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Association of HLA-DQ and IFNL4 polymorphisms with susceptibility to hepatitis B virus infection and clearance

Jia-Hao Fan,*,◆ Si-Hui Hou,*,◆ Qing-Ling Li,** Jun Hu,* Hong Peng,* Jin-Jun Guo*

* Department of Gastroenterology and Hepatology, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China.
** Institute of Life Sciences, Chongqing Medical University, Chongqing, China
◆ These two authors contributed equally to this work.

ABSTRACT

Background and aim. Leukocyte antigen DQ (HLA-DQ) and interferon-λ4 (IFNL4) gene polymorphisms were associated with susceptibility to chronic hepatitis B and C virus infection. This study further confirmed that variants of these genes were associated with susceptibility and spontaneous clearance of HBV infection in a Chinese population. **Material and methods.** A total of 1,069 subjects were recruited and divided into three groups i.e. 397 with CLD (HBV-related chronic liver disease), 434 with SC (spontaneous clearance), and 238 HC (healthy controls). HLA-DQrs9275319 and IFNL4rs368234815, rs12971396, rs12979860, and rs8099917SNPs were genotyped using the Sequenom MassARRAY MALDI-TOF system. **Results.** HLA-DQ rs9275319 showed a significant association with HBV infection (allele model, OR, 0.514; 95% CI, 0.359-0.738, adjusted p = 0.0003) and with natural clearance (allele model, OR, 1.659; 95% CI, 1.197-2.300, adjusted. However, there was no association between IFNL4 polymorphism and HBV susceptibility or natural clearance (all p > 0.05). The multifactor dimensionality reduction (MDR) test with permutation correction showed that a three-way interaction between IFNL4 and HLA-DQ SNPs was identified for HBV susceptibility (permutation p = 0.009 for the best factor model) and clearance (permutation p = 0.014 for the best factor model). **Conclusions.** The data from the current study provided additional evidence for an SNP-SNP interaction between HLA-DQ and IFNL4 in regulation to HBV infection and natural clearance.

Key words. Hepatitis B virus (HBV). Human leukocyte antigen DQ (HLA-DQ). Interferon-λ4 (IFNL4). Single nucleotide polymorphisms (SNPs). Viral infection risk.

INTRODUCTION

Hepatitis B virus (HBV) infects approximately 400 million people in the world and the highest HBV incidence occurs in China. Chinese national surveys have indicated that a prevalence of approximately 10% of the entire Chinese population is infected with chronic HBV.[1] After HBV infection, 5-10% of adults will develop chronic HBV infection,[2] which consequently causes a broad spectrum of diseases, such as chronic hepatitis, liver cirrhosis (LC), and even hepatocellular carcinoma (HCC).[3] Hepatic diseases are responsible for approximately 5% of deaths in China, indicating that HBV infection is a significant public health problem and challenge in this country. To date, the outcomes of HBV infection are multifactorial and include HBV subtypes, host immunization status and genetic factors. There is compelling evidence, which shows that host genetic factors play a role and contribute to variability of HBV-related liver disease.[4-6] Segregation analysis and twin studies support the role of intrinsic host factors in influencing the host response to HBV infection.[7] A recent genomic-wide association study (GWAS) of HBV-related HCC in a Chinese population showed an association between human leukocyte antigen DQ (HLA-DQ) rs9275319 with a risk of developing HBV-related HCC using a two-stage design with 2514 chronic HBV carriers.[8] Other candidate gene association studies also suggested that the HLA class II locus was associated with HBV-related disease.[9-12] HLA-DQ encodes a cell surface receptor type protein in antigen presenting cells and presents different antigens to T-lymphocytes, which consequently guides

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B-lymphocytes to produce antibodies to initiate the host defense system against foreign antigens or immune tolerance to self-antigens.[8] Thus, further study of the HLA-DQ SNP (rs9275319) could help us to better understand the association of HLA-DQ SNP with HBV infection or clearance.

