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Role of CYP27B1+2838 promoter polymorphism in the treatment of chronic hepatitis B HBeAg negative with PEG-interferon

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Summary

In HBV-infected patients, the vitamin D deficiency has been related to chronic liver diseases, progression of hepatic fibrosis and poor response to the treatment. The CYP27B1 gene, which encodes the 1-α-hidroxylase and involved in the 1,25-dihydroxyvitamin D synthesis, was recently associated to type-1 diabetes, autoimmune disorders and treatment response in HCV. Then, we aimed to investigate the role of CYP27B1 polymorphisms in HBV treatment with PEG-IFN. We retrospectively enrolled 190 patients with chronic hepatitis B HBeAg negative treated for 48 weeks with PEG-IFN α-2a. We examined the role of rs4646536 CYP27B1 SNP (CYP27B1+2838) according to virological and serological response. Our results showed that the TT genotype of CYP27B1+2838 was significantly prevalent in patients with end-of-therapy virological response (37.6%) vs CT/CC (9.4%) ($P < 0.001$). Virological relapse was prevalent in patients with CT/CC genotype (12.6%) vs TT genotype (2.1%) ($P < 0.001$). TT genotype was also related to HBsAg loss ($P = 0.004$) and anti-HBs appearance ($P = 0.002$). In the multivariate analysis, the TT genotype resulted to be a good positive predictor of sustained virological response (OR = 5.632, IC = 1.938–16.368, $P = 0.001$) and serological response (OR = 6.161, IC = 1.856–20.457, $P = 0.003$). The CYP27B1+2838 polymorphism may be useful as pretreatment factor to selection of patients with higher probability of response to therapy.

Abbreviations

1,25-dihydroxyvitamin D: 25(OH)D3

ALT: alanine aminotransferase

cccDNA: covalently closed circular DNA

CHB: chronic hepatitis B

HBeAg: hepatitis e antigen

HBsAg: hepatitis B surface antigen

HBV: hepatitis B virus

HCC: hepatocellular carcinoma

HCV: hepatitis C virus

IQR: inter-quartile range

NA: nucleos(t)ide analogues

PEG-INF: pegylated interferon

qHBsAg: quantitative HBsAg

SNP: single nucleotide polymorphism

SVR: sustained virological response

UNL: upper normal level

Background

Chronic hepatitis B (CHB) is a major cause of liver disease worldwide and it is associated with an increased risk of cirrhosis and hepatocellular carcinoma (HCC). Despite the introduction of effective vaccination programs, there are more than 350 million people with hepatitis B virus (HBV) persistent infection [1]. A complete eradication of HBV is rarely achieved because of the viral persistence in the shape of its covalently closed circular DNA (cccDNA) in host hepatocytes [2], then the main goal of therapy is to prevent the development of cirrhosis, HCC and liver failure [3]. The main objective of treatment of CHB is the seroclearance of HBsAg, but it is a very difficult aim to obtain. Other more easily attainable outcomes are HBV-DNA suppression, improvement of liver histology and alanine aminotransferase (ALT) normalization [4].

Treatment options include currently available nucleos(t)ide analogues (NA) with direct antiviral effect and HBV-DNA suppression; however, this treatment has no effect on cccDNA, and consequently, it does not get HBV eradication [5]. Therapy with PEG-IFN evidences, instead, an important stimulation of cytotoxic T cell which, through the lyses of infected hepatocytes and by producing cytokines, can control the viral replication [6]. However, the therapy with PEG-IFN is influenced by a limited tolerability due to its side effects, and response is dependent from HBV genotype [7]. In this setting, the identification of pretreatment parameters of response to PEG-IFN is essential to optimize this therapy on the basis of a patient stratification. All the patients should be selected for PEG-IFN administration based on their individual probability of response [8, 9].

The role of 1,25-dihydroxyvitamin D (1,25-(OH)2D3) has been recently demonstrated as immune modulator in autoimmune diseases [10], sepsis [11] and cancer [12]. Moreover, vitamin D deficiency was also associated with low response to PEG-IFN and ribavirin in HCV genotype 1 [13-16] and an inhibitory in vitro effect of vitamin D on the HCV-RNA replication [17] was demonstrated. The prevalence of vitamin D deficiency in patients with chronic liver diseases varies from 64% to 92% [18], and it is related to the degree of liver impairment [19]. The exact interaction between the vitamin D deficiency and HCV infection is still debated, as it is not clear whether the vitamin D lack plays a role to susceptibility to HCV infection or, inversely, the viral infection leads to low levels of vitamin [16].

