GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 16, Number 9, 2012

Pp. 1115–1121
DOI: 10.1089/gtmb.2012.0148

Single-Nucleotide Polymorphism at *CYP27B1*-1260, but Not *VDR Taq* I, Is Possibly Associated with Persistent Hepatitis B Virus Infection

Qianqian Zhu,[1] Na Li,[1] Qunying Han,[1] Zhu Li,[1] Guoyu Zhang,[1] Fang Li,[1] Pingping Zhang,[1] Jinghong Chen,[2] Yi Lv,[3,4] and Zhengwen Liu[1,4]

Background: Vitamin D, beyond its role in calcium and bone metabolism, exhibits immunomodulatory effects on innate and adaptive immune pathways and is suggestively related to liver diseases. *Objective:* This study investigated the association of single-nucleotide polymorphisms in genes involved in vitamin D functions with hepatitis B virus (HBV) infection. *Methods:* Five hundred Chinese Han subjects, including 274 chronic HBV patients, 68 HBV infection resolvers, and 158 healthy controls without HBV infection, were studied. The *CYP27B1*-1260 promoter and the *VDR Taq* I polymorphisms were genotyped by polymerase chain reaction–restriction fragment length polymorphism. *Results:* Although there was no difference between HBV patients and healthy controls, HBV patients and healthy controls had a higher frequency of the *CYP27B1*-1260 genotype CC (15.0% vs. 2.9%, $p = 0.004$ and 13.3% vs. 2.9%, $p = 0.006$, respectively) and allele C (38.3% vs. 25.7%, $p = 0.006$ and 39.2% vs. 25.7%, $p = 0.006$, respectively) compared with resolvers. The genotype and allele frequencies of the *VDR Taq* I polymorphism had no difference between patients, resolvers, and healthy controls. *Conclusion:* These results suggest that the *CYP27B1*-1260 promoter polymorphism is possibly associated with the persistence, but not susceptibility to HBV infection in Chinese HBV patients, and that the *VDR Taq* I polymorphism is not suggested to be related to chronic HBV infection.

Introduction

Hepatitis B virus (HBV) infection is a major public health problem, with more than 350 million people being chronically infected worldwide (Custer *et al*., 2004). Chronic HBV infection is associated with a variety of clinical outcomes, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), depending on the interactions of multiple viral and host factors (Lavanchy, 2004; Wright, 2006). Host factors associated with both innate and adaptive immune mechanisms are believed to play crucial roles in the consequence of HBV infection (Chang and Lewin, 2007; Das and Maini, 2010).

Vitamin D, beyond its well-known role in calcium and bone metabolism, possesses important immunomodulatory functions in innate and adaptive immune pathways (von Essen *et al*., 2010; Hewison, 2011). In view of this, vitamin D deficiency has been proposed to be involved in autoimmune disorders, metabolic diseases, chronic viral infection, and tumors, including those affecting liver such as autoimmune hepatitis (Saron *et al*., 2009), alcoholic liver disease (Malham *et al*., 2011), nonalcoholic fat liver disease (Targher *et al*., 2007; Barchetta *et al*., 2011), chronic hepatitis C virus (HCV) infection (Arteh *et al*., 2010; Petta *et al*., 2010), and HCC (Chiang *et al*., 2011).

Vitamin D_3 can be synthesized in the skin and acquired in the diet or in vitamin supplements. Then, it is converted by 25-hydroxylase in the liver or other cells to 25-hydroxyvitamin D_3 [25(OH)D]. 25(OH)D is further hydroxylated to bioactive 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2$D] in the kidney, skin, and immune cells by the enzyme encoded by *CYP27B1* (cytochrome P450, family 27, subfamily B, peptide 1) on chromosome 12q13.1-13.3 (Jones *et al*., 1998). 1,25$(OH)_2$D is the physiologically most active hormonal form of vitamin D and is also believed to influence the immune system. It binds to the vitamin D receptor (VDR) located in the nucleus of a variety of cell types, including cells such as peripheral blood monocytes, T cells, and antigen-presenting cells in the immune system

[1]Department of Infectious Diseases, First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Shaanxi, China.
[2]Key Laboratory of Environment and Genes related to Diseases, Institute of Endemic Diseases, School of Medicine, Xi'an Jiaotong University, Ministry of Education, Shaanxi, China.
[3]Department of Hepatobiliary Surgery, First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Shaanxi, China.
[4]Institute of Advanced Surgical Technology and Engineering, Xi'an Jiaotong University, Shaanxi, China.

