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IL28B Polymorphisms Predict Interferon-Related Hepatitis B Surface Antigen Seroclearance in Genotype D Hepatitis B e Antigen–Negative Patients With Chronic Hepatitis B

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Interleukin (IL)28B polymorphisms have been associated with interferon (IFN)-induced viral clearance in patients with chronic hepatitis C. Whether this is also true for patients with the difficult-to-cure hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) is unknown. One hundred and one HBeAg-negative patients (92% genotype D) with compensated CHB (84% males, 46 years; hepatitis B virus [HBV] DNA: 6.0 log cp/mL; alanine aminotransferase [ALT]: 136 IU/L; 42% with cirrhosis) were followed up for a median of 11 years (range, 1-17) after a median of 23 months (range, 10-48) of either standard or pegylated (Peg)-IFN-alpha therapy. A post-treatment response was defined as hepatitis B surface antigen (HBsAg) clearance with or without antibody to hepatitis B surface antigen (anti-HBs) seroconversion. The rs12979860 (C>T) genotype in the IL28B locus was assessed in serum samples by using Custom TaqMan SNP Genotyping Assays (Applied Biosystems, Carlsbad, CA). During a median of 11 years of post-treatment follow-up, 21 patients (21%) cleared serum HBsAg, including 15 who developed >10 IU/mL of anti-HBs titers. Forty-eight patients (47%) had CC genotype, 42 (42%) had CT, and 11 (11%) had TT, with the allelic frequency being 68% for C allele and 32% for T allele. The rate of serum HBsAg clearance was 29% (n = 14) in CC compared to 13% (n = 7) in non-CC, genotype carriers (P = 0.039). Baseline HBV DNA levels <6 log cp/mL (odds ratio [OR], 11.9; 95% confidence interval [CI]: 2.8-50.6; P = 0.001), ALT levels >136 IU/L (OR, 6.5; 95% CI: 1.8-22.5; P = 0.003), duration of IFN (OR, 1.16; 95% CI: 1.02-1.31; P = 0.021), and genotype CC (OR, 3.9; 95% CI: 1.1-13.2; P = 0.025) independently predicted HBsAg clearance. *Conclusions*: IL28B polymorphism is an additional predictor of off-therapy IFN-related HBsAg seroclearance to be used in the pretreatment stratification of HBeAg-negative patients chronically infected by genotype D of HBV. (HEPATOLOGY 2013;57:890-896)

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Persistent infection with the hepatitis B virus (HBV) is associated with an increased risk of cirrhosis, hepatocellular carcinoma (HCC), and anticipated liver-related mortality worldwide.[1-4] To attenuate or prevent these long-term sequelae of HBV, interruption of HBV replication by means of inhibitors of HBV polymerase nucleos(t)ide analogs or interferon (IFN) is the only practical approach. IFN therapy, which is recommended as a first-line therapy in patients with less-advanced liver disease and moderately replicating HBV. However, IFN has limited application in virtue of its suboptimal tolerability and restricted antiviral activity in general, and this is particularly true in the hepatitis B e antigen (HBeAg)-

Abbreviations: ALT, alanine aminotransferase; anti-HBe, antibody to hepatitis e antigen; anti-HBs, antibody to hepatitis B surface antigen; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CI, confidence interval; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; OR, odds ratio; PCR, polymerase chain reaction; Peg-IFN-α, pegylated IFN-alpha; SVR, sustained virological response;