Furthermore, association of interferon gene variants with viral invasion has also been reported in several studies along with the effect of HLA-DQ polymorphisms on HBV infection.[13-16] For example, interferon-λ4 (IFNL4) encodes the interferon lambda 4 (IFN-λ4) protein that can induce antiviral activity by activation of the JAK-STAT pathway.[17,18] A previous GWAS showed that IFNL4 SNPs were associated with impaired HCV clearance in the African ancestry and ss469415590 (namely rs368234815) was better than rs12979860 in predicting host HCV clearance.[19] Other candidate gene association studies corroborated these results.[20-22] On the other hand, a genome-wide association study showed that the polymorphism of *HLA-DQ* had a significant association with chronic HCV infection in Japanese and this association might have relationship with the genotype of the *IFNL4* polymorphism in Japanese.[23] Thus, in this study, we explored SNPs in these two genes as well as gene-gene interaction between HLA-DQ and IFNL4 to assess their association with HBV susceptibility and natural clearance in Chinaese. We also added three other IFNL4 SNPs (rs12971396, rs12979860 and rs8099917) that are localized at exon 5, intron 1, and upstream of IFNL4 gene, respectively[19] as there are controversial views on hepatitis B risk and their association.[16,24-26]

Table 1. Diagnosis criteria for Healthy control group (HC), HBV spontaneous clearance group (SC), and HBV-related chronic liver disease (CLD).

Healthy control group (HC)
1. Anti-HBs, HBsAg and anti-HBc negative and no HBV vaccination history.
2. Anti-HCV and HCV RNA negative.
3. Anti-HDV and/or HDAg negative.
4. ALT < 40 and AST < 45 IU/L.

HBV spontaneous clearance group (SC)
1. HBsAg negative plus anti-HBs and anti-HBc positive.
2. HBV-DNA negative, HDAg negative and/or anti-HDV negative.
3. Anti HCV and HCV RNA negative.
4. ALT < 40 IU/L and AST < 45 IU/L at enrollment.

Chronic hepatitis B group (CHB)
1. Two positive tests for HBsAg (at least 6months) and anti-HBcAg apart.
2. Anti-HCV and HCV RNA negative.
3. Anti-HDV and/or HDAg negative.
4. ALT and/or AST levels greater than 2 times upper limits of normal range for testing hospital before or current (ALT and/or AST >80 IU/L); and, HBV-DNA Load > 1,000(copy/mL).
5. No clinical evidence of liver cirrhosis.

HBV-related liver cirrhosis group (LC)
1. HBsAg and anti-HBc positive.
2. Anti-HCV and HCV RNA negative.
3. Anti-HDV and/or HDAg negative.
4. LC confirmed by biopsy and sonography or CT or MRI.
5. Liver cirrhosis with clinical presentation of gastroesophageal varication or a history of bleeding, or ascites, or edema, or encephalopathy, or serum albumin < 35 g/L, total bilirubin > 35 μmol/L.

HBV-related hepatocellular carcinoma group (HCC)
1. HBsAg and anti-HBc positive.
2. Anti-HCV and HCV RNA negative.
3. Anti-HDV and/or HDAg negative.
4. HCC confirmed by biopsy or elevated AFP and sonography or CT or MRI.

MATERIAL AND METHODS

Study subjects

In this study, we enrolled a total of 1069 subjects from the First and Second Affiliated Hospital of Chongqing Medical University (Chongqing, China) between January 2011 and August 2014. All subjects were unrelated Han Chinese without age or gender restrictions. They were classified into three groups i.e.:

- CLD (HBV-related chronic liver disease) group (n = 397).
- SC (spontaneous clearance) group (n = 434), and
- HC (healthy control) group (n = 238).

Subjects in the CLD group included those diagnosed as having chronic hepatitis B (CHB), HBV-related liver cirrhosis (LC), and HBV-related HCC. Diagnosis of each condition was listed in table 1. The exclusion criteria included:

- Infection with human immunodeficiency virus (HIV) or another hepatitis virus.
- Lack of data on HBV serological markers.
- Those vaccinated against hepatitis B.
- Other chronic liver disease, such as autoimmune hepatitis, toxic hepatitis, or primary biliary cirrhosis.
- Severe systemic disease such as systemic lupus erythematosus; and
- Non-Chinese Han origin.

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University and informed consent was obtained from each subject before participation in this study.

Collection of serological and biochemical data from each subject

A single venous blood sample of approximately 3-5 mL was collected from each subject. All serum specimens were stored at -80°C until use. Clinical, biological, and viral characteristics of the first two groups of subjects were collected and are shown in table 2. A questionnaire was used to record all personal information, including age, gender, alcohol consumption and other characteristics. Total serum bilirubin (T-Bil), serum HBV markers, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum levels of HBV-DNA were routinely assessed using standard clinical laboratory techniques.

Genomic DNA extraction and SNP selection and genotyping

Genomic DNA was extracted from peripheral blood leukocytes from whole blood using the Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, Madison, WI, USA). DNA samples were quantified using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA) and diluted to 20 ng/μL in TE buffer and then stored at -80°C until use.

