Variations at multiple genes improve interleukin 28B genotype predictive capacity for response to therapy against hepatitis C infection

Karin Neukam[a,b], Antonio Caruz[c], Antonio Rivero-Juárez[d], Pablo Barreiro[e], Dolores Merino[f], Luis M. Real[a,b], Rocío Herrero[c], Angela Camacho[d], Vicente Soriano[e], Federico A. Di Lello[a,b], Juan Macías[a,b], Antonio Rivero[d] and Juan A. Pineda[a,b]

Objective: To identify genetic factors that predict sustained virological response (SVR) to pegylated interferon (Peg-IFN)/ribavirin (RBV) in HIV/hepatitis C virus (HCV) genotype 1 or 4-coinfected patients and that enhance the predictive capacity of *IL28B* genotype in this population.

Design: Prospective cohort study.

Setting: Five tertiary care centers in Spain.

Patients: Two hundred and five HIV/HCV genotype 1 or 4-coinfected patients who received a complete course of Peg-IFN/RBV for 48 weeks.

Main outcome measures: All individuals were genotyped for 144 single-nucleotide polymorphisms (SNPs).

Results: One hundred and sixty-two (79%) patients bore HCV genotype 1. Overall SVR was achieved by 73 (36%) individuals. SNPs at the following genes were associated with SVR: *IL28B*, low-density lipoprotein receptor (*LDLR*), transforming growth factor β (*TGF*-β), aquaporine 2 (*AQP-2*), very-low-density lipoprotein receptor, Sp110 nuclear body protein, interferon alpha/beta receptor 1, 2′-5′-oligoadenylate synthase 1 and apolipoprotein B. There was a strong synergy between SNPs at *IL28B*, *TGF*-β and *AQP-2* genes: the number of patients reaching SVR with all three favorable genotypes versus unfavorable genotypes were 22 (78.6%) versus 1 (7.1%) ($P = 2.1 \times 10^{-4}$). HCV baseline viral load, *IL28B*, *TGF*-β, *AQP-2* and *LDLR* haplotypes were independently associated with SVR.

Conclusion: A number of genetic factors modify the predictive capacity of *IL28B* genotype. These can be used to identify HCV genotype 1 or 4-infected patients with a very high or a very low probability to respond to bitherapy with Peg-IFN/RBV. Predictive models based on these factors could be helpful to tailor direct acting antiviral-based therapy.

AIDS 2013, **27**:2715–2724

Keywords: aquaporine 2, hepatitis C virus, HIV, low-density lipoprotein receptor, pegylated interferon, ribavirin, transforming growth factor β

[a]Unit of Infectious Diseases and Microbiology, Hospital Universitario de Valme, [b]Instituto de Biomedicina de Sevilla (IBiS), Seville, [c]Immunogenetics Unit, Faculty of Sciences, Universidad de Jaén, Jaen, [d]Unit of Infectious Diseases, Hospital Universitario Reina Sofía, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Cordoba, [e]Department of Infectious Diseases, Hospital Carlos III, Madrid, and [f]Unit of Infectious Diseases, Hospital Juan Ramón Jiménez. Huelva, Spain.

Correspondence to Dr Juan A. Pineda, PhD, MD, Unidad de Enfermedades Infecciosas y Microbiología, Hospital Universitario de Valme, Avda. de Bellavista, 41014 Sevilla, Spain.

Tel: +34 955015684; fax: +34 955015795; e-mail: japineda@telefonica.net
Received: 15 April 2013; revised: 20 May 2013; accepted: 13 June 2013.

DOI:10.1097/01.aids.0000432459.36970.a9

Introduction

In European countries, over 50% of the cases of chronic hepatitis C among HIV-infected patients are caused by hepatitis C virus (HCV) genotype 1 [1]. The rates of sustained virological response (SVR) to dual therapy with pegylated interferon (Peg-IFN) and ribavirin (RBV) are very low in this subset under real-life conditions [2]. HIV/HCV genotype 4-infected patients represent approximately 15–20% of the HIV/HCV-coinfected population [1,2]. The response rates to Peg-IFN and RBV observed in these individuals are somewhat higher, as compared to HIV/HCV genotype 1 infections; however, approximately two thirds do not achieve SVR [2]. Triple therapy including Peg-IFN, RBV and either telaprevir or boceprevir has recently become the standard of care against chronic hepatitis C by genotype 1 in the HIV-coinfected patients [3]. SVR rates with these regimens observed in clinical trials in treatment-naive patients have reached up to 74% [4,5], and data obtained under real-life conditions are also promising [6,7]. However, response in all patients is not achieved. In the case of HIV/HCV genotype 4 coinfection, no alternative treatment option has been approved to date. Therefore, predictive tools to select patients with a very high or a very low probability to achieve SVR are necessary in the current clinical practice, especially for those infected with HCV genotype 4.

