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ARTICLE

Effects of the genetic pattern defined by low-density lipoprotein receptor and IL28B genotypes on the outcome of hepatitis C virus infection

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Abstract The aim of this study was to assess the impact of the genetic pattern (GP) defined by the single nucleotide polymorphisms (SNPs) rs14158 of low-density lipoprotein receptor (LDLR) and rs12979860 of interleukin-28B (IL28B) genes on the outcome and features of hepatitis C virus (HCV) infection in patients with and without human immunodeficiency virus (HIV) coinfection. 314 HIV/HCV-coinfected and 109 HCV-monoinfected patients treated with pegylated interferon (Peg-IFN) plus ribavirin (RBV), as well as 51 patients with HCV spontaneous clearance (SC), were included. Variations in both SNPs were determined by the TaqMan polymerase chain reaction (PCR) assay. In the 286 patients chronically infected by HCV genotypes 1 or 4, both rs14158 CC and rs12979860 CC were associated with a higher rate of sustained virological response (SVR), and these effects were complementary in both HCV-monoinfected and HIV/HCV-coinfected patients. Thus, 24 % of patients with rs14158/rs12979860 TT-TC/TT-TC, 33 % with TT-TC/CC, 44.2 % with CC/TT-TC, and 75.8 % harboring CC/CC attained SVR ($p<0.001$). SC was associated with the IL28B genotype (66.7 % CC in SC vs. 42.6 % among those with chronic infection, $p<0.001$) but not with the LDLR genotype. There was no association between GP and the plasma level of alanine aminotransferase (ALT) or the presence of advanced fibrosis. There is a complementary effect between the IL28B and LDLR genotypes on the probability of achieving SVR after Peg-IFN/RBV therapy in patients with HCV 1 or 4. Thus, the predictive value of IL28B genotype is modulated by the LDLR genotype in both HCV-monoinfected and HIV/HCV-coinfected patients. This complementary effect of both genotypes is also observed on the plasma levels of low-density lipoprotein cholesterol (LDL-C).

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Introduction

Several genome-wide association studies have shown that genomic factors play a crucial role on certain aspects of hepatitis C virus (HCV) infection [1, 2]. Thus, the single nucleotide polymorphism (SNP) rs12979860, near the interleukin 28B (IL28B) gene, has been identified as a strong predictor of sustained virological response (SVR) to pegylated interferon (Peg-IFN) plus ribavirin (RBV) in HCV-monoinfected and human immunodeficiency virus (HIV)/HCV-coinfected patients [1, 3]. Moreover, the favorable genotype (CC) of this SNP is associated with the spontaneous clearance (SC) of HCV [4]. Besides its effect on SC and on the response to Peg-IFN-based therapy, the favorable rs12979860 genotype has also been associated

with higher plasma HCV RNA levels and with more severe liver damage [1, 5, 6], but these two associations should be viewed as controversial, since other studies failed to find them [3, 7, 8]. Furthermore, the rs12979860 genotype CC is also related with higher plasma levels of low-density lipoprotein cholesterol (LDL-C) [3, 9], a well-defined predictor of SVR [10, 11].

The association between plasma LDL-C and SVR is believed to be due to the fact that low-density lipoprotein receptor (LDLR) is involved in the mechanism of HCV entry into the hepatocytes. Accordingly, plasma LDL-C could competitively block the entry of HCV, thus, interfering the viral replicative cycle. Also, as a consequence of that fact, mutations in LDLR, which are associated with the plasma levels of LDL-C [12, 13], may have an impact on the likelihood of achieving SVR with Peg-IFN/RBV [14, 15]. With regard to this, variations in the SNP rs14158 located at the 3′UTR of the LDLR gene have been reported to be associated with SVR to Peg-IFN/RBV, independently of plasma HCV viral load and IL28B genotype, in HIV/HCV-coinfected patients bearing HCV genotype 1 [16]. And, more importantly, LDLR and IL28B genotypes seem to have a complementary effect on SVR [16]. However, these findings were observed in a relatively small population, including only HIV-coinfected patients. Besides, there is no information about the influence of the genetic pattern (GP) defined by rs12979860 and rs14158 genotypes on the probability of HCV SC and on relevant features of HCV infection, such as plasma viral load, liver fibrosis, or plasmatic levels of LDL-C. Because of the above-stated reasons, the possible genetic interaction between these two SNPs require further investigations in larger cohorts, also comprising HCV-monoinfected patients and addressing aspects of HCV infection other than the likelihood of SVR with Peg-IFN/RBV.

The objective of this study was to assess the impact of the GP defined by rs14158 and rs12979860 genotypes on the outcome and features of HCV infection in HIV/HCV-coinfected and HCV-monoinfected patients.

Methods

Study patients

A population of 544 patients who tested positive for serum HCV antibodies, 51 with SC of HCV and 493 with chronic HCV infection (CHC), were prospectively recruited in the Infectious Diseases Units of three tertiary-care hospitals in Spain from May 2000 to December 2010. Patients with chronic hepatitis C included 144 HCV-monoinfected patients and 349 HIV/HCV-coinfected patients (184 of them were included in a previously published study) [16]. All of them were previously naïve for therapy against HCV and started treatment with Peg-IFN/RBV. A whole blood or peripheral blood mononuclear cell sample was collected from each patient and stored at −70 °C for subsequent genetic determinations.

