



IL21 and *IL21R* polymorphisms and their interactive effects on serum IL-21 and IgE levels in patients with chronic hepatitis B virus infection

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ABSTRACT

Interleukin (IL)-21 may affect both T-cell and B-cell responses and was suggested to be involved in response to HBV infection. This study explored *IL21* rs907715 and rs2221903 and *IL21R* T-83C and rs3093301 polymorphisms and serum IL-21 and IgE levels in 395 patients with chronic HBV infection, 75 HBV infection resolvers and 174 healthy controls. *IL21R* T-83C was not polymorphic in the study populations. *IL21* rs2221903 AG was less frequent in HBV patients than in resolvers ($p < 0.001$, OR = 0.364, 95% CI = 0.211–0.629) or in controls ($p = 0.017$, OR = 0.589, 95% CI = 0.381–0.911). *IL21R* rs3093301 TT was more frequent in HBV patients than in controls [p value after Bonferroni correction (p_c) = 0.022, OR = 1.908, 95% CI = 1.158–3.142] and more frequent in resolvers than in controls ($p_c = 0.010$, OR = 2.965, 95% CI = 1.375–6.392). The carriage of *IL21* rs2221903 AG/*IL21R* rs3093301 CT + *IL21* rs2221903 AG/*IL21R* rs3093301 TT was less frequent in patients than in resolvers ($p_c = 0.007$, OR = 0.236, 95% CI = 0.096–0.579) and more frequent in resolvers than in controls ($p_c = 0.014$, OR = 4.354, 95% CI = 1.660–11.420). *IL21* rs2221903 was, by interaction with *IL21R* rs3093301, associated with serum IL-21 and IgE levels in HBV patients. It is suggested that *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms may, independently or interactively, affect the susceptibility to and/or persistence of HBV infection potentially through altering IL-21 and IgE production.

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1. Introduction

Infection with hepatitis B virus (HBV) is associated with a significantly heterogeneous disease courses and clinical outcomes including spontaneous resolution of the infection, acute and chronic hepatitis, hepatic cirrhosis and hepatocellular carcinoma (HCC), relying on viral replication and host immune response [1,2].

Interleukin (IL)-21, which is mainly expressed by activated CD4⁺ T cells and natural killer (NK) cells, is a novel member of the type I cytokine superfamily [3]. Upon binding to its receptor (IL-21R) expressed on a variety of cells including NK cells, T cells, B cells, and dendritic cells [4–6], IL-21 plays an important role in regulating the proliferation and maturation of NK cells and the proliferation of T cells and in figuring the effector function of B cells including influencing the production of human IgE [4,7–10]. Genet-

ically, studies indicated that *IL21* and *IL21R* gene polymorphisms were associated with systemic lupus erythematosus (SLE) [11,12] and *IL21R* polymorphism may influence IgE production [13,14], suggesting the possible relevance of *IL21* and *IL21R* gene polymorphisms to the disease susceptibility and immunopathogenesis. Concerning HBV infection, in addition to T cell and NK cell mediated immune responses [15–17], B-cell activation was shown to be common in chronic HBV infection [18] and serum IgE levels were indicated to be related to the clinical course in patients with HBV infection [19–21]. Recent studies suggest that IL-21 plays a pivotal role in determining age-dependent effectiveness of immune responses to HBV infection [22] and in predicting HBeAg seroconversion in chronic hepatitis B patients treated with telbivudine [23] and that upregulated IL-21 is associated with the severity of HBV-related acute-on-chronic liver failure [24].

These data make *IL21* and *IL21R* interesting candidate genes in chronic HBV infection. Accordingly, this study examined the *IL21* rs907715 and rs2221903 and *IL21R* T-83C and rs3093301 polymorphisms and their possible effects on serum IL-21 and IgE levels in Chinese Han populations including patients with chronic HBV

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infection, individuals spontaneously recovered from HBV infection and healthy controls without HBV infection.

