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Role of interleukin-21 and interleukin-21 receptor polymorphisms in the treatment of HBeAg-positive chronic hepatitis B patients with peginterferon

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Abstract

The aim of this study was to evaluate the relationship between interleukin-21 (IL-21) and interleukin-21 receptor (IL-21R) polymorphisms and the response to peginterferon alfa (PEG-IFN α) therapy in HBeAg-positive chronic hepatitis B (CHB) patients.

A total of 143 HBeAg-positive CHB patients treated for 48 weeks with PEG-IFN α and followed up for 24 weeks post-treatment were retrospectively evaluated. Genotypes analysis was performed for IL-21 polymorphisms rs907715, rs2221903, and IL-21R polymorphisms rs3093301 and rs2285452. Serum IL-21 levels were measured by enzyme-linked immunosorbent assay.

The end of virological response (EVR) rate was 46.9% (67/143) at the end of treatment, the sustained virological response (SVR) rate was 43.4% (62/143) and the complete response (CR) rate was 32.1% (46/143) at 24 weeks post-treatment. Patients who carried IL-21 rs 2221903 genotype AA had a rather higher rate of EVR (response rate: 52.4%, odds ratio [OR] 0.42, 95% confidence interval [CI]: 0.19–0.91, $P = .021$), SVR (response rate: 47.6%, OR 0.43, 95% CI: 0.19–0.95, $P = .028$), and CR (response rate: 38.1%, OR 0.31, 95% CI: 0.12–0.79, $P = .014$) when compared to those had AG genotype. Meanwhile, IL-21rs 2221903 genotype AA was also independently associated with markedly reduced HBsAg levels ($>1\log_{10}$ IU/mL) after 24 weeks treatment and low HBsAg levels (<100 IU/mL) at the end of treatment. IL-21 rs907715 AG/GG genotype was independently associated with SVR (OR: 2.92, 95% CI: 0.98–8.6, $P = .039$; OR: 3.23, 95% CI: 1.0–10.4, $P = .039$). Patients with IL-21 rs907715 AG/GG genotype had higher serum IL-21 levels than those with rs907715 AA genotype ($P = .021$).

IL-21 rs2221903 and rs907715 polymorphisms were significantly associated with the treatment response to PEG-IFN α among Chinese HBeAg-positive CHB patients.

Abbreviations: ALT = alanine aminotransferase, CHB = chronic hepatitis B, CHC = chronic hepatitis C, CR = complete response, EVR = end of virological response, HBV = Hepatitis B virus, HCV = hepatitis C virus, IL-21 = Interleukin-21, IL-21R = interleukin-21 receptor, NCR = non-complete response, N-EVR = non-end of virological response, N-SVR = non-sustained virological response, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, PEG-IFN α = peginterferon alfa, SVR = sustained virological response.

Keywords: Hepatitis B virus infection, IL-21, peginterferon alfa, polymorphisms, virological response

1. Introduction

Chronic hepatitis B virus (HBV) infection is still a global health problem, and is a major cause of severe liver disease including hepatitis failure, cirrhosis, and hepatocellular carcinoma in China and other Asian countries.[1] Suppression of HBV replication by antiviral therapy is crucial for blocking the progression in active chronic hepatiris B (CHB) patients with high HBV DNA load and serum elevated alanine aminotransferase (ALT) levels.[2] Peginterferon alfa (PEG-IFN α) is recommended as treatment option for HBeAg-positive CHB patients. However, only no >40% of CHB patients receiving PEG-IFN α treatment could achieve sustained virological response (SVR).[3] Up to date, the reason why only a minority of patients response completely to PEG-IFN α is not well understood.

Several pretreatment predictors including low viral load, high serum ALT levels, high activity scores on liver biopsy, genotype, and female sex have been demonstrated to predict treatment response to PEG-IFN α in patients with CHB.[4,5] However, the value of these predictors in predicting response to PEG-IFN α remains limited. Therefore, ideal pretreatment predictors determining treatment response to PEG-IFN α are needed.