Drug therapy

All chronically infected patients received Peg-IFN alfa-2a at a dose of 180 μg once per week or Peg-IFN alfa-2b at a dose of 1.5 μg/kg once per week, both in combination with RBV at a daily dose of 800 mg to 1,200 mg. Subjects harboring HCV genotype 2 or 3 received HCV therapy during a period of 24 weeks, if they were HCV-monoinfected or if they were HIV/HCV-coinfected and showed undetectable plasma HCV-RNA load at week 4. Therapy was given for 48 weeks in the remaining patients. At weeks 12 and 24, HCV therapy was prematurely discontinued in non-responders.

Definition of spontaneous clearance and viral responses

Spontaneous clearance was defined as the presence of HCV antibody seropositivity with undetectable plasma HCV-RNA without prior HCV treatment. Chronic HCV infection was defined as a persistent elevation of serum transaminases for longer than 6 months, along with positive serum antibodies against HCV and detectable plasma HCV-RNA.

SVR was defined as undetectable serum HCV-RNA 24 weeks after the completion of HCV therapy. For the purpose of this analysis, SVR was assessed in an on-treatment approach, i.e., excluding those who voluntarily dropped out or discontinued therapy due to adverse events. Undetectable HCV-RNA in plasma at week 4 was considered as rapid virological response (RVR) and a decrease in plasma HCV-RNA level ≥ 2 $\log_{10}$ or below the detection limit at week 12 was considered as early virological response (EVR). End of treatment response (ETR) was defined as undetectable plasma HCV-RNA at the moment when therapy was completed. Patients without EVR, as well as those with detectable plasma HCV-RNA at week 24 after showing EVR, were considered to be non-responders. Virological breakthrough was defined as detectable plasma HCV-RNA after week 24 and before the end of treatment. Relapse was defined as the lack of SVR after having reached ETR.

Laboratory determinations

Plasma HCV-RNA load was measured by quantitative real-time polymerase chain reaction (PCR) assays (Cobas TaqMan; Roche Diagnostic Systems Inc., Pleasanton, CA, USA).

For SNP genotyping, DNA was extracted using the MagNA Pure system (Roche Diagnostics Corporation, Indianapolis, IN, USA). SNP rs12979860 (IL28B)

genotyping and rs14158 (LDLR) were done as previously reported [3, 16]. The researchers responsible for genotyping were blinded to the remaining data from the patients.

Statistical analysis

The Hardy–Weinberg equilibrium was evaluated using the Haploview software [17]. PLINK software, complemented with gPLINK (http://pngu.mgh.harvard.edu/~purcell/plink/gplink.shtml), was used to estimate and compare the single-marker and two-locus associations. A dominant model for the risk allele (TT=TC<CC) was used to determine the association between both genotypes (rs14158 and rs12979860) and SVR. For the analysis, advanced fibrosis was defined as a stage of fibrosis ≥F3, according to the Scheuer's scoring system in patients with a liver biopsy prior to therapy [18], or as a baseline liver stiffness ≥11 kPa, as determined by transient elastography (FibroScan®, Echosens, Paris, France) , in subjects without biopsy. In the same way, patients with stage of fibrosis F4 or baseline liver stiffness ≥14 kPa were classified as individuals with cirrhosis.

Frequencies were compared using the Chi-square test or Fisher's test. The Student's *t*-test and the Mann–Whitney *U*-test were used for comparing continuous variables in two groups and the Kruskal–Wallis test was used to compare continuous variables among more than two independent groups. The median (interquartile range, IQR) value was used as the cutoff value when continuous variables were categorized, unless otherwise specified.

In order to identify predictors of SVR in patients with CHC, a logistic regression analysis adjusted for age and sex was performed. Factors associated with SVR in the bivariate analysis with a p-value ≤0.2 were entered as covariates. The statistical analysis was carried out using the SPSS statistical software package release 19.0 (IBM SPSS Inc., Chicago, IL, USA).

Ethical aspects

The study was designed and performed according to the Helsinki Declaration and was approved by the Ethics Committees of the participating hospitals.

Results

Main characteristics of the study patients

Twenty-seven (5.5 %) out of 493 patients with chronic HCV infection who started therapy with Peg-IFN/RBV during the study period stopped treatment due to adverse events and 43 (8.7 %) voluntarily dropped out. Therefore, 423 (85.8 %) patients were the chronically HCV-infected population analyzed in this study.

The 51 patients with spontaneous HCV clearance and the 423 patients with chronic HCV infection were of European ancestry and Caucasian race. Forty-two (82.4 %) patients with SC were male. The median (IQR) age of this group was 43 (40–45) years. Among these patients, 12 (23.5 %) were HCV-monoinfected and 39 (76.5 %) were HIV/HCV-coinfected. Forty-one (80.4 %) had been infected with HCV through the use of intravenous drugs (IDU), 5 (9.8 %) by sexual contact, and in the remaining 5 (9.8 %), the route of infection was unknown. The main baseline features of the patients with CHC patients are shown in Table 1.

The studied SNPs were in linkage equilibrium (D′=0.21). There was no association between LDLR rs14158 and IL28B rs12979860 genotypes distribution. In this way, 105 (43.4 %) patients were LDLR CC and IL28B CC versus 75 (41.4 %) patients with LDLR TT-TC and IL28B CC (p=0.688).