2. Methods

2.1. Study subjects

A total of 644 adults including 395 patients with chronic HBV infection (male/female, 299/96; age, 44.05 ± 13.30 years), 75 HBV infection resolvers (male/female, 49/26; age, 45.49 ± 14.29 years) and 174 healthy controls (male/female, 127/47; age, 42.23 ± 13.42 years) were included in this study. The sex and age had no statistical differences between HBV patients, spontaneous resolvers and healthy controls ($p > 0.05$). Of the 395 patients with chronic HBV infection, 134 patients were diagnosed with chronic hepatitis, 125 patients were diagnosed with liver cirrhosis and 136 patients were diagnosed with HCC as had been described elsewhere [25]. Chronic HBV infection was established according to the serological positivity for HBsAg, HBeAg or anti-HBe and anti-HBc for more than 6 months. The patients with chronic HBV infection were diagnosed chronic hepatitis, liver cirrhosis or HCC according to the diagnostic criteria described elsewhere [26,27]. In brief, among the subjects with chronic HBV infection, those who had persistent or frequent fluctuation of abnormal liver function and had no evidence of liver cirrhosis and HCC on ultrasonography and/or computerized tomography (CT) and/or magnetic resonance imaging (MRI) were diagnosed as chronic hepatitis, those who had pathological manifestations of cirrhosis in liver tissue section of surgery, or exhibited abnormal liver function and manifested portal hypertension with ascites and esophageal varices on endoscopic examination, small liver size, irregular liver surface and splenomegaly on ultrasonography and/or CT and/or MRI, and excluded the existence of HCC were diagnosed as cirrhosis, and those who had either pathological examination of liver tissue section of surgery or characteristics of HCC image of angiography, ultrasonography and CT and/or MRI with or without the elevation of α -fetoprotein level were diagnosed as HCC. The 75 subjects defined as HBV infection resolvers were those who spontaneously recovered from HBV infection and were negative for HBsAg, HBeAg and anti-HBe and positive for anti-HBs and anti-HBc IgG. The 174 healthy controls were those who were negative for HBsAg, HBeAg, anti-HBe and anti-HBc without a history of hepatitis and with or without positive anti-HBs depending on the history of hepatitis B vaccination. Infection of other hepatitis viruses such as hepatitis A virus, hepatitis C virus and hepatitis E virus and human immunodeficiency virus, autoimmune disorders and other diseases unrelated to HBV infection were excluded. All the participants were of Han nationality, unrelated in kinship and resided in the same geographical area. Informed consent was obtained from all the individuals. The study protocol conformed to the Declaration of Helsinki and was approved by the Ethics Committee of First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University.

2.2. Genotyping of *IL21* and *IL21R* gene polymorphisms

Genomic DNA was isolated from whole blood using TIANamp Genomic DNA Kit [Tiagen Biotech (Beijing) Co., Ltd., China] according to the manufacturer's instruction. Genotyping of the *IL21* and *IL21R* gene polymorphisms was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

A 222-bp PCR fragment including the *IL21* rs907715 polymorphism was amplified using the primers: 5'-CAATGGCTTGGTGTTTGGTAT-3' (upstream) and 5'-CTCTTTTCACTTGAGCATTC-3' (downstream), a 236-bp PCR fragment including the *IL21* rs2221903 polymorphism was amplified using the primers: 5'-

GGTACCTGGACACTGACGCCCA-3' (upstream) and 5'-AAGGCAGTTT AGTGGCGACAGCC-3' (downstream), a 229-bp PCR fragment including the *IL21R* T-83C polymorphism was amplified using the primers: 5'-GGTGTAGAGCCTGATGGGACCATTCGTGTCACTTTCGA-3' (upstream) and 5'-CCGTGCTTCATGAGAAAGATG-3' (downstream), and a 183-bp PCR fragment including the *IL21R* rs3093301 polymorphism was amplified using the primers: 5'-CCCTCCCTCTTCTTGTAG-3' (upstream) and 5'-TCCTCCTACCTCGGCCTCTCAAAGTGCTGGGATGACACGC-3' (downstream).

PCR was conducted in a total volume of 25 μ l containing 3.0 μ l (0.3 μ g) of DNA, 1.0 μ l (10 μ M) of each primer, 12.5 μ l of 2 $\times$ Master Mix (2 $\times$ Taq PCR Mix, Xi'an Runde Biotechnology Ltd., Xi'an, China), and 7.5 μ l of double distilled H₂O. Samples were denatured at 94 °C for 3 min followed by 30 (for *IL21* rs907715 and rs2221903) or 35 (for *IL21R* T-83C and rs3093301) cycles at 94 °C for 30 s, 55 °C (for *IL21* rs907715), 60 °C (for *IL21* rs2221903), 58 °C (for *IL21R* T-83C) or 56 °C (for *IL21R* rs3093301) for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min. The fragments were digested using *HpyCH4V*, *Mbo* II, *Aat* II and *Mlu* I (New England Biolabs (Beijing) LTD, Beijing, China) for *IL21* rs907715, *IL21* rs2221903, *IL21R* T-83C and *IL21R* rs3093301 polymorphisms, respectively, according to the manufacturer's instructions, and analyzed by separating with 2% agarose gels stained with ethidium bromide. The genotypes of each polymorphism were determined according to the digestion patterns.

2.3. Determination of serum *IL-21* and IgE levels

Serum *IL-21* and IgE concentrations were determined using commercially available *IL-21* Kit (BioVendor Laboratory Medicine, Inc. Guangzhou, China) and ELISA Kit for Human Immunoglobulin E (Uscn Life Science Inc. Wuhan, China), respectively, according to the instructions of the manufacturers.