It is well known that the PEG-IFN α-based anti-HBV treatment efficiency is closely related to host immune status. Many investigators have attempted to identify some immunological biomarkers used as predictors for antiviral response. Recently, it was reported that high serum IL-21 levels at the week 12 could predict HBeAg seroconversion in HBeAg-positive CHB patients

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undergoing telbivudine treatment.[6] However, the role of IL-21 in predicting response to PEG-IFN α in CHB patients is still not well known.

Cytokine IL-21 is a type I cytokine which mainly produced by CD4+T cell subsets, including T helper 17 cells, follicular helper T cells, and natural killer (NK) T cells.[7,8] By binding to its receptor (IL-21R), which expressed on a variety of cells including NK cells, T cells, B cells, and dendritic cells,[9–11] IL-21 plays a pivotal role in regulating both cellular and humoral immune response.[12–15] Several studies have demonstrated that IL-21 plays an important role in controlling chronic virus infection including lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), hepatitis C virus (HCV), and HBV.[16–19] Recently, we reported that HBcAg-specific IL-21-producing CD4+T cells are associated with relative viral control in patients with CHB, and additional IL-21 could enhance function of CD8+T cells of CHB patients in vitro.[20] Moreover, other reports have shown that CXCR5+CD4 +T cells from HBeAg seroconverters could promote anti-HBe production by autologous B cells in vitro in an IL-21-dependent manner.[21] These results strongly indicate an important role of IL-21 in sustaining HBV-specific CD8+T and B-cell responses which are crucial for HBV clearance. Several studies have demonstrated that genetic polymorphisms of IL-21 and/or IL-21R were closely associated with susceptibility to or the disease progression of chronic HBV infection.[22–24] For example, IL-21 rs2221903 and IL-21R rs3093301 polymorphisms are risk factors for carrying HBV, and IL-21 rs2221903 and IL-21R rs3093301 polymorphisms may affect the susceptibility to and/or persistence of HBV infection potentially through altering IL-21 and IgE production.[23] In chronic hepatitis C (CHC) patients, serum IL-21 levels and IL-21R polymorphisms can predict virological response to PEG-IFN and ribavirin in HCV therapy.[25] So, it is speculated that IL-21 and/or IL-21R polymorphisms might serve as a good therapeutic predictor for CHB patients with PEG-IFN α treatment. In this study, we evaluate whether IL-21 rs907715 and rs2221903 and IL-21R rs3093301 and rs2285452 polymorphisms are associated with outcome of PEG-IFN α treatment in HBeAg-positive CHB patients.

2. Materials and methods

2.1. Study subjects

A total of 155 HBeAg-positive CHB patients receiving PEG-IFN α therapy at The Affiliated Hospital Of Xuzhou Medical University were prospectively enrolled from August 2012 to December 2015. All patients met the treatment criteria for CHB according to the Asian Pacific Association for the study of Liver Disease guideline.[26] These patients were positive for both HBsAg and HBeAg at least 6 months, and had HBV DNA levels > 2000 IU/mL, and had serum elevated ALTs being >2-fold of upper normal limit within 6 months. Exclusion criteria were as follows: receiving nucleotide analogues treatment in the previous 6 months or interferon (IFN) therapy in the past, coinfection with HIV or other hepatitis virus such as HAV, HCV, HDV or HEV, alcohol abuse, pregnancy or lactation, autoimmune disorders, decompensated cirrhosis, and other inheritable liver disease such as Wilson disease. Patients who did not finish the complete course of therapy or follow-up would be excluded for analysis.

All patients received antiviral therapy with 80 μg PEG-IFN α-2b or 180 μg PEG-IFN α-2a per week at a complete course of 48 weeks, and then completed follow-up for 24 weeks. End of virological response (EVR) was defined as HBV DNA levels <2000 IU/mL and normal ALT levels (<40 IU/L) at the end of treatment. Patients who had HBV DNA levels ≥2000 IU/mL at the end of treatment were classified into non-end of virological response (N-EVR). SVR was defined as HBV DNA levels <2000 IU/mL and normal ALT levels (<40 IU/L) at 24 weeks post-treatment. Patients who had HBV DNA levels ≥2000 IU/mL at 24 weeks after the end of treatment were classified into non-sustained virological response (N-SVR). Complete response (CR) was defined as HBV DNA <2000 IU/mL and HBeAg seroconversion (HBeAg loss and anti-HBe-positive) at 24 weeks post-treatment. Non-complete response (NCR) was defined to occur in patients who had HBD DNA ≥2000 IU/mL or/and no HBeAg seroconversion at 24 weeks after the end of treatment. Peripheral blood samples were collected at baseline, and serum samples were collected at baseline, week 12, 24, 36, and 48 during the treatment and at 24 week after PEG-IFN α therapy.

