

IL17A Gene Polymorphisms, Serum IL-17A and IgE Levels, and Hepatocellular Carcinoma Risk in Patients With Chronic Hepatitis B Virus Infection

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Interleukin (IL)-17A plays important roles in hepatitis B virus (HBV)-induced liver diseases. This study aims to investigate *IL17A* single nucleotide polymorphisms (SNPs) and the predispositions to chronic HBV infection and hepatocellular carcinoma (HCC) risk and the correlations to IL-17A and IgE levels. Three hundred ninety-five chronic HBV patients, 75 HBV infection resolvers, and 174 healthy controls were included. *IL17A* SNPs rs8193036 (C/T) and rs2275913 (A/G) and serum IL-17A and IgE levels were determined. HBV infection resolvers had higher rs8193036 allele T and allele T-containing genotypes than HBV patients or controls. Compared with chronic hepatitis, HCC patients had more frequent rs2275913 genotype GG (odds ratios [OR] 3.317, 95% confidence interval [CI] 1.663–6.617, $P = 0.001$) and allele G (OR 1.844, 95% CI 1.311–2.595, $P < 0.001$), and more frequent haplotypes CG (OR 1.868, 95% CI 1.256–2.778, $P = 0.002$) and TG (OR 1.788, 95% CI 1.031–3.101, $P = 0.037$) of rs8193036 and rs2275913. Comparison of HCC patients with cirrhosis yielded similar findings. Apart from male gender and older ages, IL-17A level (OR 1.020, 95% CI 1.003–1.036, $P = 0.019$) and rs2275913 genotypes AG and GG (OR 1.704, 95% CI 1.214–2.390, $P = 0.006$) were factors significantly associated with HCC risk in multivariate analysis in comparison with HBV patients without HCC. These factors remained significant in multivariate analysis in relation to cirrhosis. *IL17A* rs2275913 genotype GG was associated with significantly increased IL-17A and IgE levels. *IL17A* polymorphisms may influence HCC risk in chronic HBV infection via regulating IL-17A production. © 2012 Wiley Periodicals, Inc.

Key words: *IL17A* polymorphism; IL-17A; IgE; Chronic HBV infection; HCC; Association

INTRODUCTION

Infection with hepatitis B virus (HBV) is associated with a variety of clinical diseases including acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1]. The host immunological and genetic factors are regarded as critical determinants of the outcomes of HBV infection in view of the noncytopathic effect of HBV replication in hepatocytes [1–4]. Numerous immuno-inflammatory cells and cytokines/chemokines have been shown to be involved in the disease course and the development of various clinical consequences of HBV infection [2–7].

Interleukin (IL)-17 is a novel family of proinflammatory cytokines that consists of six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F [8,9]. IL-17A is the plenty characterized member of the IL-17 cytokine family and its expression facilitates the definition of a novel lineage of CD4⁺ T helper cells, namely Th17 cells [10–13]. Th17 cells and the IL-17 cytokine have potent pro-inflammatory properties and play an active role in immuno-

inflammatory responses associated with inflammatory diseases, autoimmune disorders, and cancers [9,14–20]. Studies also support the involvement of Th17 cells and IL-17 molecule in the disease course of HBV infection, showing higher frequency of Th17 cells and overexpressing of IL-17A in HBV-associated liver diseases, including chronic hepatitis, liver cirrhosis, and HCC. It has been, for instance, demonstrated that increased Th17 cells frequencies

Abbreviation: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IL, Interleukin; ALT, alanine aminotransferase; SNPs, single nucleotide polymorphisms; OR, odds ratios; CI, confidence interval.

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in peripheral blood and liver of patients with chronic HBV infection were positively associated with serum alanine aminotransferase (ALT) levels and liver inflammation grade in severe chronic hepatitis B patients [21–23]. Increased expression of IL-17 in the liver of patients with HBV-related chronic liver diseases was closely correlated with the degree of fibrosis [24]. Furthermore, increased levels of Th17 cells in peripheral blood of HCC patients were correlated with the tumor size, portal vein tumor thrombus, metastasis, and tumor staging [25]. Significantly increased levels of Th17 cells in the tumor tissues of HCC patients were associated with overall survival and disease-free survival as well as HCC metastasis [26]. IL-17A was demonstrated to be able to promote HCC cell migration and invasion [27].

Recent genetic study has linked polymorphisms in *IL17A* gene to the susceptibility and/or pathogenesis of immuno-inflammatory diseases such as rheumatoid arthritis [28], ulcerative colitis [29], inflammatory bowel disease [30], asthma [31], acute graft-versus-host disease after unrelated bone marrow transplantation, or T cell-depleted allogeneic stem cell transplantation [32,33], and cancers such as gastric cancer [34], and breast cancer [35]. However, it remains unknown whether genetic polymorphisms in *IL17A* influence the development of liver diseases associated with HBV infection. The current study, therefore, determined single-nucleotide polymorphisms (SNPs) in the *IL17A* gene and correlated the genotypes of these SNPs with serum IL-17A levels and clinical diseases in patients with chronic HBV infection in comparison with individuals spontaneously recovered from HBV infection and healthy control individuals from Chinese Han population. The associations of *IL17A* polymorphisms with serum IgE levels were also determined taking into account the relatedness of IgE levels to the clinical course in patients with HBV infection [36–38], the role of IL-17A in promoting IgE production [39], and the possible contribution of IgE to antitumor immune responses [40,41].