(Bhalla *et al*., 1983; Provvedini *et al*., 1983; Bikle, 2009), thereby inducing heterodimerization of the VDR with the retinoid X receptor. This heterodimer binds to vitamin D response elements and activates or represses the transcription of specific target genes, mediating the maintenance of calcium homeostasis, hormone secretion, cytokine production, and other functions of vitamin D (Christakos *et al*., 1996, 2003).

It is reasonable to hypothesize that genetic variations in the genes involved in vitamin D metabolism and activity, via the effect on bioactive metabolite and the VDR, may affect the susceptibility or disease progression and outcomes of HBV infection. Single-nucleotide polymorphisms (SNPs) in the *VDR* gene on chromosome 12q12-q14 have been shown to be associated with distinct clinical phenotypes, except HCC (Huang *et al*., 2010), and severity of liver disease in HBV infection (Suneetha *et al*., 2006) with conflicting findings (Li *et al*., 2006). The *CYP27B1*-1260 promoter polymorphism has been demonstrated to be associated with autoimmune diseases (Lopez *et al*., 2004; Bailey *et al*., 2007; Sundqvist *et al*., 2010). Moreover, this polymorphism was recently shown to influence the $1,25(OH)_2D$ serum levels and treatment response in chronic HCV infection (Lange *et al*., 2011). However, the role of *CYP27B1* polymorphisms in chronic HBV infection remains unknown. In this study, we, for the first time to our knowledge, investigated the *CYP27B1*-1260 promoter polymorphism (rs10877012) and further examined the *VDR Taq* I polymorphism (rs731236) in Chinese populations, including patients with chronic HBV infection, individuals with spontaneous clearance of HBV infection, and healthy individuals without HBV infection.

Materials and Methods

Patients and controls

HBV patients were recruited from the First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University. Chronic HBV infection was defined as being positive for HBsAg and anti-HBc for more than 6 months with or without HBeAg positivity. The clinical diagnoses in the patients were performed based on the diagnostic criteria described elsewhere (Talwalkar and Gores, 2004; Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association, 2011). The individuals with spontaneous clearance of HBV infection (HBV infection resolvers) and healthy controls without HBV infection were recruited from those for general physical examination and blood donors. All the HBV infection resolvers were positive for anti-HBs and anti-HBc with normal liver biochemistry. The healthy control individuals were negative for HBV seromarkers or only positive for ant-HBs due to hepatitis B vaccination with normal liver biochemistry and without a history of HBV infection. The HBV patients and infection resolvers had never been treated with any antiviral or immunomodulating therapy. Patients aged <18 years, pregnant women, patients who had other hepatotrophic viral infection (hepatitis A virus, HCV, hepatitis D virus, or hepatitis E virus), autoimmune hepatitis and drug-induced hepatitis or alcoholic hepatitis, patients with severe complications of the cardiovascular, renal, or respiratory system, and patients with concurrent infection of human immunodeficiency virus-1 were all excluded. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the institutional human research committee, and informed consent was obtained from all the individuals.

Genomic DNA extraction and genotyping of polymorphisms

Genomic DNA was extracted from EDTA-treated whole blood using the TIANamp Genomic DNA Kit [Tiangen Biotech (Beijing) Co., Ltd., Beijing, China], according to the manufacturer's instructions. Then, the DNA was aliquoted and stored at $-20°C$ for further use.

