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RESEARCH ARTICLE

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Polymorphisms of CYP27B1 are associated with IFN efficacy in HBeAg-positive patients

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Background: Host single nucleotide polymorphisms were associated with antiviral therapy in CHB patients. The CYP27B1 gene, encoding 25(OH)D_3-1α hydroxylase, might activate 25(OH)D_3 to 1,25(OH)$_2D_3$ in kidney resulted in influencing the efficacy of interferon (IFN). The aim of the study was to investigate the association between CYP27B1 polymorphisms and the response to IFN in HBeAg-positive patients.

Methods: Eighty-seven HBeAg-positive CHB patients infected with HBV genotype B or C were included in the study. All patients were treated with IFN at least 1 year. According to the response to PEG-IFN therapy, they were divided into three groups: 16 complete responses (CR), 42 partial responses (PR), and 29 nonresponses (NR). Sanger-sequencing was utilized to genotype the CYP27B1 SNPs(rs4646536 and rs10877012).

Results: In logistic regression analysis, the frequency of rs4646536 CC genotype was observed to be higher in the NR group. Besides, the GG genotype of rs10877012 differed significantly among the three groups. The GG genotype was prevalent in patients with CR, and patients with TT genotype result in NR at the end of IFN treatment. The most common haplotype TG was independently associated with CR, after adjustment, and haplotype CT appeared to be associated with NR and PR, rather than CR. The data also showed that patients with baseline 1,25(OH)$_2D_3$ > 39.39 pg/mL had higher CR rates at the end of IFN therapy.

Conclusion: These results suggested CYP28B1 gene polymorphisms may be independently associated with the efficacy of IFN in HBeAg-positive patients.

KEYWORDS
1,25(OH)$_2D_3$, chronic hepatitis B, CYP27B1 polymorphism, interferon, response

1 | INTRODUCTION

Hepatitis B virus (HBV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) which leads to high mortality.[1] A complete eradication of HBV is rarely achieved because of the persistence covalently closed circular DNA (cccDNA) in host hepatocytes.[2] At present, interferon (IFN) is used as one of the first-line treatments for chronic hepatitis B (CHB). Researchers evidenced that therapy with IFN might control the viral replication. In detail, the function of IFN was to stimulate the cytotoxic T cell, lysing the infected hepatocytes and producing cytokines.[3] Compared with nucleos(t)ide analogues (NAs), resistance rarely occurred during PEG-IFN treatment. However, IFN therapy may cause several side-effects such as influenza-like symptoms, fatigue, neutropenia, and thrombocytopenia during treatment. Moreover, the cost of PEG-IFN was higher.[4] After PEG-IFN treatment, most patients obtained sustained virological response, HBeAg seroconversion with or without HBsAg clearance. However, only 29%-32% of HBeAg-positive CHB patients who received PEG-IFN treatment for 1 year would gain HBeAg seroconversion, and only 3%- 7% of them could eradicate the virus inside the

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liver cell.[5,6] It is indicated that sustained virological response, HBeAg seroconversion, and HBsAg seroclearance were the ideal clinical outcomes of IFN treatment.[1] However, not all CHB patients could obtain satisfactory ending by PEG-IFN treatment. Consequently, identification of molecular biomarkers that can identify CHB patients sensitive to IFN therapy would be useful in the clinic.

Increasing evidences indicate that host genetics act as an important role in the natural history of HBV-related liver disease and the response of treatment. Single nucleotide polymorphisms (SNPs) were found to be associated with antiviral therapy in CHB patients. Cheng et al[7] found that rs3077 GG and rs9277535 GG were strongly related to a higher response rate to IFN treatment. Zhu et al[8] reported that HLA-DR and HLA-DQ were also associated with treatment response to IFN therapy in CHB patients.