The study was conducted in full compliance with the ethical principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines and applicable local regulatory requirements. All subjects have signed informed consent, and the consent forms were approved by the Affiliated Hospital of Xuzhou Medical University Ethics Committee.

2.2. Serological assays and HBV DNA assays

Serum HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb were determined quantitatively using an electrochemiluminescence immunoassay on the Roche Elecsys 2010 immunoassay analyser (Roche, Basel, Switzerland). Serum HBV DNA levels were detected by real-time polymerase chain reaction (PCR) using Applied Biosystems 5700, which has a detection limit of 500 IU/mL. The ALT levels were determined by using a continuous monitoring assay.

2.3. Serum IL-21 concentration

The serum IL-21 level was measured by an enzyme-linked immunosorbent assay (ELISA) assay (Human IL-21 Platinum ELISA kit #BMS2043, eBioscience, San Diego, CA), according to the instructions of the manufacturers.

2.4. Genotyping of IL-21 and IL-21R gene polymorphisms

Genomic DNA was extracted from peripheral blood sample by using TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instruction. Genotype of the IL-21 and IL-21R gene polymorphisms was carried out using PCR-restriction fragment length polymorphism method.

A 236-bp PCR fragment including the IL-21 rs2221903 polymorphism was amplified using the primers: 5'-GGTACCTG-GACACTGACGCCCA-3' (upstream) and 5'-AAGGCAGTT-TAGTGGCGACAGCC-3' (downstream), a 222-bp PCR fragment including the IL-21 rs907715 polymorphism was amplified using the primers: 5'-CAATGGCTTGGTGTTTGGTAT-3' (upstream), and 5'-CCTCTTTTCACTTGGA GCATTC-3' (downstream), a 229-bp PCR fragment including the IL-21R rs2285452 polymorphism was amplified using the primers: 5'-CTGGGCTGTGATGTGA AGAC-3' (upstream) and 5'-TGGCAGGTGATAAGGAACAA-3' (downstream), and a 183-bp PCR fragment including the IL-21R rs3093301 polymorphism was amplified using the primers: 5'-CCCTCCCTCTTTCTTTGTTAG-3' (upstream) and 5'-TCCTCCTACCTCGGCCTCTCAAAGTG-3' (downstream).

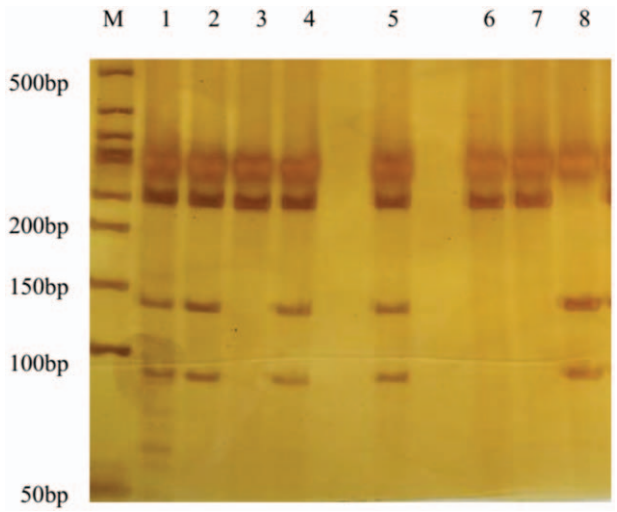

Figure 1. The representative results of polymerase chain reaction-restriction fragment length polymorphisms analysis of IL-21 gene polymorphisms. Polymorphisms of rs907715 were found in chronic hepatitis B patients. Line 1, 2, 4, 5 = AG genotype, line 3, 6, 7 = AA genotype, line 8 = GG genotype, M = maker.