The polymorphisms within the vitamin D receptor (VDR) and 1-α-hydroxylase (CYP27B1) were recently associated with virological response to PEG-IFN and ribavirin in HCV treatment [13, 14, 20], with onset of oral cancer [21], type 1 diabetes [22] and autoimmunity disorders [23]; moreover, a possible role in the natural course of HBV infection was recently reported by Zhu et al. [24], but no data were available about the treatment with PEG-IFN in HBV.

The aim of our study was to evaluate a possible association between the CYP27B1 rs4646536 promoter polymorphism and response to PEG-IFN therapy in a cohort of HBeAg-negative patients with different HBV genotypes.

Materials and Methods

Patient population

A total of 190 patients were retrospectively enrolled, with CHB HBeAg negative, consecutively treated between 2004 and 2010 with PEG-IFN α-2a for 48 weeks at the Infectious Diseases Unit of the Amedeo di

Savoia Hospital, Turin, Italy. All patients were positive for HBsAg and negative for HBeAg. Inclusion criteria were as follows: 18 years of age, persistent level of HBV-DNA > 2000 IU/mL, ALT > 1 UNL (upper normal level), no previous treatment with standard interferon or NA, any contraindication to PEG-IFN administration. Exclusion criteria were as follows: treatment with standard interferon, concomitant or previous NA assumption, coinfection with hepatitis C or D, HIV, pregnancy or lactation, decompensated cirrhosis, alcohol abuse, autoimmune disorders or HCC. We excluded also patients with incomplete course of therapy or follow-up.

The treatment with PEG-IFN α-2a 180 μg/week lasted 48 weeks; a follow-up for 2 year after treatment completion was performed. We performed a genotype analysis for the CYP27B1 rs4646536 C>T polymorphism (CYP27B1+2838) for all patients in this study. HBV genotype determination and transient elastography (Fibroscan®, Echosens, Paris, France) have been performed before start of treatment. We tested HBV-DNA, quantitative HBsAg (qHBsAg) and ALT monthly during the treatment and every 6 months during the follow-up. We performed the test for anti-HBsAg antibodies (anti-HBs) at the end of therapy and during the follow-up. This study was conducted in compliance with the Declaration of Helsinki and in accordance with local regulations. All patients gave written informed consent according to the local ethic committees standards for genotyping analysis.

Study end points

The 'end-of-treatment virological response' was defined as HBV-DNA < 2000 IU/mL (10 000 copies/mL) at the end of therapy; the 'sustained virological response' was defined as HBV-DNA < 2000 IU/mL (10 000 copies/mL) at 6 months after the end of therapy. We evaluated the serological response according to HBsAg loss and anti-HBs appearance (at the end of therapy or during the follow-up). The 'virological relapse' was defined as HBV-DNA > 2000 IU/mL (10 000 copies/mL) after previous end-of-treatment response.

We studied the virological and serological response according to the role of different HBV and CYP27B1 rs4646536 genotypes.

Assays

Serum HBV-DNA levels were quantified with the real-time PCR COBAS AmpliPrep/COBAS TaqMan HBV Test 2.0 (Roche Molecular Systems, Branchburg, NJ, USA). HBV genotypes were determined with the INNOLIPA reverse hybridization assays (Innogenetics, Gent, Belgium). HBsAg, HBeAg, anti-HBs, anti-HBe were detected by the Elecsys instrumental platform (Roche Diagnostics, Monza, Italy); qHBsAg was quantified with ARCHITECT HBsAg (Abbott Diagnostics, Sligo, Ireland). Fibrosis stage (F) was determined before treatment start with Fibroscan using the stiffness values in KPa.

CYP27B1+2838 (rs4646536) polymorphism genotyping

Genomic DNA was isolated from blood samples. We evaluated the rs4646536 C>T CYP27B1, rs12979860 T>C and rs8099917 T>G IL28B single nucleotide polymorphisms (SNP) in patients who have agreed to undergo genetic analyses and for whom blood samples were available. Genotypes were assessed with Taqman Drug Metabolism Genotyping Assays (TaqMan MGM probes, FAM and VIC dye-labelled, Applied Biosystems by Life Technologies, Carlsbad, CA, USA), using a real-time polymerase chain reaction allelic discrimination system (Bio-Rad real-time thermal cycler CFX96) and a standard procedure (primers, probes and PCR conditions available on request).

