Antiviral Research 102 (2014) 35–43

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Role of *IL28-B* polymorphisms in the treatment of chronic hepatitis B HBeAg-negative patients with peginterferon

Lucio Boglione [1], Jessica Cusato, Sarah Allegra, Isabella Esposito, Francesca Patti, Giuseppe Cariti, Giovanni Di Perri, Antonio D'Avolio *,[1]

Unit of Infectious Diseases, University of Turin, Department of Medical Sciences, Amedeo di Savoia Hospital, Turin, Italy

ARTICLE INFO

Article history:
Received 4 October 2013
Revised 15 November 2013
Accepted 27 November 2013
Available online 4 December 2013

Keywords:
Hepatitis B
IL28-B
PEG-IFN
HBeAg-negative
HBV genotype
Predictive factor

ABSTRACT

Interleukin (*IL*)*28-B* polymorphism has been related to interferon response in the treatment of hepatitis C, but its role in chronic hepatitis B (CHB) therapy is still poorly understood.

We aimed to investigate the effect of *IL28-B* polymorphisms in the treatment with pegylated-interferon (PEG-IFN) of patients with CHB.

We retrospectively analyzed 190 patients with chronic hepatitis B e antigen (HBeAg) negative, genotype A (22%), B (12%), C (10%), D (33%), E (20%), treated with PEG-IFN alfa-2a for 48 weeks; genotype analysis was performed for IL28-B polymorphisms rs12979860, rs8099917 and rs12980275 according to virological, serological and biochemical response.

During 2 years of follow-up 12 patients (6.3%) cleared hepatitis B surface antigen (HBsAg) with seroconversion, 40 (21%) obtained a negative viral load and 104 (54.7%) gained a biochemical response. We found a difference of distribution of rs12979860 CC genotype among different ethnicity ($p = 0.013$). Rs12979860 CC genotype was significantly associated with serological and virological response ($p < 0.001$); rs8099917 TT and rs12980275 AA genotypes were mostly related with virological response ($p < 0.001$). In multivariate logistic analysis rs12979860 CC was predictive of virological response (OR = 4.290; CI = 1.589–11.580, $p = 0.004$) and serological response (OR = 10.129; CI = 2.440–42.044; $p < 0.001$). Rs8099917 TT was predictive only of virological response (OR = 3.746, CI = 1.235–11.355; $p = 0.020$). The E genotype was a negative predictive factor of virological response (OR = 0.057; CI = 0.014–0.238; $p < 0.001$).

IL28-B polymorphisms are related to different response in the treatment of CHB HBeAg-negative with PEG-IFN, and the E genotype is a novel negative predictive factor.

1. Introduction

Chronic hepatitis B 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 240 million people with hepatitis B virus (HBV) persistent infection (WHO, 2013). 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 (Locarnini, 2004), so the main goal of therapy is to prevent the development of cirrhosis, HCC and liver failure (Feld et al., 2009). The main objective of the treatment of CHB is seroclearance of HBsAg, but this goal is difficult; other more easily attainable outcomes are HBV-DNA suppression, improvement of liver histology and alanine aminotransferase (ALT) normalization (Lok and McMahon, 2009).

Treatment options include currently available nucleos(t)ide analogues (NA) with direct anti-viral effect and HBV-DNA suppression, but this treatment has no effect on cccDNA and consequently it does not lead to HBV eradication (Urban et al., 2010). Instead, the therapy with PEG-IFN evidences, an important stimulation of cytotoxic T-cell which, through the lyses of infected hepatocytes and by producing cytokines, can control viral replication (Wong et al., 1993). However, the therapy with PEG-IFN is influenced by a limited tolerability due to its side-effects and the response is dependent from HBV genotype (Fung and Lok, 2004). The

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogues; CHB, chronic hepatitis B; PEG-INF, pegylated interferon; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; HBeAg, hepatitis e antigen; SVR, sustained virological response; cccDNA, closed circular DNA; UNL, upper normal level; qHBsAg, quantitative HBsAg; IQR, inter-quartile range; SNP, single nucleotide polymorphism.

* Corresponding author. Address: Amedeo di Savoia Hospital, C.so Svizzera 164, 10149 Turin, Italy. Tel./fax: +39 011 4393882.

E-mail address: antonio.davolio@unito.it (A. D'Avolio).

[1] Both authors contributed equally to this work.

0166-3542/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2013.11.014

identification of pre-treatment parameters of response to PEG-IFN is essential to optimize this therapy on the basis of a patient stratification. All patients should be selected for PEG-IFN administration based on their individual probability of response (Wong and Lok, 2006). Recently, the influence of single nucleotide polymorphism (SNP) rs12979860 and rs8099917 in the *IL28-B* region of chromosome 19 has been shown to be associated with response to treatment with PEG-IFN and ribavirin in patients with chronic hepatitis C virus (Ge et al., 2009). Carriers of rs12979860 C/C and rs8099917 T/T polymorphisms showed higher rates of rapid virological response (RVR), early virological response (EVR) and SVR (D'Avolio et al., 2011, 2012; Suppiah et al., 2009; Tanaka et al., 2009), so *IL28-B* genotyping plays a decisive role in the clinical practice of HCV treatment (Suppiah et al., 2009). The role of *IL28-B* in the modulation of immune response could suggest a wide effect on other viral infections (Balagopal et al., 2010), but its role in CHB is still poor understood. A recent study evidences that C/C genotype of rs12979860 is not associated with HBV recovery or human immunodeficiency virus (HIV) disease progression (Martin et al., 2010), while in HCV this genotype is strictly related to spontaneous seroclearance (Ge et al., 2009; Thomas et al., 2009). Sonneveld et al. (2012b) examined the effect of *IL28-B* gene polymorphisms rs12979860 and rs12980275 in the response to PEG-IFN in patients with hepatitis B HBeAg-positive; the results of this study showed that the two polymorphisms were independently associated with seroconversion to anti-HBeAg. Lampertico et al. (2013)) analyzed the role of *IL28-B* rs12979860 in the treatment with standard or pegylated interferon in a cohort of HBeAg-negative patients with D genotype and they evidenced that the rate of serum HBsAg clearance was significantly higher in CC versus non-CC genotype carriers ($p = 0.039$). However, despite the recent published papers about *IL28-B* and HBV, the role of *IL28-B* SNPs are still remaining in debating (Chen et al., 2012; de Niet et al., 2013; Holmes et al., 2013b; Kim et al., 2013; Lampertico et al., 2013; Lee et al., 2013a,b; Seto et al., 2013; Sonneveld et al., 2013, 2012a,b).