CYP27B1-1260 (rs10877012) and *VDR Taq* I (rs731236) polymorphisms were genotyped by DNA amplifications with polymerase chain reaction (PCR) and specific primer sets, followed by the restriction fragment length polymorphism method. The primers for *CYP27B1-1260* were forward 5′-GTGTTCCCTAAGTGTTGTCTC-3′; reverse 5′-GCTGACTC GGTCTCCTCTG-3′ (Yang and Xiong, 2008). The primers used for the *VDR Taq* I polymorphism were forward 5′-CA GAGCATGGACAGGGAGC-3′; reverse 5′-AGGAGAGGCA GCGGTACTG-3′ (Huang *et al*., 2010). The PCR was performed in a volume of 25-μL reaction containing 12.5 μL (0.3 μg) of 2 × Taq PCR Mix (Xi'an Runde Biotechnology, Xi'an, China), 2 μL genomic DNA (0.05 μg/μL), 1.0 μL (10 μM) of each primer, and 8.5 μL sterile double-distilled water. The amplification was performed with the following program. The mixture was first heated at 94°C for 3 min and then amplified for 30 cycles by denaturation at 94°C for 30 s, annealing at 55°C (for *CYP27B1*-1260 polymorphism) or 63°C (for the *VDR Taq* I polymorphism) for 30 s and extension at 72°C for 1 min in each cycle, and a final extension at 72°C for 10 min. The amplified DNA was digested with 0.5 μL *Tfi*I and *Taq* I endonucleases (Fermentas China Co., Ltd., Shenzhen, China), respectively, for *CYP27B1*-1260 and *VDR Taq* I polymorphisms overnight using the buffers and temperatures as recommended by the manufacturers. Digested fragments were separated on a 1.5% agarose gel and visualized by ethidium bromide staining. Individuals were determined as homozygous or heterozygous genotypes according to the digestion pattern (Yang and Xiong, 2008; Huang *et al*., 2010).

Statistical analysis

SPSS software version 13.0 (SPSS, Inc., Chicago, IL) was used for statistical analyses. The assumption of Hardy–Weinberg equilibrium was tested for all SNPs using a χ^2 test comparing observed to expected genotype frequencies to assure population representation of the HBV patients, HBV infection resolvers, and healthy controls. The genotype/allele frequency between groups was analyzed using a chi-square analysis of variance and a Fisher's exact test. A p-value <0.05 indicates statistical significance.

Results

Characteristics of the participants and Hardy–Weinberg equilibrium

A total of 500 unrelated Chinese Han subjects (274 patients with chronic HBV infection, 68 HBV infection resolvers, and 158 healthy control individuals without HBV infection) were included in the study. The 274 HBV patients had a mean age of 45.51 ± 13.28 (mean ± SD) years, and 76.6% of them were male. The 68 HBV infection resolvers had an average age of

46.69 ± 13.72 years, and 63.2% of them were males. The 158 healthy controls had an average age of 43.11 ± 13.28 years, and 74.7% of them were male. The distribution of age and gender between these three groups had no significant difference ($p = 0.099$ and $p = 0.076$, respectively, Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/gtmb). Of the 274 patients, 94 were diagnosed with chronic hepatitis, 90 with liver cirrhosis, and 90 with HCC (Supplementary Table S1).

The genotype frequencies of *CYP27B1*-1260 and *VDR Taq* I polymorphisms were in accordance with the Hardy–Weinberg equilibrium in the HBV patients, HBV infection resolvers, and healthy controls (Supplementary Table S1).

Genotype and allele frequencies of the CYP27B1-*1260 polymorphism*

The distribution of genotype and allele of the *CYP27B1*-1260 polymorphism between the HBV patients, resolvers, and healthy controls were significantly different (Table 1). The *CYP27B1*-1260 genotype AC between HBV patients and HBV infection resolvers had no significant difference ($p = 0.252$). However, the HBV patients had a higher frequency of the genotype CC compared with resolvers (15.0% vs. 2.9%, $p = 0.004$). The *CYP27B1*-1260 genotypes AC and CC between HBV patients and healthy controls had no significant difference ($p = 0.356$ and $p = 0.943$, respectively). The *CYP27B1*-1260 genotypes AC between resolvers and controls had no significant difference ($p = 0.083$), but the healthy controls had a higher frequency of genotype CC compared with the resolvers (13.3% vs. 2.9%, $p = 0.006$, Table 1).

The *CYP27B1*-1260 allele C frequency was higher in HBV patients compared with resolvers (38.3% vs. 25.7%, $p = 0.006$). The *CYP27B1*-1260 allele C frequency had no significant difference between HBV patients and controls ($p = 0.789$). The healthy controls also had a higher frequency of the *CYP27B1*-1260 allele C compared with resolvers (39.2% vs. 25.7%, $p = 0.006$, Table 1).