Impact of LDLR and IL28B genotypes on spontaneous HCV clearance

The rs12979860 genotype distribution was significantly different between patients with SC and those with CHC. Thus, subjects with SC showed TT in 1 (2 %), TC in 16 (31.4 %), and CC in 34 (66.6 %), whereas the corresponding figures among CHC carriers were TT in 57 (13.5 %), TC in 186 (44 %), and CC in 180 (42.6 %) (p<0.001). The distribution of the rs14158 genotype in patients with SC was TT in 4 (7.8 %), TC in 16 (31.4 %), and CC in 31 (60.8 %), while in those who had CHC, there was TT in 19 (4.5 %), TC in 162 (38.3 %), and CC in 242 (57.2 %) (p=0.980). The GP characterized by the carriage of rs14158 CC/rs12979860 CC was significantly more frequent among the patients with SC, although this fact was due to the differences in the IL28B genotype (Fig. 1).

Association between LDLR, IL28B genotypes, and response to HCV therapy

Two hundred and thirty-two out of 423 (54.8 %) CHC patients achieved SVR. Ninety-one (40.3 %) of the patients with genotype 1, two (100 %) with genotype 2, 111 (82.2 %) harboring genotype 3, and 28 (46.7 %) of those infected with genotype 4 (p<0.001) achieved SVR. The frequencies of virological events according to HIV serostatus are shown in Table 1.

In the overall population with CHC infection, 140 (57.9 %) patients harboring rs14158 genotype CC and 92 (50.8 %) patients with genotype TT-TC attained SVR (p=0.151). The respective figures for the rs12979860 genotype were 130 (72.2 %) for CC and 102 (42 %) for TT-TC (p<0.001). However, when the population was stratified according to the HCV genotype, rs12979860 and rs14158 genotypes predicted SVR only in those with HCV 1 or 4, but not in subjects harboring genotypes 2 or 3. Thus, the rate of SVR in

Table 1 Characteristics of the patients with chronic hepatitis C according to human immunodeficiency virus (HIV) infection (n=423)

Parameter	HIV/HCV (n=314)	HCV (n=109)	p-Value
Age (years)[a]	41.5 (38.5–44.8)	42.3 (37.6–48.5)	0.367
Male gender, no. (%)	251 (79.9)	91 (83.5)	0.417
Body mass index (kg/m^2)[a]	23.4 (21.8–26.3)	25.8 (22.9–28.6)	0.001
IDU, no. (%)	275 (89.3)	75 (68.8)	<0.001
Virological response			
Non-response, no. (%)	93 (29.6)	23 (21.1)	0.086
RVR, no. (%)[b]	87 (30.5)	43 (42.6)	0.028
EVR, no. (%)	241 (77.7)	93 (86.1)	0.062
Viral breakthrough, no. (%)	20 (6.4)	2 (1.8)	0.066
ETR, no. (%)	201 (64)	84 (77.1)	0.012
Relapse, no. (%)[c]	39 (19.4)	14 (16.7)	0.588
SVR, no. (%)	162 (51.6)	70 (64.2)	0.022
HCV genotype, no. (%)			
1	165 (52.5)	61 (56)	
2	1 (0.3)	1 (0.9)	
3	102 (32.5)	33 (30.3)	
4	46 (14.6)	14 (12.8)	0.777
Plasma HCV-RNA load ($\log_{10}$ IU/mL)[a]	6.1 (5.5–6.4)	6.1 (5.3–6.8)	0.625
Plasma HCV-RNA load >600,000 IU/mL	202 (64.5)	68 (62.4)	0.687
Baseline serum ALT (IU/L)[a]	69 (46–104)	71 (42–130)	0.515
Advanced fibrosis, no. (%)[d]	118 (46.1)	27 (27.3)	0.001
rs12979860, no. (%)			
TT	40 (12.7)	17 (15.6)	
TC	142 (45.2)	44 (40.4)	
CC	132 (42)	48 (44.1)	0.606
rs14158, no. (%)			
TT	13 (4.1)	6 (5.5)	
TC	121 (37.6)	41 (37.6)	
CC	180 (57.3)	62 (56.9)	0.837
Genetic pattern			
rs14158 TT-TC/rs12979860 TT-TC	79 (24.8)	27 (25.2)	
rs14158 CC/rs12979860 TT-TC	103 (32.8)	34 (31.2)	
rs14158 TT-TC/rs12979860 CC	55 (17.5)	20 (18.3)	
rs14158 CC/rs12979860 CC	77 (24.5)	28 (25.7)	0.775
Baseline plasma LDL cholesterol (mg/dL)[a,e]	90 (70–112)	91 (75–116)	0.355
Baseline plasma HDL cholesterol (mg/dL)[a]	42 (33–54)	48 (40–62)	0.003
Baseline plasma total cholesterol (mg/dL)[a]	163 (140–187)	165 (140–197)	0.725
Baseline plasma triglycerides (mg/dL)[a]	113 (82–182)	97 (72–143)	0.016

IDU intravenous drug user, *RVR* rapid virological response, *EVR* early virological response, *ETR* end of treatment response, *SVR* sustained virological response, *HCV* hepatitis C virus, *ALT* alanine aminotransferase, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

[a] Median (quartile 1–quartile 3)