The aim of our paper is the evaluation of the effect of *IL28-B* rs12979860, rs8099917 and rs12980275 SNPs on the serological, virological and biochemical response to treatment with PEG-IFN in HBeAg-negative patients with different HBV genotypes.

2. Materials and methods

2.1. Patient population

We retrospectively enrolled 190 HBeAg-negative CHB patients treated between 2005 and 2010 with PEG-IFN α-2a for at least 48 weeks at the Infectious Diseases Unit of the Amedeo di Savoia Hospital, Turin, Italy. From an initial cohort of 216 patients with CHB HBeAg-negative treated with IFN at our unit, 3 were excluded because of no PEG-IFN administration, no completed course of therapy of at least 48 weeks ($n = 14$), no data about HBV genotype and stored DNA available ($n = 3$), no at least 2 years of follow-up or validated outcome ($n = 6$). All patients were positive for HBsAg and negative for HBeAg. Inclusion criteria were: 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: 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; we performed a follow-up for at least 2 year after treatment completion. We evaluated the following genotypes in all patients: rs12979860, rs8099917 and rs12980275 SNPs of *IL28-B* gene. HBV genotype was performed before starting the therapy, transient elastography (Fibroscan®) was performed before start of treatment to assess the degree of liver fibrosis. We tested HBV-DNA, quantitative HBsAg (qHBsAg) and ALT monthly during the treatment and every 3 months during the follow-up. We performed antibodies for HBsAg (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 released written informed consent according to standards of the local ethic committees for *IL28-B* genotyping.

2.2. Study end points

End points have been strictly considered according to EASL guideline (EASL, 2009): 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 12 months after the end of therapy. We have evaluated the serological response according to HBsAg loss with or without anti-HBs appearance during the therapy or in follow-up; we have reported also the decline of qHBsAg during the treatment.

We have defined the "virological relapse" as HBV-DNA > 2000 IU/mL (10,000 copies/mL) after previous end-of-treatment response. The "serological relapse" was defined as HBsAg reappearance after previous clearance.

We have studied the virological and serological response according to the role of different HBV genotypes and *IL28-B* genotype.

2.3. Assays

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

2.4. IL28-B genotyping

Genomic DNA was isolated from blood samples. We have evaluated rs12979860, rs8099917 and rs12980275 *IL28-B* polymorphisms in patients who have agreed to undergo genetic analyses and for whom blood samples were available. Genotypes were assessed with Taq Man Drug Metabolism Genotyping Assays (TaqMan MGM probes, FAM and VIC dye-labeled, Applied Biosystem**s** by Life Technologies, Carlsbad, California, 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).

2.5. Statistical analysis

In descriptive statistics continuous variables were summarized as median (Inter-quartile range (IQR): 25th–75th percentiles). Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro–Wilk test and categorical data were compared using a Mann–Whitney or Kruskal–Wallis statistical test. To investigate continuous data, a Spearman Rank correlation was utilized. The association was

calculated using the χ^2-test. Multivariate logistic regression analysis with stepwise forward selection was performed with *p*-values of less than 0.05 as the criteria for model inclusion. Continuous variables are expressed as dichotomous using median values.

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

3. Results

3.1. Baseline characteristics

Table 1 reports baseline characteristics of study population. A total of 190 patients were included; male were 70.5%, median age was 41 years. Geographic origin was: Italy (34%), East-Europe (20%), China (23%), Central Africa (22%). The HBV genotype distribution was: A (22%), B (12%), C (10%), D (33%), 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 (56.5 years) and lower in B and E genotypes (32, 36 years) ($p < 0.001$).

IL28-B genotypes rs12979860 and rs12980275 showed a different distribution among ethnic groups: the CC and AA genotypes were prevalent in Italian and Chinese, while they were less frequent in African ($p = 0.013$; $p = 0.007$). Only 5 patients (2.6%) were classified discordantly to rs12979860 and rs12980275 frequencies.

IL28-B distribution was: 47%, 36%, 15% for CC/TC/TT at rs12979860, 47%, 38%, 14% for AA/GA/GG at rs12980275 and 2%, 23%, 74% for GG/TG/TT at rs8099917 without any significant difference in the distribution among different geographic group ($p = 0.116$) (Table 2).

3.2. Treatment response according to HBV genotype

Results of treatment are shown in Table 3 and Fig. 1. We observed, for all genotypes, an end-of-therapy virological response of 46.8%, 34.2% at 24 weeks of follow-up and 29.4% at 48 weeks after treatment. Virological relapse at 48 weeks of follow-up was 37%. We noticed a significant difference of virological outcomes among HBV genotypes: the sustained virological response was obtained in 48.8%, 62.5%, 30%, 34.9%, 2.5% of A, B, C, D, 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 for all outcomes ($p < 0.001$). HBsAg loss rate after 48 weeks of follow-up was 9.3% in A genotype and 8.3% in B; moreover a reduction >1 Log of qHBsAg during the treatment was observed in 37.2% and 41.7% of genotype A and B, respectively. In D genotype the HBsAg loss rate was 3.1% at 24 weeks of follow-up and 1.6 at 48 weeks, with a reduction >1 Log of qHBsAg for 20.6% of patients. One patient (5%) with C genotype obtained the HBsAg loss. No patient with E genotype reached the HBsAg seroclearance. We found a significant correlation between HBV genotype and treatment outcomes: serological response ($p = 0.001$), virological response ($p < 0.001$). Other significant correlations were 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 ($p < 0.001$).

3.3. Treatment response according to IL28-B genotype

Results are shown in Table 4 and Fig. 2. We observed that virological response was significantly higher in patients with CC genotype at rs12979860 than in patients with TT/CT genotypes ($p < 0.001$). We noticed no significant differences between the two groups ($p = 0.144$) in HBsAg seroclearance, while we found a significant difference in patients with reduction of qHBsAg >1 Log during the treatment ($p < 0.001$).

TT genotype at rs8099917 was significantly prevalent in patients with virological response vs TG/GG ($p = 0.002$ for end-of-therapy virological response, $p < 0.001$ for 24 weeks of follow-up, $p = 0.003$ for 48 weeks of follow-up). No significant differences were found between the two groups according to HBsAg loss and qHBsAg reduction during therapy ($p = 0.343$).

AA genotype for rs12980275 SNP was significantly prevalent in patients with virological response ($p < 0.001$) and in patients with reduction of qHBsAg >1 Log during the treatment ($p < 0.001$), than GG/GA genotype.

Table 1
Baseline characteristics of the study population.

Characteristic	All patients *n*, (%) 190	Genotype A *n*, (%) 43 (22.6)	Genotype B *n*, (%) 24 (12.6)	Genotype C *n*, (%) 20 (10.5)	Genotype D *n*, (%) 63 (33.2)	Genotype E *n*, (%) 39 (20.5)	Genotype F *n*, (%) 1 (0.5)
Age (yr) 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)
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 – –

Table 2
IL28-B genotype distribution [n, (%)] among different geographical origin.