As it is the case for dual therapy, the standard recommended treatment duration for triple therapy in HIV/HCV-coinfected patients remains 48 weeks [3]. The finding of reliable predictors for SVR could, on the one hand, help us to identify candidates who may benefit from dual therapy and, on the other hand, allow the development of shorter treatment schedules with direct-acting antivirals (DAAs). Because tolerance to Peg-IFN and RBV is poor, especially in the setting of antiretroviral therapy, the benefit of shorter regimens is even higher in HIV-coinfected patients. Likewise, source-limited settings are in high need of predictors of SVR to dual therapy. Pharmacogenetic determinations represent cost-effective tools to predict the probability of SVR. In this context, the single-nucleotide polymorphism (SNP) rs12979860 near the interleukin 28B (*IL28B*) gene is a potent predictor for SVR to dual therapy in HIV/HCV genotype 1 or 4-coinfected patients [2,8,9]. Likewise, it has a lower, but evident, impact on the outcome of first-generation protease inhibitor-based therapy in prior treatment-naive patients without HIV coinfection [10–12]. The predictive capacity of *IL28B* genotype can be enhanced by its combination with viral and host factors [13–16]. In this context, the determination of genetic variations of the SNP rs14158, at the low-density lipoprotein receptor (*LDLR*) gene, increases the *IL28B* predictive performance [13], which may be caused by the HCV viral replication cycle being affected by cholesterol and fatty acid biosynthesis. However, the predictive value obtained for HIV/HCV genotype 1 or 4-infected patients even using *IL28B* and rs14158 genotyping is suboptimal, as the probability of SVR in patients identified as likely responders using these parameters hardly reaches 70% [14–16].

The study aimed to identify genes other than *IL28B* and *LDLR* whose variations predict response to Peg-IFN and RBV, and which may allow us to enhance the predictive value of *IL28B* genotype in HIV/HCV genotype 1 or 4-infected patients.

Methods

Study population

Hepatitis C virus genotype 1 or 4-infected patients were selected from a cohort that was prospectively followed at the Units of Infectious Diseases of five Spanish hospitals between June 2000 and December 2010. The inclusion criteria in this cohort were: older than 18 years, prior HCV treatment-naive, initiation of dual therapy with Peg-IFN and RBV, coinfection with HIV and stored whole blood samples available for genetic determinations. Responses were analyzed in an on-treatment approach, that is, those who interrupted treatment because of adverse events or who voluntarily dropped out were excluded. The scheduled visits were at baseline, every 4 weeks during the first 6 months of treatment and every 12 weeks thereafter. In order to evaluate SVR, a visit 24 weeks after stopping therapy was conducted.

Treatment regimens and definition of response

Pegylated interferon alfa-2a or alfa-2b was administered at doses of 180 μg or 1.5 μg/kg once per week, respectively, in combination with weight-adjusted RBV (1000 mg/day for $<$75 kg and 1200 mg/day for $\geq$75 kg). The scheduled treatment duration was 48 weeks for all patients. Stopping rules were applied following international guidelines in force at the moment of treatment. SVR was defined as undetectable plasma HCV RNA 24 weeks after the completion of therapy. A decline of plasma HCV RNA of less than 2 $\log_{10}$ at week 12 or lack of undetectable HCV RNA at treatment week 24 after having presented an at least 2 $\log_{10}$ decline but without reaching undetectability was considered as nonresponse. Undetectable HCV RNA at the end of therapy without achieving SVR was considered as relapse. Viral breakthrough was defined as detectable plasma HCV RNA during treatment after having reached undetectability.

Selection and genotyping of single-nucleotide polymorphisms and HCV RNA quantification

Genomic DNA was isolated from whole blood samples or from isolated peripheral blood mononuclear cells using the automated MagNA Pure DNA extraction method

(Roche Diagnostics Corporation, Indianapolis, Indiana, USA) or the Quick Pure Blood DNA extraction Kit (Macherey-Nagel, Düren, Germany). Selected SNPs included SNPs belonging to genes involved in the cholesterol metabolism and transport or in the immune response and which were identified as tag SNPs after an analysis of the genotype data obtained from Hapmap CEU population (www.hapmap.org) using Haploview V4.1 software (https://www.broad.harvard.edu/haploview/haploview). Likewise, SNPs from genes which had previously been found to be associated with SVR or with liver fibrosis in HCV-infected patients were tested [17–19]. All SNPs were genotyped using a custom Golden Gate Veracode genotyping assay (Illumina, San Diego, California, USA) according to the manufacturer's instructions. Those SNPs with the following characteristics were discarded: a minor allele frequency below 0.05, a Hardy–Weinberg equilibrium *P* less than 0.01 and genotyping of less than 80%. Genotyping of *IL28B* and determination of plasmatic HCV RNA was conducted as described elsewhere [8,9]. The researchers responsible for genotyping were blinded to other patient data.

Data analysis

Haploview V4.1 Software (https://www.broad.harvard.edu/haploview/haploview) was used to calculate the Hardy–Weinberg equilibrium for all SNPs. A first analysis of standard case-control allelic associations (1 degree freedom), as well as of different genetic models using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/gplink.shtml) was performed. Those SNPs that were associated with SVR showing a *P*-value less than 0.05 were selected for further analysis. Genotypes within one SNP that showed similar rates of SVR were combined. The linkage disequilibrium values between the genetic markers studied (measured in Lewontin's standardized disequilibrium coefficient D′), haplotype frequencies and haplotype-based association analyses were calculated using Haploview V4.1 for those genes with at least two SNPs available. Chromosome phases for LDLR haplotypes were estimated using PLINK software.