[b] Available in 285 HIV/HCV-coinfected and 101 HCV-monoinfected patients

[c] Calculated over 201 HIV/HCV-coinfected and 85 HCV-monoinfected patients who attained ETR

[d] Fibrosis stage in biopsy ≥F3 or liver stiffness ≥11 kPa if biopsy had not been carried out; available in 256 HIV/HCV-coinfected and 99 HCV-monoinfected patients

[e] Available in 285 HIV/HCV-coinfected and 75 HCV-monoinfected patients

patients with HCV genotypes 1 or 4 and rs14158 CC was 48.8 % versus 31.4 % in those bearing rs14158 genotype TT-TC (p=0.003), whereas in subjects harboring HCV genotypes 2 or 3, they were 78.4 % and 87.3 % (p=

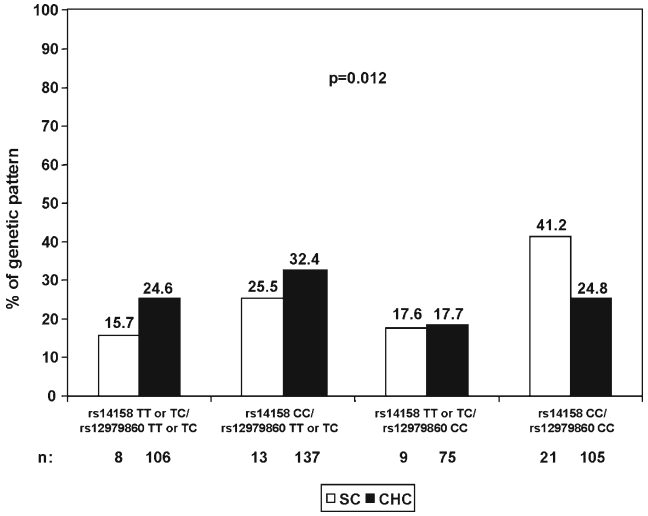

Fig. 1 Genetic pattern (GP) distribution among patients with spontaneous clearance (SC) (n=51) and chronic HCV infection (CHC) (n=423)

0.171), respectively. In the same way, the rate of SVR in patients with HCV genotypes 1 or 4 and rs12979860 CC was 62.9 % versus 29.3 % in those with TT-TC (p<0.001). The corresponding figures in subjects infected with HCV genotypes 2 or 3 were 85.3 % versus 79 % (p=0.334). Figure 2 shows the virological events in patients infected with genotypes 1 or 4.

Relationship between the genetic pattern including LDLR and IL28B genotypes and sustained virological response

In the overall CHC population, 44 (41.5 %) patients with rs14158 TC-TT/rs12979860 TC-TT, 58 (42.3 %) with rs14158 CC/rs12979860 TC-TT, 48 (64 %) with rs14158 TC-TT/rs12979860 CC, and 82 (78.1 %) with rs14158 CC/rs12979860 CC achieved SVR (p<0.001). However, the effect of the LDLR genotype on the relationship between IL28B genotype and SVR was restricted to subjects bearing HCV genotypes 1 or 4 but not in HCV genotypes 2 or 3 carriers (Fig. 3). This effect was observed in both HCV-monoinfected and HIV/HCV-coinfected patients with genotypes 1 or 4 (Fig. 4). In an intention-to-treat approach, where those who discontinued therapy voluntarily or due to adverse events were also considered, a similar trend was observed. Thus, among those individuals infected by genotypes 1 or 4, 18 (22.2 %) patients with rs14158 TC-TT/rs12979860 TC-TT, 35 (31.8 %) with rs14158 CC/rs12979860 TC-TT, 19 (40.4 %) with rs14158 TC-TT/rs12979860 CC, and 47 (70.1 %) with rs14158 CC/rs12979860 CC achieved SVR (p<0.001).

In a logistic regression analysis conducted in patients with HCV genotypes 1 or 4, a statistical interaction between rs12979860 and the GP (p<0.001) was detected. The Nagelkerke R^2 values were 0.317 for the model with IL28B alone, while it was 0.347 when the GP was included instead of IL28B. Then, the analysis was continued using only the GP as the covariate. The multivariate analysis showed that the GP was a predictor of SVR independent of HIV, plasma HCV-RNA load, and baseline plasma level of LDL-C in subjects carrying HCV genotypes 1 or 4 (Table 2).

Impact of the genetic pattern including LDLR and IL28B genotypes on other features of HCV infection

The proportion of patients with baseline HCV-RNA load below 600,000 IU/mL tended to be higher among those harboring rs14158 CC than among TT-TC carriers (39 % vs. 32 %, respectively, p=0.140). Moreover, patients with rs14158 CC showed higher baseline plasma levels of LDL-C than TT-TC [91 (74–115) mg/dl vs. 89 (68–110) mg/dl, respectively, p=0.162]. In the same way, the baseline plasma level of LDL-C was higher in patients with rs12979860 CC [96 (74–121) mg/dL] than in those with TT-TC [88 (69–106)