SNP		All patients $n = 190$	Italy $n = 66$	East-Europe $n = 38$	China $n = 44$	Africa $n = 42$	p value
rs12979860							
	TT	30 (15.8)	10 (15.2)	6 (15.8)	5 (11.4)	9 (21.4)	$p = 0.013$
	TC	70 (36.8)	18 (27.3)	16 (42.1)	13 (29.5)	23 (54.8)	
	CC	90 (47.4)	38 (57.6)	16 (42.1)	26 (59.1)	10 (23.8)	
rs8099917							
	TT	141 (74.2)	48 (72.7)	24 (63.2)	33 (75.0)	36 (85.7)	$p = 0.116$
	TG	44 (23.2)	15 (22.7)	12 (31.6)	11 (5.8)	6 (14.3)	
	GG	5 (2.6)	3 (1.6)	2 (1.1)	0 (0)	0 (0)	
rs12980275							
	GG	27 (14.2)	12 (18.2)	5 (13.2)	5 (11.4)	5 (11.9)	$p = 0.007$
	GA	73 (38.4)	18 (27.3)	17 (44.7)	12 (27.3)	26 (61.9)	
	AA	90 (47.4)	36 (54.5)	16 (42.1)	27 (14.2)	11 (26.2)	

number, (%).

Table 3
Number (percentage) of patients and outcomes of treatment according to HBV genotypes.

Outcomes	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)
Virological response at 24 weeks post-treatment	65 (34.2)	21 (48.8)	15 (62.5)	6 (30)	22 (34.9)	1 (2.5)
Virological response at 48 weeks post-treatment	56 (29.4)	19 (44)	14 (58.3)	4 (20)	18 (28.5)	1 (2.5)
Virological relapse* at 48 weeks post-treatment	33 (37)	5 (20.8)	4 (22.2)	7 (63.3)	13 (41.9)	4 (80)
Serological						
HBsAg loss at 24 weeks post-treatment	10 (5.3)	5 (11.6)	2 (8.3)	1 (5)	2 (3.1)	0 (0)
HBsAg loss at 48 weeks post-treatment	8 (4.2)	4 (9.3)	2 (8.3)	1 (5)	1 (1.6)	0 (0)
Serological relapse* at 48 weeks post-treatment	2 (20)	1 (20)	0 (0)	0 (0)	1 (50)	0 (0)
↓ qHBsAg > 1 Log during therapy	43 (22.6)	16 (37.2)	10 (41.7)	2 (10.5)	13 (20.6)	2 (6.5)

number, (%).

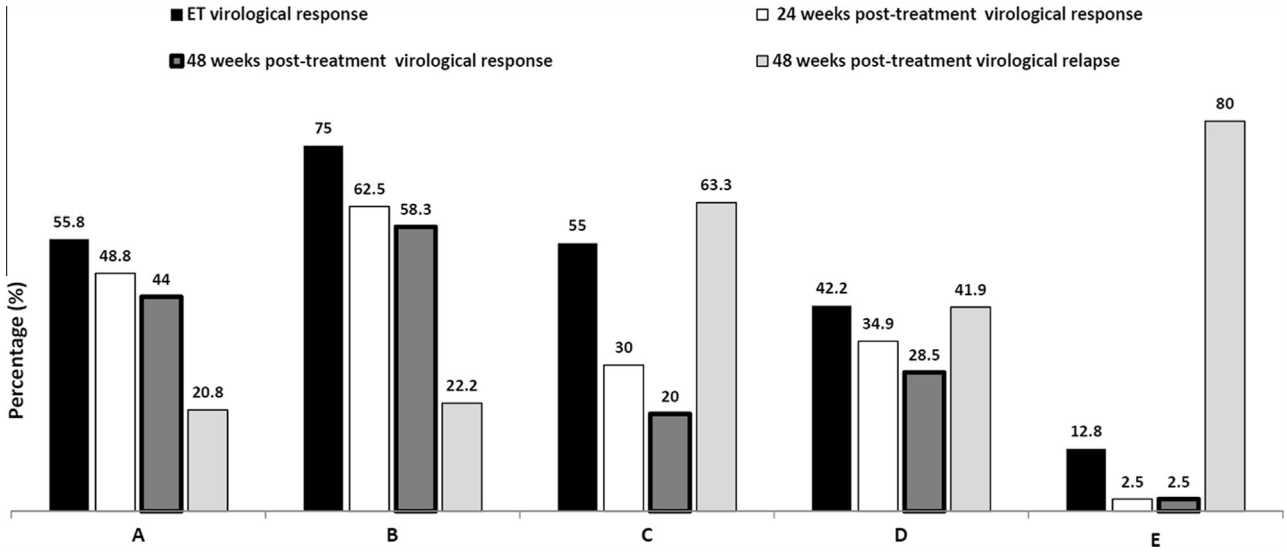

Fig. 1. Serological and virological responses to PEG-IFN according to HBV genotypes.

Table 4
Number (percentage) of patients and outcomes of treatment according to *IL28-B* genotype.

Outcomes	*IL28-B* polymorphisms (*n*, %)								
	rs12979860			rs8099917			rs12980275		
	CC *n* = 90	TT/CT *n* = 100	*p* value	TT *n* = 141	TG/GG *n* = 49	*p* value	AA *n* = 90	GG/GA *n* = 100	*p* value
Virological									
End-of-therapy virological response	69 (76.6)	20 (20)	<0.001	72 (51)	17 (34.6)	0.002	69 (76.6)	20 (20)	<0.001
At 24 weeks post-treatment	60 (66.6)	5 (5)	<0.001	55 (39)	10 (20.4)	<0.001	60 (66.6)	5 (5)	<0.001
At 48 weeks post-treatment	54 (60)	2 (2)	<0.001	51 (36.1)	5 (10.2)	0.003	54 (60)	2 (2)	<0.001
Virological relapse* at 48 weeks post-treatment	7 (7.7)	26 (26)	<0.001	9 (6.3)	24 (49)	<0.001	7 (7.7)	26 (26)	<0.001
Serological									
HBsAg loss at 24 weeks post-treatment	9 (10)	1 (1)	0.144	6 (4.2)	4 (8.1)	0.374	9 (10)	1 (1)	0.144
HBsAg loss at 48 weeks post-treatment	8 (8.8)	0 (0)	0.144	6 (4.2)	2 (4)	0.374	9 (10)	1 (1)	0.144
↓ qHBsAg > 1 Log during therapy	38 (42.2)	5 (5)	<0.001	31 (21.9)	12 (29.4)	0.343	38 (42.2)	5 (5)	<0.001
Serological relapse* at 48 weeks post-treatment	0 (0)	2 (2)	<0.004	0 (0)	2 (4)	0.003	0 (0)	2 (2)	0.004

number, (%).