PCR was carried out in a volume of 20 μL containing 3.0 μL DNA, 1.0 μL of each primer, 12.5 μL 2*Taq PCR MasterMix (TianGen Biotech Co. Ltd, Beijing, China) and 7.5 μL ddH_2O. PCR was carried out with initial denaturation for 3 minutes at 94°C, followed by 30 (IL-21 rs907715 and rs2221903) or 35 (IL-21R rs2285452 and rs3093301) cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 60°C (IL-21 rs2221903), 55°C (IL-21 rs907715), 56°C (IL-21R rs3093301 and rs2285452), extension for 30 seconds at 72°C, and a final extension for 10 minutes at 72°C. The products were digested with restriction enzyme HpyCH4 V, MobII, MluI, BsrGI (New England Biolabs) at 37°C for 2 hours, and analyzed on a 8% polyacrylamide gel electrophoresis. The representative endonuclease digestion figure was showed in Figure 1. The genotypes of each polymorphism were determined according to the digestion patterns.

2.5. Statistical analyses

Statistical analyses were carried out using a SPSS19.0 software (SPSS Inc., Chicago, IL). The χ^2 test or independent-Sample *t* test was used to compare parameters between groups. The χ^2 test was performed to estimate the Hardy–Weinberg equilibrium (HWE). Logistic regression was performed to examine the relationship between treatment response and variants compared to the IL-21 or IL-21R genotypes. Haplotype analysis was performed using SHEsis platform.[27] Serum IL-21 levels were expressed with median (range) and compared using the rank sum test. A *P* value $<.05$ was considered significant.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the subjects in this study are shown in Tables 1–3, and Supplementary Table S1 to Table S2, http://links.lww.com/MD/C267, Supplemental Content. A total of 155 HBeAg-positive CHB patients, 143 patients completed the full course of PEG-IFN α therapy and 24 weeks of follow-up, whereas 12 (7.7%) patients stopped PEG-IFN α treatment because of intolerance, unexpected pregnancy, or melancholia. Of the 143 HBeAg-positive patients, 67 (46.9%) patients achieved EVR at the end of treatment, whereas 62 (43.4%) patients achieved SVR and 46 (32.1%) patients achieved CR at 24 week post-treatment. The lower HBsAg levels were associated with EVR, SVR or CR, whereas age, baseline ALT levels and baseline HBV DNA levels had no significant differences between EVR/N-EVR, SVR/N-SVR, and CR/NCR groups, respectively.

Table 1

Clinical characteristics at baseline of patients in EVR and N-EVR group.

Characteristic	Total	EVR	N-EVR	*P*
Patients, n (%)	143	67 (46.9)	76 (53.1)	
Sex (male/female)	80/63	41/26	39/37	.235
Age, y	28.7 ± 7.7	23.2 ± 2.6	29.0 ± 6.4	.501
Family history (%)	57 (39.8)	24 (35.8)	33 (43.4)	.354
ALT, U/L	218.0 ± 89.91	220.5 ± 99.84	200.5 ± 108.78	.529
HBsAg (log_{10}IU/mL)	3.96 ± 0.62	3.17 ± 0.60	4.33 ± 0.23	.001
HBVDNA (log_{10}IU/mL)	6.39 ± 1.17	6.35 ± 1.29	6.75 ± 1.06	.069

ALT = alanine aminotransferase, EVR = end of virological response, N-EVR = non-end of virological response.

Table 2

Clinical characteristics at baseline of patients in SVR group and NSVR group.

Characteristic	Total	SVR	N-SVR	*P*
Patients, n (%)	143	62 (43.4)	81 (56.6)	
Sex (male/female)	80/63	34/28	46/35	.816
Age, y	28.7 ± 7.7	23.5 ± 2.4	29.4 ± 7.4	.561
Family history (%)	57 (39.8)	23 (37.1)	34 (41.9)	.555
ALT, U/L	218.0 ± 89.91	215.5 ± 149.85	200.8 ± 98.78	.729
HBsAg (log_{10}IU/mL)	3.96 ± 0.62	3.57 ± 0.59	4.05 ± 0.48	.001
HBVDNA (log_{10}IU/mL)	6.39 ± 1.17	6.43 ± 1.24	6.71 ± 1.09	.196

ALT = alanine aminotransferase, N-SVR = non-sustained virological response, SVR = sustained virological response.