We initially identified eight SNPs, which represented all haplotypes of the HapMap sets according to a previous study[19] and after combining these with the MAF > 0.05 from Chinese Han samples in Beijing, China, we selected five SNP loci for genotyping in this study. These included rs368234815, rs12979860, rs8099917, rs12971396, and rs4803221. Due to a high disequilibrium between SNP rs12971396 and rs4803221 in the HapMap-CHB ($r^2 = 1.0$), we selected the SNP rs12971396 as a representative of these two loci. We utilized the Sequenom MassARRAY MALDI-TOF system (Biomiao Biological, Beijing, China) to genotype these SNPs. Locus detection was evaluated using a > 95% success rate. Genotyping for all SNPs was repeated in 5% of samples for verification and quality control. Quality control testing revealed that the genotype data had an error rate of < 0.1%.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) of genotype frequency was performed using Stata 12.0 (Stata Corp, College Station, TX, USA). Differences in age and laboratory parameters, such as AST, ALT, and T-Bil were compared between groups using Student *t* test or Mann-Whitney *U* test. The χ^2 test was performed to assess differences in distribution of gender between cases and controls (CLD *vs.* HC or CLD *vs.* SC). A binary logistic regression test was conducted to estimate the relative risk of these SNPs with HBV infection and clearance in both an allelic model (major allele A *vs.* minor B) and a genetic model. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated. Each genotype was assessed with the dominant model(AA *vs.* AB + BB). Haploview 4.2 software (http://www.broad.mit.edu/haploview/haploview) and PHASE software (v2.0.2) were employed to analyze linkage disequilibrium (LD) and haplotype frequencies. All statistical tests were two-sided, and a probability level of $p < 0.05$ was considered statistically significant. P-values were corrected for multiple testing by Bonferroni's corrections. Statistical analysis was performed using SPSS 19.0 (SPSS Inc. Chicago, IL, USA).

MDR analysis of SNP-SNP interaction

A Multifactor Dimensionality Reduction (MDR) program (v3.0.2) was performed to analyze the gene-gene interactions of HLA-DQ and IFNL4 SNPs with HBV susceptibility or natural clearance. This program was recommended as a method to improve the identification of polymorphism combinations associated with the risk

Table 2. Characteristics of study subjects.

Parameters	CLD (n = 397)	SC (n = 434)	HC (n = 238)	P value
			CLD *vs.* SC/CLD *vs.* HC	
Age (years)	51.5 ± 13.7	52.2 ± 13.9	50.3 ± 13.2	0.512/0.486
Male/Female, n (%)	289 (72.8)/108 (27.2)	295 (68.0)/139 (32.0)	160 (67.2)/78 (32.8)	0.129/0.136
ALT (IU/L)	64.0 (32.0, 214)	16.0 (11.0, 24.0)	16.0 (11.0, 23.0)	< 0.001/< 0.001
AST (IU/L)	56.0 (25.0, 153.0)	20.0 (17.0, 25.0)	20.0 (16.0, 23.0)	< 0.001/< 0.001
T-Bil (μmol/L)	24.1 (14.3, 70.9)	10.7 (8.0, 14.8)	10.4 (7.6, 13.5)	< 0.001/< 0.001
HBV-DNA (IU/L)	5.02E3 (1.00E3, 4.28E5)	-	-	-

CLD: HBV-related chronic liver disease. SC: spontaneous clearance. HC: healthy control. ALT: alanine aminotransferase. AST: aspartate transferase. T-Bil: total bilirubin. Continuous variables as mean ± standard or median (range), categorical variables as n (%). Differences in age, ALB, ALT and T-Bil were compared between groups using the Student t test or Mann-Whitney U test. χ^2 test was used to assess the differences in the distribution of gender between the case group and control group.

of diseases by reducing the dimensionality of multilocus information.[27] Permutation test of MDR program was used to determine statistical significance for SNP-SNP interaction. Similarly, $p < 0.05$ corrected by Bonferroni's corrections was considered statistically significant in Permutation test. The MDR program is an open-source software and freely available from http://www.epistasis.org.

RESULTS

Demographic and clinical characteristics of these subjects

In this study, we recruited 397 individuals who had HBV-related chronic liver disease, 434 who had spontaneous HBV clearance, and 238 who were healthy controls. Table 2 summarizes their laboratory and demographic characteristics, including age, gender, ALT, AST, T-Bil and HBV-DNA load. As expected, there was no statistical difference in age and gender between cases and controls (CLD *vs.* SC or CLD *vs.* HC). However, abnormal laboratory results of ALT, AST, and T-Bil, and HBV DNA load were present in the CLD group.