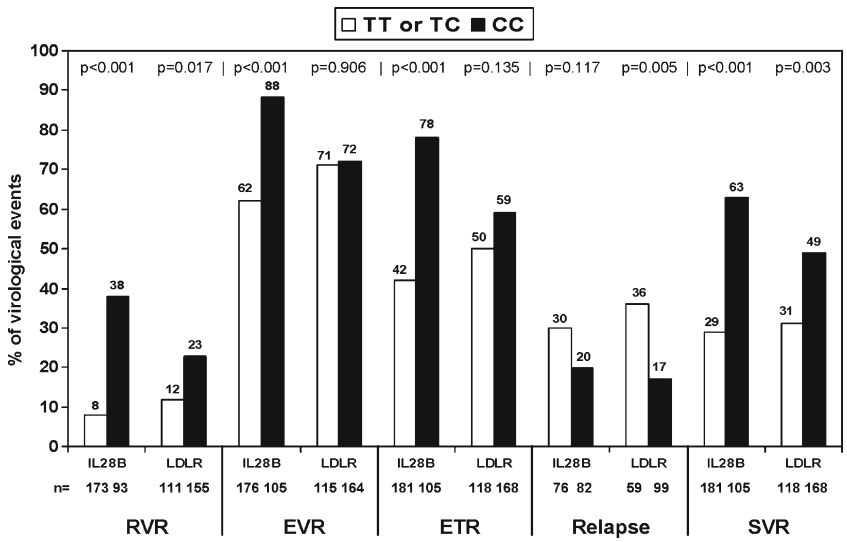

Fig. 2 Frequency of virological events according to interleukin-28B (IL28B) and low-density lipoprotein receptor (LDLR) genotypes in patients infected with hepatitis C virus (HCV) genotypes 1 or 4 (n=286)

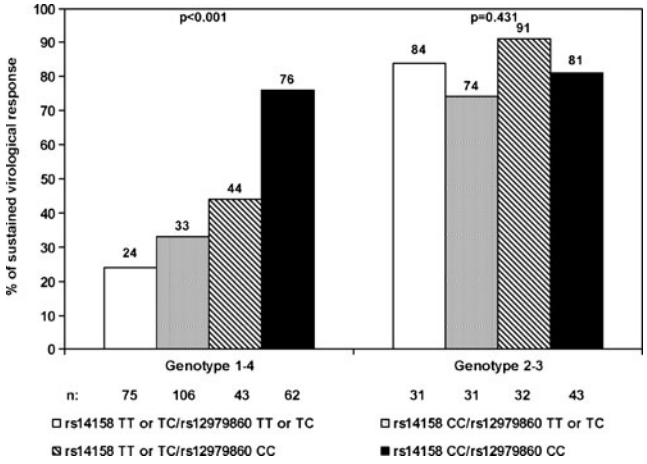

Fig. 3 Rate of sustained virological response (SVR) according to the GP (LDLR/IL28B) and HCV genotype (n=423)

mg/dL] (p=0.001). The proportion of patients with HCV-RNA load below 600,000 IU/mL was 35 % in subjects with rs12979860 CC versus 36.8 % in those with the TT-TC genotype (p=0.70).

There was no association between the GP and the baseline plasma level of ALT, plasma HCV-RNA load, body mass index, and the presence of advanced fibrosis or cirrhosis. However, the combination of LDLR genotype and IL28B genotype also showed a complementary effect on the baseline plasma levels of LDL-C (Fig. 5).

Discussion

The results presented here confirm that there is an interactive effect between IL28B and LDLR genotypes on the probability of achieving SVR after Peg-IFN/RBV therapy in patients with HCV genotypes 1 or 4. Thus, the

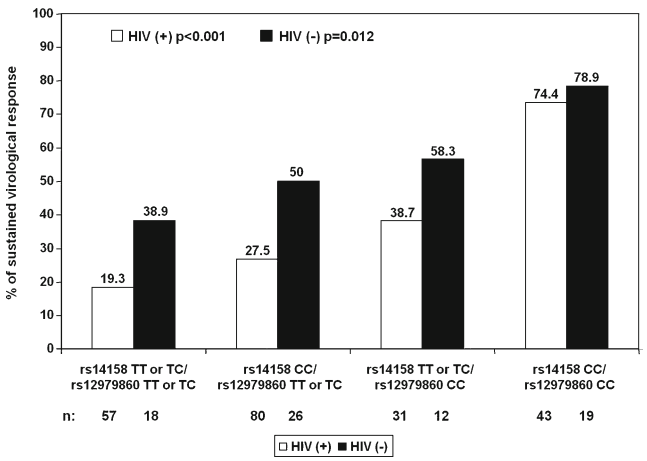

Fig. 4 Rate of SVR according to the GP, stratified by human immunodeficiency virus (HIV) serostatus, in patients with HCV genotypes 1 or 4 (n=286)

combination of these genotypes markedly increases the capacity of IL28B to predict SVR, in both HCV-monoinfected and HIV/HCV-coinfected patients. This complementary effect of both genotypes is also observed on plasma levels of LDL-C, a predictor of SVR, but it is not seen on the likelihood of SC of HCV infection, mainly due to the lack of impact of the LDLR genotype on SC.