Continuous variables were compared by means of the Student's *t*-test or the Mann–Whitney *U*-test, when applicable, and are presented as median [interquartile range (IQR)]. For the analysis of categorical variables, the chi-square test or the Fisher's exact test was used in 2×2 tables. SVR was the primary outcome variable. A multivariate logistic regression analysis, including as covariables age, sex, as well as those factors that were associated with SVR with a *P*-value less than 0.05 in the univariate analysis, was conducted. For additive genetic factor analysis, a simple unweighted genetic risk score (GRS) was calculated with those SNPs that were independently associated with SVR. In the subpopulation of *IL28B* genotype CC carriers, protective genotypes were added (+1 point for each favorable genotype; unfavorable genotypes were rated as 0). Likewise, in the subpopulation of patients presenting *IL28B* genotype non-CC, unfavorable genotypes were subtracted (−1 point for each unfavorable genotype; favorable genotypes were rated as 0). Descriptive and inferential analyses were carried out using the SPSS statistical software package release 19.0 (IBM Corporation, Somers, New York, USA).

Ethical issues

The study was designed and performed according to the Helsinki Declaration, and was approved by the Ethics Committee of the Hospital Universitario de Valme.

Results

Overall study population

A total of 262 patients fulfilled the inclusion criteria for this study. Of these, 57 (21.8%) individuals were excluded because they could not be assessed in an on-treatment approach. Finally, 205 patients were selected (Table 1). One hundred and sixty-two (79%) were infected with HCV genotype 1 and 43 (21%) bore HCV genotype 4. Sixty-seven (32.8%) presented a baseline plasma HCV RNA below 600 000 IU/ml.

Response to pegylated interferon and ribavirin

Sustained virological response was achieved by 73 (35.6%) individuals. Undetectable HCV RNA at week 4 of treatment was observed in 32 (16.8%) of the 190 patients in whom this information was available. Eighty-nine (43.4%) patients were nonresponders and 30 (14.6%) patients relapsed. Thirteen (6.3%) individuals experienced a viral breakthrough. Fifty-five (34%) of the patients infected with HCV genotype 1 versus 18 (41.9%)

Table 1. Baseline characteristics of the study population.

Parameter	Value
Age (years)*	41.6 (38.5–44.8)
Male sex, no. (%)	170 (82.9)
BMI (kg/m^2)*	23.4 (21.6–26.2)
Intravenous drug user, no. (%)	174 (84.9)
HCV genotype 1, no. (%)	162 (79)
IL28B rs12979860 CC, no. (%)	79 (38.5)
Plasma HCV viral load ($\log_{10}$ IU/ml)*	6.21 (5.52–6.72)
Advanced fibrosis, no. (%)[1]	80 (47.6)
Alanine aminotransferase (U/l)*	66 (44–99.5)
Total cholesterol (mg/dl)*	168 (148–196)
Low-density lipoprotein cholesterol (mg/dl)*	92.6 (74.9–116)
Triglycerides (mg/dl)*	117 (83–172)
Pegylated interferon alfa-2a, no. (%)	146 (71.2)
Daily dose of ribavirin (mg/kg)*	14.9 (13.1–16.9)
Undetectable plasma HIV RNA, no. (%)	167 (81.5)
CD4$^+$ cell count (cells/μl)*	521 (388–730)

*Median (IQR); [1]determined by liver biopsy (F ≥3 according to the Scheuer Index) or a liver stiffness value at least 11 kPa, available in 168 patients.

of those individuals bearing HCV genotype 4 achieved SVR ($P=0.336$). The rate of patients achieving SVR according to *IL28B* rs12979869 genotype was: 44 (55.7%) for CC, 20 (20.8%) for CT and 9 (30%) for TT, respectively ($P=8.1\times10^{-6}$). Individuals who achieved SVR presented a baseline median HCV RNA (IQR) of 5.7 (5.1–6.5) $\log_{10}$ IU/ml versus 6.4 (5.9–6.8) $\log_{10}$ IU/ml for those who did not ($P=4.5\times10^{-5}$). The median (IQR) plasmatic LDL cholesterol levels were 105 (84.6–120) mg/dl among patients with SVR versus 89 (70.5–113) mg/dl for those without SVR ($P=0.007$).