Statistical analysis

In descriptive statistics, continuous variables were summarized as median (interquartile range (IQR): 25–75th percentiles). Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro–Wilk's test and categorical data were compared using a Mann–Whitney U-test or Kruskal–Wallis statistical test. To investigate continuous data, a Spearman's rank correlation was utilized. The association was calculated using the χ2-test. Multivariate linear regression analysis with stepwise forward selection was performed with P-values of < 0.05 as the criteria for model inclusion.

Statistical analyses were conducted by using SPSS software package ver. 18.0 (SPSS, Chicago, IL, USA).

Results

Baseline characteristics

Table 1 reports baseline characteristics of study population. A total of 190 patients have been included; male were 70.5%, median age was 41 years. Geographic origin was Italy (34%), East Europe (20%), China (23%) and Central Africa (22%). The HBV genotype distribution was A (22%), B (12%), C (10%), D (33%) and E (20%); only one patient owned the F genotype. The median stiffness was 8.5 KPa; 5 patients (2%) showed compensated cirrhosis. Median HBV-DNA was 4.82 Log IU/mL, qHBsAg 3.91 IU/mL, ALT 73 IU/mL. Median age was significant higher in patients with D genotypes (56 years) and lower in B and E genotypes (32 years) (P < 0.001).

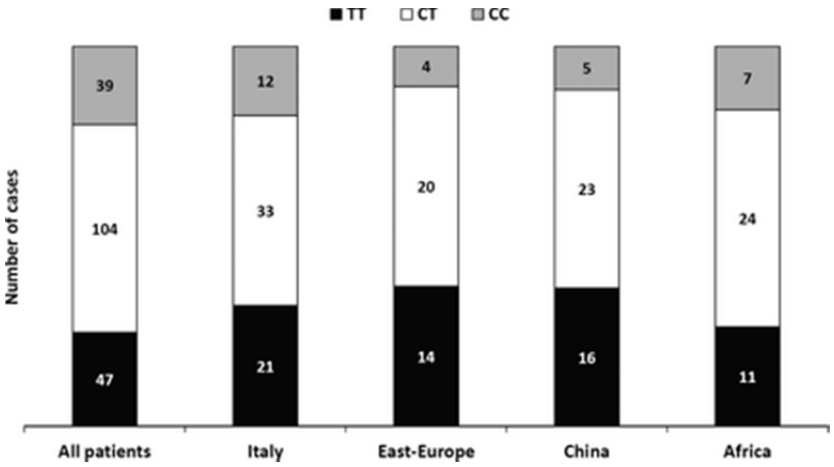
Table 1. Baseline characteristics of the study population

Characteristic	All patients	Genotype A	Genotype B	Genotype C	Genotype D	Genotype E	Genotype F
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
	190	43 (22.6)	24 (12.6)	20 (10.5)	63 (33.2)	39 (20.5)	1 (0.5)
Age (year) median [IQR]; (Range)	41.5 [33.3–55.3]; (21–76)	37.4 [30.9–49.2]; (22–57)	32.5 [27.6–40.3]; (21–60)	37.4 [32.6–47.0]; (23–65)	56.5 [45.7–63.0]; (33–65)	36.9 [28.6–42.9]; (24–56)	56 – –
Male sex n (%)	134 (70.5)	24 (55.8)	17 (70.8)	10 (50)	46 (73)	36 (92.3)	1 (100)
BMI median [IQR]; (Range)	21.7 [20.5–24]; (16.5–29)	22.0 [21.0–24.0]; (16.5–28.5)	21.0 [20.0–21.3]; (18.0–23.5)	21.0 [19.5–23.1]; (17.0–25.0)	24.0 [21.0–26.0]; (17.0–29.0)	21.0 [19.5–22.0]; (17.0–27.0)	24.0 – –
Geographic origin n (%)							
Italy	66 (34.7)	4 (9.3)	0 (0)	0 (0)	61 (96.8)	0 (0)	1 (100)