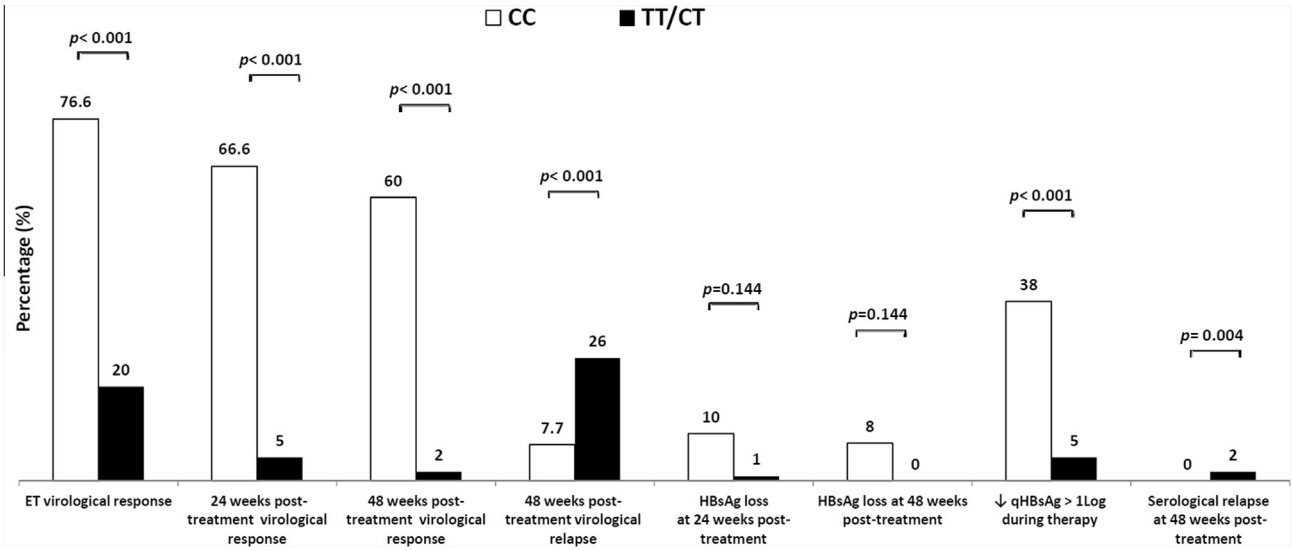

Fig. 2. Serological and virological responses according to rs12979860 polymorphism.

Virological relapse was significantly higher in TT/CT genotypes for rs12979860 SNP than CC ($p < 0.001$), in TG/GG genotypes at rs8099917 than TT ($p < 0.001$), in GG/GA genotypes at rs12980275 than AA ($p < 0.001$).

We evidenced a correlation with the CC genotype of rs12979860 and serological and virological response ($p < 0.001$), with reduction of HBV-DNA and qHBsAg (Log IU/mL) after 12, 24 and 48 weeks of treatment ($p < 0.001$), with a reduction of qHBsAg >1 Log during the treatment ($p < 0.001$).

We noticed a similar correlations between AA genotype of rs12980275 and serological response ($p = 0.019$), virological response ($p = 0.001$), reduction of HBV-DNA and qHBsAg (Log IU/mL) at 12 weeks ($p = 0.028$ and $p = 0.004$), at 24 weeks ($p < 0.001$ and $p = 0.002$), at 48 weeks ($p < 0.001$).

The TT genotype of rs8099917 evidenced the relationship between virological response ($p < 0.001$), reduction of HBV-DNA (Log IU/mL) at 12 weeks ($p = 0.021$), of HBV-DNA and qHBsAg (Log IU/mL) at 24 weeks ($p = 0.030$ and $p = 0.009$, respectively), HBV-DNA (Log IU/mL) at 48 weeks ($p = 0.001$).

3.4. HBV-DNA and qHBsAg kinetics during the treatment according to IL28-B rs12979860

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 CC carriers versus CT/TT ($p < 0.001$). We reported also the values of decrease of HBV-DNA and qHBsAg (Log IU/mL) (B and D) after 12, 24 and 48 weeks of treatment; values at end-of-therapy were significantly different between the CC carriers versus CT/TT ($p < 0.001$).

3.5. Univariate and multivariate analysis between clinical outcomes and biological variables

Table 5 shows the results of univariate and multivariate logistic regression analysis. Univariate analysis for sustained virological response identifies the following significant factors: age < 41 years, stiffness > 8.5 kPa, B genotype, E genotype, HBV-