LDLR gene expression is regulated at transcriptional and post-transcriptional levels by changes in mRNA stability [19, 20]. In this way, changes in 3′UTR could reduce the LDLR mRNA half-life, the production of LDLR [20], the LDL-C entry into hepatocytes, and, consequently, increase plasma LDL-C and total cholesterol levels [12, 16]. A higher baseline level of LDL-C has been associated with higher rates of SVR in several studies [10, 11]. LDL-C is thought to interfere with HCV replication, as it could compete with HCV for LDLR on the surface of hepatocytes [21]. In this study, patients with rs14158 CC tended to show a lower plasma HCV-RNA load than those with genotypes TT-TC. This finding is in agreement with the above-stated hypothesis. Specific functional assays should be carried out to assess more precisely how variations in rs14158 impact on the expression of the LDLR gene.

Both SNPs genotypes studied herein could have a different impact on the HCV replication. Thus, IL28B seems to act through interferon-stimulated genes [22], whereas LDLR variations would cause inefficient viral entry [23]. Because of this, it is not surprising that their effects on viral kinetics during the treatment of chronic hepatitis C caused by HCV 1 or 4 are also different. As previously reported [24], favorable IL28B genotype has a marked effect on the early phases of treatment, leading to higher rates of RVR and EVR. This effect leads to a strong impact on ETR and SVR. However the risk of relapse is hardly influenced by the IL28B genotype. The impact of the LDLR genotype on early kinetics is less relevant, whereas it is strongly associated with the likelihood of relapse. This is a plausible explanation for the complementary effect of both SNPs. Furthermore, the favorable IL28B genotype is associated with higher plasma levels of LDL-C, which may play a role in the effect of IL28B variations on SVR. As this study has shown, the combination of IL28B and LDLR genotype also seems to have an effect on the plasma levels of LDL-C. However, as observed in Fig. 5, this effect is not well defined, as the trend of the median values among the different genetic patterns is not linear. It is, thus, likely that the underlying mechanism for our observations is more complex and cannot only be explained by plasmatic levels of LDL-C.

In this study, no association between the GP and plasma HCV-RNA load was observed. This may be due to the fact that the effects of both genotypes on this parameter are different. Thus, while rs14158 CC tends to be associated with lower plasma HCV-RNA load, the IL28B genotype has

Table 2 Predictors of sustained virologic response (SVR) in the univariate and multivariate analysis in patients with hepatitis C virus (HCV) genotypes 1 or 4 (n=286)

Parameter	SVR, n (%)	Unadjusted OR (95 % CI)	p-Value univariate	Adjusted OR (95 % CI)	p-Value multivariate
Age (years)[a]					
≤ 42	68 (44.7)	0.85 (0.64–1.13)		0.99 (0.94–1.06)	
>42	51 (38.1)		0.253		0.751
Gender					
Male	100 (41.8)	0.97 (0.66–1.13)		1.48 (0.61–3.58)	
Female	19 (40.4)		0.857		0.389
HIV					
Yes	77 (36.5)	1.54 (1.17–2.01)		3.61 (1.66–7.87)	
No	42 (56)		0.003		0.001
HCV genotype					
1	91 (40.3)	0.86 (0.63–1.18)			
4	28 (46.7)		0.371	–	–
Genetic pattern					
rs14158 TT-TC/rs12979860 TT-TC	18 (24)			0.09 (0.03–0.25)	<0.001
rs14158 CC/rs12979860 TT-TC	35 (33)			0.14 (0.06–0.37)	<0.001
rs14158 TT-TC/rs12979860 CC	19 (44.2)			0.40 (0.13–1.19)	0.100
rs14158 CC/rs12979860 CC[b]	47 (75.8)	2.81 (1.79–4.4)[c]	<0.001	1	<0.001
IDU					
Yes	95 (40.9)	1.17 (0.85–1.63)			
No	24 (48)		0.360	–	–
Advanced liver fibrosis[d]					
Yes	36 (36)	1.25 (0.91–1.72)		1.19 (0.57–2.47)	
No	65 (45.1)		0.154		0.648
Baseline HCV-RNA load (IU/mL)					
≤600,000	61 (64.2)	0.47 (0.36–0.61)		0.20 (0.09–0.43)	
>600,000	57 (30)		<0.001		<0.001
Baseline LDL-C[e]					
≤100 mg/dL	44 (32.1)	1.3 (1.04–1.61)		1.01 (1.01–1.03)	
>100 mg/dL	49 (47.6)		0.015		0.025

IDU intravenous drug user

[a] Categorized by median

[b] Reference category

[c] rs14158 CC/rs12979860 CC versus non-rs14158 CC/rs12979860 CC

[d] Fibrosis stage in biopsy ≥F3 or liver stiffness ≥11 kPa if biopsy had not been carried out; available in 244 patients

[e] Available in 240 patients

been associated with higher HCV burden in some studies [1, 6] or had no effect in others [25, 26], as happened in the present investigation.