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negative patients who predominate in the Mediterranean area.[2-14] These limitations notwithstanding, IFN therapy has gained popularity to treat HBV, because several permanent responders will ultimately achieve seroclearance of hepatitis B surface antigen (HBsAg), which is a proxy of a cure of the infection.[14,15] In view of its limited therapeutic index, many efforts have been made to optimize IFN treatment through pretreatment patient selection based on outcome predictors, but the use of both host- and virus-related variables that predict a response to therapy have not proved to be clinically useful. Indeed, constitutional variables, such as gender and age, and such disease-related predictors as elevated baseline transaminases, low baseline viremia, and moderate liver cell inflammation[16] are either underrepresented in our geographical area (e.g., age and gender) or may vary with time (e.g., HBV DNA and liver inflammation), thus precluding their systematic use in pretreatment patient stratification. The polymorphisms near the IL28B gene, which encodes for IFN-λ-3 and may be implicated in the modulation of innate immune response, were found to be significantly associated with treatment-related resolution of chronic viral hepatitis C (CHC) and offer a chance to improve therapeutic algorithms of patients with CHC based on IFN.[17] Because hepatitis C patients with the rs12979860 CC genotype of interleukin (IL)28B, representing approximately 30% of the Caucasian population, have the greatest chances of a sustained virological response (SVR) to IFN, compared to those carrying the T allele,[18-23] we asked whether IL28B polymorphisms also have any predictive value for IFN therapy outcome in the difficult-to-cure HBeAg-seronegative patients with chronic hepatitis B (CHB). To answer this question, we retrospectively analyzed, for this genetic polymorphism, 101 patients with CHB who were followed up for a median of 11 years after a course of IFN and then correlated findings with permanent seroclearance of HBsAg.

Patients and Methods

Patients. From an initial cohort of 168 consecutive HBeAg-negative CHB patients treated with IFN-based therapy with regular follow-up at our liver unit, 101 individuals were selected who had completed a course of IFN-α-2b (Intron A; Schering-Plough, Milan, Italy), IFN-α-2a (Roferon; Roche, Milan, Italy) 6 MU three times a week (n = 69) or Peg-IFN-α-2a at 180 μg per week (PEGASYS; Roche) (n = 32) with a validated outcome and available repository DNA. Of these, 67 were excluded from analysis because of an incomplete course of IFN (n = 13), lack of a validated outcome (n = 7), missing clinical or virological data or follow-up (n = 45), or absence of adequate stored human cell DNA material for the genetic analysis or refusal to provide genetic testing consent (n = 2). All patients were positive for HBsAg and hepatitis e antigen (anti-HBe) antibody, but negative for HBeAg. Entry criteria included an HBV DNA level of >10.000 copies/mL and an alanine aminotransferase (ALT) level >1 times, but ≤10 times, the upper limit of normal. Patients were excluded if they tested positive for antibody to hepatitis D virus (HDV) or hepatitis C virus (HCV), human immunodeficiency virus (HIV), or had been treated previously with IFN. Exclusion criteria for antiviral treatment were as follows: pregnancy or lactation; drug or alcohol abuse (>40 g ethanol/day); presence of HCC; platelet count less than 100,000/mm^3; white blood cell count less than 3,000/mm^3; serum markers of autoimmunity; renal failure; previous episodes of hepatic decompensation; esophageal varices; or other serious medical illnesses. The extent of liver fibrosis was measured by liver biopsy according to Ishak's score.[24]

In all patients, ALT, aspartate aminotransferase (AST), and HBV DNA levels were measured every 3 months during treatment and every 6 months thereafter. HBsAg and anti-HBs were measured every 3 months during treatment and every year thereafter. We defined treatment outcomes according to HBsAg clearance with or without HBsAg seroconversion after the end of treatment or during follow-up. An end of therapy, response was undetectable viremia by a non–polymerase chain reaction (PCR)-based assay or HBV DNA <2,000 IU/mL by PCR assay. An SVR was HBV DNA suppression, as previously defined, starting 6 months after completion of therapy.

To participate in the study, written informed consent for genetic testing, including IL28B

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Potential conflict of interest: Dr. Lampertico advises and is on the speakers' bureau of Bristol-Myers Squibb, Roche, Gilead, and GlaxoSmithKline. Dr. Colombo advises, is on the speakers' bureau of, and received grants from Merck, Roche, Bristol-Myers Squibb, Gilead, Novartis, and Vertex. Dr. Vigano is on the speakers' bureau of Roche and Bristol-Myers Squibb. He is also in the speakers' bureau of and recieved grants from Gilead.

polymorphisms, was obtained from all individuals, and the study protocol was evaluated and approved by the local institutional review committee.