Characteristic	All patients	Genotype A	Genotype B	Genotype C	Genotype D	Genotype E	Genotype F
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
	190	43 (22.6)	24 (12.6)	20 (10.5)	63 (33.2)	39 (20.5)	1 (0.5)
East Europe	38 (20.0)	36 (83.7)	0 (0)	0 (0)	2 (3.2)	0 (0)	0 (0)
China	44 (23.2)	0 (0)	24 (100)	20 (100)	0 (0)	0 (0)	0 (0)
Central Africa	42 (22.1)	3 (7%)	0 (0)	0 (0)	0 (0)	39 (100)	0 (0)
Fibrosis score (Stiffness KPa) median [IQR]; (Range)	8.5 [7.4–10.9]; (5.9–16.1)	8.2 [7.2–9.6]; (4.9–12.5)	9.7 [7.9–10.4]; (7.2–18.2)	8.5 [7.6–8.9]; (7.1–9.6)	7.5 [6.4–10.2]; (6.1–14.5)	9.2 [7.8–12.5]; (7.2–18.6)	6.7 – –
HBV-DNA BL (Log IU/mL) median [IQR]; (Range)	4.82 [4.26–5.28]; (3.5–8.11)	4.85 [4.43–5.53]; (4.07–8.11)	4.93 [4.45–5.77]; (4.08–7.15)	4.75 [4.26–5.33]; (3.99–5.85)	4.85 [4.26–5.02]; (3.66–5.85)	4.49 [3.91–5.22]; (3.50–6.16)	5.91 – –
qHBsAg BL (Log IU/mL) median [IQR]; (Range)	3.91 [3.79–3.99]; (3.03–4.49)	3.95 [3.88–4.04]; (3.51–4.45)	3.97 [3.89–4.05]; (3.64–4.33)	3.98 [3.86–4.04]; (3.79–4.33)	3.90 [3.68–3.96]; (3.03–4.14)	3.82 [3.63–3.99]; (3.26–4.49)	3.92 – –
ALT BL (IU/mL) median [IQR]; (Range)	73 [59–91]; (42–161)	78 [62–99]; (51–161)	72.5 [52.5–102.5]; (42–126)	68 [56–81.7]; (48–91)	72 [62–91]; (43–144)	72 [59–91]; (43–148)	107 – –

CYP27B1 rs4646536 genotype distribution was 47 (24.8%) TT, 104 (54.7%) CT and 39 (20.5%) CC. No significant difference in this distribution among different geographic group was detected (Fig. 1) (P = 0.246).

Figure 1.



CYP27B1+2838 (rs4646536) genotype distribution among the different ethnicity of the patients.

Treatment response according to HBV genotype

Results of treatment are shown in the Table 2. We observed, for all genotypes, virological response rates of 46.8%, 34.2% and 29.4%, at the end of treatment, at 24 and 48 weeks of follow-up, respectively. Virological relapse rate was 14.7%. We evidenced a significant difference in virological response among HBV genotypes: the sustained virological response was obtained in 48.8%, 62.5%, 30%, 34.9% and 2.5% of A, B, C, D and E genotype, respectively ($P < 0.001$ for E genotype vs others). The A and B genotype showed a better serological response than C, D and E genotype ($P < 0.001$). HBsAg loss occurred in 11.6%, 8.3%, 3.1% and 5% of patients with A, B, D and C genotypes, respectively. No patient with E genotype reached the HBsAg seroclearance.

Table 2. Outcomes of treatment according to HBV genotypes

Outcomes n (%)	HBV genotypes					
	All n = 190	A n = 43	B n = 24	C n = 20	D n = 63	E n = 39
Virological						
End-of-therapy virological response	89 (46.8)	24 (55.8)	18 (75)	11 (55)	31 (42.2)	5 (12.8)
Sustained virological response	61 (32.1)	21 (48.8)	11 (45.8)	6 (30)	22 (34.9)	1 (2.5)
Virological relapse	28 (14.7)	3 (6.9)	7 (29.1)	5 (25)	9 (14)	4 (10.2)
Serological						
HBsAg loss	10 (5.3)	5 (11.6)	2 (8.3)	1 (5)	2 (3.1)	0 (0)
anti-HBs+	6 (3.1)	4 (9.3)	1 (4.2)	0 (0)	1 (1.6)	0 (0)