Single-nucleotide polymorphisms selected

One hundred and forty-four SNPs were selected for the primary analysis (Supplement 1, http://links.lww.com/QAD/A374). Of these, 26 were excluded as a result of either a minor allele frequency below 0.05 [20 (13.9%) SNPs], a *P*-value of the Hardy–Weinberg equilibrium below 0.01 [1 (0.7%) SNP] or a rate of genotyping below 80% [5 (3.5%) SNPs]. Thus, 118 (81.9%) of the previously selected SNPs were analyzed (Supplement 1, http://links.lww.com/QAD/A374). SNPs at the following genes were associated with SVR in the analysis conducted using PLINK software: *LDLR* (rs10415811, $P=0.047$; rs11672123, $P=0.0067$; rs1433099, $P=0.0019$; rs2569540, $P=0.0044$; rs5930; $P=0.046$), transforming growth factor β (*TGF*-β; rs1800469, $P=0.0087$), aquaporine 2 (*AQP-2*; rs2878771, $P=8\times10^{-4}$), very-low-density lipoprotein receptor (*VLDLR*; rs1454626, $P=0.015$), Sp110 nuclear body protein (*SP110*; rs919178, $P=0.045$), interferon alpha/beta receptor 1 (*IFNAR1*; rs2243592, $P=0.036$), 2'-5'-oligoadenylate synthase 1 (*OAS1*; rs1131454, $P=0.047$) and apolipoprotein B (*APO-B*; rs11126598, $P=0.0094$). Genotyping was achieved in 100% (*AQP-2*, *VLDLR*, *SP110*, *IFNAR1*, *OAS1* and *APO-B*) and 97.1% (*TGF*-β) of the cases, respectively. The *VLDLR* rs7043199 was discarded because of the low prevalence of its favorable genotype TT (2.9%), so that subsequent analysis exclusively refers to rs1454626.

Treatment response according to newly identified single-nucleotide polymorphisms

According to the SVR rates observed for each genotype, the SNP genotyping results were classified as favorable and unfavorable. Fig. 1 presents the rates of SVR, as well as the distribution of each genotype in the study population. SVR rates according to variations of rs1131454 in the *IFNAR1* gene were 18 (49%) for genotype CC and 55 (33%) for genotype AG/AA ($P=0.067$).

In spite of the number of SNPs in specific genes identified as predictors for SVR in this study, haplotypic analysis could only be performed for *LDLR*, *VLDLR* and *TGF*-β genes due to the number of SNPs analyzed in these genes (Supplement 1, http://links.lww.com/QAD/A374). In the *LDLR* gene, seven linkage disequilibrium blocks were detected (Fig. 2). An extended haplotype (GGAAG) in linkage disequilibrium block number 6 defined by rs2738464, rs2738465, rs1433099, rs2738466 and rs7258950 (Fig. 2) was associated with SVR ($P=0.0046$). After categorization, 31 (50.8%) of the patients with *LDLR* haplotype GGAAG versus 42 (29.4%) of those with the non-GGAAG haplotype showed SVR ($P=0.003$). Among the HCV genotype 1-infected patients, 22 (50%) of those presenting *LDLR* haplotype GGAAG versus 33 (28.2%) of those with other *LDLR* haplotypes reached SVR ($P=0.009$). The corresponding figures for HCV genotype 4 carriers were 9 (52.9%) versus 9 (34.6%), respectively ($P=0.23$). The interaction between *LDLR* haplotype and *IL28B* genotype on SVR rates is shown in Table 2. No haplotype of *VLDLR* or *TGF*-β was associated with SVR.

Interaction of newly identified single-nucleotide polymorphisms with the predictive value of *IL28B* rs12979860

Forty-four (55.7%) of the patients presenting *IL28B* genotype CC versus 29 (23%) of those with genotype CT/TT attained SVR ($P=2\times10^{-6}$). The corresponding figures among the subpopulations infected with HCV genotype 1 and 4 were 34 (51.5%) versus 21 (21.9%; $P=9\times10^{-4}$) and 10 (76.9%) versus 8 (26.7%; $P=0.002$), respectively. SVR rates according to the newly identified genes stratified for *IL28B* genotype are listed in Table 3. After categorization for *IL28B*, *TGF*-β and *AQP-2* genotypes, the number of patients reaching SVR with favorable genotypes (*IL28B* CC, *TGF*-β non-AA and *AQP-2* non-GG) versus unfavorable genotypes (*IL28B* non-CC, *TGF*-β AA and *AQP*-2 GG) were 22 out of 28 (78.6%) versus 1 out of 13 (7.1%) ($P=2.1\times10^{-5}$). According to HCV genotype, the numbers of individuals with the former combinations of genotypes reaching SVR were 16 (76.2%) versus 1 (9.1%) in HCV genotype 1 ($P=3\times10^{-4}$) and 6 (85.7%) versus 0 in HCV genotype 4 ($P=0.023$), respectively. Furthermore, an interaction between *IL28B* and *VLDLR*, *SP110*, *IFNAR1* and *APO-B*, respectively, was observed (Table 2).

Multivariate analysis

According to the univariate analysis, SNPs in *TGF*-β, *AQP-2*, *VLDLR* and *APO-B* genes, as well as the *LDLR* haplotype GGAAG, were entered into a multivariate logistic regression model adjusted for age, sex, presence of advanced fibrosis, HCV baseline viral load and *IL28B* genotype. *SP110* and *OAS1* were excluded as a result of their considerably weak association with SVR and, mainly, because of the low prevalence of the unfavorable genotype (Fig. 1). In the multivariate analysis *TGF*-β non-AA, *AQP-2* non-GG and *LDLR* GGAAG were independently associated with SVR along with *IL28B* CC genotype and low baseline HCV RNA load (Table 3).