Serum Assays. HBsAg, antibody to HBsAg (anti-HBs), HBeAg, and anti-HBe were detected by a microparticle enzyme immunoassay (AXSYM; Abbott Laboratories, North Chicago, IL). Antibody to HDV was assessed by enzyme-linked immunosorbent assay (Sorin Biomedica, Saluggia, Italy). Antibody to HIV was detected by HIV-1 third-generation assay (AXSYM HIV 1/2; Abbott Laboratories), and antibody to HCV was detected by a second-generation test system (Ortho Diagnostic Systems, Raritan, NJ). Until 2007, serum HBV DNA level was detected by non-PCR assays (Digene Hybrid Capture System; Digene Diagnostics, Inc., Beltsville, MD) and branched DNA (Bayer Corp., Tarrytown, NJ), and starting from 2007, HBV DNA was measured by a real-time PCR assay (COBAS TaqMan; Roche), which has a lower limit of quantification of 12 IU/mL. HBV genotypes were retrospectively looked for from frozen serum sample by line-probe assay (INNO-LiPA HBV Genotyping; Innogenetics N.V., Ghent, Belgium), containing specific probes for the six major genotypes (A-F). ALT and AST levels were measured by automated optimized colorimetry at 37°C.

rs12979860 Single-Nucleotide Polymorphism Genotyping. Genotyping was conducted in a blinded fashion, relative to HBV treatment status and other characteristics, on DNA specimens collected from each individual, using the 5' nuclease assay with allele-specific TaqMan probes (TaqMan SNP Genotyping Assay on a 7900HT real-time PCR instrument; Applied Biosystems, Carlsbad, CA).

Statistical Analysis. Data were expressed as counts and percentages for qualitative variables and as median and range for discrete variables. Comparisons between groups were carried out using nonparametric analysis of variance. Associations between different qualitative parameters were explored using the chi-square test or Fishers's exact test, as appropriate. Univariable (i.e., chi-square test) and multivariable (i.e., logistic regression) analyses were used to determine predictors of response. Primary analysis of the association between rs12979860 and treatment response considered the following: age; gender; serum ALT levels; serum HBV DNA; HBV genotype; cirrhosis; duration of treatment; and type of IFN. Gender, cirrhosis, type of IFN, and genotype were included as categorical variables; age, serum ALT, duration of treatment, and serum HBV DNA were included as continuous variables. Those pretreatment factors found to be significant in the overall logistic regression model were assessed in stratified analyses to show rates of response across different categories of these characteristics. Statistical analysis was done with *STATA* software (Stata Statistical Software: Release 7.0; StataCorp LP, College Station, TX).

Table 1. Baseline Demographic, Clinical, and Virological Features of the 101 Patients With CHB Enrolled in the Study

Patient Features	
Age, years*	46 (26-63)
Males, no. (%)	85 (84)
Cirrhosis, no. (%)	42 (42)
ALT, IU/L*	136 (32-932)
HBV DNA, $\log_{10}$ copies/mL*	6.0 (3.3-9.0)
HBV genotype D, no. (%)	93 (92)
IFN standard, no. (%)	69 (68)
Treatment duration, months*	23 (10-48)
Post-treatment follow-up, years*	11 (1-17)
IL28B genotype (%)	
CC	48 (47)
CT	42 (42)
TT	11 (11)

*Median (range).

Results

All patients were of European ancestry (Table 1), most were men, infected with genotype D of HBV and with advanced liver fibrosis, and IFN was given for a median of 23 months (range, 10-48). The IL28B polymorphism was genotype CC in 48 patients, CT in 42, and TT in 11, with the allelic frequency being 68% for C allele and 32% for T allele. Overall, CC, CT, and TT carriers were comparable in terms of age, gender, serum ALT levels, serum HBV DNA, HBV genotype, cirrhosis, duration of treatment, type of IFN, and duration of follow-up (Table 2). The proportion of patients with <12, 12-24, and >24 months of treatment was similar in the three IL28B groups: 8%, 61%, and 31% for the CC group; 14%, 69%, and 17% for the CT group; and 9%, 45.5%, and 45.5% for the TT group (CC versus CT versus TT, $P = 0.28$; CC versus non-CC, $P = 0.52$).