Treatment response according to CYP27B1 genotype

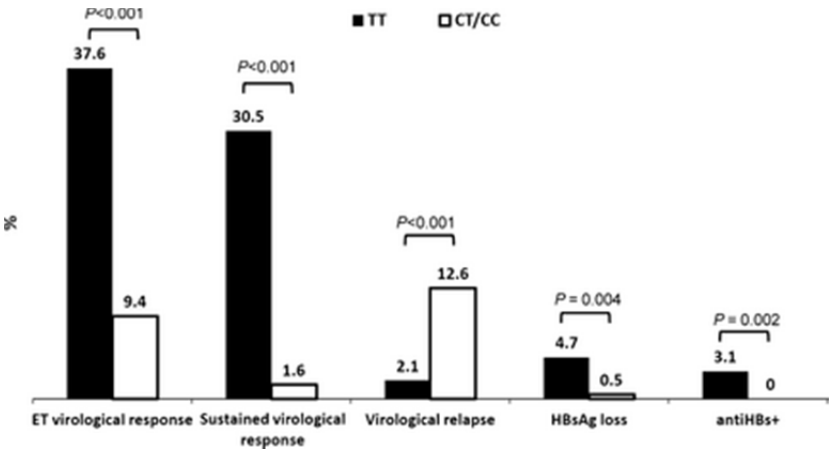
Results of treatment are shown in the Table 3. The TT genotype of CYP27B1 rs4646536 was prevalent in patients with end-of-therapy virological response (37.6%) vs CT/CC (9.4%) (P < 0.001). The sustained virological response was gained in 58 patients with TT genotype (30.5%) vs 3 with CT/CC genotype (P < 0.001). Virological relapse was prevalent in patients with CT/CC genotype (12.6%) vs TT genotype (2.1%) (P < 0.001).

Table 3. Outcomes of treatment according to CYP27B1 rs4646536

Outcomes n (%)	CYP27B1 (rs4646536)		P-value
	TT	CT/CC	
Virological			
End-of-therapy virological response	71 (37.6)	18 (9.4)	<0.001
Sustained virological response	58 (30.5)	3 (1.6)	<0.001
Virological relapse	4 (2.1)	24 (12.6)	<0.001
Serological			
HBsAg loss	9 (4.7)	1 (0.5)	0.004
anti-HBs+	6 (3.1)	0 (0)	0.002

HBsAg loss was obtained in 9 patients with TT genotype (4.7%) vs 1 (0.5%) with CT/CC genotype (P = 0.004); anti-HBs was reached in 6 patients with TT genotype (3.1%) vs none in CT/CC genotype (P = 0.002) (Fig. 2).

Figure 2.



Virological response (end-of-treatment and sustained), virological relapse, serological response (HBsAg loss and antiHBs+) to PEG-IFN according to CYP27B1+2838 (rs4646536) genotype.

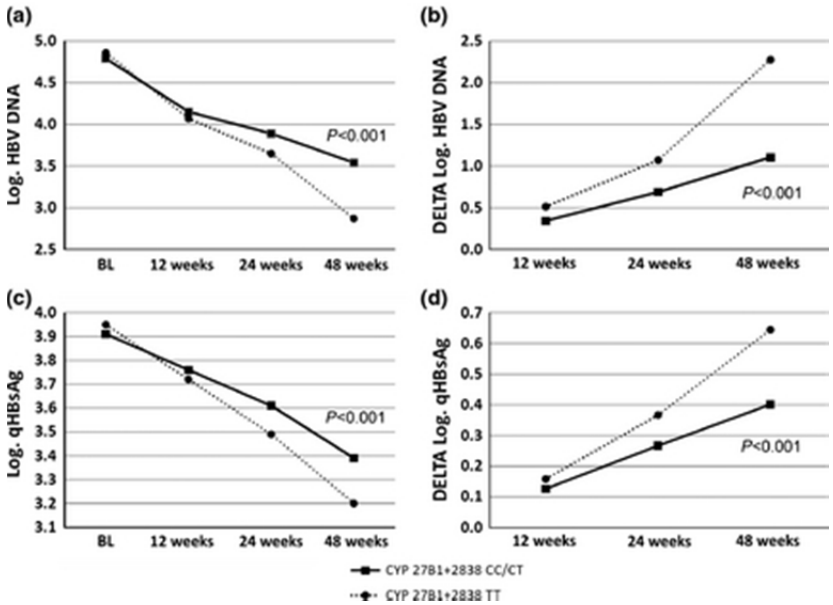
Relationship between HBV genotype and treatment response

We found a significant correlation between HBV genotype and treatment outcomes: serological response ($P = 0.001$) and virological response ($P < 0.001$). Other significant correlation was with HBV-DNA and qHBsAg (Log IU/mL) at baseline ($P < 0.001$), with reduction of HBV-DNA and qHBsAg (Log IU/mL) after 12 weeks of treatment ($P = 0.005$ and $P = 0.003$), after 24 weeks ($P < 0.001$), after 48 weeks ($P < 0.001$).