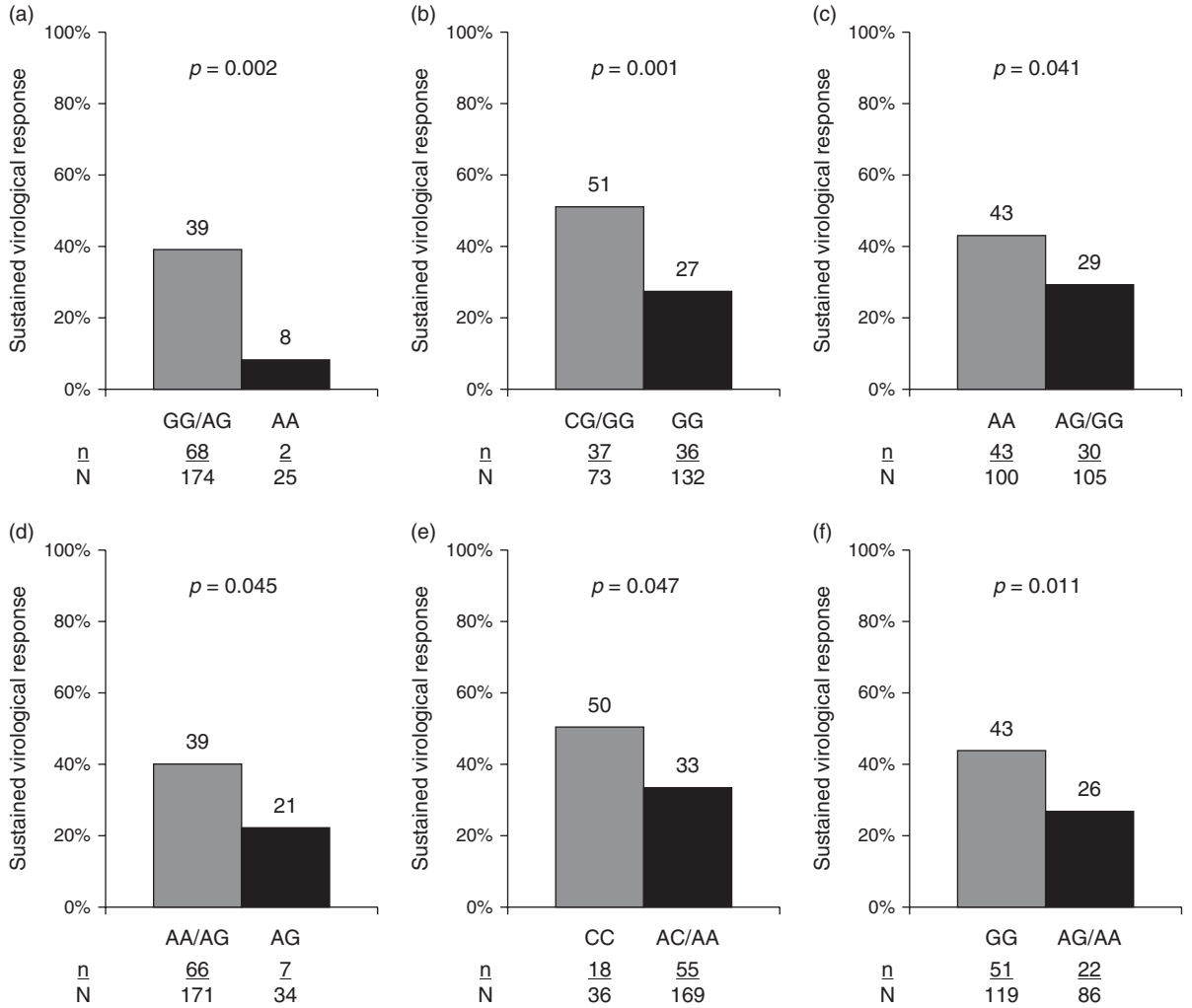

Fig. 1. Rates of sustained virological response (SVR) according to genotypes of the SNPs newly identified and distribution of each genotype in the study. (a) Transforming growth factor β (*TGF*-β); (b) aquaporine 2 (*AQP-2*); (c) very-low-density lipoprotein receptor (VLDLR); (d) Sp110 nuclear body protein (SP110); (e) 2′-5′-oligoadenylate synthase 1 (OAS1); (f) apolipoprotein B (APO-B). n = Number of patients achieving SVR for each genotype. N = Number of patients bearing the specific genotype in the overall population.