2.4. Statistical analysis

Statistical analyses were carried out using a SPSS13.0 software (SPSS Inc., Chicago, IL, USA). The chi-square test or student's *t*-test was used to compare parameters between groups. The chi-square test or Fisher's exact test was used to estimate the Hardy–Weinberg equilibrium (HWE) and to compare the genotype and allele frequencies between the groups. Odds ratios (OR) with 95% confidence interval (CI) were calculated by logistic regression analyses adjusted for age and sex. Haplotype analysis was performed using SHEsis platform [28]. Serum *IL-21* and IgE levels were expressed with median (range) and compared using the rank sum test. A *p* value less than 0.05 was considered significant. A corrected *p* value (*p_c*) was calculated by Bonferroni correction for multiple comparisons when the uncorrected *p* value was less than 0.05.

3. Results

3.1. Hardy–Weinberg equilibrium of the polymorphisms

The distributions of the genotypes in all the polymorphisms except *IL21R* T-83C which was not polymorphic in the study populations were in Hardy–Weinberg equilibrium (Supplementary Table S1), suggesting the representativeness of the study populations.

3.2. Genotype, allele and haplotype frequencies of *IL21* polymorphisms

The distribution of genotypes and alleles of *IL21* rs907715 polymorphisms showed no significant difference among the patients, resolvers and healthy controls (Table 1). However, *IL21* rs2221903 polymorphism was differently distributed among the

Table 1Genotype, allele and haplotype frequencies of *IL21* polymorphism in hepatitis B virus (HBV) patients, HBV infection resolvers and healthy controls.

		Patients (n = 395)	Resolvers (n = 75)	Controls (n = 174)	p	Patients vs. Resolvers		Patients vs. Controls		Resolvers vs. Controls	
						p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)
rs907715											
Genotype											
GG	145 (36.7)	22 (29.3)	60 (34.5)	Reference							
GA	176 (44.6)	37 (49.3)	76 (43.7)	0.533	0.262	0.722 (0.407–1.278)	0.836	1.044 (0.697–1.562)	0.375	1.328 (0.709–2.485)	
AA	74 (18.7)	16 (21.4)	38 (21.8)	0.495	0.321	0.702 (0.348–1.416)	0.391	0.806 (0.492–1.320)	0.722	0.871 (0.407–1.865)	
Allele											
G	466 (59.0)	93 (62.0)	196 (56.3)	Reference							
A	324 (41.0)	57 (38.0)	152 (43.7)	0.470	0.491	1.134 (0.792–1.624)	0.401	0.897 (0.695–1.157)	0.239	0.790 (0.534–1.169)	
rs2221903											
Genotype											
AA	331 (83.8)	49 (65.3)	131 (75.3)	Reference							
AG	64 (16.2)	26 (34.7)	43 (24.7)	<0.001	<0.001	0.364 (0.211–0.629)	0.017	0.589 (0.381–0.911)	0.107	1.617 (0.899–2.908)	
GG	0 (0)	0 (0)	0 (0)								
Allele											
A	726 (91.9)	124 (82.7)	305 (87.6)	Reference							
G	64 (8.1)	26 (17.3)	43 (12.4)	0.001	<0.001	0.420 (0.257–0.689)	0.023	0.625 (0.415–0.941)	0.140	1.487 (0.876–2.526)	
rs907715 and rs2221903 haplotype											
AA	298 (37.8)	57 (38.0)	137 (39.4)	Reference							
AG	26 (3.2)	12 (8.0)	15 (4.3)	0.058	0.017 ^a	0.414 (0.198–0.869)	0.504	0.797 (0.409–1.552)	0.114	1.923 (0.847–4.364)	
GA	428 (54.1)	67 (44.7)	168 (48.3)	0.375	0.304	1.222 (0.833–1.792)	0.251	1.171 (0.894–1.534)	0.843	0.959 (0.630–1.458)	
GG	38 (4.9)	14 (9.3)	28 (8.0)	0.076	0.054	0.519 (0.264–1.020)	0.078	0.624 (0.368–1.058)	0.613	1.202 (0.590–2.449)	

Data are presented as n (%). OR, Odds Ratio; 95%CI, 95% Confidence Intervals.