Most recently, it has been reported that vitamin D (VD) deficiency was associated with the low response rate to IFN plus ribavirin in HCV genotype 1. The theory was to inhibit the effect of VD on the HCV-RNA replication.[9] The relationship between VD and liver disease is reciprocal. Liver and kidneys are required to activate VD by 25(OH)D_3-1α hydroxylase, respectively.[10] CYP27B1 gene encoded 25(OH)D_3-1α hydroxylase which might activate 25(OH)D_3 to 1,25(OH)$_2D_3$ in kidney resulted in influencing efficacy of IFNR. The role of CYP27B1 had been demonstrated as an immune-related gene in autoimmune disease, metabolic disease, and schizophrenia, especially in the development of HBV and HCV. The polymorphisms in CYP27B1 (rs10877012 genotype CC)were found to be associated with the treatment response of IFN therapy in HBeAg-negative CHB patients. And the polymorphisms within CYP27B1 (rs10877012 genotype AA) were associated with virological response to IFN plus ribavirin in HCV treatment.[11,12] However, little is known about the CYP27B1 polymorphisms in HBeAg-positive CHB patients treated with IFN.

The role of 1,25(OH)$_2D_3$ has been demonstrated as an important immune modulator in IFN-signaling pathways.[13] It might upregulate the expression of β-IFN and MxA resulted in potent and sustained antiviral activity.[13] Through VD receptor and Jak-Stat signaling pathways, 1,25(OH)$_2D_3$ would convert into calcitriol-mediated, and the response rate of IFN could be increased. Therefore, 1,25(OH)$_2D_3$ could enhance the inhibitory effect of IFN in HCV replication. But lacking researches had investigated the relationship between 1,25(OH)$_2D_3$ and the efficacy of IFN in CHB patients.

The aim of our study was to evaluate a possible association between the CYP27B1 polymorphisms and responses to IFN therapy in a cohort of HBeAg-positive patients and, in addition, to determine the associations between 1,25(OH)$_2D_3$ serum level as well as polymorphisms within genes encoding the 1α-hydroxylase with the response of IFN-based treatment in patients chronically infected with HBV.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 87 HBeAg-positive CHB patients who were ≥ 16 years old and received PEG-IFN (180 μg/week; Pegasys®; Roche, Shanghai, China) for 48 weeks were enrolled in between May 2012 and September 2015. All patients were recruited in the Center of Liver diseases of the First Affiliated Hospital of Fujian Medical University. The clinical diagnosis of CHB was based on the guideline of prevention and treatment for chronic hepatitis B (2010 version) by Chinese Society of Hepatology and Chinese Society of Infectious Diseases.[14] The inclusion criteria were as follows: (i) HBsAg positive for at least 6 months; (ii) HBeAg positive; (iii) persistent level of HBV DNA ≥20 000 IU/mL; and (iv) ALT ≥2 and ≤10 × upper limit of normal without any other antiviral therapy. Patients treated with other antiviral drugs, co-infected with HCV, HIV, or HDV, cirrhosis, hepatocellular carcinoma (HCC) autoimmune hepatitis, association with other severe underlying disease were excluded. Patients with incomplete course of therapy or follow-up were excluded as well.

2.2 | Definitions of treatment responses

Treatment response was determined after 48 weeks of treatment based on the guideline of prevention and treatment for chronic hepatitis B (2010 version).[14] HBsAg seroclearance was defined as HBsAg <0.05 IU/mL; HBsAg seroconversion was defined as HBsAg seroclearance with anti-HBs >10 mIU/mL. HBeAg seroclearance was defined as HBeAg < 1 S/CO; the seroconversion of HBeAg was defined as a loss HBeAg with the development of anti-HBe on at least two consecutive follow-up visits. Sustained virological response was defined as HBV DNA <500 IU/mL.

Patients were divided into complete response (CR) group, partial response (PR) group, and nonresponse (NR) group. Detailed criteria were described as follows: CR was defined as normalization of serum ALT levels, serum HBV DNA level undetectable by PCR, and HBeAg seroclearance with or without HBeAg seroconversion, and HBsAg seroclearance or not; PR was situated between CR and NR, such as the decrease in the level of HBV DNA of more than 1 $\log_{10}$ IU/mL but still more than 500 IU/mL with HBeAg clearance or not; and NR when lacking either a biochemical or a virological response during therapy.