Genotype and allele frequencies of the VDR Taq I *polymorphism*

The genotype and allele frequencies of the *VDR Taq* I polymorphism had no significant difference between HBV patients, HBV infection resolvers, and healthy controls (Table 2). Further, pairwise comparisons between the groups of different individuals also showed no statistical significance in the genotype and allele frequencies of the *VDR Taq* I polymorphism (Table 2).

Association of the CYP27B1-*1260 and* VDR Taq I *polymorphisms with clinical variables*

The distribution of genotypes and alleles of the *CYP27B1*-1260 and *VDR Taq* I polymorphisms had no significant differences between the patients with different clinical diagnoses (Table 3).

The distribution of genotypes and alleles of the *CYP27B1*-1260 and *VDR Taq* I polymorphisms had no significant differences between HBeAg-positive and HBeAg-negative patients (data not shown). No difference of the genotype and allele frequencies in both polymorphisms according to HBV DNA levels was observed in HBV-infected individuals when

Table 1. Genotype and Allele Frequencies of the *CYP27B1*-1260 Polymorphism in Hepatitis B Virus Patients, Hepatitis B Virus Infection Resolvers, and Healthy Controls

CYP27B1-1260 polymorphism	*Patients* (n = 274)	*Resolvers* (n = 68)	*Controls* (n = 158)	p	*Patients vs. resolvers*		*Patients vs. controls*		*Resolvers vs. controls*	
					p	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*
Genotype										
AA	105 (38.3%)	35 (51.5%)	55 (34.8%)	Reference	Reference		Reference		Reference	
AC	128 (46.7%)	31 (45.6%)	82 (51.9%)	0.222	0.252	1.376 (0.796–2.381)	0.356	1.223 (0.797–1.877)	0.083	0.594 (0.329–1.074)
CC	41 (15.0%)	2 (2.9%)	21 (13.3%)	0.013	0.004	6.833 (1.571–29.718)	0.943	1.023 (0.551–1.899)	0.006	0.150 (0.033–0.678)
Allele										
A	338 (61.7%)	101 (74.3%)	192 (60.8%)	Reference	Reference		Reference		Reference	
C	210 (38.3%)	35 (25.7%)	124 (39.2%)	0.014	0.006	1.793 (1.177–2.732)	0.789	1.039 (0.783–1.381)	0.006	0.537 (0.344–0.838)

Data are presented as *n* (%).
OR, odds ratio; CIs, confidence intervals.

Table 2. Genotype and Allele Frequencies of the *VDR Taq* I Polymorphism in Hepatitis B Virus Patients, Hepatitis B Virus Infection Resolvers, and Healthy Controls

VDR Taq *I polymorphism*	*Patients* (n = 274)	*Resolvers* (n = 68)	*Controls* (n = 158)	p	*Patients vs. resolvers*		*Patients vs. controls*		*Resolvers vs. controls*	
					p	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*
Genotype										
TT	239 (87.2%)	63 (92.6%)	139 (88.0%)	Reference	Reference		Reference		Reference	
Tt	35 (12.8%)	5 (7.4%)	18 (11.4%)	0.457	0.213	1.845 (0.694–4.903)	0.691	1.131 (0.617–2.072)	0.350	0.613 (0.218–1.725)
tt	0 (0.0%)[a]	0 (0.0%)[a]	1 (0.6%)[a]		—	—	—	—	—	—
Allele										
T	513 (93.6%)	131 (96.3%)	296 (93.7%)	Reference	Reference		Reference		Reference	
t	35 (6.4%)	5 (3.7%)	20 (6.3%)	0.470	0.228	1.788 (0.687–4.652)	0.973	1.010 (0.572–1.781)	0.258	0.565 (0.208–1.538)

[a]Analysis was not performed.
Data are presented as *n* (%).
VDR, vitamin D receptor.

Table 3. Genotype and Allele Frequencies of the *CYP27B1*-1260 and *VDR Taq* I Polymorphisms in Hepatitis B Virus Patients with Different Clinical Diagnosis