^a p_c (p value after Bonferroni correction) = 0.051.

patients, resolvers and healthy controls (Table 1). *IL21* rs2221903 genotype AG frequency in patients, controls and resolvers was 16.2%, 24.7% and 34.7%, respectively. This rs2221903 genotype AG frequency in HBV patients was lower than that in controls ($p = 0.017$, OR = 0.589, 95% CI = 0.381–0.911) or that in resolvers ($p < 0.001$, OR = 0.364, 95% CI = 0.211–0.629) with no difference between resolvers and controls (Table 1). The rs2221903 allele G frequency in patients, controls and resolvers was 8.1%, 12.4% and 17.3%, respectively. This rs2221903 allele G frequency in HBV patients was lower than that in controls ($p = 0.023$, OR = 0.625, 95% CI = 0.415–0.941) or that in resolvers ($p < 0.001$, OR = 0.420, 95% CI = 0.257–0.689) with no difference between resolvers and controls (Table 1).

The frequency of *IL21* rs907715 and rs2221903 haplotype had no significant difference among HBV patients, HBV infection resolvers and healthy controls (Table 1).

The genotype and allele frequencies of *IL21* rs907715 and rs2221903 polymorphisms and the frequency of *IL21* rs907715 and rs2221903 haplotype had no significant difference according to the clinical diagnoses of the patients (Supplementary Table S2).

3.3. Genotype and allele frequencies of *IL21R* polymorphisms

IL21R T-83C locus was not polymorphic in the subjects genotyped in this study. *IL21R* rs3093301 polymorphism was significantly different between HBV patients, infection resolvers and healthy controls (Table 2). The genotype TT frequency in patients, resolvers and controls was 26.3%, 32.0% and 19.5%, respectively. Although this genotype TT frequency between patients and resolvers had no significant difference, it was higher in HBV patients than that in controls ($p_c = 0.022$, OR = 1.908, 95% CI = 1.158–3.142) and it was higher in resolvers than that in controls ($p_c = 0.010$, OR = 2.965, 95% CI = 1.375–6.392, Table 2).

The *IL21R* rs3093301 allele T frequency in patients, controls and resolvers was 50.3%, 41.7% and 56.0%, respectively. The frequency of this allele between HBV patients and resolvers had no significant difference. This allele frequency was higher in HBV patients than in controls ($p = 0.007$, OR = 1.421, 95% CI = 1.102–1.834) and was much higher in resolvers than in controls ($p = 0.003$, OR = 1.782, 95% CI = 1.211–2.622, Table 2).

The frequency of *IL21R* rs3093301 genotype and allele had no significance according to clinical diagnoses (Supplementary Table S3).

3.4. Interaction of *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms

In view of the interaction between *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms, the carriage of *IL21* rs2221903/*IL21R* rs3093301 AG/CT in HBV patients (27/395, 6.8%) was significantly lower than that in resolvers (13/75, 17.3%, $p_c = 0.007$, OR = 0.198, 95% CI = 0.074–0.528) and was more frequent in resolvers than in controls (16/174, 9.2%, $p_c = 0.028$, OR = 4.469, 95% CI = 1.563–12.774) although was not significant between patients and controls (Table 3). The carriage of *IL21* rs2221903/*IL21R* rs3093301 AA/TT was more frequent in resolvers (18/75, 24.0%) than in controls (26/174, 14.9%, $p_c = 0.035$, OR = 3.808, 95% CI = 1.453–9.981) and there were no significant difference between patients (84/395, 21.3%) and resolvers or between patients and controls. The carriage of *IL21* rs2221903/*IL21R* rs3093301 AG/CT + AG/TT was less frequent in patients (47/395, 11.9%) than in resolvers (19/75, 25.3%, $p_c = 0.007$, OR = 0.236, 95% CI = 0.096–0.579), more frequent in resolvers than in controls (24/174, 13.8%, $p_c = 0.014$, OR = 4.354, 95% CI = 1.660–11.420) and no significant difference between patients and controls (Table 3).

No combined association between *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms and clinical diagnoses was observed (Data not shown).

3.5. Associations of *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms with serum IL-21 and IgE levels

Serum IL-21 levels in HBV patients [237.933 (111.38–2996.29) pg/ml] were significantly elevated compared with healthy controls [68.346 (27.72–360.11) pg/ml, $p < 0.001$] or HBV infection resolvers [119.154 (24.39–310.34) pg/ml, $p < 0.001$]. Serum IgE levels in HBV patients [240.070 (130.09–7192.88 pg/ml)] were also significantly increased compared with healthy controls [71.363 (45.89–503.75) pg/ml, $p < 0.001$] or HBV infection resolvers [117.202 (28.74–320.3) pg/ml, $p < 0.001$].

Table 2Genotype and allele frequencies of *IL21R* polymorphism in hepatitis B virus (HBV) patients, HBV infection resolvers and healthy controls.