METHODS

Patients and Controls

Three hundred ninety-five patients with chronic HBV infection, recruited at the First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University in Xi'an, Shaanxi Province, China, and fulfilling all inclusion and exclusion criteria described below, were included in this study. All patients were randomly selected. Patients were all those who had a history of HBV infection for more than 6 months. Patients were excluded from the study if they had any of the following conditions: (1) aged less than 18 yrs; (2) pregnant women; (3) concomitant other causes of liver disease or mixed etiologies (hepatitis A virus, hepatitis C virus, hepatitis D

virus, or hepatitis E virus, autoimmune hepatitis, primary biliary cirrhosis, Wilson's disease, drug induced hepatitis, alcoholic hepatitis, or nonalcoholic fatty liver disease); (4) complications of the cardiovascular, renal or respiratory system, mental diseases and concurrent infection of human immunodeficiency virus; and (5) treatment with antiviral agents such as nucleotides or immunomodulators such as interferon- α . Of the 395 HBV patients, based on the diagnostic criteria described elsewhere [42–45], 134 were diagnosed as chronic hepatitis, 125 as liver cirrhosis, and 136 as HCC. Considering that HBV patients with normal ALT levels and high HBV DNA may have significant histological disease [46–48], the lack of association between ALT elevation and liver injury in HBV infection [49], and the relatively small number of patients with normal ALT levels and high HBV DNA load in the recruited patients, 21 patients with HBeAg positivity and normal or minimally elevated ALT levels were included in the 134 chronic hepatitis patients for analysis.

Seventy-five individuals from the same area as the HBV patients who were spontaneously recovered from HBV infection with positive anti-HBs and anti-HBc antibodies and normal liver function were included as HBV infection resolvers. One hundred seventy-four healthy subjects, who were negative for HBV markers or only positive for anti-HBs due to hepatitis B vaccination with normal liver biochemistry and without a history of HBV infection, from the same area as the HBV patients and infection resolvers, were recruited as healthy controls. All the subjects, including patients, resolvers, and healthy controls, are unrelated Chinese Han individuals. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the Institutional Ethics Committee and informed consent was obtained from each subject included in the study.

Genotyping of *IL17A* rs8193036 and rs2275913 Polymorphisms

Genomic DNA was extracted from 200 μ l of EDTA-treated peripheral blood using TIANamp Genomic DNA Kit (Tiangen biotech (Beijing) Co., Ltd., Beijing, China) according to manufacturer's instruction. Genotyping of *IL17A* rs8193036 and rs2275913 polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers were designed according to the *IL17A* sequence (NT_007592.15) using Oligo6.0 and primer5.0 software (Molecular Biology Insights, Inc., Cascade, CO), and synthesized and purified by Beijing AuGCT DNA-SYN Biotechnology Co., Ltd. (Beijing, China).

The primers used for *IL17A* rs8193036 polymorphism are upstream: 5'-CCTTCCTGTCTAGTCCT-CTG-3' and downstream: 5'-GGAATTGAAAATGGG-GATAGAG-3'. The primers used for *IL17A* rs2275913

polymorphism are upstream: 5'-GCATGCAGGGTT-GGAACATGCCT-3' and downstream: 5'-TGGCGTG-TCGCAGTGGGTTC-3'.

A 3.0 μ l (0.3 μ g) quantity of genomic DNA was amplified in each 25- μ l reaction containing 12.5 μ l of 2 $\times$ Master Mix (2 $\times$ Taq PCR Mix; Xi'an Runde Biotechnology Ltd., Xi'an, China), 1.0 μ l (10 μ M) of each primer, and 7.5 μ l of double distilled H₂O. The reaction mixture was first heated at 94°C for 3 min and amplification was done for 30 (for rs8193036) or 35 (for rs2275913) cycles in PTC-200 TM Peltier thermal cycler (MJ Research, Waltham, MA) by denaturation at 94°C for 30 s, annealing at 56°C (for rs8193036) or 60°C (for rs2275913) 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 restriction fragments of rs8193036 and rs2275913 polymorphisms were obtained using *Mbo*II and *Xmn*I (New England Biolabs (Beijing) Ltd., Beijing, China), respectively, according to the instructions of the manufacturer. Digested fragments were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide and revealed under UV light using UV-transilluminator (UVP Model White/2 UV; UVP Inc., Upland, CA). The genotypes of each polymorphism in the individuals were determined according to the digestion pattern.

Determination of Serum IL-17A and IgE Levels

Serum levels of IL-17A were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available human IL-17A ELISA (Bio-Vendor Laboratories Ltd., Guang Zhou, China). Serum IgE levels were measured by ELISA Kit for Human Immunoglobulin E (Uscn Life Science Inc., Wuhan, China). The procedures were performed closely following the manufacturers' instructions. Mean intra- and inter-batch coefficients of variation based on the standard samples of different concentrations supplied by the manufacturers were 7.67% and 13.37% for IL-17A and 9.85% and 13.48% for IgE, respectively. The lab staffs were not blinded to the case-control status but they were unaware of the patients' clinical characteristics when read the IL-17A and IgE ELISA.