Association of HLA-DQ rs9275319 with HBV infection and clearance

There was no departure from the Hardy-Weinberg distribution in each genotype of these 5 SNP loci in all groups ($p > 0.05$). This finding indicates that there was no significant SNP-specific deviation observed and all subjects were suitable for further statistical analysis. We then assessed the association of HLA-DQ rs9275319 with HBV susceptibility and clearance and found that the rs9275319 TT was most frequently identified among all groups (86.2% in the CLD group, 77.6% in the SC group, and 75.9% in the HC group). Carriage of the rs9275319 C allele was a protective factor for chronic HBV infection and clearance (the allele model: $p = 0.002$, OR, 1.659; 95%CI, 1.197-2.300, respectively; Table 3). In the dominant genetic model, there was significant divergence for HBV infection ($p = 0.001$; OR, 0.465; 95%CI, 0.293-0.739), whereas there was no significant association between the CLD and SC group ($p = 0.058$; OR, 1.505; 95%CI, 0.986-2.296). After Bonferroni's corrections, rs9275319 still showed a significant association with chronic HBV infection and clearance (Table 3).

Association of IFNL4 polymorphisms with chronic HBV infection and clearance

The prevalence of rs368234815 ΔG/ΔG, rs12971396 GG, rs12979860AA, and rs8099917GG was very low, or even undetectable in any of these three groups of subjects. We assessed whether IFNL4 polymorphisms were associated with CLD subjects compared to healthy controls or spontaneous clearance patients by logistic regression models and did not find any association between these SNPs and HBV infection (allele model: $p = 0.082$ for rs368234815; $p = 0.063$ for rs12971396; $p = 0.517$ for rs12979860; $p = 0.695$ for rs8099917) or HBV natural clearance (allele model: $p = 0.358$ for rs368234815; $p = 0.105$ for rs12971396; $p = 0.640$ for rs12979860; $p = 0.640$ for rs8099917; Table 4). Moreover, there was no statistical difference found using the dominant model. Although carriage of the rs12971396 minor G allele had some association for HBV susceptibility ($p = 0.063$ for the allele model; $p = 0.056$ for the dominant model), there was no statistical significance after Bonferroni's corrections ($p = 0.315$ for the allele model; $p = 0.280$ for the dominant model) (Table 4).

Haplotype analysis of IFNL4 polymorphisms with HBV infection and clearance

Due to a complete linkage disequilibrium between rs368234815 and rs12979860 (r-squared=1.0),[19] as a

Table 3. Association of HLA-DQ genotypes with HBV susceptibility and clearance.

Polymorphism	CLD (n = 397)	SC (n = 434)	HC (n = 238)	P value/OR (95% CI)	
				CLD *vs.* HC	CLD *vs.* SC
HLA-DQ (rs9275319)	n = 347	n = 375	n = 224		
TT/TC/CC	299/45/3	291/75/9	170/48/6	Reference	Reference
C allele model	51 (0.07)	93 (0.12) (0.359, 0.738)	60 (0.13)	0.0003*[1]/0.514 (1.197, 2.300)	0.002*[2]/1.659
Dominant model (TT/TC+CC)	299/48	291/84	170/54	0.001*[3]/0.465 (0.293, 0.739)	0.058/1.505 (0.986, 2.296)

*Logistic regression analyses adjusted for age and gender. CLD: HBV-related chronic liver disease. SC: spontaneous clearance. HC: healthy control. CI: confidence interval. OR: odds ratio. After Bonferoni correction, *[1]p = 0.001; *[2]p = 0.01; *[3]p = 0.005.*

Table 4. Association of IFNL4 genotypes with HBV susceptibility and clearance.