Polymorphism	*Chronic hepatitis* (n = *94*)	*Cirrhosis* (n = *90*)	*HCC* (n = *90*)	p
CYP27B1-1260				
Genotype				
AA	31 (33.0%)	38 (42.2%)	36 (40.0%)	Reference
AC	47 (50.0%)	41 (45.6%)	40 (44.4%)	0.509
CC	16 (17.0%)	11 (12.2%)	14 (12.2%)	0.450
Allele				
A	109 (58.0%)	117 (65.0%)	112 (62.2%)	Reference
C	79 (42.0%)	63 (35.0%)	68 (37.8%)	0.163
VDR Taq I				
Genotype				
TT	82 (87.2%)	81 (90.0%)	76 (84.4%)	Reference
Tt	12 (12.8%)	9 (10.0%)	14 (15.6%)	0.536
tt	0	0	0	—
Allele				
T	176 (93.6%)	171 (95.0%)	166 (92.2%)	Reference
t	12 (6.4%)	9 (5.0%)	14 (7.8%)	0.559

Data are presented as *n* (%).
HCC, hepatocellular carcinoma.

the HBeAg-positive patients and HBeAg-negative patients were separately categorized (data not shown).

Discussion

In this study, we for the first time to our knowledge have investigated the role of the *CYP27B1*-1260 promoter polymorphism in chronic HBV infection. We revealed that the distribution of genotypes and alleles of *CYP27B1*-1260 polymorphisms, although not significantly different between HBV patients and healthy controls, was significantly different between individuals who spontaneously cleared HBV infection, individuals with chronic HBV infection, and healthy controls. The genotype CC and allele C of the *CYP27B1*-1260 polymorphism were more frequent in HBV patients and healthy controls in comparison with spontaneous HBV infection resolvers. These findings suggest that the *CYP27B1*-1260 polymorphism may not confer susceptibility to HBV infection, but it is associated with the persistence or chronicity of HBV infection.

The *CYP27B1*-1260 promoter polymorphism has been demonstrated to be related to autoimmune diseases (Lopez *et al*., 2004; Bailey *et al*., 2007; Sundqvist *et al*., 2010), with the CC genotype and C allele being associated with an increased risk of type 1 diabetes (Bailey *et al*., 2007) and autoimmune Addison's disease (Fichna *et al*., 2010). This polymorphism is also recently shown to influence the $1,25(OH)_2D$ serum levels and treatment response in chronic HCV infection, with the CC genotype being associated with reduced $1,25(OH)_2D$ levels and lower sustained virologic response to the treatment with interferon (IFN)-α-based regimens (Lange *et al*., 2011). It is indicated that type 1 diabetic patients carrying the *CYP27B1*-1260 genotype CC had lower CYP27B1 mRNA levels in the peripheral blood mononuclear cells compared with healthy control subjects carrying the CC genotype (Ramos-Lopez *et al*., 2007). It is hypothesized that the presence of the

CYP27B1-1260 C allele or other variants in the linkage disequilibrium with this allele may reduce the level of the active 1α-hydroxylase and the conversion of 25(OH)D to $1,25(OH)_2D$ (Bailey *et al*., 2007). In view of the preventive and beneficial effect of $1,25(OH)_2D$ on both autoimmune diseases and viral infections (Saron *et al*., 2009; Petta *et al*., 2010), it seems that the predisposing effect of genetic variation in the *CYP27B1* gene may be exerted through the downregulation of $1,25(OH)_2D$ levels. However, different immunological mechanisms are involved in autoimmune disorders and non-autoimmune diseases such as viral infections. $1,25(OH)_2D$ may suppress T helper-1 (Th1) and promote Th2 differentiation through action on CD4+ T cells (Rigby *et al*., 1987; Müller and Bendtzen, 1992; Boonstra *et al*., 2001) and when combined with interleukin (IL)-2, can directly suppress the production of proinflammatory cytokines IFN-γ, IL-17, and IL-21 and promote development of regulatory T cells expressing cytotoxic T lymphocyte antigen-4 and FoxP3 (Jeffery *et al*., 2009). The reaction of such an immune response may benefit autoimmune diseases, but exacerbate chronic viral infections such as chronic HCV and HBV infections, because the balance of Th1/Th2 response is shifted toward Th1 in autoimmune reaction (Crane and Forrester, 2005; Afzali *et al*., 2007) and skewed toward Th2 in the immune response of chronic HBV and HCV infections (Bertoletti *et al*., 1997; Rossol *et al*., 1997; Tsai *et al*., 1997). The explanation for this scenario remains elusive. The Th1/Th2 imbalance is regarded as an integral part of the complex interactions in the development of autoimmune disease (Crane and Forrester, 2005), and this may also apply for viral infections with various characteristics (Bertoletti *et al*., 1997). Other pathophysiological processes possibly involved in the complex multifactorial genetic regulation and the action of vitamin D in the autoimmune and viral disorders may also be responsible for this scenario. For instance, the viral diseases have specific pathogenic agents. *In vitro* study demonstrated a direct antiviral effect of vitamin D on HCV (Gal-Tanamy *et al*., 2011), suggesting the possibility that vitamin D, functioned as a natural antiviral mediator, may promote the resolution of viral infection. This possibility may not exist in autoimmune diseases. Of course, there are some common mechanisms in the action of vitamin D that may benefit both autoimmune and viral disorders. For example, the antiproliferative and antifibrotic effects of $1,25(OH)_2D$ on liver fibrosis found in *in vitro* and *in vivo* models (Abramovitch *et al*., 2011) may be considered as having a potential role in the mechanisms of beneficial effect of vitamin D on liver diseases related to both autoimmune and viral infections. Furthermore, it is unclear whether there are the U-shaped exposure–risk relationships between vitamin D concentrations and disease outcomes in the autoimmune and viral diseases, as have been proposed in prostate cancer (Tuohimaa *et al*., 2004), with both low and high concentrations, in comparison with the middle-range concentration, of vitamin D being associated with disease outcomes.