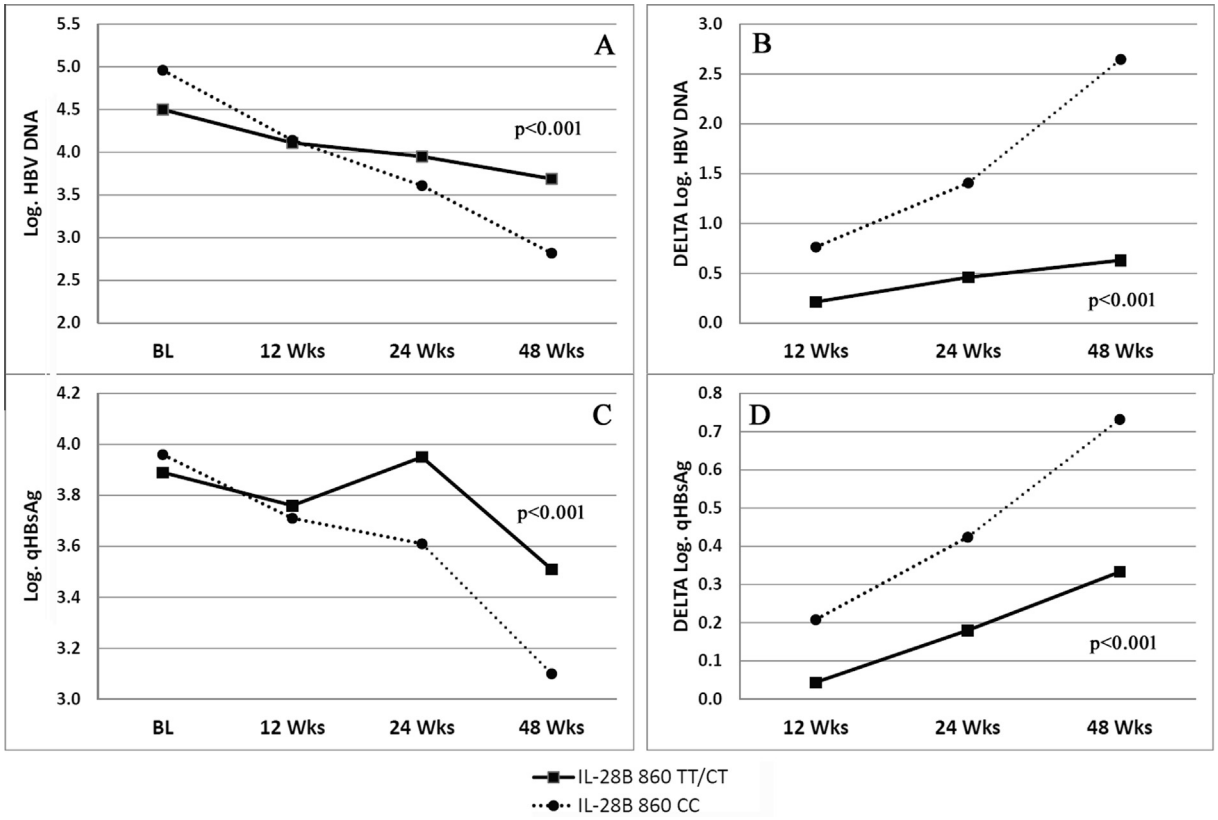

Fig. 3. Trend over time of HBV DNA (A and B) and qHBsAg (C and D) according to *IL28-B* rs12979860 polymorphism.

Table 5
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 < 41 years	0.578 (0.325–1.027) *p* = 0.061	0.402 (0.169–0.958) *p* = 0.026	0.781 (0.386–1.582) *p* = 0.493	
Sex	0.750 (0.400–1.406) *p* = 0.557		0.787 (0.354–1.748) *p* = 0.370	
BMI < 22	1.463 (0.826–2.593) *p* = 0.192	1.279 (0.524–3.119) *p* = 0.589	1.214 (0.599–2.459) *p* = 0.590	
Stiffness > 8.5 kPa	0.224 (0.122–0.412) *p* < 0.001	0.231 (0.098–0.547) *p* = 0.001	0.336 (0.158–0.713) *p* = 0.004	0.324 (0.102–1.033) *p* = 0.057
HBV genotype A	1.508 (0.761–2.988) *p* = 0.239		2.356 (1.091–5.085) *p* = 0.029	5.778 (1.446–23.090) *p* = 0.013
HBV genotype B	3.822 (1.444–10.117) *p* = 0.007	1.365 (0.345–5.406) *p* = 0.658	3.374 (1.365–8.341) *p* = 0.008	9.432 (1.446–39.788) *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	0.057 (0.014–0.238) *p* < 0.001	0.167 (0.038–0.725) *p* = 0.017	0.805 (0.095–6.805) *p* = 0.842
HBV-DNA BL < 4.8 Log IU/mL	3.609 (1.888–6.900) *p* < 0.001	2.258 (0.918–5.555) *p* = 0.076	4.930 (2.342–10.375) *p* < 0.001	3.160 (1.003–9.959) *p* = 0.200
ALT BL > 73 IU/mL	3.090 (1.709–5.587) *p* < 0.001	2.574 (1.018–6.506) *p* = 0.046	12.910 (4.365–38.180) *p* < 0.001	9.198 (1.003–9.959) *p* = 0.004
ΔLog HBV-DNA > 1 Log IU/mL 12 weeks	14.167 (4.77–42.0) *p* < 0.001	2.241 (0.547–9.182) *p* = 0.262	6.600 (2.993–14.556) *p* < 0.001	0.640 (0.154–2.660) *p* = 0.539
ΔLog qHBsAg > 0.5 Log IU/mL 12 weeks	25.861 (3.384–197.651) *p* = 0.002	8.459 (0.715–100.078) *p* = 0.090	63.857 (13.819–295.092) *p* < 0.001	39.909 (5.910–269.488) *p* < 0.001
rs12979860 CC	11.649 (5.901–22.995) *p* < 0.001	4.290 (1.589–11.580) *p* = 0.004	15.273 (5.154–45.256) *p* < 0.001	10.129 (2.440–42.044) *p* < 0.001
rs8099917 TT	4.531 (2.142–9.584) *p* < 0.001	3.746 (1.235–11.355) *p* = 0.020	3.715 (1.247–11.067) *p* = 0.018	0.634 (0.080–5.031) *p* = 0.666
rs12980275 AA	7.400 (3.893–14.065) *p* < 0.001	0.178 (0,012–2.564) *p* = 0.205	9.070 (3.579–22.989) *p* < 0.001	0.783 (0.037–16.784) *p* = 0.876

DNA baseline < 4.8 Log IU/mL, ALT baseline > 73 IU/mL, ΔLog HBV-DNA > 1Log IU/mL at 12 weeks, Δ Log qHBsAg > 0.5 Log IU/mL at 12 weeks, rs12979860 CC, rs8099917 TT, rs12980275 AA. The following variables are the ones which are significantly associated with serological response: stiffness > 8.5 kPa, genotype A, B, E, HBV-DNA baseline < 4.8 Log IU/mL, ALT baseline > 73 IU/mL, Δ Log HBV-DNA > 1 Log IU/mL at 12 weeks, Δ Log qHBsAg > 0.5 Log IU/mL at 12 weeks, rs12979860 CC, rs8099917 TT, rs12980275 AA.

In multivariate analysis we found that the following variables are predictive for sustained virological response: stiffness > 8.5 kPa (OR 0.231, CI 0.098–0.547, $p = 0.001$), genotype E (OR 0.050, CI 0.014–0.242, $p < 0.001$), ALT baseline > 73 IU/mL 2.574 (OR 2.574, CI 1.018–6.506, $p = 0.046$), rs12979860 CC (OR 4.290, CI 1.589–11.580, $p = 0.004$), rs8099917 TT (OR 3.746, CI 1.235–11.355, $p = 0.020$). Dealing with serological response: genotype A (OR 5.778, CI 1.446–23.090, $p = 0.013$), genotype B (OR 9.432, CI 1.446–39.788, $p = 0.002$), ALT baseline > 73 IU/mL (OR 9.198. CI 1.003–9.959, $p = 0.004$), Δ Log qHBsAg > 0.5 Log IU/mL at 12 weeks (OR 39.909, CI 5.910–269.488, $p < 0.001$), rs12979860 CC (OR 10.129, CI 2.440–42.044, $p < 0.001$).