2.3 | SNPs selection

HapMap dataset was used to select the tagger SNPs (TagSNPs). TagSNPs were selected from Haploview software 4.2 (Mark Daly's lab of Broad Institute, Cambridge, MA, USA) with a pairwise correlation $r^2 = 1$ and minor allele frequency (MAF) ≥ 0.05, which was according to HapMap Data Rel 27 PhaseII+III, Feb 09, on NCBI B36 assembly, dbSNP b126). Two SNPs (rs4646536 and rs10877012) were identified on the region of CYP27B1 gene on chromosome 12.

2.4 | CYP27B1 (rs4646536 and rs10877012) polymorphism genotyping

Genomic DNA was isolated from blood samples using the DNA Extraction Kit (Tiangen Biotech Co, Ltd, Beijing, China). We evaluated

the SNPs in patients who have agreed to undergo genetic analyses and for whom blood samples were available. The genotypes of rs4646536 and rs10877012 were analyzed by direct sequencing.

2.5 | 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$-1α hydroxylase measurement

1,25(OH)$_2$D$_3$ was quantified by enzyme-linked immunosorbent assay (Human 1,25- hydroxylase VD$_3$ ELISA Kit; Kanghua Biotechnology Company Ltd., China), and 25(OH)D$_3$-1α hydroxylase was determined by ELISA [Human 25-hydroxyVD-1 alpha hydroxylase, mitochondrial (CYP27B1) ELISA Kit; CUSABIO Biotechnology Company Ltd, China]. These were retrospectively analyzed from serum samples which were obtained at baseline before treatment initiation.

2.6 | Laboratory measurements

Serum HBV DNA, HBeAg, and HBsAg were quantified in samples taken at baseline, week 12, and at the end of IFN treatment. HBsAg and HBeAg were quantified with the Chemiluminescence Microparticle Immuno Assay on Abbott Architect I2000. ALT level was measured by rate assay on Olympus AU2700. Serum levels of HBV DNA were quantified by qPCR (the lower limit of quantification 500 IU/mL) on ABI7500. HBV genotypes were determined by real-time PCR-Fluorescence Probing on ABI7500.

2.7 | Statistical analysis

Statistical analysis was performed using IBM SPSS version 18.0 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism software version 5.0 (GraphPad Software, San Diego, CL, USA). Associations between variables were tested using Student's *t* test and chi square test. The genetic association analysis consisted of an additive model as well as a haplotype block analysis. Binary logistic regression modeling was used to identify independent factors associated with the outcome at the end of treatment. All statistical tests were two-sided and evaluated at the 0.05 level of significance.

3 | RESULTS

3.1 | Patient characteristics

A total of 87 HBeAg-positive CHB patients treated with IFN were collected. The characteristics of the enrolled patients are shown in Table 1. At the end of treatment, 16 patients achieved CR, 42 patients achieved PR and 29 of them achieved NR. There was no significant difference in distribution of age, gender, HBV genotype, HBsAg, HBV DNA, ALT, and AST (*P* value >.05) among the three groups of patients.

3.2 | Prevalence of CYP27B1 Genotypes

There were significant differences among the groups both in allele frequencies and genotype distributions. CYP27B1 genotype frequencies were 20.7%, 42.5%, and 36.8% for TT, TC, and CC at rs4646536, respectively; 58.6%, 4.6%, and 36.8% for GG, GT, and TT at rs10877012, respectively. The allele frequencies were 42% and 58% for T and C at rs4646536, respectively; 49% and 51% for G and T at rs10877012, respectively.

3.3 | Relationship between CYP27B1 polymorphisms and treatment response

We found a significant correlation between CYP27B1 polymorphisms and treatment outcome (Table 2). The proportion of individuals with CC genotype at rs4646536 was higher in the NR group than in the CR group (*P* = .005). Besides, higher frequency of rs4646536 C allele was observed in the NR group (68.97%) than in the CR group (40.62%).