End-of-treatment response as well as SVR were significantly higher in CC than in non-CC patients (end-of-treatment response: 69% versus 45%, $P = 0.014$; SVR: 31% versus 13%, $P = 0.025$) (Table 3). At univariate analysis, duration of IFN (odds ratio [OR], 1.09; 95% confidence interval [CI]: 1.01-1.17; $P = 0.02$) and genotype CC (OR, 2.86; 95% CI: 1.26-6.49; $P = 0.01$) were associated with an increased likelihood of end-of-treatment response. Baseline HBV DNA levels (OR, 0.70; 95% CI: 0.50-0.98;

Table 2. Demographic, Clinical, and Virological Features of Patients According to rs12979860 Genotype

Patient Features	CC (n = 48)	CT (n = 42)	TT (n = 11)	*P* Value†	*P* Value‡
Age, years*	45 (26-61)	47 (28-63)	48 (29-62)	0.31§	0.60§
Males, no. (%)	38 (79)	37 (88)	10 (91)	0.15‖	0.41¶
Cirrhosis, no. (%)	20 (42)	19 (45)	3 (27)	0.57‖	0.56¶
ALT, IU/L*	153 (32-720)	135 (32-932)	88 (44-235)	0.07§	0.08§
HBV DNA, $\log_{10}$ copies/mL*	6.3 (3.3-9.0)	6.0 (4.0-9.0)	5.3 (5.0-7.9)	0.58§	0.54§
HBV genotype D, no. (%)	45 (94)	38 (90)	10 (91)	0.41‖	0.84¶
IFN standard, no. (%)	32 (67)	29 (69)	8 (73)	0.44‖	0.92¶
IFN treatment, months*	23 (10-48)	23 (12-33)	22 (12-32)	0.60§	0.78§
Post-treatment follow-up years*	11 (1-16)	12 (1-17)	11 (2-16)	0.86§	0.98§

*Median (range).
†CC versus non-CC patients.
‡CC versus CT versus TT patients.
§Kruskal-Wallis' test.
‖Fisher's exact test.
¶Chi-square test.

$P = 0.03$), duration of IFN (OR, 1.09; 95% CI: 1.00-1.18; $P = 0.03$), and genotype CC (OR, 2.99; 95% CI: 1.24-7.21; $P = 0.01$) independently predicted end-of-treatment response. An SVR was associated with age (OR, 0.94; 95% CI: 0.89-0.99; $P = 0.02$), HBV DNA levels (OR, 0.53; 95% CI: 0.34-0.84; $P = 0.007$), duration of post-treatment follow-up (OR, 1.10; 95% CI: 1.00-1.21; $P = 0.03$), and CC genotype (OR, 2.98; 95% CI: 1.09-8.13; $P = 0.03$). At multivariate analysis, age (OR, 0.92; 95% CI: 0.87- 0.98; $P = 0.01$), baseline HBV DNA levels (OR, 0.44; 95% CI: 0.27-0.74; $P = 0.002$), and genotype CC (OR, 3.7; 95% CI: 1.19-11.5; $P = 0.02$) independently predicted an SVR.

In the post-treatment follow-up, 95 patients (94%) were seen at least every year at the outpatient clinic, and 75 (74%) fulfilled the predefined strict post-treatment surveillance program of yearly HBsAg testing. Among the 26 patients with suboptimal HBsAg testing rates, 16 (62%) had this marker being performed for at least 5 years. During a median of 11 years of post-treatment follow-up, 21 patients (21%) cleared HBsAg and 15 developed >10 IU/mL of anti-HBs titers. HBsAg clearance rates were 29% (14 patients) in CC, 7% (3 patients) in CT, and 36% (4 patients) in TT.