Additive genetic factor analysis

In accordance with the multivariate analysis, *TGF*-β, *AQP-2* and *LDLR* haplotype were selected for this calculation. In patients presenting *IL28B* genotype CC, the mean GRS for patients with SVR was 1.88 versus 1.18 presented by those individuals without SVR ($P = 1.3 \times 10^{-4}$). In the subpopulation of patients with *IL28B* non-CC genotype, mean GRS was −1 in patients presenting SVR versus −1.62 in those without SVR ($P = 3.5 \times 10^{-5}$). The rates of SVR according to different GRS among patients with favorable *IL28B* genotype were zero out of four patients with GRS 0, 38.9% (14 patients) with GRS 1, 82.6% (19 patients) with GRS 2 and 75% (9 patients) with GRS 3 ($P = 4 \times 10^{-4}$). Among patients with *IL28B* genotype non-CC, the rates of SVR were 55.6% (5 patients) with GRS 0, 34.6% (18 patients) with GRS −1, 8.9% (5 patients) with GRS −2 and none out of six patients with GRS −3 ($P = 0.001$).

Discussion

The study has identified genetic variations in the *TGF*-β and *AQP-2* genes as independent predictors of SVR to Peg-IFN and RBV in HIV/HCV genotype 1 or 4-infected patients. Additionally, a haplotype on the *LDLR* gene has also been found to be associated with SVR. These pharmacogenomic parameters improve the predictive capacity of *IL28B* genotype and may therefore play a role in the development of a tool to accurately predict response to therapy against HCV.

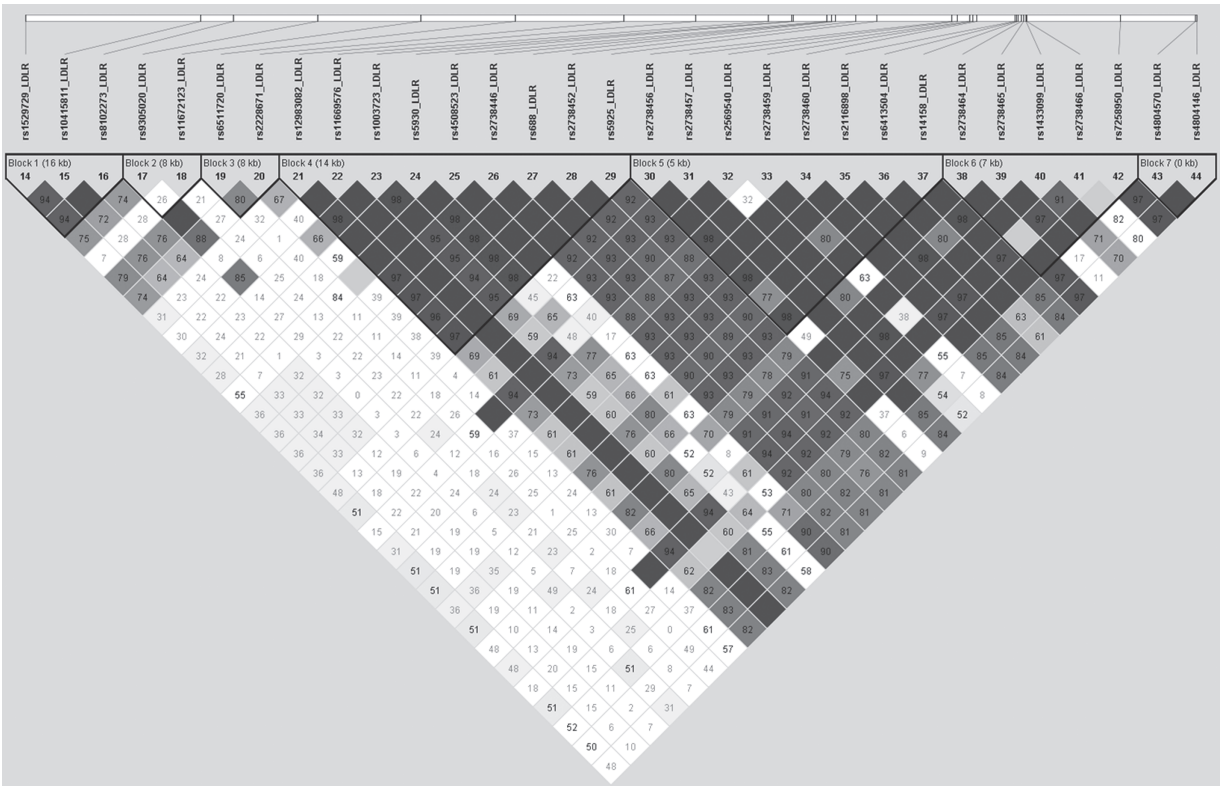

Fig. 2. Genomic position of the SNPs on the LDLR gene and linkage disequilibrium blocks analyzed. LDLR, low-density lipoprotein receptor; SNPs, single-nucleotide polymorphisms.

The *IL28B* genotype is a potent predictor of response to dual therapy in HIV/HCV-coinfected patients [2,8,9], which is commonly used in daily practice. However, the meaning of a favorable or unfavorable *IL28B* genotype in terms of the likelihood of SVR is very different depending on viral [16] and host factors, such as plasma levels of IP-10 [15] or LDLR genotype [13,14]. This study demonstrates that other genomic factors may determine the SVR rates associated with *IL28B* variations and that some of those factors with a greater impact should be considered along with *IL28B* genotype when used in clinical routine.