The frequency of allele T at rs10877012 was significantly higher in the NR group (*P* < .001, *OR*=0.101). The GG genotype was prevalent in patients with CR (*P* = .001, OR = 0.087), and patients with TT genotype were more likely to obtain NR of IFN treatment (*P* = .005, OR = 8.615).

The relationship between haplotype and the efficacy of IFN in CHB patients was demonstrated by the multivariate analyses of CYP27B1 haplotype (rs4646536 and rs10877012). The most common haplotype was TG that (major alleles of the two genetic variants)

TABLE 1 Characteristics of the study cohort

Characteristics	CR	PR	NR	Total	*P* Value
N	16	42	29	87	-
Gander (M/F)	8/8	30/12	22/7	60/27	.183
Ages (yrs)	28.12 ± 4.91	27.14 ± 4.25	26.66 ± 8.95	27.16 ± 6.26	.757
Genotype (B/C)	9/7	27/15	16/13	52/35	.561
HBsAg ($\log_{10}$ IU/mL)	3.87 ± 0.72	4.00 ± 0.60	4.24 ± 0.54	4.04 ± 0.61	.080
HBeAg (S/CO)	600.64 ± 639.78	859.46 ± 496.77	1044.80 ± 520.83	873.64 ± 548.82	.031*
ALT (U/L)	282.06 ± 279.00	297.24 ± 234.89	199.79 ± 156.18	261.97 ± 223.18	.181
AST (U/L)	132.75 ± 103.55	144.88 ± 136.92	114.90 ± 119.65	132.66 ± 125.09	.616
HBV DNA ($\log_{10}$ IU/mL)	6.93 ± 0.82	7.06 ± 0.95	7.13 ± 1.07	7.06 ± 0.97	.803

Data were expressed as median±SD or median (interquartile range).
*Significant *P*-value (<.05).

TABLE 2 Association of SNPs genotypes with outcomes of IFN treatment in HBeAg-positive CHB patients

SNP		CR (n = 16)	PR (n = 42)	NR (n = 29)	*P*	CR vs PR		PR vs NR		CR vs NR	
						P	OR (95% CI)	P	OR (95% CI)	*P*	OR (95% CI)
rs4646536											
Genotype	TT	5 (31.2)	8 (19.1)	5 (17.2)	.059		1		1		1
	TC	9 (56.3)	20 (47.6)	8 (27.6)		.729	0.720 (0.184-2.824)	.720	1.562 (0.391-6.248)	1.000	1.125 (0.236-5.371)
	CC	2 (12.5)	14 (33.3)	16 (55.2)		.192	0.229 (0.036-1.462)	.510	0.547 (0.145-2.063)	.063	0.125 (0.018-0.855)
Dominate	TT vs TC+CC	5 (31.3)/11 (68.7)	8 (19.0)/34 (81.0)	5 (17.2)/24 (82.8)	.537	.482	0.518 (0.140-1.915)	.847	0.885 (0.258-3.040)	.455	0.458 (0.110-1.916)
Recessive	CC vs TC+TT	2 (12.5)/14 (87.5)	14 (33.3)/28 (66.7)	16 (55.2)/13 (44.8)	.014	.188	0.286 (0.057-1.436)	.067	2.462 (0.930-6.514)	.005*	8.615 (1.65-44.972)
Allele	T	19 (59.4)	36 (42.9)	18 (31.0)	.000*	.146	0.513 (0.224-1.174)	.154	0.600 (0.297-1.214)	.009*	3.248 (1.323-7.976)
	C	13 (40.6)	48 (57.1)	40 (69.0)							
rs10877012											
Genotype	GG	14 (87.5)	26 (61.9)	11 (37.9)	.018*		1		1		1
	GT	0 (0)	2 (4.8)	2 (6.9)		.545	1.077 (0.972-1.193)	.579	0.423 (0.053-3.396)	.222	1.182 (0.937-1.490)
	TT	2 (12.5)	14 (33.3)	16 (55.2)		.114	0.265 (0.053-1.338)	.050*	2.701 (0.988-7.385)	.003*	10.182 (1.919-54.015)
Dominate	GG vs GT+TT	14 (87.5)/2 (12.5)	26 (61.9)/16 (38.1)	11 (37.9)/18 (62.1)	.005*	.110	0.232 (0.047-1.158)	.047*	0.376 (0.142-0.997)	.001*	0.087 (0.017-0.459)
Recessive	TT vs GT+GG	2 (12.5)/14 (87.5)	14 (33.3)/28 (66.7)	16 (55.2)/13 (44.8)	.014*	.188	0.286 (0.057-1.436)	.067	0.406 (0.154-1.075)	.005*	8.615 (1.650-44.972)
Allele	G	28 (87.5)	34 (53.1)	24 (41.4)	.000*	.001*	6.176 (1.942-19.643)	.195	0.623 (0.304-1.273)	.000*	9.917 (3.076-31.975)
	T	4 (12.5)	30 (46.9)	34 (58.6)							