Polymorphism	CLD (n = 397)	SC (n = 434)	HC (n = 238)	P value/OR (95% CI)	
				CLD *vs.* HC	CLD *vs.* SC
IFNL4 (rs368234815)	**n = 397**	**n = 434**	**n = 238**		
TT/TT-ΔG/ΔGG	374/23/0	417/17/0	232/6/0	Reference	Reference
ΔG allele	23 (0.03)	17 (0.02)	6 (0.01)	0.082/2.303 (0.899, 5.901)	0.358/0.729 (0.371, 1.431)
Dominant model (TT/TT-ΔG + ΔGG)	374/23	417/17	234/4	0.079/2.343 (0.907, 6.053)	0.352/0.723 (0.365, 1.431)
IFNL4 (rs12971396)	**n = 390**	**n = 421**	**n = 222**		
CC/CG/GG	358/32/0	375/46/0	194/28/0	Reference	Reference
G allele	32 (0.04)	46 (0.05)	28 (0.06)	0.063/0.593 (0.341, 1.028)	0.105/1.506 (0.918, 2.470)
Dominant model (CC/CG + GG)	358/32	375/46	194/28	0.056/0.575 (0.325, 1.014)	0.095/1.543 (0.927, 2.567)
IFNL4 (rs12979860)	**n = 385**	**n = 430**	**n = 228**		
GG/AG/AA	344/40/1	381/48/1	200/27/1	Reference	Reference
A allele	42 (0.05)	50 (0.06)	29 (0.06)	0.517/0.848 (0.516, 1.395)	0.640/1.110 (0.716, 1.720)
Dominant model (GG/AG + AA)	344/41	381/49	200/28	0.564/0.858 (0.510, 1.444)	0.596/1.132 (0.716, 1.789)
IFNL4 (rs8099917)	**n = 383**	**n = 418**	**n = 221**		
TT/TG/GG	347/35/1	376/42/0	198/22/1	Reference	Reference
G allele	37 (0.05)	42 (0.05)	24 (0.05)	0.695/0.898 (0.524, 1.538)	0.640/1.119 (0.699, 1.791)
Dominant model (TT/TG + GG)	347/36	376/42	198/23	0.742/1.081 (0.678, 1.724)	0.613/1.107 (0.746, 1.644)

CLD: HBV-related chronic liver disease. SC: spontaneous clearance. HC: healthy control. Logistic regression analyses adjusted for age and gender. CI: confidence interval. OR: odds ratio. After Bonferoni correction.

Table 5. Haplotype frequencies of IFNL4 SNPs in all three groups of subjects.

Haplotype	CLD (n = 383)		HC (n = 221)		SC (n = 418)		P value/OR (95% CI)	
	Frequency (%)	n	Frequency (%)	n	Frequency (%)	n	CLD *vs.* HC	CLD *vs.* SC
CGT[1]	94.57	362	92.19	204	92.27	386	1	
GAG	3.68	14	5.01	11	5.93	25	0.418/0.72 (0.32, 1.61)	0.128/1.67 (0.86, 3.27)
Others	1.75	7	2.80	6	1.80	7	0.453/0.66 (0.22, 1.98)	0.905/0.94 (0.33, 2.70)

[1]The most common haplotype as the reference. CLD: HBV-related chronic liver disease. SC: spontaneous clearance. HC: healthy control. From left, SNPs order of haplotype: rs12971396, rs12979860, rs8099917. Others: haplotype frequency < 2%.

Table 6. Gene-gene interaction of IFNL4 and HLA-DQ in relation to HBV infection.

Factor model	TA	CVC	Permutation P
CLD *vs.* HC			
rs9275319	0.5242	9/10	0.306
rs12971396, rs9275319	0.5528	10/10	0.012-0.013
rs12971396, rs12979860, rs9275319*	0.5642	10/10	0.009
CLD *vs.* SC			
rs9275319	0.5507	10/10	0.029
rs12971396, rs9275319	0.5562	10/10	0.015
rs12971396, rs12979860, rs9275319*	0.5571	10/10	0.014

*TA: testing accuracy. CVC: cross-validation consistency. CLD: HBV-related chronic liver disease. SC: spontaneous clearance. HC: healthy control. *The best factor model.*

representative of the two sites, rs12979860 was considered in the following statistical analysis. Haplotypes were constructed and analyzed for their associations with HBV infection and clearance (Table 5). When compared with the most frequent CGT haplotype, there was no significant correlation with HBV infection or natural clearance for the haplotype GAG (CLD *vs.* HC: p = 0.418; CLD *vs.* SC: p = 0.128) or other minor frequency haplotype (CLD *vs.* HC: p = 0.453; CLD *vs.* SC: p = 0.905; Table 5).

Interaction of HLA-DQ and IFNL4 polymorphisms with HBV infection and clearance

The MDR program (v3.0.2) was applied to analyze interactions of HLA-DQ (rs9275319) and IFNL4 polymorphisms with HBV infection and clearance. We identified significant effects of a three-way (rs12971396, rs12979860, and rs9275319) interaction on HBV susceptibility (Permutation) and clearance (Permutation, p = 0.014; Table 6), indicating that this gene-gene interaction could alter susceptibility to HBV infection and clearance.

