		$n = 80$	$n = 58$	$\chi^2 = 4.626$ [a]NS		
	431 bp (6TA)	11 (6.9)	17 (14.7)	$\chi^2 = 4.465$ [b]$P < 0.05$		
	433 bp (7TA)	138 (86.3)	93 (80.2)	$\chi^2 = 1.820$ [b]NS		1.215 (0.463, 3.185)
	435 bp (8TA)	11 (6.9)	6 (5.2)	$\chi^2 = 0.337$ [b]NS		
Han		$n = 130$	$n = 35$	$\chi^2 = 1.597$ [a]NS		
	431 bp (6TA)	18 (6.9)	8 (11.4)	$\chi^2 = 1.542$ [b]NS		
	433 bp (7TA)	212 (81.5)	55 (78.6)	$\chi^2 = 0.314$ [b]NS		1.038 (0.906, 1.188)
	435 bp (8TA)	30 (11.5)	7 (10.0)	$\chi^2 = 0.131$ [b]NS		

Data presented as *n* of genes (%).
[a]*P*-value from χ^2-test with two degrees of freedom.
[b]*P*-value from χ^2-test with one degree of freedom.
[c]*P*-value from χ^2-test in the same genotype between Yi and Yao groups with two degrees of freedom.
*$P < 0.01$, significant difference in the frequency of 8TA between the Yi and Yao groups; χ^2-test.
CI, confidence interval; bp, base pairs; NS, no significant difference ($P \geq 0.05$).

The pair-wise evaluation did not reveal any linkage disequilibrium with other SNPs, or that the genotypes (GG, GA or AA) of the *CCR5Δ32* polymorphism were associated with HBV infection in Chinese subjects (data not shown).

Discussions

In the present investigation, the potential relationship between the *UGT1A1* rs8175347, *UGT1A6* rs2070959, *CCR5-59029* and *CCR5Δ32* genetic polymorphisms and the clinical course of HBV infection was examined in the Yi, Yao and Han ethnic groups living in Guizhou Province, China. Although the importance of these genes in connection with various diseases is widely acknowledged, their potential role in HBV infection is seldom studied.[3,5,6]

The distributions of the *UGT1A1* rs8175347 polymorphisms 6TA, 7TA and 8TA in the Yi, Yao and Han groups

Table 5. The frequency of the *UGT1A6* rs2070959 genotypes among the three Chinese ethnic groups stratified according to hepatitis B virus (HBV) infection status.

Ethnic groups	Genotype	HBV infected	HBV uninfected	Statistical significance		Odds ratio (95% CI)
Yi*		$n = 53$	$n = 48$	$\chi^2 = 2.764$ [a]NS		
	GG	1 (1.9)	3 (6.3)	$\chi^2 = 1.261$ [b]NS		
	AG	19 (35.8)	22 (45.8)	$\chi^2 = 1.041$ [b]NS		
	AA	33 (62.3)	23 (47.9)	χ^2=2.099 [b]NS	$\chi^2 = 6.232$ [c]$P < 0.05$	1.793 (0.811, 3.964)
Yao		$n = 80$	$n = 58$	$\chi^2 = 1.027$ [a]NS		
	GG	11 (13.8)	6 (10.3)	$\chi^2 = 0.361$ [b]NS		
	AG	32 (40.0)	28 (48.3)	$\chi^2 = 0.927$ [b]NS		
	AA	37 (46.3)	24 (41.4)	$\chi^2 = 0.323$ [b]NS		1.219 (0.616, 2.143)
Han#		$n = 130$	$n = 35$	$\chi^2 = 3.194$ [a]NS		
	GG	21 (16.2)	4 (11.4)	$\chi^2 = 1.441$ [b]NS	χ^2 = 8.67 [d]$P < 0.01$	
	AG	42 (32.3)	17 (48.6)	$\chi^2 = 3.175$ [b]*P* NS		
	AA	67 (51.5)	14 (40.0)	$\chi^2 = 1.469$ [b]NS		1.595 (0.747, 3.407)