The differences in genotype frequencies between any two groups were analyzed using logistic regression models. ORs were calculated as reported within the 95% CI.
CR, compete response; PR, partial response; NR: nonresponse; OR, odds ratio; CI, confidence interval.
Data were presented as number (percentage) for every group.
*Significant *P*-value (<.05).

TABLE 3 Association of haplotypes with IFN therapy in HBeAg-positive CHB patients

				CR vs PR		PR vs NR		CR vs NR	
Haplotype	CR	PR	NR	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)
CG	9 (28.1)	18 (21.4)	7 (12.3)	.446	0.697 (0.275-1.767)	.165	0.516 (0.200-1.328)	.063	0.359 (0.119-1.084)
CT	4 (12.5)	30 (35.7)	33 (57.9)	.014*	3.889 (1.245-12.145)	.009*	2.473 (1.241-4.929)	.000*	9.618 (2.978-31.058)
TG	19 (59.4)	36 (42.9)	17 (29.8)	.111	0.513 (0.224-1.174)	.116	0.566 (0.277-1.155)	.006*	0.290 (0.117-0.718)

Data were presented as number (percentage) for every group. The differences in haplotype frequencies between any two groups were analyzed using χ^2 test. ORs were calculated as reported within the 95% CI. A two-sided *P* value<.05 was considered as statistically significant. All frequencies <0.05 had been ignored in analysis.

CR, compete response; PR, partial response; NR, nonresponse; OR, odds ratio; CI, confidence interval.

*Significant *P*-value (<.05).

was independently associated with CR, rather than NR after adjustment [*P* = .006, OR = 0.290, 95%CI (0.117~0.718)]. In addition, haplotype CT appeared to be associated with NR and PR, rather than CR [*P* < .001, OR = 9.618, 95%CI (0.119-1.084)] (Table 3).

3.4 | Relationship between $1,25(OH)_2D_3$ and treatment response

Our data showed that baseline concentration of $1,25(OH)_2D_3$ was significant difference among the three groups. The mean $1,25(OH)_2D_3$ level at baseline of treatment was significantly higher in the CR group (132.70 ± 106.32 pg/mL) than that in the PR group (81.10 ± 72.83 pg/mL) and NR group (70.97 ± 59.17 pg/mL) (*P* = .012, Figure 1). The ROC curve was made to analyze the correlation between $1,25(OH)_2D_3$ and treatment response. The predictive value [indicated by area under the curve (AUC)] of $1,25(OH)_2D_3$ level was 0.672 [*P* = .024,95%*CI*(0.533~0.811)] at baseline. It showed that the CR rate among patients was classified by baseline $1,25(OH)_2D_3$ level using the optimal cutoff indicated by the inflection point of the ROC in both cohorts. It predicted that patients with baseline $1,25(OH)_2D_3$ > 39.39 pg/ml had higher CR rates at the end of IFN therapy (Table 4).