By univariate analysis, baseline HBV DNA, ALT, duration of treatment, post-treatment follow-up, and IL28B polymorphisms, but not age, gender, cirrhosis, HBV genotype, and type of IFN, were significantly associated with HBsAg clearance (Table 4). At multivariate analysis, baseline HBV DNA levels (OR, 0.31; 95% CI: 0.15-0.62; $P = 0.001$), ALT levels (OR, 1.0; 95% CI: 1.0-1.0; $P = 0.03$), duration of IFN therapy (OR, 1.20; 95% CI: 1.04-1.39; $P = 0.012$), and genotype CC (OR, 3.6; 95% CI: 1.05-12.5; $P = 0.04$) predicted HBsAg seroclearance (Table 5). When baseline levels of HBV DNA and ALT were expressed as dichotomous variables by using median values, HBV DNA levels <6 log cp/mL (OR, 11.9; 95% CI: 2.8-50.6; $P = 0.001$), ALT levels >136 IU/L (OR, 6.5; 95% CI: 1.8-22.5; $P = 0.003$), duration of IFN therapy (OR, 1.16; 95% CI: 1.02-1.31; $P = 0.021$), and genotype CC (OR, 3.9; 95% CI: 1.1-13.2; $P = 0.025$) were confirmed to predict HBsAg seroclearance. By combining HBV DNA, ALT, and IL28B genotype, patients were classified into four different groups characterized by increasing rates of HBsAg loss (Table 6).

Table 3. Rates of Virological Response to IFN Therapy According to IL28B Genotype

Response	CC (n = 48)	CT/TT (n = 53)	*P* Value
End-of-therapy virological response (%)	33 (69)	24 (45)	0.01
SVR (%)	15 (31)	7 (13)	0.02
HBsAg clearance (%)	14 (29)	7 (13)	0.04

Table 4. Demographic, Clinical, and Virological Features of Patients With and Without HBsAg Seroclearance

	HBsAg Seroclearance		
Patient Features	Yes (n = 21)	No (n = 80)	*P* Value
Age, years*	40 (26-62)	47 (26-63)	0.11†
Males, no. (%)	17 (81)	68 (85)	0.43‡
Cirrhosis, no. (%)	9 (43)	33 (41)	0.54‡
ALT, IU/L*	201 (45-720)	129 (32-932)	0.04†
HBV DNA, $\log_{10}$ copies/mL*	5.0 (4.0-9.0)	6.3 (3.3-9.0)	0.002†
HBV genotype D, no.* (%)	19 (90)	74 (92)	0.53‡
IFN standard, no. (%)	18 (86)	51 (63)	0.05‡
Duration of IFN treatment, months*	24 (16-48)	22 (10-33)	0.01†
Post-treatment follow-up, years*	12 (1-16)	7 (1-17)	0.02†
IL28B CC genotype (%)	14 (67)	34 (43)	0.04‡

*Median (range).
†Kruskal-Wallis' test.
‡Fisher's exact test.

Table 5. Baseline Factors That Predicted End-of-Treatment Response, SVR, and HBsAg Clearance by Univariate and Multivariate Analysis