3.2. HWE of the polymorphisms

The frequencies of IL-21 and IL-21R genotypes in all of these polymorphisms in the study were within HWE (Supplementary Table S3, Supplemental Content, http://links.lww.com/MD/C267) (*P* range from .051 to .636).

3.3. Relationship between IL-21 and IL-21R polymorphism and VR

Among 143 patients who finished 48 weeks' course of PEG-IFN α treatment and 24 weeks post-treatment, the distribution of IL-21

Table 3

Clinical characteristics at baseline of patients in CR group and NCR group.

Characteristic	Total	CR	NCR	*P*
Patients, n (%)	143	46 (32.1)	97 (67.9)	
Sex (male/female)	80/63	23/23	57/40	.324
Age (years)	28.7 ± 7.3	30.5 ± 7.0	27.1 ± 7.5	.179
Family history (%)	57 (39.8)	18 (39.1)	39 (40.2)	.902
ALT, U/L	218 ± 89.91	240.2 ± 90.84	190.3 ± 89.84	.200
HBsAg (log10IU/mL)	3.96 ± 0.62	3.70 ± 0.83	4.22 ± 0.54	.050
HBVDNA (log10IU/mL)	6.39 ± 1.17	6.26 ± 1.02	6.50 ± 1.21	.910

ALT = alanine aminotransferase, CR = complete response, NCR = non-complete response.

Table 4

Association between IL-21 and IL-21R genotypes and the EVR.

Tag-SNP	Genotype	EVR n = 67	N-EVR n = 76	EVR%	*P*	Multivariate analysis	
						OR (95%CI)	*P*
IL-21							
rs907715	AA	10 (14.9)	12 (15.4)	45.5			
	GG	17 (25.4)	24 (31.6)	41.5	0.760	0.85 (0.29–2.41)	.483
	AG	40 (59.7)	40 (52.6)	50.0	0.706	1.20 (0.46–3.09)	.446
rs2221903	AA	55 (82.1)	50 (65.8)	52.4			
	AG	12 (17.9)	26 (34.2)	31.6	0.028	0.42 (0.19–0.91)	.021
IL-21R							
rs3093301	CC	18 (26.9)	32 (42.1)	36.0			
	TT	16 (23.9)	13 (17.1)	55.2	0.097	2.18 (0.86–5.55)	.077
	CT	33 (49.3)	31 (40.8)	51.6	0.097	1.89 (0.88–4.03)	.071
rs2285452	AA	48 (71.6)	60 (78.9)	44.4			
	GG	1 (1.5)	2 (2.6)	33.3	1.000	0.62 (0.05–7.11)	.588
	AG	18 (26.9)	14 (18.4)	56.3	0.240	1.60 (0.72–3.55)	.165

CI = confidence interval, EVR = end of virological response, IL-21 = interleukin 21, IL-21R = interleukin-21 receptor, N-EVR = non-end of virological response, OR = odds ratio.

genotypes was 15.4%, 55.9%, 28.7% for AA/AG/GG at rs907715, 73.4%, 26.6% for AA/AG at rs2221903, IL-21R genotype distribution was 75.5%, 22.4%, 2.1% for AA/AG/GG at rs2285452, 34.9%, 44.8%, 20.3% for CC/CT/TT at rs3093301.

At the end of treatment, a total of 67 patients (46.8%) achieved EVR. Patients with IL-21 rs 2221903 genotype AA achieved this response in 52.4% (55/105) versus 31.6% (12/38) in patients with genotype AG ($P = .028$). By multivariate analysis, IL-21 rs2221903 (additive odds ratio [OR] 0.42, 95% confidence interval [CI]: 0.19–0.91, $P = .021$) was independently associated with EVR at the end of treatment (Table 4).