DISCUSSION

Chronic HBV infection is a major health problem in China and beyond. Effective control of HBV infection not only prevents HBV-related hepatitis and cirrhosis, but also reduces HCC incidence and death. Researchers in the field are especially interested in the association of the host gene variants with the risk and clearance of HBV infection.[4-6,13-16] Thus, our current study assessed such an association in 1,069 subjects and found that HLA-DQ rs9275319 C allele was associated with a decrease in HBV infection risk and an increased HBV clearance. However, IFNL4 variants were not associated with HBV infection or natural clearance.

Classic HLA loci span 4 Mb on the short arm of chromosome 6p21, which includes MHC class I, II, and III genes. The HLA-DQ, a classical HLA class II, encodes a protein with two non-covalently bound glycoprotein chains, the 34 Kd α and the 28 Kd β chains. This protein mediates the host immune response against infection through its combination with antigen peptides to CD4+ T-lymphocytes.[28] Our current data showed that carriage of the rs9275319 C allele was associated with a reduced HBV infection, which is consistent with data from a previous GWAS of HBV-related HCC in the Han Chinese population.[8] Moreover, our current study further confirmed that rs9275319 non-TT genotype was associated with an increased effect on HBV natural clearance but this was not statistically significant. To the best of our knowledge, our current data corroborate the GWAS data reported by Jiang and colleagues and demonstrate that the rs9275319 loci plays a key role in modulating HBV infection and clearance. However, direct experimental validation is still required.

A di-nucleotide variant ss469415590, namely rs368234815 (TT/ΔG), localized upstream of IFNL3 (IL-28B) on chromosome 19q13.13, has been reported to be associated with chronic HCV infection.[19-21] A frame-shift variant of ss469415590 [ΔG] generates a novel gene, denoted by IFNL4. The IFNL4-ΔG encodes an interferon-λ4 protein, which closely resembles IFN-λ3 and can activate the JAK-STAT signaling pathway. However, as a novel type of interferon, it has been postulated as having a paradoxical function distinct from other type III IFNs against HCV infection.[18] However, our current study did not show the same prominent impact of both rs368234815 and rs12979860 on hepatitis B as has been observed for hepatitis C.[19] Meanwhile, there was no association between IFNL4 rs12971396 and rs8099917 SNPs and hepatitis B infection. We speculate that ethnicity plays an important role in association of these SNPs with HBV infection (different genetic background causes different disease susceptibilities) and HBV is a DNA virus that is different from HCV.

In our study, the gene-gene interaction analysis of IFNL4 and HLA-DQ imply that host gene-gene interactions do alter HBV infection or clearance. Although the molecular mechanisms underpinning this association has not been elucidated, we speculate that there are several potential contributing factors. The most important one might be that the encoding products of mutant IFNL4 have no direct effect on the immune of HBV infection or clearance. However, the IFNL4 and HLA-DQ polymorphisms might affect each other in complementary during any phase of HBV infection. Moreover, a another GWAS had not observed this association of HLA-DQ and IFNL4 polymorphisms with spontaneous resolution of HCV infection in african ancestry.[29] So we speculate that ethnicity, environmental factors, virus type, limited sample size or other coexisting genetic might also be possible confounders. Direct experimental validations are required as well.

To the best of our knowledge, our current study of the rs12971396 SNP is the first to investigate interactions of HLA-DQ and IFNL4 polymorphisms with HBV immunity. However, there are several limitations in the current study, which must be addressed. For example, we were unable to verify our findings using another independent population, although the chance of false positive findings has been reduced by using a very strict correction for multiple testing. Furthermore, our study was limited to the sample size for effective analyses of the progression of chronic HBV infection.

In conclusion, our current study did provide proof-of-principle evidence to support the suggestion that HLA-DQ

combined with IFNL4 polymorphisms are correlated with HBV infection risk and natural clearance. Further study will verify our current data before we can utilize this information for prevention and management of chronic HBV infection in clinic.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this work.

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Correspondence and reprint request:

Jin-Jun Guo, Ph.D.
Department of Gastroenterology and Hepatology, The Second Affiliated Hospital of Chongqing Medical University, 76 Linjiang Road, Yuzhong District, Chongqing 400010, China
Tel.: +862363693326. Fax: +862368486780
E-mail: guojinjun1972@163.com