Baseline Variable	End-of-Treatment Response OR (95% CI); *P* Value		SVR OR (95% CI); *P* Value		HBsAg Clearance OR (95% CI); *P* Value	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
Age, years	1.01 (0.97-1.05); 0.54		0.94 (0.89-0.99); 0.02	0.92 (0.87-0.98); 0.01	0.95 (0.90-1.00); 0.10	
Male	0.70 (0.23-2.12); 0.53		0.80 (0.23-2.80); 0.73		0.75 (0.21-2.61); 0.65	
Cirrhosis	0.58 (0.26-1.29); 0.18		0.75 (0.28-2.00); 0.57		1.06 (0.40-2.82); 0.89	
ALT levels, IU/L	1.00 (0.99-1.00); 0.61		1.00 (0.99-1.00); 0.16		1.00 (1.00-1.00); 0.02	1.00 (1.00-1.00); 0.03
HBV-DNA levels, log cp/mL	0.74 (0.54-1.01); 0.06	0.70 (0.50-0.98); 0.03	0.53 (0.34-0.84); 0.007	0.44 (0.27-0.74); 0.002	0.53 (0.33-0.84); 0.008	0.31 (0.15-0.62); 0.001
HBV genotype D	1.26 (0.29-5.38); 0.74		0.82 (0.15-4.38); 0.81		0.77 (0.14-4.12); 0.76	
IFN standard	0.53 (0.22-1.28); 0.16		1.76 (0.58-5.30); 0.31		3.41 (0.92-12.5); 0.06	
IFN treatment, months	1.09 (1.01-1.17); 0.02	1.09 (1.00-1.18); 0.03	1.07 (0.99-1.17); 0.08		1.16 (1.04-1.30); 0.008	1.20 (1.04-1.39); 0.01
Post-treatment follow-up, years	1.00 (0.93-1.07); 0.90		1.10 (1.00-1.21); 0.03		1.11 (1.01-1.22); 0.02	
CC genotype IL28B	2.86 (1.26-6.49); 0.01	2.99 (1.24-7.21); 0.01	2.98 (1.09-8.13); 0.03	3.72 (1.19-11.5); 0.02	2.70 (0.98-7.42); 0.04	3.63 (1.05-12.5); 0.04

Table 6. HBsAg Loss Rates in Patients With Different Baseline Clinical and Genomic Profile

Patient Features	CC (%)	CT/TT (%)
Overall	29	13
HBV DNA low and ALT high	60	38
HBV DNA low and ALT low	28	11
HBV DNA high and ALT high	23	0
HBV DNA high and ALT low	0	0

HBV DNA levels (low: <6 log cp/mL; high: >6 log cp/mL) and ALT levels (high: >136 IU/L; low: <136 IU/L) are shown.

Discussion

Optimization of IFN treatment of HBeAg-negative patients with CHB, based on an accurate pretreatment patient selection by response predictors, might help in overcoming such barriers to treatment as the need for parenteral administration, limited effectiveness, and poor tolerability. Our findings of an association between IL28B polymorphisms and likelihood of an SVR to IFN in the difficult-to-cure patients with CHB partially meet these expectations. Indeed, we demonstrated increased rates of SVR and HBsAg seroclearance in HBeAg-negative patients with CHB who carry the CC genotype of rs12979860 IL28B, compared to those carrying either the CT or TT genotype. Thus, our findings suggest that IL28B polymorphism adds to the already known list of pretreatment predictors of IFN therapy outcome that might be used for optimizing the management of the difficult-to-cure HBeAg-negative patients with chronic infection with the genotype D of HBV. This genetic pretreatment predictor, in fact, has major advantages over classical constitutional or virus-related predictors of treatment outcome that, as with age and gender, are either underrepresented in our geographical area or, as with viremia and ALT, may vary during the natural course of hepatitis, thus imposing a close patient monitoring to detect the appropriate timing of IFN therapy.[14,16,25] Because in our geographical regions the less IFN-sensitive genotype D of HBV prevails among HBeAg-negative CHB, pretreatment patient stratification by a trustable, user-friendly treatment-outcome predictor might improve the cost-effectiveness ratio of IFN therapy. This might be the case of IL28B polymorphisms in the prediction of IFN-associated HBsAg seroclearance in HBeAg-negative patients with CHB, because rates of seroclearance were 3.9-fold higher in CC patients than in T-allele carriers (TT and CT). Our findings that baseline ALT and HBV DNA levels predicted an SVR to IFN independently on IL28B not only confirmed previous studies in patients with CHB, but also clearly indicates that our study was not biased

by the retrospective selection of patients, thus well representing the complex pattern of the practice of HBV in our region. Interestingly, the combination of baseline values of ALT, HBV DNA, and IL28B polymorphisms allowed us to identify a set of patients with a high chance (60%) of losing serum HBsAg after IFN therapy. Although we acknowledge that the identification of a predictor of IFN treatment outcome in such a difficult-to-cure set of patients as HBeAg-seronegative patients infected by genotype D of HBV is clinically relevant, we also recognize that the prediction power of this combined set of variables is somehow diluted in an 11-year off-therapy period. On the other hand, no other predictor of IFN therapy outcome of comparable power is available to optimize the management of HBeAg-negative patients with CHB, including the on-therapy kinetics of serum HBsAg, which better performs as a negative predictor of treatment response.[26,27]