Statistical Analysis

Statistical analysis was performed by SPSS software version 16.0 (SPSS Inc., Chicago, IL). The frequency of genotypes and alleles was determined by direct gene counting method. Hardy-Weinberg equilibrium was tested for each polymorphism included in this study with χ^2 -test. The linkage disequilibrium and haplotype construction were performed from the observed genotypes using SHEsis method [50]. For the association between genotype, allele, and haplotype of the polymorphisms and the groups, univariate logistic regression analysis was used to

calculate the *P*-value, odds ratios (OR), and 95% confidence interval (CI) after adjusting for age and gender. In the analyses, the most frequent genotype were considered as reference.

The age, gender, HBV DNA, ALT, aspartate aminotransferase (AST), total bilirubin, serum albumin, HBeAg status, IL-17A level, IgE level, *IL17A* rs8193036, and *IL17A* rs2275913 polymorphisms were selected as candidate risk factors for HCC. Univariate analyses and then multiple logistic regressions were used to assess the factors independently associated with the development of HCC in HBV patients or HBV-associated cirrhosis. A *P*-value less than 0.05 was regarded to be statistically significant.

RESULTS

Demographics of the Study Subjects, Patient Features, and Hardy-Weinberg Equilibrium

The age and gender between the 395 patients with chronic HBV infection (age, 44.05 $\pm$ 13.30 yrs; male/female, 299/96), 75 HBV infection resolvers (age, 45.49 $\pm$ 14.29 yrs; male/female, 49/26) and 174 healthy controls (42.23 $\pm$ 13.42 yrs; male/female, 127/47) had no significant difference (*P* = 0.160 and *P* = 0.168, respectively). The clinical diagnoses of the patients included 134 chronic hepatitis, 125 liver cirrhosis, and 136 HCC. The observed values of genotype frequencies of *IL17A* rs8193036 and rs2275913 demonstrated no significant differences in comparison with the corresponding expected values in each group (*P* > 0.05), indicating that the genotype and allele distribution of the two SNPs in the patients, HBV infection resolvers and healthy controls were all in Hardy-Weinberg equilibrium.

Genotypes, Alleles, and Haplotypes of *IL17A* Polymorphisms in HBV Patients, HBV Infection Resolvers and Healthy Controls

The frequencies of *IL17A* rs8193036 genotypes and alleles were significantly different between patients, resolvers, and controls although those of *IL17A* rs2275913 had no significance between the three groups (Table 1). The frequency of *IL17A* rs8193036 genotype CT in HBV infection resolvers was higher than that in HBV patients (OR 2.617, 95% CI 1.533–4.467, *P* < 0.001) or healthy controls (OR 2.673, 95% CI 1.486–4.809, *P* = 0.001) though it was not different between HBV patients and controls. The genotype TT in resolvers was higher than that in patients (OR 2.516, 95% CI 1.039–6.093, *P* = 0.049) though it was not different between patients and healthy controls and between resolvers and controls. When genotypes CT and TT were combined, the genotypes CT + TT in resolvers was also higher than that in patients (OR 2.600, 95% CI

Table 1. Genotype, Allele, and Haplotype Frequency of IL17A Polymorphisms in Hepatitis B Virus (HBV) Patients, HBV Infection Resolvers, and Healthy Controls

	Patients (n = 395)	Resolvers (n = 75)	Controls (n = 174)	Resolvers versus patients		Patients versus controls		Resolvers versus controls	
				P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
rs8193036									
Genotype									
CC	229 (58.0)	26 (34.7)	100 (57.5)	Reference					
CT	138 (34.9)	41 (54.7)	59 (33.9)	<0.001	<0.001	2.617 (1.533–4.467)	0.914	1.021 (0.695–1.501)	0.001
TT	28 (7.1)	8 (10.6)	15 (8.6)	0.110	0.049	2.516 (1.039–6.093)	0.549	0.851 (0.417–1.592)	0.137
CT + TT	166 (42.0)	49 (65.3)	74 (42.5)	<0.001	<0.001	2.600 (1.552–4.355)	0.911	0.980 (0.683–1.405)	0.001
Allele									
C	596 (75.4)	93 (62.0)	259 (74.4)	Reference					
T	194 (24.6)	57 (38.0)	89 (25.6)	0.003	0.001	1.883 (1.304–2.719)	0.714	0.947 (0.709–1.266)	0.005
rs2275913									
Genotype									
AA	110 (27.8)	20 (26.7)	50 (28.7)	Reference					
AG	197 (49.9)	40 (53.3)	85 (48.9)	0.883	0.711	1.117 (0.622–2.005)	0.808	1.053 (0.692–1.603)	0.619
GG	88 (22.3)	15 (20.0)	39 (22.4)	0.983	0.862	0.938 (0.454–1.937)	0.922	0.975 (0.589–1.604)	0.922
Allele									
A	417 (52.8)	80 (53.3)	185 (53.2)	Reference					
G	373 (47.2)	70 (46.7)	163 (46.8)	0.988	0.902	0.978 (0.689–1.388)	0.907	1.015 (0.789–1.307)	0.972
Haplotype (rs8193036/rs2275913)									
CA	314 (39.8)	51 (33.7)	133 (38.2)	Reference					
CG	282 (35.6)	42 (28.3)	126 (36.2)	0.840	0.699	0.917 (0.591–1.422)	0.720	0.948 (0.708–1.269)	0.564
TA	103 (13.0)	29 (19.7)	52 (14.9)	0.095	0.032	1.733 (1.044–2.879)	0.378	0.839 (0.568–1.239)	0.187
TG	91 (11.6)	28 (18.3)	37 (10.6)	0.035	0.014	1.894 (1.130–3.176)	0.853	1.042 (0.676–1.606)	0.022
									0.993 (0.677–1.458)
									0.869 (0.540–1.399)
									1.454 (0.833–2.539)
									1.974 (1.097–3.551)

OR, odds ratio; 95% CI, 95% confidence intervals.