HBV-DNA and qHBsAg kinetics during the treatment according to CYP27B1 genotype

In the Fig. 3, were reported the kinetics of HBV-DNA (Log IU/mL) (A) and qHBsAg (Log IU/mL) (C) during the treatment with PEG-IFN. We observed a significant difference of HBV-DNA and qHBsAg values at end of therapy between the TT genotype carriers vs CT/CC ($P < 0.001$). We reported also the values of HBV-DNA and qHBsAg decrease (Log IU/mL) (Fig. 3, b and d) after 12, 24 and 48 weeks of treatment. Moreover, values at end of therapy were significantly different between the TT genotype carriers vs CT/CC ($P < 0.001$).

Figure 3.



Decline of HBV-DNA and qHBsAg (a, c) and difference (Delta) from baseline levels after 12, 24 and 48 weeks of treatment (b, d) according to CYP27B1+2838 (rs4646536) genotype.

Univariate and multivariate analysis between clinical outcomes and biological variables

Table 4 shows the results of univariate and multivariate logistic regression analysis: univariate analysis for sustained virological response identifies the following significant factors: age, liver stiffness, B genotype, E genotype, HBV-DNA baseline, qHBsAg baseline, ALT baseline, rs4646536 TT genotype, IL28B rs12979860 CC genotype, IL28B rs8099917 TT genotype. The following variables are the ones which are significantly associated with serological response: age, liver stiffness, genotype A, B, E, HBV-DNA baseline, qHBsAg baseline, ALT baseline, rs4646536 TT genotype, IL28B rs12979860 CC genotype, IL28B rs8099917 TT genotype.

Table 4. Univariate and multivariate analysis between clinical outcomes and biological variables

Baseline variable	Sustained virological response OR (95% CI) P-value		Serological response OR (95% CI) P-value	
	Univariate	Multivariate	Univariate	Multivariate
Age (years)	0.984 (0.963–1.007) P = 0.165	0.980 (0.943–1.018) P = 0.291	0.978 (0.951–1.007) P = 0.133	1.011 (0.964–1.060) P = 0.656
Sex	0.750 (0.400–1.406) P = 0.557		0.787 (0.354–1.748) P = 0.370	
BMI	1.037 (0.936–1.149) P = 0.489		0.988 (0.870–1.122) P = 0.851	
Liver stiffness (kPa)	0.381 (0.263–0.553) P = 0.489	0.411 (0.238–0.708) P < 0.001	0.396 (0.245–0.642) P < 0.001	0.417 (0.291–1.057) P = 0.073
HBV genotype A	1.508 (0.761–2.988) P = 0.239		2.356 (1.091–5.085) P = 0.029	7.936 (2.058–30.606) P = 0.003
HBV genotype B	3.822 (1.444–10.117) P = 0.007	1.702 (0.395–7.340) P = 0.476	3.374 (1.365–8.341) P = 0.008	11.459 (2.397–54.791) P = 0.002
HBV genotype C	1.375 (0.542–3.488) P = 0.503		0.399 (0.089–1.800) P = 0.232	
HBV genotype D	1.309 (0.542–3.488) P = 0.384		0.638 (0.289–1.409) P = 0.266	
HBV genotype E	0.084 (0.028–0.248) P < 0.001	0.030 (0.005–0.162) P < 0.001	0.167 (0.038–0.725) P = 0.017	0.321 (0.103–0.760) P = 0.021
HBV-DNA BL (Log IU/mL)	4.544 (2.599–7.945) P < 0.001	3.489 (1.518–8.019) P = 0.003	3.743 (2.109–6.642) P < 0.001	1.329 (0.599–2.951) P = 0.484
qHBsAg BL (Log IU/mL)	15.722 (3.494–70.745)	0.630 (0.053–7.445)	50.596 (6.805–376.212)	0.935 (0.026–33.651)