<i>IL21R</i> rs3093301	Patients (<i>n</i> = 395)	Resolvers (<i>n</i> = 75)	Controls (<i>n</i> = 174)	<i>p</i>	Patients vs. Resolvers		Patients vs. Controls		Resolvers vs. Controls	
					<i>p</i>	OR (95%CI)	<i>p</i>	OR (95%CI)	<i>p</i>	OR (95%CI)
<i>Genotype</i>										
CC	101 (25.6)	15 (20.0)	63 (36.2)	Reference						
CT	190 (48.1)	36 (48.0)	77 (44.3)	0.057	0.461	1.276 (0.667–2.441)	0.039 ^b	1.539 (1.020–2.322)	0.053	1.964(0.987–3.908)
TT	104 (26.3)	24 (32.0)	34 (19.5)	0.007 ^a	0.215	0.644 (0.319–1.297)	0.011 ^c	1.908 (1.158–3.142)	0.005 ^d	2.965 (1.375–6.392)
<i>Allele</i>										
C	392 (49.7)	66 (44.0)	203 (58.3)	Reference						
T	398 (50.3)	84 (56.0)	145 (41.7)	0.004	0.207	0.798 (0.561–1.133)	0.007	1.421 (1.102–1.834)	0.003	1.782 (1.211–2.622)

Data are presented as n (%). OR, Odds Ratio; 95%CI, 95% Confidence Intervals.

^a p_c (p value after Bonferroni correction) = 0.014.^b p_c = 0.078.^c p_c = 0.022.^d p_c = 0.010.**Table 3**Combined effect of *IL21* (rs2221903) and *IL21R* (rs3093301) polymorphism in hepatitis B virus (HBV) patients, HBV infection resolvers and healthy controls.

<i>IL21</i> rs2221903	<i>IL21R</i> rs3093301	Patients (n = 395)	Resolvers (n = 75)	Controls (n = 174)	p	Patients vs. Resolvers		Patients vs. Controls		Resolvers vs. Controls	
						p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)
AA	CC	84 (21.3)	8 (10.7)	44 (25.3)	Reference						
AG	CC	17 (4.3)	7 (9.3)	19 (10.9)	0.013 ^A	0.008 ^{a1}	0.231 (0.074–0.724)	0.045 ^{a2}	2.134 (1.009–4.513)	0.223	0.494 (0.157–1.556)
AA	CT	163 (41.3)	23 (30.7)	61 (35.1)	0.183	0.360	1.482 (0.635–3.454)	0.159	1.400 (0.876–2.236)	0.105	0.482 (0.197–1.178)
AG	CT	27 (6.8)	13 (17.3)	16 (9.2)	0.002 ^B	0.001 ^{b1}	0.198 (0.074–0.528)	0.736	0.884 (0.431–1.812)	0.004 ^{b2}	4.469 (1.563–12.774)
AA	TT	84 (21.3)	18 (24.0)	26 (14.9)	0.016 ^C	0.068	0.444 (0.183–1.078)	0.070	1.692 (0.956–2.997)	0.005 ^{c1}	3.808 (1.453–9.981)
AG	TT	20 (5.0)	6 (8.0)	8 (4.6)	0.071	0.079	0.317 (0.099–1.018)	0.661	1.310 (0.534–3.212)	0.026 ^d	4.125 (1.125–15.124)
AA	CT+TT	247 (62.5)	41 (54.7)	87 (50.0)	0.046 ^E	0.167	1.743 (0.786–3.867)	0.076	1.487 (0.959–2.307)	0.023 ^{e1}	2.592 (1.119–6.003)
AG	CT+TT	47 (11.9)	19 (25.3)	24 (13.8)	0.003 ^F	0.001 ^{f1}	0.236 (0.096–0.579)	0.935	0.975 (0.529–1.798)	0.002 ^{f2}	4.354 (1.660–11.420)

Data are presented as n (%). OR, Odds Ratio; 95%CI, 95% Confidence Intervals.

^A p_c (p value after Bonferroni correction) = 0.091; ^{a1} p_c = 0.056; ^{a2} p_c = 0.315.^B p_c = 0.014; ^{b1} p_c = 0.007; ^{b2} p_c = 0.028.^C p_c = 0.112; ^{c1} p_c = 0.035.^d p_c = 0.182.^E p_c = 0.322; ^{e1} p_c = 0.161.^F p_c = 0.021; ^{f1} p_c = 0.007; ^{f2} p_c = 0.014.

No significant association of serum IL-21 levels with either *IL21* rs2221903 or *IL21R* rs3093301 polymorphisms was observed (Table 4). However, although there was no significant difference in healthy controls and infection resolvers, HBV patients with *IL21* rs2221903 genotype AG was associated with elevated serum IgE levels compared with those with genotype AA (p = 0.001, Table 4). *IL21R* rs3093301 polymorphisms had no significant association with serum IgE levels in all the three groups (Table 4).