Data of genotype, allele, and haplotype are presented as n (%).

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1.552–4.355, $P < 0.001$) or healthy controls (OR 2.547, 95% CI 1.451–4.470, $P = 0.001$, Table 1). The allele T frequency in HBV infection resolvers was higher than that in HBV patients (OR 1.883, 95% CI 1.304–2.719, $P = 0.001$) or healthy controls (OR 1.784, 95% CI 1.186–2.683, $P = 0.005$) but there was no significance between HBV patients and healthy controls (Table 1).

Haplotypes from *IL17A* rs8193036 and rs2275913 polymorphisms were constructed and compared. The frequency of haplotype CG (rs8193036, rs2275913) had no significance. HBV infection resolvers had higher haplotype TA frequency compared with HBV patients (OR 1.733, 95% CI 1.044–2.879, $P = 0.032$) but the frequency had no significant difference between patients and healthy controls or between resolvers and controls. HBV infection resolvers had higher haplotype TG frequency compared with HBV patients (OR 1.894, 95% CI 1.130–3.176, $P = 0.014$) or healthy controls (OR 1.974, 95% CI 1.097–3.551, $P = 0.022$) although there was no significant difference between patients and controls (Table 1).

Genotypes, Alleles, and Haplotypes of *IL17A* Polymorphisms in Chronic HBV Patients With Different Clinical Diagnoses

Among HBV patients with different clinical diagnoses, although *IL17A* rs8193036 polymorphism was not significantly different, the frequencies of *IL17A* rs2275913 genotype and allele were significantly different. Although there was no significance between chronic hepatitis and liver cirrhosis, *IL17A* rs2275913 genotype GG was more frequent in HCC patients than in chronic hepatitis (OR 3.317, 95% CI 1.663–6.617, $P = 0.001$) or cirrhosis (OR 4.412, 95% CI 2.077–9.370, $P < 0.001$). The allele G frequency of *IL17A* rs2275913 between chronic hepatitis and cirrhosis was not significant but HCC patients had higher allele G frequency compared with chronic hepatitis (OR 1.844, 95% CI 1.311–2.595, $P < 0.001$) or cirrhosis (OR 1.981, 95% CI 1.398–2.807, $P < 0.001$, Table 2).

The frequency of haplotype CG of *IL17A* rs8193036 and rs2275913 in HCC patients was higher than that in chronic hepatitis (OR 1.868, 95% CI 1.256–2.778, $P = 0.002$) or cirrhosis (OR 1.954, 95% CI 1.312–2.911, $P = 0.001$) although there was no significance between chronic hepatitis and cirrhosis. Likewise, the frequency of haplotype TG in HCC was higher than that in chronic hepatitis (OR 1.788, 95% CI 1.031–3.101, $P = 0.037$) or cirrhosis (OR 2.577, 95% CI 2.417–4.686, $P = 0.002$) although it was not significant between chronic hepatitis and cirrhosis (Table 2).

Factors Associated With HCC Risk

When the analysis was performed in comparison with all other HBV patients without HCC for factors

associated with HCC risk, gender, age, HBV-DNA level, ALT level, total bilirubin, HBeAg status, IL-17A level, IgE level, and *IL17A* rs2275913 polymorphism were factors associated with HCC risk by univariate analysis and only male gender (OR 4.316, 95% CI 2.299–8.100, $P < 0.001$), older age (OR 1.064, 95% CI 1.041–1.087, $P < 0.001$), IL-17A level (OR 1.020, 95% CI 1.003–1.036, $P = 0.019$), and *IL17A* rs2275913 AG and GG (OR 1.704, 95% CI 1.214–2.390, $P = 0.006$) were significantly associated with HCC risk in multivariate analysis (Table 3).

When the analysis was carried out in relation to cirrhosis patients, gender, age, albumin concentration, IL-17A level, IgE level, and *IL17A* rs2275913 polymorphism were factors associated with HCC risk by univariate analysis and only male gender (OR 3.645, 95% CI 1.803–7.370, $P < 0.001$), older age (OR 1.051, 95% CI 1.023–1.080, $P < 0.001$), IL-17A level (OR 1.024, 95% CI 1.009–1.038, $P = 0.032$), and *IL17A* rs2275913 AG and GG (OR 1.068, 95% CI 1.023–1.115, $P = 0.002$) were significantly associated with HCC risk in multivariate analysis (Table 4).