At 24 weeks post-treatment, a total of 62 patients (43.3%) achieved SVR. Both IL-21 rs2221903 and rs907715 were significantly associated with SVR. Patients with IL-21 rs2221903 genotype AA achieved SVR in 47.6% (51/105) versus 28.9% (11/38) in patients with genotype AG ($P = .036$), whereas patients with IL-21 907715 genotype AA achieved lower rates of SVR (22.7%), compared to 20 of 41 (48.8%) patients with genotype GG and 37 of 80 (46.2%) patients with genotype AG achieving SVR ($P = .044$, $P = .047$). By multivariate analysis, both IL-21 rs2221903 (additive OR 0.43, 95% CI: 0.19–0.95, $P = .028$) and rs907715 (additive OR 3.23, 95% CI: 1.00–10.43, $P = .039$; OR 2.92, 95% CI: 0.98–8.69, $P = .039$) were independently associated with SVR respectively at 24 weeks post-treatment (Table 5).

3.4. Relationship between IL-21 and IL-21R genotypes and complete virological response

We observed 32.1% (46/143) of patients reaching CR at 24 week post-treatment. As shown in Table 6, the frequency of IL-21 2221903 genotype AA was significantly higher in CR group than in NCR group (38.1% vs. 15.8%, $P = .012$). In a multivariate analysis, the IL-21 rs 2221903 AA genotype was also revealed independently related to CR at 24 weeks post-treatment after adjustment for other factors (OR 0.30, 95% CI 0.11–0.79, $P = .014$) (Table 6)

3.5. Relationship between IL-21, IL-21R genotypes and HBsAg response

A total of 12.5% (18/143) patients achieved reduction of qHBsAg $>1\log_{10}$ after 24 weeks of treatment. We observed that AA genotype for IL-21 rs2221903 polymorphisms was significantly prevalent in patients with reduction of qHBsAg $>1\log_{10}$

Table 5

Association between IL-21 and IL-21R genotypes and SVR.

Tag-SNP	Genotype	SVR n = 62	N-SVR n = 81	SVR%	P	Multivariate analysis	
						OR (95%CI)	*P*
IL-21							
rs907715	AA	5 (8.1)	17 (21.0)	22.7			
	GG	20 (32.3)	21 (25.9)	48.8	0.044	3.23 (1.00–10.43)	.039
	AG	37 (59.7)	43 (53.1)	46.2	0.047	2.92 (0.98–8.69)	.039
rs2221903	AA	51 (82.3)	54 (66.7)	47.6			
	AG	11 (17.7)	27 (33.3)	28.9	0.036	0.43 (0.19–0.95)	.028
IL-21R							
rs3093301	CC	17 (27.4)	33 (40.7)	34.0			
	TT	14 (22.6)	15 (18.5)	48.2	0.210	1.81 (0.71–4.61)	.155
	CT	31 (50.0)	33 (40.7)	48.4	0.121	1.82 (0.85–3.91)	.087
rs2285452	AA	42 (67.6)	66 (81.5)	38.9			
	GG	2 (3.2)	1 (1.2)	66.7	0.561	3.14 (0.27–35.7)	.345
	AG	18 (29.0)	14 (17.3)	56.2	0.081	2.02 (0.90–4.48)	.062

CI = confidence interval, IL-21 = interleukin 21, IL-21R = interleukin-21 receptor, N-SVR = non-sustained virological response, OR = odds ratio, SVR = sustained virological response.

Table 6

Association between IL-21 and IL-21R genotypes and CR.

Tag-SNP	Genotype	CR n = 46	N-CR n = 97	CR%	P	Multivariate analysis	
						OR (95% CI)	P
IL-21							
rs907715	AA	7 (13.0)	15 (15.5)	31.8			
	GG	10 (23.9)	31 (32.0)	24.4	0.527	0.69 (0.22–2.17)	.562
	AG	29 (63.1)	51 (52.5)	36.3	0.700	1.21 (0.44–3.33)	.804
rs2221903	AA	40 (87.0)	65 (67.0)	38.1			
	AG	6 (13.0)	32 (33.0)	15.8	0.012	0.30 (0.11–0.79)	.014
IL-21R							
rs3093301	CC	13 (28.3)	37 (38.1)	26.0			
	TT	9 (19.6)	20 (20.6)	31.0	0.630	1.28 (0.46–3.51)	.795
	CT	24 (52.2)	40 (41.2)	37.5	0.193	1.70 (0.76–3.83)	.229
rs2285452	AA	32 (0.696)	76 (0.784)	29.6			
	GG	1 (0.022)	2 (0.021)	33.3	1.000	1.18 (0.10–13.56)	.089
	AG	13 (0.283)	19 (0.196)	40.6	0.242	1.62 (0.71–3.68)	.283