The findings presented in this study raise a number of questions, since few or no datum is available on the association of SVR with the protein encoded by the corresponding genes of some of the herein described SNPs. In this context, *TGF*-β is a cytokine with multiple functions that has been associated with the development of hepatic fibrosis [20,21]. However, the role of *TGF*-β on the outcome of treatment against HCV is unclear and data are scarce and contradictory [22–27]. In this regard, high *TGF*-β levels have been described to diminish response to dual therapy in this population [22]. On the contrary, a study observed a direct relationship between *TGF*-β levels and response to Peg-IFN in HIV-infected patients with acute hepatitis C [23]. The results of the present study supports that *TGF*-β is involved in viral clearance. However, it is unknown how this influence is exerted. Similarly, AQP-2 seems to be involved in the development of fibrosis [28], but no datum is currently available on its impact on HCV treatment outcome. The main function of AQP-2 is the vasopressin-dependent reabsorption of water by forming water-specific membrane channels in the renal collecting duct. Dysfunction of AQP-2 caused by mutations on the *AQP-2* gene can lead to diabetes insipidus [29]. There might be an association between cholesterol metabolism and AQP-2, as statins interfere with its expression [30].

Plasma lipoproteins, including VLDLR [31] and APO-B [32], play an important role on HCV infectivity and on the outcome of therapy against HCV. Genetic variations in the *VLDLR* gene were observed to impact on SVR herein, although no independent association was observed in the multivariate analysis. The identification of a *LDLR* haplotype associated with SVR supports the findings of a previous study in which an influence of a specific SNP (rs14158) in the 3′UTR of the *LDLR* gene on SVR was described [13]. Likewise, this study shows that using this haplotype, the predictive capacity of isolated SNPs in *LDLR* genes is improved.

The predictive performance of *IL28B* genotype can be markedly enhanced by using other genomic predictors concomitantly. This has been proven using the

Table 2. Interaction of newly identified SNPs and *IL28B* rs12979860 genotype regarding rates of SVR.

		IL28B rs12979860			
		CC		CT/TT	
Gene	Genotype	No. of SVR (%)	*P*	No. of SVR (%)	*P*
SNPs					
TGF-β rs1800469	GG/AG	42 (63)	0.001	26 (24)	0.251
	AA	0		2 (12)	
AQP-2 rs2878771	CC/CG	22 (73)	0.014	15 (35)	0.023
	GG	22 (45)		14 (17)	
VLDLR rs1454626	AA	29 (71)	0.005	14 (24)	0.858
	AC/CC	15 (40)		15 (22)	
SP110 rs919178	AA/AG	39 (60)	0.171	27 (26)	0.108
	GG	5 (39)		2 (9.5)	
OAS1 rs1131454	GG	13 (72)	0.604	24 (22)	0.108
	AG/AA	31 (51)		5(28)	
IFNAR1 rs2243592	CC	11 (73)	0.127	7 (32)	0.280
	AC/AA	33 (52)		22 (21)	
APO-B rs11126598	GG	33 (66)	0.015	18 (26)	0.368
	AG/AA	11 (38)		11 (19)	
Haplotypes					
LDLR∗	GGAAG	15 (71)	0.09	16 (40)	0.002
	Non-GGAAG	29 (50)		13 (15)	

APO-B, apolipoprotein B; *AQP-2*, aquaporine 2; IFNAR1, interferon alpha/beta receptor 1; *LDLR*, low-density lipoprotein receptor; OAS1, 2′-5′-oligoadenylate synthase 1; SP110, Sp110 nuclear body protein; *TGF-β*, transforming growth factor *β*; *VLDLR*, very-low-density lipoprotein receptor. ∗Defined by rs2738464, rs2738465, rs1433099, rs2738466 and rs7258950.

Table 3. Univariate and multivariate analysis to identify factors associated with sustained virologic response (SVR).

Parameter	SVR, *n* (%)	*P* (univariate)	AOR (95% CI)	*P* (multivariate)
Age				
≤41.6 years	42 (41.2)	0.098	1 (0.91–1.1)	0.961
>41.6 years	31 (30.1)			
Sex				
Male	61 (35.9)	0.857		0.776
Female	12 (34.3)		0.844 (0.26–2.73)	
Advanced fibrosis[1]				
No	35 (39.8)	0.093	2.11 (0.86–5.15)	0.102
Yes	22 (27.5)			
Baseline HCV RNA load				
≤600 000 IU/ml	39 (58.2)	1.7×10^{-6}	5.87 (2.19–15.7)	4.3×10^{-4}
>600 000 IU/ml	33 (24.1)			
IL28B rs12979860				
CC	44 (55.7)	1.9×10^{-6}	8.88 (3.32–23.7)	1.3×10^{-4}
CT/TT	29 (23)			
TGF-β rs1800469				
GG/AG	68 (39.1)	0.002	6.927 (1.29–37.3)	0.024
AA	2 (8)			
AQP-2 rs2878771				
CC/CG	37 (50.7)	0.001	3.781 (1.51–9.49)	0.005
GG	36 (27.3)			
VLDLR rs1454626				
AA	43 (43)	0.041	1.49 (0.63–3.56)	0.368
AC/CC	30 (28.6)			
APO-B rs11126598				
GG	51 (42.9)	0.011	1.56 (0.65–3.75)	0.321
AG/AA	22 (25.6)			
LDLR haplotype				
GGAAG	31 (50.8)	0.003	5.078 (1.79–14.4)	0.002
Non-GGAAG	42 (29.4)			