Data presented as *n* of subjects (%).
[a]*P*-value from χ^2-test with two degrees of freedom.
[b]*P*-value from χ^2-test with one degree of freedom.
[c]*P*-value from χ^2-test in the same genotype between Yi and Yao groups with two degrees of freedom.
[d]*P*-value from χ^2-test in the same genotype between Han and Yi groups with two degrees of freedom.
*$P < 0.05$, significant difference between the Yi and Yao groups; χ^2-test.
#$P < 0.05$, significant difference between the Han and Yi groups; χ^2-test.
CI, confidence interval; NS, no significant difference ($P \geq 0.05$).

differed significantly, indicating that these alleles exhibit both geographic and ethnic variation. In addition, occurrence of this polymorphism differed significantly, with the highest rate of 6TA being in the Yi group and the highest rate of 8TA being in the Han group.

The *UGT1A1* rs8175347 polymorphism is located in the TATA box in the promoter region of the *UGT1A1* gene, which binds the TATA-binding protein (TBP) required for precise regulation of the initiation of DNA transcription.[32] The normal TA repeat allele is 12 or 14 nucleotides long. A longer repeat lowers the binding affinity for TBP[33] and reduces both the expression and activity of *UGT1A1*, which may influence the degradation and metabolism of the viral proteins.[21] Accordingly, a normal *UGT1A1* promoter may protect against HBV infection. In this present study, the rates of 7TA carriers of *UGT1A1*

Table 6. The frequency of the *CCR5-59029* genotypes among the three Chinese ethnic groups stratified according to hepatitis B virus (HBV) infection status.

Ethnic groups	Genotype	HBV infected	HBV uninfected	Statistical significance		Odds ratio (95% CI)
Yi*		$n = 53$	$n = 48$	$\chi^2 = 4.470$ [a]NS		
	GG	16 (30.2)	22 (45.8)	$\chi^2 = 2.670$ [b]NS		
	GA	24 (45.3)	21 (43.8)	$\chi^2 = 0.024$ [b]NS	$\chi^2 = 9.59$ [c]$P = 0.01$	1.064 (0.485, 2.335)
	AA	13 (24.5)	5 (10.4)	$\chi^2 = 3.425$ [b]NS		2.795 (0.914, 8.546)
Yao		$n = 80$	$n = 58$	$\chi^2 = 6.450$ [a]$P < 0.05$		
	GG	38 (47.5)	40 (69.0)	$\chi^2 = 6.300$ [b]$P < 0.05$		
	GA	33 (41.3)	15 (25.9)	$\chi^2 = 3.510$ [b]NS		2.013 (0.963, 4.207)
	AA	9 (11.3)	3 (5.2)	$\chi^2 = 1.546$ [b]NS		2.324 (0.601, 8.994)
Han#		$n = 130$	$n = 35$	$\chi^2 = 1.100$ [a]NS		
	GG	45 (34.6)	9 (25.7)	$\chi^2 = 0.880$ [b]NS	$\chi^2 = 20.41$ [d]$P < 0.01$	1.529 (0.660, 3.542)
	GA	56 (43.1)	18 (51.4)	$\chi^2 = 0.778$ [b]NS		
	AA	29 (22.3)	8 (22.9)	$\chi^2 = 0.005$ [b]NS		

Data presented as *n* of subjects (%).
[a]*P*-value from χ^2-test with two degrees of freedom.
[b]*P*-value from χ^2-test with one degree of freedom.
[c]*P*-value from χ^2-test in the same genotype between Yi and Yao groups with two degrees of freedom.
[d]*P*-value from χ^2-test in the same genotype between Han and Yao groups with two degrees of freedom.
*$P < 0.01$, significant difference between the Yi and Yao groups; $\chi^2 = 9.59$; $P = 0.008$; χ^2-test.
#$P < 0.05$, significant difference in those with the same genotype between the Han and Yi groups; $\chi^2 = 20.41$; $P < 0.01$); χ^2-test.
CI, confidence interval; NS, no significant difference ($P \geq 0.05$).

rs8175347 were significantly higher than those of other genotypes in both the subjects with HBV infection and those without HVB infection from the Yi, Yao and Han groups. The Yi carriers of 8TA exhibited a significantly lower risk of being infected with HBV than those with this same genotype in the other two ethnic groups, which may indicate the important protection to HBV in this ethnic group.