CI = confidence interval, CR = complete response, IL-21 = interleukin 21, IL-21R = interleukin-21 receptor, NCR = non-complete response, OR = odds ratio.

than those with AG genotype ($P = .004$). whereas no significant differences were found among genotype AA, GG/AG at IL-21 rs907715 according to qHBsAg reduction $>1\log_{10}$ after 24 weeks of treatment ($P = .818$). We noticed no significant differences among genotype AA, GG/AG at IL-21R rs2285452, or among genotype TT, CT/CC at IL-21R rs3093301 in levels of qHBsAg reduction (Table 7). Furthermore, a total of 11 (7.6%) patients achieved a low HbsAg level<100 IU/mL, and 6 (4.1%) patients lost HBsAg at the end of treatment. Patients with IL-21 rs2221903 genotype AA had a significantly higher probability of achieving HbsAg level <100 IU/mL (10.5% vs. 0% $P = .037$) compared to patients with genotype AG (Table 7).

3.6. Association of IL-21 polymorphisms with serum IL-21 levels

We found a significant correlation between serum IL-21 levels and IL-21 rs907715 polymorphisms in CHB patients (Table 8). Patients with IL-21 rs907715 genotype AA was associated with more lower serum IL-21 levels than that in those with genotype AG or GG ($P = .002$, $P < .001$). However, IL-21 rs2221903 polymorphism was not associated with serum IL-21 levels in CHB patients.

4. Discussion

The increasing evidences have suggested that host genetic variation, especially which are closely associated with immune response to HBV or immunological control of HBV infection, may play an important role in outcome of antiviral treatment in CHB patients. Some studies demonstrated an important role of IL-21 in controlling chronic HBV infection, and identified IL-21 as a biomarker to predict HBeAg seroconversion in CHB patients with telbivudine therapy.[6,21–22] In the present study, we investigated whether IL-21 and IL-21R gene polymorphisms could be associated with PEG-IFN efficacy in Chinese HBeAg-positive CHB patients. The results demonstrated that rs2221903 and rs907715 of IL-21 genotype affect response to PEG-IFN-based therapy in HBeAg-positive CHB patients.

PEG-IFN α has both antivial and immunomodulatory activities, and outcome of IFN-α-based therapy strongly depends on preexisting immune response to HBV infection in CHB patients. IL-21 may partially represent anti-HBV immune status

Table 7

Association between IL-21, IL-21R genotype and HBsAg reponse in CHB patients.

Genotype	qHBsAg decline, IU/mL			low qHBsAg level, IU/mL		
	>1 Log	<1 Log	P	>100	<100	P
IL-21						
rs907715						
AA	10 (12.5)	70 (87.5)	.818	73 (91.2)	7 (8.8)	.705
GG/AG	8 (12.7)	55 (87.3)		59 (93.7)	4 (6.3)	
rs2221903						
AA	18 (17.1)	87 (82.9)	.004	94 (89.5)	11 (10.5)	.037
AG	0 (0.0)	38 (100.0)		38 (100.0)	0 (0.0)	
IL-21R						
rs2285452						
AA	14 (12.9)	94 (87.1)	.548	101 (93.5)	7 (6.5)	.449
AG/GG	4 (11.4)	31 (88.6)		31 (88.6)	4 (11.4)	
rs3093301						
CC	8 (16.0)	42 (84.0)	.073	48 (96.0)	2 (4.0)	.147
CT/TT	10 (10.7)	83 (89.3)		84 (90.3)	9 (9.7)	

CHB = chronic hepatitis B, IL-21 = interleukin 21, IL-21R = interleukin-21 receptor.

Table 8

Association between IL-21 polymorphisms and serum IL-21 levels.