Baseline variable	Sustained virological response OR (95% CI) P-value		Serological response OR (95% CI) P-value	
	Univariate	Multivariate	Univariate	Multivariate
ALT BL (IU/mL)	3.134 (1.706–5.757) P < 0.001	1.032 (1.007–1.058) P = 0.011	1.051 (1.033–1.070) P < 0.001	1.039 (1.017–1.063) P = 0.001
rs4646536 TT	3.134 (1.706–5.757) P < 0.001	5.632 (1.938–16.368) P = 0.001	4.292 (2.030–9.074) P < 0.001	6.161 (1.856–20.457) P = 0.003
IL28B rs12979860	11.649 (5.901–22.995) P < 0.001	5.614 (1.801–17.497) P = 0.003	15.273 (5.154–45.256) P < 0.001	30.999 (6.611–145.356) P < 0.001
IL28B rs8099917	4.531 (2.142–9.584) P < 0.001	4.287 (1.284–14.308) P = 0.018	3.715 (1.247–11.067) P = 0.018	0.417 (0.048–3.597) P = 0.426

In multivariate analysis, we found that the following variables are predictive for sustained virological response: liver stiffness (OR = 0.411, CI = 0.238–0.708, P < 0.001), genotype E (OR = 0.030, CI = 0.005–0.162, P < 0.001), HBV-DNA baseline (OR = 3.489, CI = 1.518–8.019, P = 0.003), ALT baseline (OR = 1.032, CI = 1.007–1.058, P = 0.011), rs4646536 TT genotype (OR = 5.632, CI = 1.938–16.368, P = 0.003), IL28B rs12979860 CC genotype (OR = 5.614, CI = 1.801–17.497, P = 0.003), IL28B rs8099917 TT genotype (OR = 4.287, CI = 1.284–14.308, P = 0.018). Dealing with serological response: HBV A genotype (OR = 7.936, CI = 2.058–30.606, P = 0.003), HBV B genotype (OR = 11.459, CI = 2.397–54.791, P = 0.002), HBV E genotype (OR = 0.321, CI = 0.103–0.760, P = 0.021), ALT baseline (OR = 1.039, CI = 1.017–1.063, P = 0.001), rs4646536 TT genotype (OR = 6.161, CI = 1.856–20.457, P = 0.003), IL28B rs12979860 CC genotype (OR = 30.999, CI = 6.611–145.356, P < 0.001).

Discussion

In the treatment of HBV infection with PEG-IFN, it is very important to identify the pretherapy predictive factors useful to select the patients with the highest probability of response, as this therapy is affected by many side effects and limited effectiveness. On-treatment predictors of response were recently reported in previous studies [9, 25-27] which highlight that main response predictive factor results to be the combination of the HBV-DNA and qHBsAg drop after 12 weeks of treatment. The response rate to treatment of HCV was strongly influenced by genetic factors such as ITPA [28-30], IL28B [13, 14, 31, 32] and CYP27B1 [13, 14, 20]. In the HBV treatment, the role of IL28B is still debated and requires further insights, despite some interesting results [9, 33-35]. The vitamin D deficiency has been related with hepatic disease progression and poor response to PEG-IFN therapy in hepatic disease [36], but the role of CYP27B1 and its polymorphisms has not been studied yet in the HBV treatment. This is in our knowledge the first study

about the impact of a CYP27B1 SNP on the response to PEG-IFN therapy in patients with HBV HBeAg negative. The CYP27B1+2838 SNP, rs4646536, has been previously related to autoimmunity disorders and type 1 diabetes [22], multiple sclerosis [37] and cancer [38]. HCV treatment evidenced a negative predictive role [13, 14] (OR = 4.5, CI = 1.54–13.1, P = 0.006) similarly to IL28B rs8099917 (OR = 3.06, CI = 1.14–8.1, P = 0.026) [14].

The role of vitamin D on the immune response was described in previous studies [39, 40], in which the immunomodulatory effect was given on the regulatory T cell [41]. This finding may be a novelty point in the HBV treatment, where the role of immune system and T cell was strongly related to persistence of infection and response to PEG-IFN [42, 43]. The CYP27B1 gene on chromosome 12q13.1-q13.3 encodes the 1α-hydroxylase, the enzyme responsible of conversion of 25(OH)D into 1α,25(OH)2D that evidenced the major biological effects on immune response [44].