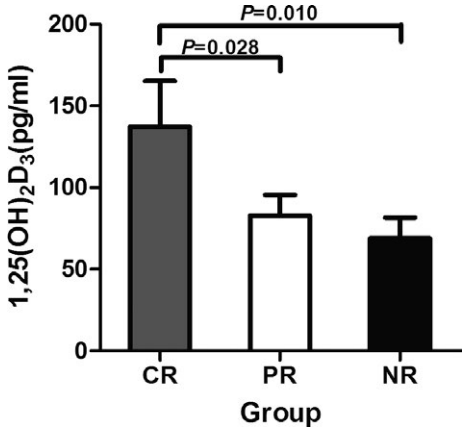

FIGURE 1 Correlation of baseline $1,25(OH)_2D_3$ level with the efficacy of IFN. Indicates baseline $1,25(OH)_2D_3$ serum concentration of patients who achieved CR, PR or NR at the end of IFN therapy. CR rates was associated with $1,25(OH)_2D_3$ level in HBeAg-postivie CHB patients

CYP27B1 encode $25(OH)D_3$-1α hydroxylase, which was active $25(OH)D_3$ to $1,25(OH)_2D_3$ in the proximal convoluted tubules of kidney. It would affect the antiviral outcome of IFN. Rs10877012 was located in the promoter region of CYP27B1 gene, and it was demonstrated as important loci related to the expression regulation of $25(OH)D_3$-1α hydroxylase. Our data showed that rs10877012 was associated with the level of $25(OH)D_3$-1α hydroxylase (*P* = .035). The GG genotype was related to lower $25(OH)D_3$-1α hydroxylase level (56.25 ± 15.29 pg/mL) than that in patients with TT genotype (74.53 ± 43.59 pg/mL) (*P* = .016, Figure 2).

4 | DISCUSSION

As IFN is generally poorly tolerated and only leads to a successful response in a subgroup of patients, it is essential to select patients who have the high probability to achieve a response before initiating therapy. The response rate to treatment of CHB was strongly influenced by genetic factors such as HLA-DR, HLA-DP, HLA-DQ, IL-28B, and IFNAR.[7,8,15-20] In the CHB treatment, the role of CYP27B1 is still ambiguous. The relationship between VD and liver disease is reciprocal. Liver and kidneys are required to activate VD by $25(OH)D_3$-1α hydroxylase, respectively.[10] The VD deficiency has been related to hepatic disease progression.[21] However, the role of CYP27B1 and its polymorphisms has not been studied yet in the treatment of HBeAg-positive CHB patients by IFN. In the current study, we have shown for the first time that polymorphisms of the CYP27B1 gene are associated with response to IFN 12 months post-treatment in CHB patients.

In the treatment of CHB, the role of $1,25(OH)_2D_3$ has been demonstrated as an important immune modulator in IFN-signaling pathways, in which the immunomodulatory effect was given the regulatory T cell.[13] The CYP27B1 gene encodes $25(OH)D_3$-1α hydroxylase which could catalyze $25(OH)D_3$ into $1,25(OH)_2D_3$. The $1,25(OH)_2D_3$ might upregulate the expression of β-IFN and MxA resulted in potent and sustained antiviral activity in IFN-signaling pathway.[22] Moreover, polymorphisms within CYP27B1 gene (rs10877012 CC genotype) cause persistent infection of HBV among Chinese.[23] However, AA genotype at rs10877012 was associated with an increased amount of response rates in patients with HCV who were treated with IFN plus ribavirin.[24] In addition, PEG-IFN treatment evidenced a positive predictive

TABLE 4 Association of baseline $1,25(OH)_2D_3$ level with the efficacy of IFN in HBeAg-positive CHB patients

Threshold (pg/mL)	CR (n = 16)	PR (n = 42)	NR (n = 29)	Total (n = 87)	CR rates (%)	*P*
≥39.39	15	25	17	57	26.32	.031
<39.39	1	17	12	30	3.33	

CR, compete response; PR, partial response; NR, nonresponse.