Although there was no significant difference in infection resolvers and healthy controls, *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms had combined effects on both serum IL-21 and IgE levels in HBV patients. HBV patients with *IL21* rs2221903/*IL21R* rs3093301 AA/CC had elevated IL-21 levels compared with HBV patients with *IL21* rs2221903/*IL21R* rs3093301 AA/CT (p = 0.016), patients with *IL21* rs2221903/*IL21R* rs3093301 AA/TT (p = 0.038) or patients with *IL21* rs2221903/*IL21R* rs3093301 AA/CT + AA/TT (p = 0.011). HBV patients with *IL21* rs2221903/*IL21R* rs3093301 AG/CC had increased IL-21 levels compared with patients with *IL21* rs2221903/*IL21R* rs3093301 AA/CT (p = 0.013) or patients with *IL21* rs2221903/*IL21R* rs3093301 AA/TT (p = 0.017). HBV patients with *IL21* rs2221903/*IL21R* rs3093301 AA/CC also had elevated IgE levels compared with HBV patients with *IL21*

rs2221903/*IL21R* rs3093301 AA/CT (p < 0.001), patients with *IL21* rs2221903/*IL21R* rs3093301 AA/TT (p < 0.001), or patients with *IL21* rs2221903/*IL21R* rs3093301 AA/CT + AA/TT (p < 0.001, Table 5).

4. Discussion

In this study, we examined *IL21* rs907715 and rs2221903 and *IL21R* T-83C and rs3093301 polymorphisms and serum IL-21 and IgE levels in chronic HBV patients, HBV infection resolvers and healthy controls. We found that *IL21R* T-83C was not polymorphic in our populations and that *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms were associated with chronic HBV infection. Although serum IL-21 levels had no significant association with either *IL21* rs2221903 or *IL21R* rs3093301 polymorphisms, the carriage of *IL21* rs2221903 genotype AG in HBV patients was associated with significantly elevated serum IgE levels compared with the carriage of genotype AA in the patients. The carriage of *IL21* rs2221903/*IL21R* rs3093301 AA/CC in HBV patients was associated with significantly elevated IL-21 and IgE levels compared with the carriage of AA/CT or AA/TT in the patients, indicating an interactive

Table 4Association of *IL21* rs907715 and rs2221903 and *IL21R* rs3093301 polymorphisms with serum IL-21 and IgE levels in HBV patients, HBV infection resolvers and healthy controls.

		IL-21 (pg/ml)			IgE (pg/ml)		
		Patients	Controls	Resolvers	Patients	Controls	Resolvers
<i>IL21</i> rs907715							
	AA	233.1 (135.8–894.5)	64.6 (43.1–360.1)	107.9 (49.9–271.9)	220.2 (135.8–1697.8)	70.3 (53.2–503.8)	128.2 (36.2–274.9)
	AG	247.9 (111.4–2996.3)	68.8 (27.7–329.6)	140.8 (24.4–303.9)	258.5 (135.5–7192.9)	73.4 (50.9–450.5)	123.0 (24.4–303.9)
	GG	237.9 (130.0–904.0)	69.5 (39.8–280.4)	108.7 (35.5–310.3)	233.3 (130.1–2193.4)	68.9 (45.9–461.5)	107.7 (30.9–320.3)
<i>p</i>		0.856	0.763	0.620	0.340	0.609	0.609
<i>IL21</i> rs2221903							
	AA	237.4 (120.8–2996.3)	68.4 (27.7–301.9)	124.2 (24.4–310.3)	231.5 (130.1–7192.9)	74.9 (45.9–53.8)	117.2 (28.7–320.3)
	AG	248.3 (111.4–1724.5)	66.6 (28.1–360.1)	104.4 (24.8–271.9)	297.6 (141.6–1315.7)	67.5 (48.6–293.2)	118.2 (30.9–274.9)
<i>p</i>		0.768	0.860	0.375	0.001	0.219	0.933
<i>IL21R</i> rs3093301							
	CC	254.8 (120.8–2996.3)	65.9 (28.1–360.1)	106.9 (49.8–273.7)	265.6 (135.5–7192.9)	71.5 (51.7–503.8)	81.2 (38.4–268.5)
	CT	232.9 (130.0–1558.4)	69.9 (27.7–266.8)	115.0 (24.8–310.3)	219.9 (130.1–1697.8)	73.7 (51.3–461.5)	116.7 (24.8–310.3)
	TT	245.4 (111.4–1469.4)	66.9 (49.9–329.6)	136.0 (24.4–310.3)	251.3 (136.2–1326.5)	69.6 (45.9–248.5)	133.4 (28.7–320.3)
<i>p</i>		0.322	0.746	0.505	0.141	0.740	0.491

role of *IL21R* rs3093301 CC with *IL21* rs2221903 in inducing the elevation of IL-21 and IgE production in the HBV patients.