The LDLR genotype, although having a weaker impact than the IL28B genotype on the likelihood of SVR, may be useful in clinical practice. Indeed, it clearly enhances the predictive value of the IL28B genotype for SVR. In this manner, patients bearing HCV 1 or 4 with rs12979860 CC have a likelihood of SVR of 75.8 % if rs14158 is CC, but only 44.2 % if rs14158 is TT-TC. Conversely, patients with both unfavorable genotypes have a rate of SVR of 24 %, which increases to 33 % if rs14158 is CC, despite IL28B being TT-TC. The complementary effect of IL28B and LDLR genotypes, observed in patients with HCV genotypes 1 or 4, was not found in those bearing HCV genotypes 2 or 3. The reason for this finding may be that HCV genotypes 2 and 3 are highly susceptible to Peg-IFN/RBV therapy and, thus, the influence of the host factors could be less significant. Despite this limitation, the GP defined by the IL28B and LDLR genotypes allow us to predict SVR in a high proportion of patients infected with HCV 1 or 4, over two-thirds in this study, who are precisely those in whom

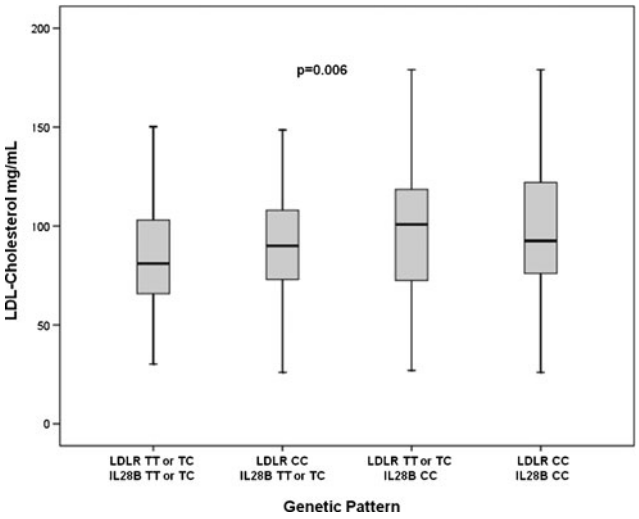

Fig. 5 Baseline plasma levels of low-density lipoprotein cholesterol (LDL-C) according to the genetic pattern in patients with CHC (n=423)

predicting SVR is more necessary, because the chance to attain it is lower.

The IL28B genotype has been strongly associated with SC in previous studies [4, 27, 28]. On the contrary, the rs14158 genotype was not associated with SC of HCV in two previous surveys [14, 15]. In the current study, the favorable GP was significantly more frequent among patients with SC. Nonetheless, this effect seemed to be mainly driven by the action of the IL28B CC genotype, which was clearly more common in individuals with SC (66.7 %) compared to those with CHC infection (42.6 %). Conversely, the frequency of rs14158 CC was similar in patients with SC and CHC (58.1 % vs. 60.8 %). It is possible that SC may principally depend on the immune response of the host, and specifically on the endogenous interferon activity, rather than on the fact that HCV entry is interfered or not, which is the effect of the LDLR genotype.

A limitation of this study is that SNPs at the LDLR gene other than rs14158 were not tested, and some of them may have a more significant phenotypic effect [12]. We selected this SNP because it was the only one that has been previously associated with SVR [14]. Nevertheless, this fact does not reduce the interest of the findings reported herein, since the application of the GP defined by rs12979860 and rs14158 has proven to be useful, and it should be considered for clinical application, in order to improve the management of patients. One further limitation is that the number of HCV-monoinfected patients in this study is relatively low and larger populations of these patients will be necessary to confirm these results. Nevertheless, most therapy response predictors are shared by HIV/HCV-coinfected and HCV-monoinfected patients. Moreover, the present study shows a very similar effect of the GP in HIV/HCV-coinfected and HCV-monoinfected patients, which lead us to think that the GP will be equally useful in both populations.

Triple therapy combining Peg-IFN, RBV, and a direct-acting antiviral (DAA) drug is currently the standard of care for HCV-infected patients carrying genotype 1 [29, 30]. With these regimens, very high rates of SVR are achieved and the impact of the host genetic features on the therapy outcome may be less significant. However, even in this setting, the findings of this study will likely continue to be relevant. Thus, this GP may be useful to identify patients who are very likely to respond to bitherapy with Peg-IFN plus RBV, which may reduce adverse effects, expenses, and, in the case of HIV/HCV-coinfection, drug interactions. In addition, GP could also help us to tailor the length of treatment. Moreover, DAA drugs currently available are active only against HCV genotype 1, but not for genotype 4, which accounts for a non-negligible proportion of HCV infection in some areas, such as Spain. However, specific studies addressing the predictive value of the GP including IL28B and LDLR genotypes in patients receiving triple therapy including DAA drugs should be undertaken.

In summary, the LDLR genotype modulates the effect of the IL28B genotype on SVR and plasma LDL-C levels, but not on other aspects of HCV infection, such as SC. The GP obtained from the combination of both genotypes is applicable to the clinical practice. The use of this pharmacogenomic marker may improve the management of patients, especially that of HIV/HCV-coinfected patients. In any case, it will be necessary to analyze the usefulness of the GP defined by the IL28B and LDLR genotypes to individualize the optimal composition and duration of treatment regimens including DAA drugs.

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Conflict of interest The authors have no conflicts of interest to declare.