4. Discussion

The role of *IL28-B* polymorphisms in the treatment of chronic HBV is still poor understood; however, a recent study underlined that polymorphisms of *IL28-B* are independently associated with serological response to pegylated interferon alfa in patients with CHB HBeAg-positive (Sonneveld et al., 2012b); in this cohort of patients the CC genotype of rs12979860 and AA genotype of rs12980275 were related to HBeAg seroconversion; the prevalent genotype were C and B, with low number of A and D and six patients with genotype unknown. In contrast, a recent study did not evidenced a role of *IL28-B* rs12979860 in the treatment with PEG-IFN and adefovir in patients with HBeAg-positive and negative (de Niet et al., 2013). Another recent paper evidenced the role of CC genotype of rs12979860 in HBsAg seroclearance in a population of patients with CHB HBeAg-negative treated with IFNα-2a, IFNα-2b or PEG-IFNα-2a and 92% of D genotype (Lampertico et al., 2013). An important pitfall of this study could be the lack of stratification of results according the others HBV genotypes (Sonneveld et al., 2013). This is relevant in our opinion, because the role of HBV genotype has been recently strictly related to PEG-IFN response in HBeAg-positive (Flink et al., 2006) and HBeAg-negative (Lin and Kao, 2011), and ethnicity also influences both the HBV genotype and the *IL28-B* distribution (Rauch et al., 2010), but it is still not understood which of these two elements plays the predominant role according to virological and serological response. For example, in the study of (Holmes et al., 2013a) no correlation were found between the *IL28-B* rs12979860 CC genotype and clinical outcome in patients with HBeAg-positive and negative; in this study the number of patients was small in the two groups and the ethnicity prevalent was the asian, with predominance of HBV B genotype and CC genotype of rs12979860; this finding may have influenced the results, because the HBV B genotype evidenced a good response to PEG-IFN and the low prevalence of CT/TT carriers in this population had probably underestimated the role of *IL28-B*. In our study we examined the impact of rs12979860, rs8099917, rs12980275 *IL28-B* gene polymorphisms on the virological and serological response in a cohort of patients affected by CHB HBeAg-negative, treated with PEG-IFNα-2a for 48 weeks, with different HBV genotype. We have only 63 Italian patients with D genotype (33%); all the other patients are immigrants from East-Europe (Moldavia, Russia, Romania, Albania), from China, from Central Africa (Benin, Togo, Burkina Faso, Cameroun, Gabon, Gambia, Sierra Leone, Senegal, Guinea) with A, B, C, E genotype, respectively. This particular characteristic of our population is strongly related to the activity of our center of care of infectious diseases, where the immigrants come often for other health problems, such sexual transmitted diseases or tropical infectious. In fact, the median age of patients in our cohort is significantly higher in D genotype (56.5 years) than A, B, C and E ($p < 0.001$); we found also a significant differences of *IL28-B* distribution across the different ethnicity of patients: the CC genotype of rs12979860 and AA genotype of rs12980275 are prevalent in Italians, Chinese, East-european people than in Africans (we only have 3 patients from Nigeria with A genotype and 39 from Central-Africa with E genotype) ($p = 0.013$ and $p = 0.007$, respectively; Table 2); on the contrary, we have not found significant differences in the rs8099917 TT genotype distribution ($p = 0.116$). This finding may explicate a global poor response of patients from Central-Africa and E genotype. We underline besides that no data about the treatment with PEG-IFN in patients with CHB and E genotype were currently available; we reported our experience of treatment with a combined approach (entecavir and interferon with sequential administration) in a small cohort of patients with E genotype and HBeAg-positive with high HBV load and we found a low global response (Boglione et al., 2013). In our multivariate logistic regression E genotype is the only negative predictive factor for virological, serological and biochemical response, independently on *IL28-B*. On the other hand, A and B genotype are strong predictors of serological response (OR = 5.778 and 9.432).

Our study, however, aims to clarify the role of *IL28-B* polymorphism in the treatment of CHB with PEG-IFN; in the HCV therapy CC genotype of rs12979860 was related to early and rapid virological response (Ge et al., 2009; Thompson et al., 2010) and rs8099917 TT genotype is associated with sustained virological response (D'Avolio et al., 2011, 2012; Rauch et al., 2010). In our analysis the CC genotype of rs12979860 is associated with end-of-treatment and sustained virological response ($p < 0.001$); the lack of an association with HBsAg loss is probably due, in our opinion, to the small number of patients with this difficult outcome and to the limited period of follow-up. However CC genotype is strongly associated with decline of qHBsAg loss >1 Log during the treatment and with qHBsAg <100 IU/mL and 10 IU/mL at the end of treatment: this is a very important finding, because an association among this end-point and seroconversion after 3 years was recently observed (Brunetto et al., 2009). We found that AA genotype of rs12980275 and TT genotype of rs8099917 are less strongly associated with virological response and with qHBsAg decline; this is an important novelty point and a substantial difference with HCV. In multivariate analysis CC genotype shows to be the best predictor of virological response (OR = 4.290), with ALT baseline (OR = 2.574) and TT genotype (OR = 3.746); dealing with serological response the best predictor is the decline of qHBsAg >0.5 Log after 12 weeks of therapy (OR = 63.857): this finding was supported by previous studies that evidenced the predictive role of kinetics of qHBsAg during the treatment (Rijckborst et al., 2010; Sonneveld et al., 2010). The CC genotype of rs12979860 has a strongly predictive role (OR = 10.129), while TT and AA genotypes show no significant associations. Our data suggest that the best positive predictors of serological response are: decline of qHBsAg >0.5 Log after 12 weeks of therapy (OR = 63.857), CC genotype of rs12979860(OR = 10.129), B genotype (OR = 9.432), ALT baseline > 73 IU (OR = 9.198) and A genotype (OR = 2.365).

The combined role of HBV and *IL28-B* genotypes is a key point in this topic. A comparison with the other studies is limited by heterogeneity of population, treatment and HBeAg status of the patients (de Niet et al., 2013; Holmes et al., 2013b; Lampertico et al., 2013; Sonneveld et al., 2013, 2012a,b). However, we confirmed in Italian patients with D genotype the role of CC genotype at rs12979860 in virological and serological response already observed in a similar

cohort by (Lampertico et al., 2013). In this genotype with poor response to PEG-IFN might be useful the selection of patients with higher probability of response using *IL28-B*. This finding is also observed in patients with A genotype, while in B genotype the impact of *IL28-B* seems to be less relevant.

Our study evidences some limitations: first, it is retrospective; second, the ethnicity heterogeneous is strictly related to HBV and *IL28-B* genotypes and this affects the different influence of *IL28-B* in different populations; third the number of patient with B and C genotype is smaller than other groups; fourth, a longer follow-up is needed to assess the long-term HBsAg seroclearance after 2 years; further studies with large number of patients are required to clarify the exact impact of *IL28-B* on the PEG-IFN treatment.