$1,25(OH)_2D$ unleashes its biological effects through the VDR, which may also be expressed on immunologically relevant cell populations such as peripheral blood monocytes, activated T lymphocytes, and antigen-presenting cells (Bhalla *et al*., 1983; Provvedini *et al*., 1983; Bikle, 2009). Polymorphisms of *VDR* may therefore influence the immunomodulation via $1,25(OH)_2D$. There are few studies that have examined the association of *VDR* polymorphisms with HBV infection. One study indicated that the *Bsm* I, *Apa* I, and *Taq* I polymorphisms of the *VDR* gene were associated with distinct clinical phenotypes in HBV carriers, but not with HCC development (Huang *et al*., 2010). An association between the *VDR Taq* I T allele and asymptomatic hepatitis B was also reported (Shan *et al*., 2006). In contrast, another study showed that the *VDR Fok* I polymorphism was independently associated with chronic HBV infection, but the *Taq* I polymorphism had no association with the outcome of HBV infection (Li *et al*., 2006). In line with this, we did not find any difference in the *VDR Taq* I polymorphism between chronic HBV patients, HBV infection resolvers, and healthy controls. The *VDR Taq* I polymorphism results in a silent mutation in exon 9 of the *VDR* gene and does not affect the stability of the VDR mRNA (Verbeek *et al*., 1997). Therefore, the functional effects of the *VDR Taq* I polymorphism reported so far cannot explain how VDR differently contributes to the pathogenesis of HBV infection, although the conflicting findings may result from genetic heterogeneity that exists for *VDR* gene polymorphisms in divergent populations.

We did not show the difference of polymorphisms in both *CYP27B1*-1260 and *VDR Taq* I between chronic HBV patients with different clinical diagnoses. It is speculated that the polymorphisms in a gene such as *CYP27B1*-1260 may modulate certain, but not all of the characteristics pertaining to the pathogenesis of a disease. Such-like a finding is documented by a recent report showing that previously identified multiple sclerosis susceptibility-associated SNPs, including loci of chromosome 12q13-14 (Australia and New Zealand Multiple Sclerosis Genetics Consortium [ANZgene], 2009), do not influence disease severity measures in multiple sclerosis patients (Jensen *et al*., 2010).

It should be noted that this study was carried out in a relatively small sample size of populations. The numbers of individuals, especially the numbers of spontaneous resolvers, recruited in our study were small, but the frequencies of *CYP27B1*-1260 and *VDR Taq* I genotypes in all the three groups, including HBV patients, HBV infection resolvers, and healthy controls, were distributed in accordance with the Hardy–Weinberg equilibrium, indicating the representation of the study subjects and reducing a probability of sample error. Moreover, this study was not an exhaustive examination of variants in the genes, and the selected SNPs did not provide full tagging coverage. Therefore, additional studies in a larger number of populations with more polymorphic sites are warranted to confirm and extend our findings, as this is the first study to investigate the role of the *CYP27B1* polymorphism in HBV infection.