Several studies have revealed associations of *UGT1A6* polymorphism with both drug metabolism[34] and colon cancer.[35–37] Indeed, rs2070959 in the *UGT1A6* gene is one of the major polymorphisms that influences the biodisposition of aspirin.[38] It has been indicated that the GG genotype can decrease *UGT1A6* activity[21] and may render individuals more susceptible to the development of breast cancer.[39] In the

present study, no significant association between the GG or AG genotypes of the *UGT1A6* rs2070959 and HBV infection was observed in the three groups. For the AA genotype, however, increased frequencies were associated with HBV infection in all three ethnic groups. Among them, the Yi group showed the highest risk for HBV infection as compared with the Yao and Han groups.

Numerous CCR5-positive cells are associated with chronic hepatitis,[40] suggesting that this CCR5 receptor may play an important role in the progression of HBV-induced pathological injury. The *CCR5Δ32* allele appears to protect against immunological diseases[41] and is also associated with recovery from HBV infection.[27] A *CCR5Δ32* mutation is disadvantageous in fighting infectious diseases, whose clearance is dependent on interferon (INF)-γ producing T lymphocytes.[42] Thus, individuals carrying at least one copy of the gene encoding a non-functional receptor (*CCR5Δ32*) are twice as likely to recover from hepatitis B, an effect that appears to be codominant. CCR5 may not regulate the immune response of T cells and can enhance the response. For example, in the concanavalin A-induced fulminant hepatitis murine model, which is a model for T cell-mediated hepatitis, CCR5 deficiency prevented apoptosis and up-regulated the function of hepatic natural killer T (NKT) cells.[43] Such effects would favour recovery from HBV infection since NKT cells play an important role in controlling HBV replication.[44] In an HBV-infected Korean cohort, 138 of the 377 individuals who spontaneously recovered carried the CCR5-32 bp deletion mutation.[45]

The occurrence of the *CCR5Δ32* mutation varies geographically and ethnically, being 10–15% in Caucasians,[46] 2–5% in the Middle East and Indian subcontinent,[47] and 20.93% in the Israeli population.[48] In China, the incidence of *CCR5Δ32* in different ethnic groups varies significantly.[49] Interestingly, where the *CCR5Δ32* mutation is relatively rare, the prevalence of HBV infection is higher; and when the incidence of *CCR5Δ32* is high, as in Europe and the United States, the prevalence of HBV is low.[47] It has been proposed that this might explain the difference between Chinese and Caucasians with respect to susceptibility to HBV infection.

The promoter variant *CCR5-59029G* might have lower activity and potentially attenuate recovery from HBV, in agreement with the protective effect of lower CCR5 levels. A significantly larger number of $CD4^+$ cells expressing CCR5 and carrying two *59029A* alleles has been detected previously.[50] The CCR5 promoter 59029 of the GG genotype may reduce the ability of this receptor to ameliorate inflammation of the liver, demonstrating that such polymorphism may influence the outcome of HBV infection.

In this current study, the *CCR5-59029* G allele was associated with resolution of HBV infection in the Yi and Yao ethnic groups. These findings were consistent with one earlier report,[29] but disagreed with another;[51] perhaps due to demographic factors and/or differences in experimental design. CCR5 is a specific marker for the Th_1-type reaction and may thus be involved in anti-viral response.[52] While inflammatory factors such as interleukin-2, INF-γ and tumour necrosis factor can potentiate inflammation of the liver,[53] the *CCR5-59029* GG genotype may play a protective role in HVB infection.

In conclusion, there was a significantly different frequency of *UGT1A1* rs8175347 among the Yi, Yao and Han groups; and the rates of 7TA carries of *UGT1A1* rs8175347 were significantly higher than those of the other genotypes. Individuals with genotype AA of *UGT1A6* rs2070959 in the Yi group had a higher risk for HBV infection. The frequency of genotype GG in

CCR5-59029 in the Yao group was significantly higher than in the Yi group. The mutant *CCR5Δ32* allele was not associated with HBV infection. These findings may provide genetic and epidemiological evidence for a possible association of the *UGT1A* and *CCR5-59029* polymorphisms with HBV infection in Chinese Yi and Yao populations.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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