Serum IL-17A and IgE Levels and the Associations With *IL17A* Polymorphisms

Serum IL-17A levels in HBV patients (1.59–463.67, median 20.09 pg/ml) were elevated compared with those in resolvers (1.48–19.79, median, 6.224 pg/ml, $P < 0.001$) or healthy controls (1.63–37.00, median, 5.469 pg/ml, $P < 0.001$) with no difference between resolvers and controls ($P = 0.699$). According to clinical diagnoses, serum IL-17A levels in HCC patients were higher than those in cirrhosis ($P = 0.005$) or chronic hepatitis ($P = 0.012$) with no significance between cirrhosis and chronic hepatitis (Figure 1A). Analogously, serum IgE levels in HBV patients (130.09–7192.88, median 240.07 pg/ml) were elevated compared with those in resolvers (28.74–320.30, median, 117.20 pg/ml, $P < 0.001$) or healthy controls (45.89–503.75, median, 71.36 pg/ml, $P < 0.001$) with no difference between resolvers and controls ($P = 0.780$). According to clinical diagnoses, serum IgE levels in HCC patients were higher than those in cirrhosis ($P < 0.001$) and chronic hepatitis ($P < 0.001$) with no significance between cirrhosis and chronic hepatitis (Figure 1B).

Serum IL-17A and IgE levels had no significant association with *IL17A* rs8193036 genotypes in HBV patients, HBV infection resolvers and healthy controls (data not shown). However, serum IL-17A levels were significantly associated with *IL17A* rs2275913 polymorphism, with genotype GG having higher IL-17A levels than genotype AG or AA in the HBV patients, resolvers and healthy controls (all $P < 0.001$, Figure 2A). Similarly, serum IgE levels were significantly associated with *IL17A* rs2275913 polymorphism, with genotype GG having higher IgE levels than genotypes AG or AA in the HBV

Table 2. Genotype, Allele, and Haplotype Frequencies of IL17A Polymorphisms in Hepatitis B Virus (HBV) Patients With Different Clinical Diagnoses

	CH (n = 134)	LC (n = 125)	HCC (n = 136)	P-value	CH versus LC		HCC versus CH		HCC versus LC	
					P-value	OR (95% CI)	P-value	OR (95 % CI)	P	OR (95% CI)
rs8193036										
Genotype										
CC	75 (56.0)	78 (62.4)	76 (55.9)	Reference						
CT	48 (35.8)	40 (32.0)	50 (36.8)	0.598	0.409	1.248 (0.738–2.112)	0.915	1.028 (0.618–1.709)	0.349	1.283 (0.761–2.163)
TT	11 (8.2)	7 (5.6)	10 (7.3)	0.611	0.332	1.634 (0.602–4.439)	0.816	0.897 (0.360–2.237)	0.458	1.466 (0.531–4.051)
Allele										
C	198 (73.9)	196 (78.4)	202 (74.3)	Reference						
T	70 (26.1)	54 (21.6)	70 (25.7)	0.420	0.228	1.283 (0.855–1.926)	0.919	0.980 (0.667–1.440)	0.267	1.258 (0.838–1.887)
rs2275913										
Genotype										
AA	46 (34.3)	40 (32.0)	24 (17.6)	Reference						
AG	62 (46.3)	68 (54.4)	67 (49.3)	0.056	0.404	0.793 (0.459–1.369)	0.017	2.071 (1.134–3.783)	0.109	0.609 (0.331–1.119)
GG	26 (19.4)	17 (13.6)	45 (33.1)	<0.001	0.452	1.330 (0.632–2.798)	0.001	3.317 (1.663–6.617)	<0.001	4.412 (2.077–9.370)
Allele										
A	154 (57.5)	148 (59.2)	115 (42.3)	Reference						
G	114 (42.5)	102 (40.8)	157 (57.7)	<0.001	0.689	1.074 (0.757–1.524)	<0.001	1.844 (1.311–2.595)	<0.001	1.981 (1.398–2.807)
Haplotype (rs8193036/rs2275913)										
CA	114 (42.6)	115 (45.9)	85 (31.1)	Reference						
CG	84 (31.3)	81 (32.5)	117 (43.2)	0.001	0.825	0.956 (0.641–1.426)	0.002	1.868 (1.256–2.778)	0.001	1.954 (1.312–2.911)
TA	40 (14.9)	33 (13.3)	30 (11.2)	0.700	0.456	1.223 (0.721–2.075)	0.983	0.994 (0.573–1.724)	0.475	0.813 (0.461–1.435)
TG	30 (11.3)	21 (8.3)	40 (14.6)	0.005	0.243	1.441 (0.779–2.665)	0.037	1.788 (1.031–3.101)	0.002	2.577 (2.417–4.686)

CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; OR, odds ratio; 95% CI, 95% confidence intervals. Data of genotype, allele, and haplotype are presented as n (%).