References

1. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ et al (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401
2. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N et al (2009) Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109
3. Pineda JA, Caruz A, Rivero A, Neukam K, Salas I, Camacho A et al (2010) Prediction of response to pegylated interferon plus

ribavirin by IL28B gene variation in patients coinfected with HIV and hepatitis C virus. Clin Infect Dis 51:788–795
4. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C et al (2009) Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461:798–801
5. Barreiro P, Pineda JA, Rallón N, Naggie S, Martín-Carbonero L, Neukam K et al (2011) Influence of interleukin-28B single-nucleotide polymorphisms on progression to liver cirrhosis in human immunodeficiency virus-hepatitis C virus-coinfected patients receiving antiretroviral therapy. J Infect Dis 203:1629–1636
6. Labarga P, Soriano V, Caruz A, Poveda E, Di Lello F, Hernandez-Quero J et al (2011) Association between IL28B gene polymorphisms and plasma HCV-RNA levels in HIV/HCV-co-infected patients. AIDS 25:761–766
7. Aghemo A, Marabita F, De Nicola S, Rumi MG, Cheroni C, Scavelli R et al (2011) Fibrosis progression in patients with chronic hepatitis C is not influenced by IL28B polymorphisms. J Hepatol 54(Suppl 1):S519
8. Fornasiere E, Cmet S, Bitetto D, Cussigh A, Fumolo E, Bignulin S et al (2011) Interleukin 28B rs12979860 C/T polymorphism and serum cholesterol as predictors of fibrosis progression in patients with chronic hepatitis C and persistently normal transaminases. J Hepatol 54(Suppl 1):S456–S457
9. Li JH, Lao XQ, Tillmann HL, Rowell J, Patel K, Thompson A et al (2010) Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. Hepatology 51:1904–1911
10. del Valle J, Mira JA, de los Santos I, López-Cortés LF, Merino D, Rivero A et al (2008) Baseline serum low-density lipoprotein cholesterol levels predict response to hepatitis C virus therapy in HIV/hepatitis C virus coinfected patients. AIDS 22:923–930
11. Harrison SA, Rossaro L, Hu K-Q, Patel K, Tillmann H, Dhaliwal S et al (2010) Serum cholesterol and statin use predict virological response to peginterferon and ribavirin therapy. Hepatology 52:864–874
12. Muallem H, North KE, Kakoki M, Wojczynski MK, Li X, Grove M et al (2007) Quantitative effects of common genetic variations in the 3′UTR of the human LDL-receptor gene and their associations with plasma lipid levels in the Atherosclerosis Risk in Communities study. Hum Genet 121:421–431
13. Petit JM, Minello A, Duvillard L, Jooste V, Monier S, Texier V et al (2007) Cell surface expression of LDL receptor in chronic hepatitis C: correlation with viral load. Am J Physiol Endocrinol Metab 293:416–420
14. Hennig BJW, Hellier S, Frodsham AJ, Zhang L, Klenerman P, Knapp S et al (2002) Association of low-density lipoprotein receptor polymorphisms and outcome of hepatitis C infection. Genes Immun 3:359–367
15. Mas Marques A, Mueller T, Welke J, Taube S, Sarrazin C, Wiese M et al (2009) Low-density lipoprotein receptor variants are associated with spontaneous and treatment-induced recovery from hepatitis C virus infection. Infect Genet Evol 9:847–852
16. Pineda JA, Caruz A, Di Lello FA, Camacho A, Mesa P, Neukam K et al (2011) Low-density lipoprotein receptor genotyping enhances the predictive value of IL28B genotype in HIV/hepatitis C virus-coinfected patients. AIDS 25:1415–1420
17. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265
18. Scheuer PJ (1991) Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 13:372–374
19. Nakahara M, Fujii H, Maloney PR, Shimizu M, Sato R (2002) Bile acids enhance low density lipoprotein receptor gene expression via a MAPK cascade-mediated stabilization of mRNA. J Biol Chem 277:37229–37234
20. Wilson GM, Vasa MZ, Deeley RG (1998) Stabilization and cytoskeletal-association of LDL receptor mRNA are mediated by distinct domains in its 3′ untranslated region. J Lipid Res 39:1025–1032
21. Enjoji M, Nakamuta M, Kinukawa N, Sugimoto R, Noguchi K, Tsuruta S et al (2000) Beta-lipoproteins influence the serum level of hepatitis C virus. Med Sci Monit 6:841–844
22. Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD et al (2010) IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology 52:1888–1896
23. Molina S, Castet V, Fournier-Wirth C, Pichard-Garcia L, Avner R, Harats D et al (2007) The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. J Hepatol 46:411–419
24. Rallón NI, Soriano V, Naggie S, Restrepo C, Goldstein D, Vispo E et al (2011) IL28B gene polymorphisms and viral kinetics in HIV/hepatitis C virus-coinfected patients treated with pegylated interferon and ribavirin. AIDS 25:1025–1033
25. Lin C-Y, Chen J-Y, Lin T-N, Jeng W-J, Huang C-H, Huang C-W et al (2011) IL28B SNP rs12979860 is a critical predictor for on-treatment and sustained virologic response in patients with hepatitis C virus genotype-1 infection. PLoS One 6:e18322
26. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T et al (2010) Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 138:1338–1345
27. di Iulio J, Ciuffi A, Fitzmaurice K, Kelleher D, Rotger M, Fellay J et al (2011) Estimating the net contribution of interleukin-28B variation to spontaneous hepatitis C virus clearance. Hepatology 53:1446–1454
28. Nattermann J, Timm J, Nischalke HD, Olbrich A, Michalk M, Tillmann HL et al (2011) The predictive value of IL28B gene polymorphism for spontaneous clearance in a single source outbreak cohort is limited in patients carrying the CCR5Δ32 mutation. J Hepatol 55:1201–1206
29. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH et al (2011) Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 364:2405–2416
30. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS et al (2011) Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 364:1195–1206