In summary, this study suggests that the *CYP27B1*-1260 promoter polymorphism is possibly associated with the chronicity, but not the susceptibility of HBV infection in Chinese HBV patients, and that chronic HBV patients have a similar distribution of the *VDR Taq* I polymorphism compared with the normal population and HBV infection resolvers. Given that vitamin D may exert its multiple biological and possibly pathogenic effects on HBV infection through complex mechanisms of action, including genetic regulation, further studies may shed light on clarifying the role of vitamin D and its function-associated genetic factors in HBV infection and designing approaches to prevent and treat chronic HBV infection or delay its progression.

Acknowledgments

This work was supported in part by funding from the National Natural Science Foundation of China (Grant no. 30972758). We thank Dr. Hamad Haider for his careful editing of the English language.

Author Disclosure Statement

No conflict of interest to declare.

References

Abramovitch S, Dahan-Bachar L, Sharvit E, *et al*. (2011) Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 60:1728–1737.

Afzali B, Lombardi G, Lechler RI, Lord GM (2007) The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 148:32–46.

Arteh J, Narra S, Nair S (2010) Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Sci 55:2624–2628.

Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene) (2009) Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet 41:824–828.

Bailey R, Cooper JD, Zeitels L, *et al*. (2007) Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes 56:2616–2621.

Barchetta I, Angelico F, Del Ben M, *et al*. (2011) Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med 9:85.

Bertoletti A, D'Elios MM, Boni C, *et al*. (1997) Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 112:193–199.

Bhalla AK, Amento EP, Clemens TL, *et al*. (1983) Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. J Clin Endocrinol Metab 57:1308–1310.

Bikle D (2009) Nonclassic actions of vitamin D. J Clin Endocrinol Metab 94:26–34.

Boonstra A, Barrat FJ, Crain C, *et al*. (2001) 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 167:4974–4980.

Chang JJ, Lewin SR (2007) Immunopathogenesis of hepatitis B virus infection. Immunol Cell Biol 85:16–23.

Chiang KC, Yeh CN, Chen MF, Chen TC (2011) Hepatocellular carcinoma and vitamin D: a review. J Gastroenterol Hepatol 26:1597–1603.

Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association (2011) The guideline of prevention and treatment for chronic hepatitis B (2010 version). Chin J Gastroenterol 16:351–366.

Christakos S, Dhawan P, Liu Y, *et al*. (2003) New insights into the mechanisms of vitamin D action. J Cell Biochem 88: 695–705.

Christakos S, Raval-Pandya M, Wernyj RP, Yang W (1996) Genomic mechanisms involved in the pleiotropic actions of 1,25-dihydroxyvitamin D3. Biochem J 316 (Pt 2):361–371.

Crane IJ, Forrester JV (2005) Th1 and Th2 lymphocytes in autoimmune disease. Crit Rev Immunol 25:75–102.

Custer B, Sullivan SD, Hazlet TK, *et al*. (2004) Global epidemiology of hepatitis B virus. J Clin Gastroenterol 38 (10 Suppl 3): S158–S168.

Das A, Maini MK (2010) Innate and adaptive immune responses in hepatitis B virus infection. Dig Dis 28:126–132.

Fichna M, Zurawek M, Januszkiewicz-Lewandowska D, *et al*. (2010) Association of the CYP27B1 C(-1260)A polymorphism with autoimmune Addison's disease. Exp Clin Endocrinol Diabetes 118:544–549.

Gal-Tanamy M, Bachmetov L, Ravid A, *et al*. (2011) Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. Hepatology 54:1570–1579.

Hewison M (2011) Vitamin D and innate and adaptive immunity. Vitam Horm 86:23–62.

Huang YW, Liao YT, Chen W, *et al*. (2010) Vitamin D receptor gene polymorphisms and distinct clinical phenotypes of hepatitis B carriers in Taiwan. Genes Immun 11:87–93.

Jeffery LE, Burke F, Mura M, *et al*. (2009) 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol 183:5458–5467.

Jensen CJ, Stankovich J, Van der Walt A, *et al*. (2010) Australian and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene), Rubio JP, Butzkueven H. Multiple sclerosis susceptibility-associated SNPs do not influence disease severity measures in a cohort of Australian MS patients. PLoS One 5:e10003.

Jones G, Strugnell SA, DeLuca HF (1998) Current understanding of the molecular actions of vitamin D. Physiol Rev 78:1193–231.