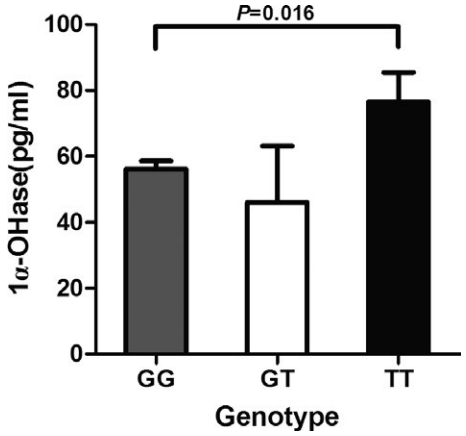

FIGURE 2 Correlation of $25(OH)D_3$-α hydroxylase with rs10877012 genotype. The GG genotype at rs10877012 was related to lower $25(OH)D_3$-α hydroxylase level than that in patients with TT genotype

role similarly to rs4646536 in patients with HBV HBeAg negative.[25] However, robust pretreatment predictors for response to IFN were currently lacking for HBeAg-positive patients.

In the current study, investigating only HBeAg-positive CHB patients, we have shown that rs4646536 CC genotype and rs10877012 TT genotype were associated with NR to IFN in HBeAg-positive patients; and patients with rs10877012 GG genotype would obtain CR at the end of treatment. HBeAg-negative CHB patients with the TT genotype of rs4646536 resulted to be a good positive predictor of sustained virological response and serological response.[25] We evaluated also the role of the haplotypes of CYP27B1 as the new pretreatment variables previously associated with response in the treatment with IFN. In our data, the haplotype CT is mostly predictive pretreatment factor for NR in HBeAg-positive CHB patients.

Serum $1,25(OH)_2D_3$ had been demonstrated to activate IFN-signaling pathways and stimulate the expression of antiviral proteins. Our data showed that the level of $25(OH)D_3$-1α hydroxylase and $1,25(OH)_2D_3$ might confirm the important function of rs10877012 at the promoter of CYP27B1. Serum $25(OH)D_3$-1α hydroxylase level in mutant TT genotype was significantly higher than wild-type GG genotype. The TT genotype would enhance the efficiency of the $25(OH)D_3$ converted into $1,25(OH)_2D_3$. Finally, it improved the antiviral efficiency of IFN. But the result was incompatible to draw the conclusion that rs10877012 TT genotype was related to NR. Researchers discovered that the mutation of G57V and L333F would affect the activity of $25(OH)D_3$-1α hydroxylase in kidney cells.[26] Thus, the activity of $25(OH)D_3$-1α hydroxylase not only influenced by just a single-site mutation, more factors which affected the enzyme activity was needed to explore in the future. Several literatures showed that combining the IFN with $1,25(OH)_2D_3$ would significantly enhance the ability to inhibit HCV replication;[27] and silencing the expression of VD receptor would obviously stimulate the phosphorylation of STAT in IFN pathways, which result in increasing the expression of antiviral protein levels.[27] Those data favors the result of our study that CHB patients with higher baseline $1,25(OH)_2D_3$ level would obtain CR at the end of IFN treatment.

There were some differences between our results and literatures. That may due to the different criteria of grouping, the diverse strategy in IFN treatment, and the different subjects enrolled in the study. Obviously, our study has certain limits that may be, at least partially, responsible for some results discordant to those already published in other studies: First, few cases were included in our study; second, the different basis for grouping, may be due to the different guidance in different countries; third, the patient type was limited. Our cohort only included HBeAg-positive CHB patients with genotype B or C, the HBeAg-negative patients, or patients with genotype A and genotype D which were prevalent in the occident need to be considered in the following studies; furthermore, ethnic variation may be another important reason. However, based on the important predictive value of CYP27B1 gene polymorphisms in IFN therapy, the results of our research will be a reference for the future study.

In conclusion, our study reveals that polymorphisms in the CYP271B1 may associate with the response to IFN therapy. In future research, multicenter studies with more samples needed to carry out and to fully validate the significance of these findings.

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