IL21 rs2221903 and *IL21R* rs3093301 polymorphisms have been shown to be associated with SLE. *IL21* rs2221903 genotype GG and allele G were associated with increased susceptibility to SLE [11], indicating their predisposing effect on this disorder. In the present study, a protective role of *IL21* rs2221903 genotype AG and allele G, or a predisposing role of genotype AA and allele A, was suggested in chronic HBV infection. Haplotype analysis also suggested the possible protective effect of rs2221903 allele G in linkage disequilibrium with rs907715 locus on chronic HBV infection. The protective and predisposing alleles in SLE and chronic HBV infection have an opposite distribution. This is possibly a differential exemplification between the autoimmune pathogenesis in SLE and the immune pathogenesis in chronic HBV infection because the immunopathogenesis of SLE and chronic HBV infection is thought to be involved by different T helper (Th)1 and Th2 dominance [29–31]. Similarly, *IL21R* rs3093301 allele T was higher in HBV patients and was much higher in infection resolvers than in healthy controls. This *IL21R* rs3093301 allele was associated with increased susceptibility to SLE [12]. In view of the differential Th1/Th2 dominance in the immunopathogenesis of SLE and chronic HBV infection, this *IL21R* rs3093301 allele is suggested to be favorable for resolving HBV infection. The higher frequency of this allele in HBV patients and infection resolvers compared with that in controls is related to the resolution of rather than to the susceptibility to chronic HBV infection because the occurrence of HBV infection may be related mainly to the chances of exposure rather than the susceptibility among the populations studied. Considering gene-gene interaction between *IL21* and *IL21R*, we found that the simultaneous presence of the genotypes *IL21* rs2221903 AG and *IL21R* rs3093301 CT or TT was related to the tendency of HBV infection resolution.

In the present study, increased serum IL-21 levels were shown in chronic HBV patients, confirming the involvement of IL-21 in the immunopathogenesis of chronic HBV infection documented by previous studies [22–24]. Serum IL-21 levels were shown to have no significant association with either *IL21* rs2221903 or *IL21R* rs3093301 polymorphisms when the analysis was performed separately with either of the polymorphisms, indicating that the associations of these polymorphisms with chronic HBV infection were, at least in adult HBV patients, not implicated through independent effect on IL-21 production from either of the polymorphisms studied. However, HBV patients with *IL21* rs2221903 genotype AG had higher serum IgE levels than those with genotype AA, suggesting that *IL21* rs2221903 polymorphism may affect chronic HBV infection at least partially through influencing IgE

production. This is consistent with the previous studies revealing the common B-cell activation in chronic HBV infection [18] and the relatedness of serum IgE levels to the clinical course in patients with HBV infection [19–21]. *IL21R* polymorphism T-83C was previously identified as a possible target locus influencing IgE synthesis [13,14]. This locus is not polymorphic in the Chinese populations included in this study. Because the other *IL21R* polymorphism rs3093301 genotyped in the present study showed no association with serum IgE levels, it seemed that this polymorphic locus can not replace the role of T-83C in influencing IgE production as has been documented in the populations from European studies [13,14].

Notably, the present study indicated a combined effect of *IL21* rs2221903 and *IL21R* rs3093301 on both serum IL-21 and IgE levels in chronic HBV patients. Interaction of the homozygous genotype CC at *IL21R* rs3093301 with the genotypes at *IL21* rs2221903 is related to increased IL-21 and IgE production in the HBV patients but not in the controls and resolvers. This may suggest an intrinsic link of individuals with different *IL21* and *IL21R* polymorphisms to the response of chronic HBV infection. Because *IL21R* rs3093301 CC was more frequent in patients than in resolvers, it is suggested that an interactive effect of *IL21R* rs3093301 CC with *IL21* rs2221903 on increased serum IL-21 and IgE levels was of potential for the persistence of HBV infection. IL-21 and IL-21R were demonstrated to be critical for maintaining and regulating CD8⁺ T cell and its activity in controlling chronic viral infection [32–34]. It is implied that there are possible links between *IL21* and *IL21R* polymorphisms and the control of chronic HBV infection.

The chronicity of HBV infection is closely related to the ages of the hosts at the acquisition of infection, with the acquisition of infection perinatally or in early childhood being related to high incidence of chronicity [26,35]. In animal model for HBV infection, IL-21 was shown to be part of an effective primary hepatic immune response to HBV and the increased levels of IL-21 transcripts were associated with viral clearance in acutely infected adult humans [22]. The present study included only adult subjects and was unable to investigate the possible effect of the polymorphisms in various age groups of the patients. Therefore, it is still unknown whether the *IL21* and *IL21R* polymorphisms have age-dependent relevance to the response of HBV infection.