In conclusion we underline the importance of optimization of treatment with PEG-IFN in CHB because of the limited response and poor tolerability; the on-treatment predictors (HBV-DNA and qHBsAg decline after 12 weeks of therapy) evidenced a good negative predictive value (Rijckborst et al., 2010; Sonneveld et al., 2010), but pretreatment patient selection is needed. Our data underline an interesting role of CC genotype of rs12979860 as the best pretreatment predictor of virological and serological response; the TT genotype of rs8099917 may play a complementary role in the selection of patients, but seems to be related only to virological response. Finally, we emphasize the role of HBV genotype that emerges from this paper: A and B genotype confirmed a positive predictive role in serological response, but E genotype, for the first time, evidences a strong negative predictive value in virological and biochemical response independently to *IL28-B* polymorphisms and this fact suggests that the administration of PEG-IFN in these patients should be avoided, using an alternative approach with NAs therapy.

Conflict of interest

The authors disclose no conflicts.

Funding

This study was not supported.

References

Balagopal, A., Thomas, D.L., Thio, C.L., 2010. IL28B and the control of hepatitis C virus infection. Gastroenterology 139, 1865–1876.
Boglione, L., D'Avolio, A., Cariti, G., Milia, M.G., Simiele, M., De Nicolò, A., Ghisetti, V., Di Perri, G., 2013. Sequential therapy with entecavir and PEG-INF in patients affected by chronic hepatitis B and high levels of HBV-DNA with non-D genotypes. J. Viral. Hepat. 20, e11–e19.
Brunetto, M.R., Moriconi, F., Bonino, F., Lau, G.K., Farci, P., Yurdaydin, C., Piratvisuth, T., Luo, K., Wang, Y., Hadziyannis, S., Wolf, E., McCloud, P., Batrla, R., Marcellin, P., 2009. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 49, 1141–1150.
Chen, J., Wang, L., Li, Y., Cai, B., Fu, Y., Liao, Y., Zhang, J., 2012. Association analysis between SNPs in IL-28B gene and the progress of hepatitis B infection in Han Chinese. PLoS One 7, e50787.
D'Avolio, A., Ciancio, A., Siccardi, M., Baietto, L., Simiele, M., Cariti, G., Calcagno, A., Smedile, A., Cusato, J., Bonora, S., Rizzetto, M., Di Perri, G., 2011. Ribavirin pharmacokinetics and interleukin 28B plus cytochrome P450 27B1 single-nucleotide polymorphisms as predictors of response to pegylated interferon/ribavirin treatment in patients infected with hepatitis C virus genotype 1/4. Hepatology 54, 2278–2279.
D'Avolio, A., Ciancio, A., Siccardi, M., Smedile, A., Simiele, M., Cusato, J., Baietto, L., Marucco, D.A., Cariti, G., Calcagno, A., Gonzalez de Requena, D., Sciandra, M., Troshina, G., Caviglia, G.P., Bonora, S., Rizzetto, M., Di Perri, G., 2012. Negative predictive value of IL28B, SLC28A2, and CYP27B1 SNPs and low RBV plasma exposure for therapeutic response to PEG/IFN-RBV treatment. Ther. Drug Monit. 34, 722–728.
de Niet, A., Takkenberg, R.B., Benayed, R., Riley-Gillis, B., Weegink, C.J., Zaaijer, H.L., Koot, M., Jansen, P.L., Beld, M.G., Lopatin, U., Reesink, H.W., 2013. Genetic variation in IL28B and treatment outcome in HBeAg-positive and -negative chronic hepatitis B patients treated with Peg interferon alfa-2a and adefovir. Scand. J. Gastroenterol. 47, 475–481.
EASL, 2009. EASL clinical practice guidelines: management of chronic hepatitis B. J. Hepatol. 50, 227–242.
Feld, J.J., Wong, D.K., Heathcote, E.J., 2009. Endpoints of therapy in chronic hepatitis B. Hepatology 49, S96–S102.
Flink, H.J., van Zonneveld, M., Hansen, B.E., de Man, R.A., Schalm, S.W., Janssen, H.L., 2006. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. Am. J. Gastroenterol. 101, 297–303.
Fung, S.K., Lok, A.S., 2004. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology 40, 790–792.
Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., Shianna, K.V., Urban, T.J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., Sulkowski, M., McHutchison, J.G., Goldstein, D.B., 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461, 399–401.
Holmes, J.A., Nguyen, T., Ratnam, D., Heerasing, N.M., Tehan, J.V., Bonanzinga, S., Dev, A., Bell, S., Pianko, S., Chen, R., Visvanathan, K., Hammond, R., Iser, D., Rusli, F., Sievert, W., Desmond, P.V., Bowden, D.S., Thompson, A.J., 2013a. IL28B genotype is not useful for predicting treatment outcome in asian chronic hepatitis b patients treated with pegylated-interferon-alpha. J. Gastroenterol. Hepatol..
Holmes, J.A., Nguyen, T., Ratnam, D., Heerasing, N.M., Tehan, J.V., Bonanzinga, S., Dev, A., Bell, S., Pianko, S., Chen, R., Visvanathan, K., Hammond, R., Iser, D., Rusli, F., Sievert, W., Desmond, P.V., Bowden, D.S., Thompson, A.J., 2013b. IL28B genotype is not useful for predicting treatment outcome in Asian chronic hepatitis B patients treated with pegylated interferon-alpha. J. Gastroenterol. Hepatol. 28, 861–866.
Kim, S.U., Song, K.J., Chang, H.Y., Shin, E.C., Park, J.Y., Kim do, Y., Han, K.H., Chon, C.Y., Ahn, S.H., 2013. Association between IL28B polymorphisms and spontaneous clearance of hepatitis B virus infection. PLoS One 8, e69166.
Lampertico, P., Vigano, M., Cheroni, C., Facchetti, F., Invernizzi, F., Valveri, V., Soffredini, R., Abrignani, S., De Francesco, R., Colombo, M., 2013. IL28B polymorphisms predict interferon-related hepatitis B surface antigen seroclearance in genotype D hepatitis B e antigen-negative patients with chronic hepatitis B. Hepatology 57, 890–896.
Lee, D.H., Cho, Y., Seo, J.Y., Kwon, J.H., Cho, E.J., Jang, E.S., Kwak, M.S., Cheong, J.Y., Cho, S.W., Lee, J.H., Yu, S.J., Yoon, J.H., Lee, H.S., Kim, C.Y., Shin, H.D., Kim, Y.J., 2013a. Polymorphisms near interleukin 28B gene are not associated with hepatitis B virus clearance, hepatitis B e antigen clearance and hepatocellular carcinoma occurrence. Intervirology 56, 84–90.
Lee, I.C., Lin, C.H., Huang, Y.H., Huo, T.I., Su, C.W., Hou, M.C., Huang, H.C., Lee, K.C., Chan, C.C., Lin, M.W., Lin, H.C., Lee, S.D., 2013b. IL28B polymorphism correlates with active hepatitis in patients with HBeAg-negative chronic hepatitis B. PLoS One 8, e58071.
Lin, C.L., Kao, J.H., 2011. The clinical implications of hepatitis B virus genotype: recent advances. J. Gastroenterol. Hepatol. 26 (Suppl. 1), 123–130.
Locarnini, S., 2004. Molecular virology of hepatitis B virus. Semin. Liver Dis. 24 (Suppl. 1), 3–10.
Lok, A.S., McMahon, B.J., 2009. Chronic hepatitis B: update 2009. Hepatology 50, 661–662.
Martin, M.P., Qi, Y., Goedert, J.J., Hussain, S.K., Kirk, G.D., Hoots, W.K., Buchbinder, S., Carrington, M., Thio, C.L., 2010. IL28B polymorphism does not determine outcomes of hepatitis B virus or HIV infection. J. Infect. Dis. 202, 1749–1753.
Rauch, A., Kutalik, Z., Descombes, P., Cai, T., Di Iulio, J., Mueller, T., Bochud, M., Battegay, M., Bernasconi, E., Borovicka, J., Colombo, S., Cerny, A., Dufour, J.F., Furrer, H., Gunthard, H.F., Heim, M., Hirschel, B., Malinverni, R., Moradpour, D., Mullhaupt, B., Witteck, A., Beckmann, J.S., Berg, T., Bergmann, S., Negro, F., Telenti, A., Bochud, P.Y., 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 138, 1338–1345 (1345 e1331-1337).
Rijckborst, V., Hansen, B.E., Cakaloglu, Y., Ferenci, P., Tabak, F., Akdogan, M., Simon, K., Akarca, U.S., Flisiak, R., Verhey, E., Van Vuuren, A.J., Boucher, C.A., ter Borg, M.J., Janssen, H.L., 2010. Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B using HBsAg and HBV DNA levels. Hepatology 52, 454–461.
Seto, W.K., Wong, D.K., Kopaniszen, M., Proitsi, P., Sham, P.C., Hung, I.F., Fung, J., Lai, C.L., Yuen, M.F., 2013. HLA-DP and IL28B polymorphisms: influence of host genome on hepatitis B surface antigen seroclearance in chronic hepatitis B. Clin. Infect. Dis. 56, 1695–1703.
Sonneveld, M.J., Rijckborst, V., Boucher, C.A., Hansen, B.E., Janssen, H.L., 2010. Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline. Hepatology 52, 1251–1257.
Sonneveld, M.J., Wong, V.W., Woltman, A.M., Wong, G.L., Cakaloglu, Y., Zeuzem, S., Buster, E.H., Uitterlinden, A.G., Hansen, B.E., Chan, H.L., Janssen, H.L., 2012a. Polymorphisms near IL28B and serologic response to peginterferon in HBeAg-positive patients with chronic hepatitis B. Gastroenterology 142 (513–520), e511.
Sonneveld, M.J., Wong, V.W.S., Woltman, A.M., Wong, G.L.H., Buster, E.H.C.J., Hansen, B.E., Chan, H.L.Y., Janssen, H.L.A., 2012b. polymorphisms at rs12979860 and rs12980275 near IL28B predict serological response to (peg-)interferon in Hbeag-positive chronic hepatitis B. J. Hepatol. 54, S32 (S32).
Sonneveld, M.J., Brouwer, W.P., Janssen, H.L., 2013. Studies of IL28B genotype and response to peginterferon in chronic hepatitis B should be stratified by HBV genotype. Hepatology 57, 1283.
Suppiah, V., Moldovan, M., Ahlenstiel, G., Berg, T., Weltman, M., Abate, M.L., Bassendine, M., Spengler, U., Dore, G.J., Powell, E., Riordan, S., Sheridan, D.,