Moreover, polymorphisms within CYP27B1 (rs4646536 C>T and rs10877012 C>A, which are in linkage disequilibrium) gene cause altered serum levels of 25(OH)D3 and/or mRNA expression [45]; then, CYP27B1 rs4646536 CC is associated with a reduced amount of CYP27B1 mRNA compared to the healthy controls. From this point of view, our observation results biologically plausible. Rs4646536 CC variant, cause a minor calcitriol synthesis; as the activated hormonal form of vitamin D is an important immune modulator that has an impact on innate and adaptative immune pathways, reduced circulating calcitriol concentrations could lead to a lower response in HBV.

Our study indicates that genetic variation in the CYP27B1 rs4646536 is strongly related to PEG-IFN response in the HBV. In a population with heterogeneous ethnicity and baseline characteristics, we found surprisingly a similar genetic distribution of SNP (Fig. 1).

This is an important finding, because the different genetic distribution among various population was observed according to IL28B SNPs and this was the main confounding factor on the debated role of IL28B in HBV [33]. The TT genotype at rs4646536 is associated with end-of-therapy virological response and sustained virological response, while the CT/CC genotypes are related to treatment failure and virological relapse. In multivariate analysis, we evaluated also the role of IL28B rs12979860 CC and rs8099917 TT genotypes as new pretreatment variables previously associated with virological and serological response in HBV treatment with PEG-IFN [9]. In our data, the rs4646536 TT genotype is the mostly predictive pretreatment factor for sustained virological response (OR = 5.632), similar to IL28B rs12979860 CC genotype (5.614) that becomes the best predictive factor of serological response (OR = 30.999), followed by the HBV B and A genotypes (OR = 11.459 and 7.936, respectively) and rs4646536 TT genotype (OR = 6.161).

The role of HBV E genotype should also be underlined in this analysis, because is the least clarified about the treatment response to PEG-IFN [46] and in our study it is the main negative predictor for both virological and serological response (OR = 0.030 and 0.103). This is a novelty and interesting finding, because this genotype shows the worse response (virological and serological) to PEG-IFN and probably requires other therapeutic strategies. The B genotype confirmed its positive predictive role in the virological response to treatment; in these patients, the PEG-IFN administration should be the most convenient choice. In the D genotype, when the response to PEG-IFN is very poor [47], the CYP27B1 might be more useful for the selection of patient with higher probability to achievement of response. The same reasoning could be applied to C genotype, but the number of patients in this study is too small to give a definitive data.

In our study, we have not evaluated the 25-(OH) vitamin D plasma levels, but we not have considered that as a limit: in fact, the analysis of blood levels of vitamin D is related to sundry obstacles, the main of which is the presence of C-3 epimers of vitamin D compound, derived from reversal of the configuration of the C-3-bound hydroxyl group [48]. The C-3 epimers give to important interferences in the analytic measurement and their biological effect is still understood; they entail a possible overestimation of vitamin D levels of approximately 25%, and accordingly these measurement may appear normal even in patients with liver disease and 25 (OH) D deficiency [49]. Another problem concerns the measurement of vitamin D levels in the obese patients, because a part of vitamin D is seized in the adipose tissue with lack of an appropriate method of detection in this compartment [50]. Further important confounding factors may affect the vitamin D plasma levels: the sunlight exposition and seasonal variability [18], a reduced alimentary sources of vitamin D [51], intestinal malabsorption, lower production of endogenous albumin and reduced hepatic hydroxylation frequent in the cirrhosis [52]. Finally, there is no consensus about the definition of vitamin D deficiency according to plasma measurement: the historical definition is a level of 25(OH)D < 20 ng/mL [53], but others consider a level < 30 or < 40 ng/mL [54]. For all these reasons, we believe that the 25(OH) vitamin D measurement (and not 1-25-dihydroxy vitamin D) may be a confounding factor when was used in the retrospective cohorts of patients and was related to severity of liver disease or PEG-IFN response.

In our opinion, the use of genetic factors that affect the vitamin D deficiency should be the novel approach without the limitations described above.

In conclusion, we suggest that the TT genotype at rs4646536 is an additional and useful baseline predictive factor to PEG-IFN response that may play a helpful role in the pretreatment selection of candidate patients with HBV infection HBeAg negative.

Therefore, further researches and perspective studies are required to confirm this finding on a large cohort of patients.

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