Table 3. Univariate and Multivariate Analyses of Risk Factors Associated With HBV-Related HCC in Comparison With Chronic HBV Infection Without HCC

Variable	Patients without HCC (n = 259)	Patients with HCC (n = 136)	Univariate analysis P-value	Multivariate analysis	
				OR (95% CI)	P-value
Gender (male/female)	180/79	119/17	<0.001	4.316 (2.299–8.100)	<0.001
Mean age (yr)	40.51 ± 13.38	50.78 ± 10.21	<0.001	1.064 (1.041–1.087)	<0.001
HBV DNA (log IU/ml)	5.49 ± 1.75	4.87 ± 1.49	0.006	1.000 (1.000–1.000)	0.078
ALT (IU/L)	73 (2–1,632)	49 (6–3,629)	0.001	0.999 (0.998–1.001)	0.250
AST (IU/L)	75 (15–1,293)	64 (15–4,082)	0.444	—	—
Total bilirubin (μmol/L)	28.70 (2.00–708.90)	24.10 (1.40–727.20)	0.005	0.999 (0.996–1.001)	0.242
Albumin (g/L)	34.50 (14.20–50.70)	33.90 (21.10–51.30)	0.577	—	—
HBeAg (+/–)	139/120	49/87	0.001	1.302 (0.778–2.177)	0.315
IL-17A (pg/ml)	16.62 (1.59–118.57)	25.45 (8.24–463.67)	<0.001	1.020 (1.003–1.036)	0.019
IgE (pg/ml)	219.43 (130.09–2193.36)	290.07 (138.51–7192.88)	0.002	1.000 (0.999–1.000)	0.360
IL17A rs8193036 polymorphism					
CC versus CT and TT	153/106	76/60	0.519	—	—
IL17A rs2275913 polymorphism					
AA versus AG and GG	86/173	24/112	0.001	1.704 (1.214–2.390)	0.006

HCC, hepatocellular carcinoma; OR, odds ratio; 95% CI, 95% confidence intervals; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

patients, resolvers, and healthy controls (all $P < 0.001$, Figure 2B).

DISCUSSION

The present study, to the best of our knowledge, is the first study to investigate a potential influence of *IL17A* gene polymorphisms in HBV-associated liver diseases. Genotyping of the two *IL17A* SNPs rs8193036 and rs2275913 showed that the distribution of genotypes from rs8193036 polymorphism,

located at position –737 from the start codon of the *IL17A* gene, was different, and that the distribution of genotypes from rs2275913 polymorphism, located at position –197 from the start codon of the *IL17A* gene, was not different between patients with chronic HBV infection, HBV infection resolvers, and healthy controls.

The *IL17A* rs8193036 allele T, genotype CT and the combined genotypes CT and TT were more frequent in HBV infection resolvers than in HBV patients and healthy controls but no significant

Table 4. Univariate and Multivariate Analyses of Risk Factors Associated With HCC in Comparison With Cirrhosis Without HCC

Variable	LC (n = 125)	HCC (n = 136)	Univariate analysis P-value	Multivariate analysis	
				OR (95% CI)	P-value
Gender (male/female)	83/42	119/17	<0.001	3.645 (1.803–7.370)	<0.001
Mean age (yr)	46.21 ± 11.48	50.78 ± 10.21	0.001	1.051 (1.023–1.080)	<0.001
HBV DNA (log IU/ml)	5.17 ± 1.62	4.87 ± 1.49	0.176	—	—
ALT (IU/L)	48 (2–504)	49 (6–3,629)	0.449	—	—
AST (IU/L)	55 (19–483)	64 (15–4,082)	0.141	—	—
Total bilirubin (μmol/L)	24.80 (6.50–238.10)	24.10 (1.40–727.20)	0.111	—	—
Albumin (g/L)	29.70 (14.23–50.70)	33.90 (21.10–51.30)	<0.001	1.785 (0.930–3.425)	0.081
HBeAg (+/–)	56/69	49/87	0.150	—	—
IL-17A (pg/ml)	18.07 (1.59–81.50)	25.45 (8.24–463.67)	0.001	1.024 (1.009–1.038)	0.032
IgE (pg/ml)	222.12 (136.20–871.73)	290.07 (138.51–7192.88)	0.018	1.000 (0.999–1.002)	0.076
IL17A rs8193036 polymorphism					
CC versus CT and TT	78/47	76/60	0.735	—	—
IL17A rs2275913 polymorphism					
AA versus AG and GG	40/85	24/112	0.007	1.068 (1.023–1.115)	0.002

LC, liver cirrhosis without HCC; HCC, hepatocellular carcinoma (most of the cases had underlying cirrhosis); OR, odds ratio; 95% CI, 95% confidence intervals; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