Lange CM, Bojunga J, Ramos-Lopez E, *et al*. (2011) Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. J Hepatol 54:887–893.

Lavanchy D (2004) Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 11:97–107.

Li JH, Li HQ, Li Z, *et al*. (2006) Association of Taq I T/C and Fok I C/T polymorphisms of vitamin D receptor gene with outcome of hepatitis B virus infection. Zhonghua Yi Xue Za Zhi 86:1952–1956.

Lopez ER, Zwermann O, Segni M, *et al*. (2004) A promoter polymorphism of the CYP27B1 gene is associated with Addison's disease, Hashimoto's thyroiditis, Graves' disease and type 1 diabetes mellitus in Germans. Eur J Endocrinol 151:193–197.

Malham M, Jørgensen SP, Ott P, *et al*. (2011) Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than aetiology. World J Gastroenterol 17:922–925.

Müller K, Bendtzen K (1992) Inhibition of human T lymphocyte proliferation and cytokine production by 1,25-dihydroxyvitamin D3. Differential effects on CD45RA+ and CD45R0+ cells. Autoimmunity 14:37–43.

Petta S, Cammà C, Scazzone C, *et al*. (2010) Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 51:1158–1167.

Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC (1983) 1,25-dihydroxyvitamin D3 receptors in human leukocytes. Science 221:1181–1183.

Ramos-Lopez E, Brück P, Jansen T, *et al*. (2007) CYP2R1-, CYP27B1- and CYP24-mRNA expression in German type 1 diabetes patients. J Steroid Biochem Mol Biol 103:807–810.

Rigby WF, Denome S, Fanger MW (1987) Regulation of lymphokine production and human T lymphocyte activation by

1,25-dihydroxyvitamin D3. Specific inhibition at the level of messenger RNA. J Clin Invest 79:1659–1664.

Rossol S, Marinos G, Carucci P, *et al*. (1997) Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. J Clin Invest 99:3025–3033.

Saron ML, Godoy HT, Hessel G (2009) Nutritional status of patients with biliary atresia and autoimmune hepatitis related to serum levels of vitamins A, D and E. Arq Gastroenterol 46:62–68.

Shan J, Wang L, Li Z, *et al*. (2006) Relationship between polymorphisms of vitamin D receptor gene and familial aggregation of HBsAg carriers. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 28:148–153.

Sundqvist E, Bäärnhielm M, Alfredsson L, *et al*. (2010) Confirmation of association between multiple sclerosis and CYP27B1. Eur J Hum Genet 18:1349–1352.

Suneetha PV, Sarin SK, Goyal A, *et al*. (2006) Association between vitamin D receptor, CCR5, TNF-alpha and TNF-beta gene polymorphisms and HBV infection and severity of liver disease. J Hepatol 44:856–863.

Talwalkar JA, Gores GJ (2004) Diagnosis and staging of hepatocellular carcinoma. Gastroenterology 127 (5 Suppl 1):S126–S132.

Targher G, Bertolini L, Scala L, *et al*. (2007) Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 17:517–524.

Tsai SL, Liaw YF, Chen MH, *et al*. (1997) Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. Hepatology 25:449–458.

Tuohimaa P, Tenkanen L, Ahonen M, *et al*. (2004) Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer 108:104–108.

Verbeek W, Gombart AF, Shiohara M, *et al*. (1997) Vitamin D receptor: no evidence for allele-specific mRNA stability in cells which are heterozygous for the Taq I restriction enzyme polymorphism. Biochem Biophys Res Commun 238:77–80.

von Essen MR, Kongsbak M, Schjerling P, *et al*. (2010) Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nat Immunol 11:344–349.

Wright TL (2006) Introduction to chronic hepatitis B infection. Am J Gastroenterol 101 Suppl 1:S1–S6.

Yang J, Xiong F (2008) Relevance of CYP2781 gene promoter polymorphism to autoimmune thyroid diseases. J South Med Univ 28:606–608.

Address correspondence to:
Zhengwen Liu, M.D., Ph.D.
Department of Infectious Diseases
First Affiliated Hospital
School of Medicine
Xi'an Jiaotong University
No. 277 Yanta West Road
Xi'an
710061 Shaanxi
China

E-mail: liuzhengwen@medmail.com.cn