Smedile, Fragomeli, V., Muller, T., Bahlo, M., Stewart, G.J., Booth, D.R., George, J., 1993. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat. Genet. 41, 1100–1104.

Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M., Matsuura, K., Sakamoto, N., Nakagawa, M., Korenaga, M., Hino, K., Hige, S., Ito, Y., Mita, E., Tanaka, E., Mochida, S., Murawaki, Y., Honda, M., Sakai, A., Hiasa, Y., Nishiguchi, S., Koike, A., Sakaida, I., Imamura, M., Ito, K., Yano, K., Masaki, N., Sugauchi, F., Izumi, N., Tokunaga, K., Mizokami, M., 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat. Genet. 41, 1105–1109.

Thomas, D.L., Thio, C.L., Martin, M.P., Qi, Y., Ge, D., O'Huigin, C., Kidd, J., Kidd, K., Khakoo, S.I., Alexander, G., Goedert, J.J., Kirk, G.D., Donfield, S.M., Rosen, H.R., Tobler, L.H., Busch, M.P., McHutchison, J.G., Goldstein, D.B., Carrington, M., 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461, 798–801.

Thompson, A.J., Muir, A.J., Sulkowski, M.S., Ge, D., Fellay, J., Shianna, K.V., Urban, T., Afdhal, N.H., Jacobson, I.M., Esteban, R., Poordad, F., Lawitz, E.J., McCone, J., Shiffman, M.L., Galler, G.W., Lee, W.M., Reindollar, R., King, J.W., Kwo, P.Y., Ghalib, R.H., Freilich, B., Nyberg, L.M., Zeuzem, S., Poynard, T., Vock, D.M., Pieper, K.S., Patel, K., Tillmann, H.L., Noviello, S., Koury, K., Pedicone, L.D., Brass, C.A., Albrecht, J.K., Goldstein, D.B., McHutchison, J.G., 2010. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 139 (120–129), e118.

Urban, S., Schulze, A., Dandri, M., Petersen, J., 2010. The replication cycle of hepatitis B virus. J. Hepatol. 52, 282–284.

WHO, 2013. Hepatitis B, fact sheet n. 204, In: Organization, W.H. (Ed.), WHO | Hepatitis B, World Health Organization.

Wong, S.N., Lok, A.S., 2006. Treatment of hepatitis B: who, when, and how? Arch. Intern. Med. 166, 9–12.

Wong, D.K., Cheung, A.M., O'Rourke, K., Naylor, C.D., Detsky, A.S., Heathcote, J., 1993. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann. Intern. Med. 119, 312–323.