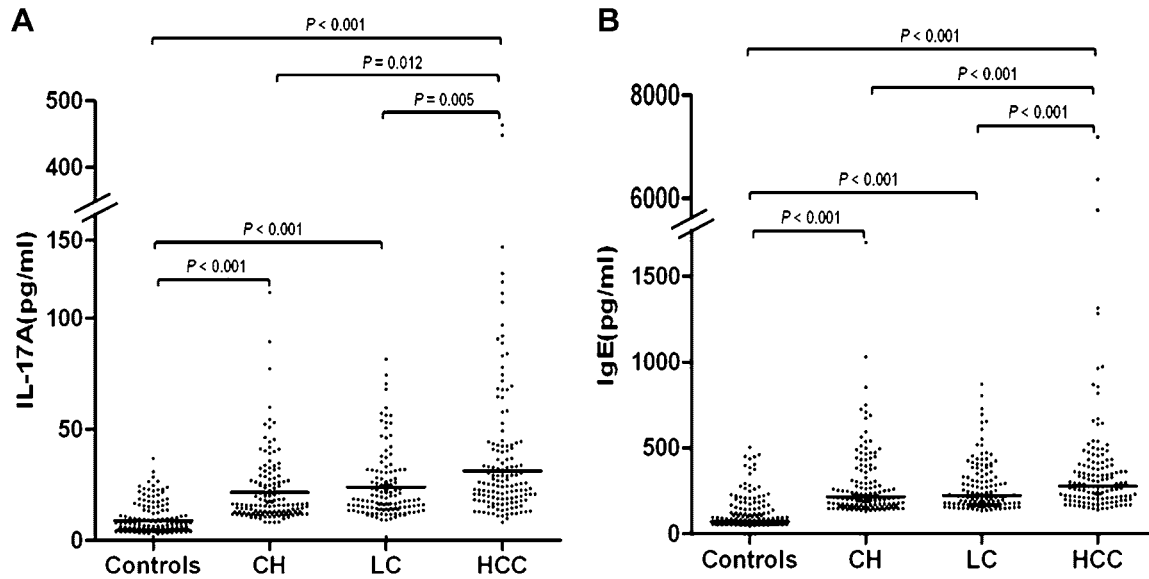


Figure 1. Serum IL-17A and IgE levels in patients with different clinical diagnoses. The (A) serum IL-17A levels and (B) serum IgE levels in patients with chronic hepatitis B virus (HBV) infection according to clinical diagnoses. CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

difference between HBV patients and healthy controls. HBV infection in China, a country with high prevalence of HBV infection, is commonly acquired during the perinatal period via mother-to-child transmission and this is related to high incidence of chronicity of the infection [51,52]. The occurrence of HBV infection depends on the opportunities of exposure to the virus, while the outcome of infection is determined by the interaction between the host and viral factors [51–53]. It is, therefore,

suggested that *IL17A* rs8193036 polymorphism may associate with the persistence or resolution instead of the susceptibility of HBV infection. Scilicet, *IL17A* rs8193036 allele T and allele T-containing genotypes (CT and TT) and haplotypes may potentiate the resolution of HBV infection, whereas the genotype CC and allele C may predispose the chronicity of infection. This result is supported by the investigation in T cell-depleted allogeneic stem cell transplantation patients suggesting that *IL17A* rs8193036 TC and

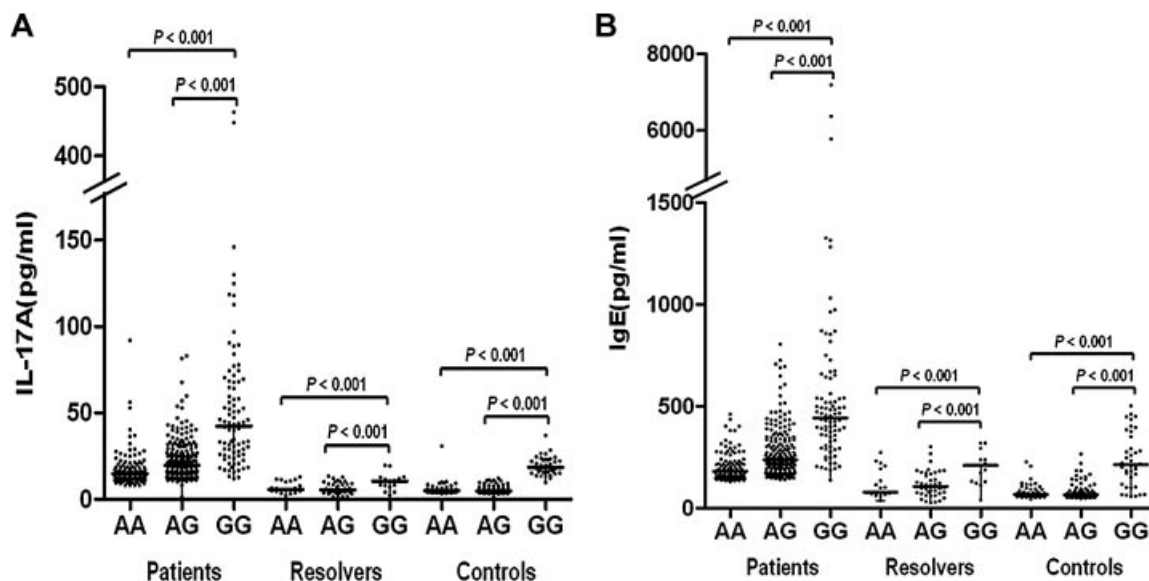


Figure 2. Associations of serum IL-17A and IgE levels with *IL17A* rs2275913 single nucleotide polymorphism. The (A) serum IL-17A levels and (B) serum IgE levels according to *IL17A* rs2275913 genotypes in patients with chronic HBV infection, HBV infection resolvers and healthy controls. Patients, patients with chronic HBV infection; resolvers, HBV infection resolvers; and controls, healthy controls.

CC genotypes were associated with increased risk of cytomegalovirus infection and patient acute graft-versus-host disease, respectively [33]. The rs8193036 polymorphism was found to be associated with neither clinical diagnoses nor serum IL-17A and IgE levels in HBV patients, HBV infection resolvers, and healthy controls, indicating the less relevance of this polymorphism to disease progression and the involvement of mechanisms other than alteration of IL-17A cytokine and IgE production pertaining to this polymorphism in promoting or temporizing the resolution of HBV infection. Replicative studies are needed to confirm and clarify the role of rs8193036 polymorphism in chronic HBV infection thanks to the small sample size of populations especially HBV infection resolvers in our study.