Chronic HBV infection is associated with the development of HCC. NK cells play a critical role in antitumor immunity and IL-21, which may be produced by NK cells in addition to activated CD4⁺ T lymphocytes [3], is also involved in NK cell proliferation, differentiation or activation [36,37]. Therefore, the possible interaction between NK cells and IL-21 as well as the possible influence

Table 5
Combined effect of *IL21* rs2221903 and *IL21R* rs3093301 polymorphisms on serum IL-21 and IgE levels in HBV patients, HBV infection resolvers and healthy controls.

	Patients			Controls			Resolvers		
	<i>IL21</i> rs2221903	<i>IL21R</i> rs3093301	<i>n</i>	IL-21 (pg/ml)	IgE (pg/ml)	<i>n</i>	IL-21 (pg/ml)	IgE (pg/ml)	<i>n</i>
AA	CC	CC	84	250.76 (120.82–2996.29) ^a	252.69 (135.46–7192.88) ^b	44	71.32 (39.83–301.88)	77.58 (51.65–503.75)	8
AG	CC	CC	17	317.08 (156.52–1724.48) ^c	342.53 (160.93–1315.73)	19	61.69 (28.05–360.11)	67.45 (53.22–173.59)	7
AA	CT	CT	163	233.37 (130.02–894.48)	216.79 (130.09–1697.77)	61	68.25 (27.72–266.78)	78.81 (53.88–461.48)	23
AG	CT	CT	27	210.03 (157.63–1558.44)	304.87 (141.61–1283.68)	16	78.18 (48.07–240.47)	66.52 (51.31–293.15)	13
AA	TT	TT	84	238.37 (122.30–1469.35)	240.56 (136.20–1326.49)	26	66.36 (49.85–280.40)	68.99 (45.89–248.53)	18
AG	TT	TT	20	268.99 (111.38–702.72)	279.47 (147.46–671.08)	8	90.01 (52.01–329.63)	86.05 (48.56–216.82)	6
AA	CT+TT	CT+TT	247	233.72 (122.3–1469.35)	219.43 (130.09–1697.77)	87	67.62 (27.72–280.4)	73.72 (45.89–461.48)	41
AG	CT+TT	CT+TT	47	227.34 (111.38–1558.44)	280.12 (141.61–1283.68)	24	78.18 (48.07–329.63)	67.60 (48.56–293.15)	19
<i>p</i> value				0.047	0.001		0.823	0.139	0.509

^a *p* = 0.016, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/CT, *p* = 0.038, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/TT, *p* = 0.011, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/CT + AA/TT.

^b *p* < 0.001, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/CT, *p* < 0.001, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/TT, *p* < 0.001, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/CT + AA/TT.

^c *p* = 0.013, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/CT, *p* = 0.017, compared with *IL21* rs2221903/*IL21R* rs3093301 AA/TT.

of *IL21* and *IL21R* polymorphisms in the development of HBV-related HCC deserves to be investigated.

B-cell activation is also common in other situation of chronic infection such as hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infection. There are data showing that the progression of HIV infection was associated with increased plasma levels of total IgE [38] although no report addressing total IgE levels in chronic HCV infection has been systematically documented. Interestingly, prior HBV infection was shown to be significantly associated with increased serum IgE levels in advanced HIV infection [39]. There are also data revealing that patients with chronic HCV infection had lower average IL-21 level [40] and that AIDS patients had significantly reduced serum IL-21 levels and the decrease of IL-21 was significantly correlated with CD4+ T-cell counts [41]. These findings are not completely consistent with those reported in chronic HBV infection [23,24] although the age of the populations studied may partially contribute to this inconsistency [22]. The clinical implications of these findings and the underlying mechanisms of disease-associated variations in IgE and IL-21 levels remain largely to be investigated.

This study is carried out in small sample size of populations and genotyped several possibly relevant polymorphisms. The findings are preliminary, and replicative studies in large cohorts of HBV patient populations are required to validate and extend these findings. The functional effects of the associations remain to be identified.

In conclusion, the data of this study suggest a contribution of *IL21* and *IL21R* to genetic susceptibility to chronic HBV infection and an interactive effect of *IL21* and *IL21R* polymorphisms on serum IL-21 and IgE levels in patients with chronic HBV infection as well as a possible involvement of IL-21 and its receptor system in the infection persistence. The confirmation of these findings in replicative studies with large cohorts of HBV patient populations and the elucidation of functional effects of the associations might improve our understanding of chronic HBV infection.

Conflicts of interest

The authors have declared no conflicting interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.humimm.2013.01.005>.

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