IL-21	genotype	Serum IL-21 levels	*P*
rs907715	AA	53.13 (13.69–399.62)	.021
	AG	84.76 (23.64–413.16)	
	GG	70.19 (50.92–307.45)	
rs2221903	AA	57.19 (24.62–193.42)	.254
	AG	67.96 (1.28–339.02)	

IL-21 = interleukin 21.

in patients with CHB, owing to its crucial roles in regulating innate and adaptive immune responses.[28] IL-21 or IL-21R polymorphisms could affect IL-21 immunomodulatory function through modulating IL-21 expression or its signaling pathway. So, it is more likely that IL-21 or IL-21R polymorphisms may be useful pre-treatment predictive factors to PEG-IFN α therapy. Indeed, our study founds indicate that polymorphisms of IL-21 rs2221903 and rs907715 genotype are strongly related to PEG-IFN α response in HBeAg-positive CHB patients. The patients harboring IL-21 rs 2221903 AA and rs907715 GG/AG had significantly higher SVR rates compared to those with rs 2221903 AG genotype and rs 907715 AA genotype (47.6% vs. 28.9%, 48.8%/46.2% vs.22.7%, respectively). Additionally, patients who carried AA genotype of IL-21 rs2221903 achieved higher rate of end-point EVR or CR when compared to AG genotype. The results above contribute to development of a personalized approach for PEG-IFN α-based anti- HBV treatment in HBeAg-positive CHB patients.

In chronic HCV infection, it was reported that patients with IL-21R rs3093390 CC genotype had a higher SVR rate than those with non-CC genotype, and IL-21R rs3093390 may serve as potential factors predictive of treatment outcomes in CHC patients with interferon-based therapy.[25] However, we did not find an association between the IL-21R polymorphisms and treatment response in HBeAg-positive CHB patients receiving PEG-IFN therapy. These results indicated that the impact of the IL-21R polymorphisms on the response to PEG-IFN treatment may be different among patients with CHC and CHB. Of course, other reasons may contribute to the discrepancy; for example, IL-21R rs2285452 and rs3093301, but not rs3093390 were analyzed in our study. It is necessary for us to investigate the relationship between IL-21R rs3093390 and outcome of IFN-based therapy in CHB patients in the future.

A pronounced on-treatment HBsAg decline in patients with a combined response to PEG-IFN was closely associated with a sustained reponse through long time off-treatment follow-up.[29] In the present study, we investigated the impact of IL-21 rs2221903 polymorphisms on HBsAg reponse in HBeAg-positive CHB patients with PEG-IFN therapy. Our findings showed that IL-21 rs2221903 genotype AA was closely associated with both marked HBsAg decline ($>1\log_{10}$) after 24 weeks of treatment and low HBsAg levels at end of treatment. It suggests that IL-21 rs2221903 genotype AA may contribute to improvement in the efficacy of PEG-IFN therapy and ideal long-term clinical outcome.

As we know that the polymorphism of rs2221903 and rs907715 locates in the promoter region of *IL-21* gene and likely affect its transcription and expression. Our ELISA results showed that the rs907715 genotype GG/AG led to more IL-21 expression, compared with genotype AA. However, rs2221903 genotype did not affect serum IL-21 levels, although the genotypes of rs2221903 are strongly linked to PEG-IFN treatment response in HBeAg-positive patients with CHB. The data suggest that predictive role of IL-21 polymorphisms on IFN response do not just depend on IL-21 production. For instance, it was reported that polymorphisms of IL-21 rs2221903 and IL-21R rs3093301 could affect IgE production in patients with CHB.[23]

Our study had some limitations, it is carried out in small sample size of populations, and only HBeAg-positive CHB patients, and genotyped several possibly relevant polymorphisms. Further studies with a longitudinal design, more follow up date are required to explore the correlation of IL-21/IL-21R polymorphisms and the response to IFN-based therapy in CHB patients.

In conclusion, the data of this study indicate that IL-21 rs 2221903 AA genotype and IL-21 rs907715 no-AA genotype were closely associated with the outcome of PEG-IFN therapy in CHB patients with HBeAg-positive and may be a promising pretreatment predictor of PEG-IFN response, which may be useful to select candidate CHB patients for IFN-based therapy.

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