The predictive value of IL28B has been recently documented also in the different scenario of HBeAg-positive patients with CHB from North Europe and Asia, where patients with other than D genotypes of HBV predominate.[28] In that study, IL28B polymorphism was, in fact, positively associated with both HBeAg seroconversion and HBsAg seroclearance, supporting the existence of a genetic control of anti-HBV response to IFN, operating across a number of clinical variables, including the phase of infection (i.e., HBeAg versus anti-HBe) and disease severity, as shown by the high rate (42%) of patients with cirrhosis present in our study. One possible explanation for the association between IL28B and IFN response is the role of IL28B in the activation of the antiviral cascade by Janus kinase/signal transducer and activator of transcription, a very well-characterized event in the HCV scenario.[29] In difficult-to-cure patients with genotype 1 of HCV, the IL28B CC genotype was associated with an early decline of serum HCV-RNA, heralding an SVR in most of the patients.[21] Along the same line, it is worth noting that CC patients with the difficult-to-cure HBeAg-negative CHB have a faster, early decline of serum HBV DNA after IFN therapy, compared to T-allele carriers, a phenomenon that could herald faster seroclearance of HBsAg in CC patients (Prof. Alberti, A., Department of Molecular Medicine, University of Padova, personal communication).

We acknowledge that the strengths of our study are counterbalanced by a number of limitations. The homogeneous criteria for patient selection, the extended duration of IFN therapy, the 11 years of median post-treatment follow-up, and the hard endpoint of HBsAg seroclearance strongly support our conclusions about the predictive value of IL28B polymorphisms. On the other hand, our findings need to be externally validated in a larger cohort of patients prospectively followed up in whom seroclearance of HBsAg can be tested by a time-dependent multivariate analysis, including those receiving Peg-IFN therapy. The predictive power of IL28B polymorphism needs also to be investigated in HBeAg-negative patients with other than genotype D infection. Indeed, in the HCV scenario, the predictive value of IL28B polymorphisms appeared to be attenuated in patients with easy-to-cure genotypes HCV 2 and 3, compared to the more difficult-to-cure HCV 1 and 4, and in patients receiving potentiated IFN therapy by combination with direct antiviral agents.[22,30,31]

All in all, our findings indicate that the IL28B polymorphism is a reliable predictor of IFN therapy outcome in HBV, which might be used for pretreatment stratification aimed at optimizing the treatment of such a difficult-to-cure patient population as HBeAg-negative carriers of genotype D of HBV.

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References

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
2. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335-352.
3. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol 2009;50: 227-242.
4. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. HEPATOLOGY 2009;50:661-662.
5. Carosi G, Rizzetto M, Alberti A, Cariti G, Colombo M, Craxì A, et al. Treatment of chronic hepatitis B: update of the recommendations from the 2007 Italian Workshop. Dig Liver Dis 2011;43:259-265.
6. Brunetto MR, Oliveri F, Rocca G, Criscuolo D, Chiaberge E, Capalbo M, et al. Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis B e antigen. HEPATOLOGY 1989;10:198-202.
7. Hadziyannis S, Bramon T, Makris A, Moussoulis G, Zignego L, Papaioannou C. Interferon alfa-2b treatment for HBeAg negative/serum HBV-DNA positive chronic active hepatitis type B. J Hepatol 1990; 11(Suppl. l):S133-S136.
8. Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. J Hepatol 2001;34:306-313.
9. Brunetto MR, Oliveri F, Coco B, Leandro G, Colombatto P, Gorin JM, Bonino F. Outcome of anti-HBe positive chronic hepatitis B in alpha interferon treated and untreated patients: a long term cohort study. J Hepatol 2002;36:263-270.
10. Oliveri F, Santantonio T, Bellati G, Colombatto P, Mels GC, Carriero L, et al. Long-term response to therapy of chronic anti-HBe-positive hepatitis B is poor independent of type and schedule of interferon. Am J Gastroenterol 1999;94:1366-1372.

11. Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. Gastroenterology 2001;121:101-109.
12. Lampertico P, Del Ninno E, Manzin A, Donato MF, Rumi MG, Lunghi G, et al. A randomized, controlled trial of a 24-month course of interferon alfa 2b in patients with chronic hepatitis B who had hepatitis B virus DNA without hepatitis B e antigen in serum. Hepatology 1997;26:1621-1625.
13. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206-1217.
14. Lampertico P, Del Ninno E, Viganò M, Romeo R, Donato MF, Sablon E, et al. Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003; 37:756-763.
15. Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology 2009;136:2169-2179.
16. Bonino F, Marcellin P, Lau GK, Hadziyannis S, Jin R, Piratvisuth T, et al. Predicting response to peginterferon alpha-2a, lamivudine, and the two combined for HBeAg-negative chronic hepatitis B. Gut 2007; 56:699-705.
17. Afdhal NH, McHutchison JG, Zeuzem S, Mangia A, Pawlotsky JM, Murray JS, et al. Hepatitis C pharmacogenetics: state of the art in 2010. Hepatology 2011;53:336-345.
18. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;46:399-401.
19. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-a and ribavirin therapy. Nat Genet 2009;41:1100-1104.
20. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-a and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105-1109.
21. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 2010;139:120-129.
22. Mangia A, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, et al. Interleukin-28B polymorphism determines treatment response of patients with hepatitis C genotypes 2 or 3 who do not achieve a rapid virologic response. Gastroenterology 2010;139:821-827.
23. McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, et al. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology 2010;138:2307-2314.
24. Ishak K, Baptista A, Bianchi L, Suchindran S, Lao XQ, Patel K, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22:696-699.
25. Moucari R, Martinot-Peignoux M, Mackiewicz V, Boyer N, Ripault MP, Castelnau C, et al. Influence of genotype on hepatitis B surface antigen kinetics in hepatitis B e antigen-negative patients treated with pegylated interferon-alpha2a. Antivir Ther 2009;14:1183-1188.
26. Rijckborst V, Hansen BE, Cakaloglu Y, Ferenci P, Tabak F, Akdogan M, et al. Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B using HBsAg and HBV DNA levels. Hepatology 2010;52:454-461.
27. Rijckborst V, Hansen BE, Ferenci F, Brunetto MR, Tabak F, Cakaloglu Y, et al. Early on-treatment HBsAg and HBV DNA levels identify HBeAg-negative patients not responding to 48 or 96 weeks of peginterferon alfa-2a therapy. Hepatology 2010;52(Suppl 4):479A.
28. Sonneveld MJ, Wong VW, Wotman AM, Wong GLH, Buster EHCJ, Hansen BE, et al. Polymorphisms at rs12979860 and rs12980275 near IL28B predict serological response to (Peg)-interferon in HBeAg-positive chronic hepatitis B. J Hepatol 2011;54 (Suppl 1):S32.
29. Sarasin-Filipowicz M, Oakeley EJ, Duong F, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. PNAS 2008;105:7034-7039.
30. Poordad F, Bronowicki JP, Gordon SC, Zeuzem S, Jacobson IM, Sulkowski MS, et al. IL28B polymorphism predicts virologic response in atients with hepatitis C genotype 1 treated with boceprevir (Boc) combination therapy. J Hepatol 2011;54(Suppl 1):S6.
31. Jacobson IM, Catlett I, Marcellin P, Bzowej NH, Muir AJ, Adda N, et al. Telaprevir substantially improved SVR rates across all IL28B genotypes in the advance trial. J Hepatol 2011;54(Suppl 1):S542-S543.