Interestingly and notably, the distribution of genotypes and alleles of *IL17A* rs2275913 polymorphisms, though not significant between HBV patients, HBV infection resolvers, and healthy controls, was significantly different between HBV patients with different clinical diseases. HCC patients had a higher frequency of *IL17A* rs2275913 genotype GG and allele G compared with patients with chronic hepatitis or cirrhosis. Multivariate analysis showed that gender, age, serum IL-17A levels, and *IL17A* rs2275913 polymorphism were factors independently associated with HCC risk in relation to either all other chronic HBV patients without HCC or cirrhosis patients without HCC. The gender and age in patients with chronic HBV infection are well-defined factors associated with HCC risk [54–57]. *IL17A* rs2275913 polymorphism was recently shown to be associated with the development of some subtypes of gastric cancer [34]. Therefore, the present study adds novel genetic information to the risk factors of HCC associated with HBV infection.

The serum IL-17A levels in HCC patients were significantly higher than those in chronic hepatitis or cirrhosis. This is consistent with previous studies showing close correlations of increased Th17 cells in peripheral blood and tumor tissues of HCC patients [25–27]. IL-17A has been shown to promote carcinogenesis and HCC metastasis and mediate immune tolerance in tumors through various pathways [27,58,59]. *IL17A* rs2275913 polymorphism was found to be significantly associated with serum IL-17A levels in this study. *IL17A* rs2275913 genotype GG, which was more frequent in HCC patients than patients without HCC in chronic HBV infection, was associated with significantly increased IL-17A levels. It is, therefore, suggested that this polymorphism may, at least in part, predispose the development of HCC associated with HBV by altering the production of IL-17A cytokine.

In the *IL17A* block, the frequency of haplotypes TA and TG of rs8193036 and rs2275913 was lower in HBV patients than in resolvers and the frequency

of haplotype TG was higher in resolvers than in controls, suggesting the potential role of rs8193036 allele T in promoting the recovery of HBV infection. The frequency of haplotype CG and TG was higher in HCC patients than in HBV patients without HCC, suggesting the predisposing role of rs2275913 allele G in HCC development. It is still not clear whether polymorphisms in the *IL17A* gene are directly responsible for regulating IL-17A production or whether they are functionally in linkage disequilibrium with other regulatory genetic loci.

B-cell activation was recently shown to be common in chronic HBV infection [60], serum IgE levels were indicated to be related to the clinical course in patients with HBV infection [36–38], IgE was suggested to be possibly contribute to antitumour immune responses [40,41], and IL-17A was revealed to play a role in promoting IgE production [39]. For these reasons, the present study determined serum IgE levels and the correlations with *IL17A* polymorphisms. Although IgE levels were elevated in HCC patients, correlated with rs2275913, and were significant in univariate analysis, multivariate analysis did not show the significance in relation to HCC development. Therefore, further studies are needed to clarify the role of IgE response in the development of HCC associated with HBV infection.

Recently, one intronic SNP (rs17401966) in kinesin family member 1B gene (KIF1B) on chromosome 1p36.22 was identified to be highly associated with HBV-related HCC by genome-wide association study (GWAS) in Chinese populations [61]. However, subsequent studies did not replicate this association in Chinese population [62] and other East Asian populations [63]. The genetic diversity among populations and the high complexity of multivariate interactions between the genomic information and HBV viral and environmental factors may be the reasons for these inconsistencies. Furthermore, there may be multiple independent disease susceptibility polymorphisms and each polymorphism may have a distinct and modest phenotypic effect. The association of *IL17A* polymorphism and IL-17A level with HBV-related HCC risk found in the present study is primarily relevant to the aspect of host immune response in chronic HBV infection. Future studies may need to address not only the identification of genes involved in HCC risk but also the interactions between disease susceptible genes and between these genes and other factors such as HBV viral and environmental factors in the development of HBV-related HCC.

Our study was performed in a relatively small number of patients with only two SNPs being examined, and the definite functional elucidation of the SNPs is still in scarcity. HBV infection is associated with a diversity of clinical diseases involving multiple factors. Our study also had some other potential limitations and alternative explanations. These

include chance, particularly in the context of multiple comparisons, and non-inclusion of other known and unknown confounders such as the specific duration of infection and family history of cancers, especially HCC, as well as socioeconomic status. Therefore, further investigation in large sample size of population is definitely warranted to replicate, confirm, and extend our results.

In conclusion, although validation studies are ultimately required, our findings in this study suggest that *IL17A* SNPs were associated with chronic HBV infection and HCC risk possibly through influencing IL-17 production. Further elucidation of the biological functions of *IL17A* SNPs may lead to the understanding of genetic component in the susceptibility of chronic HBV infection and carcinogenesis related to HBV infection as well as the precise monitoring of disease progression using genetic biomarkers and the novel therapeutic designs for chronic HBV infection and HCC by regulating IL-17A molecule based on individualized consideration of genetic background.

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