AOR, adjusted odds ratio; *APO-B*, apolipoprotein B; *AQP-2*, aquaporine 2; CI, confidence interval; *IL28B*, interleukin 28B; IU, International Unit; *LDLR*, low-density lipoprotein receptor; SVR, sustained virological response; *TGF*-β, transforming growth factor *β*; *VLDLR*, very-low-density lipoprotein receptor. [1]Available in 168 individuals.

combination of *IL28B* and the rs14158 CC genotype on the *LDLR* gene [13]. However, almost 31% of the carriers of both *IL28B* and *LDLR* favorable genotypes do not respond to therapy, whereas 14% of those harboring both unfavorable genotypes show SVR to Peg-IFN and RBV [13]. This points out the necessity to optimize this combination of genotypes. In this study, using three genotypes (*IL28B*/*AQP-2*/*TGF*-β), the probability of SVR increased to 80% for the favorable combination and it was only 7% for the triple unfavorable genotype. Unfortunately, the clinical utility of this combination is limited because the triple favorable and unfavorable genotypes are relatively uncommon; indeed, they were found only in 13.7 and 6.3% of the population analyzed herein. However, it is probable that the combination of some of the SNPs described in this study with other viral or host predictors of SVR may yield valuable predictive tools. As it can be seen with the GRS calculation, SVR rates vary considerably according to the number of risk factors both among carriers of the favorable and the unfavorable *IL28B* genotype. Importantly, the rates of SVR are higher for those patients bearing *IL28B* non-CC but no other unfavorable genotype than those patients with *IL28B* CC but no other favorable genotype. In the case of HCV genotype 1 infection, this information could be used to select those individuals who may greatly benefit from dual therapy. This is a critical point because protease inhibitor-based therapy is unlikely to be widely available in many countries due to financial restrictions in the next few years. Likewise, HCV genotype 4-infected patients with a very high probability to respond could be motivated to undergo dual therapy. On the contrary, treatment could be deferred in those patients with a very low likelihood to respond to dual therapy, if they do not present advanced fibrosis.

The duration of dual therapy against HCV may be decided on the basis of HCV kinetics on treatment [3,33,34]. Similarly, in patients with rapid viral decline, DAA-based therapy may be also shortened without reduction of the rate of SVR [35,36]. Viral kinetics in HCV-infected patients strongly depends on the pharmacogenomic host features [37–39]. Consequently, the genomic predictors identified here may correlate with viral kinetics. If so, these predictors could be useful to identify patients who qualify for shorter double or triple treatment durations from baseline, thus avoiding very early viral load determinations. Further studies are required in order to address this topic.

The study has some limitations. First, the number of patients is relatively limited to allow classification into multiple genetic profiles. This led to categories with low numbers of cases. Because of this, these data should be reproduced with a higher number of patients and in other populations. However, the main objective of this study was to identify novel SNPs that may be used to develop a predictive model that allows calculating the individual probability of response for each patient, and, in fact, we have identified SNP potentially candidates to be entered in predictive models along with viral and other host factors. Second, these results should be analyzed in HCV-monoinfected patients, since the predictive value may be different in this population. Third, the analysis presented herein is limited to HCV genotypes 1 and 4. In genotype 3-infected individuals, a higher mortality has been observed for *IL28B* CC carriers [40] and these individuals would benefit from identifying alternative predictors. However, and similar to what is observed for *IL28B* genotype in patients with or without HIV coinfection [8,9,41,42], no association between SVR and the SNPs described herein could be detected in HCV genotype 2 or 3-infected individuals (data not shown). However, an impact of a SNP on the proprotein convertase subtilisin/kexin type 9 gene on SVR uniquely in genotype 3 infection has been described recently [39], lining out the necessity to distinguish between these genotypes. Finally, in the era of new DAAs genomic predictors may be less important. However, an impact of *IL28B* genetic variations has been observed under interferon-based triple therapy [10–12] in treatment-naive patients. It also seems to play a role in interferon-free regimens [43,44], particularly with specific combinations [44]. Therefore, the value of genomic predictors is likely to remain important in the setting of DAA-based therapy. Furthermore, DAA-based therapy will not be available for all HCV-infected patients in most countries, mainly due to financial restrictions. Because of this, dual therapy may continue to be given in a significant number of patients in these settings. A combination of pharmacogenomic markers with high predictive performance may be very helpful to identify patients to be treated with dual therapy and, among them, those who may benefit from shorter courses of therapy.

In conclusion, there are a number of important genetic factors that modify the predictive capacity of *IL28B* genotype, as *TGF*-β, *AQP-2* and *LDLR* genotype. A combination of these factors can be used to identify HIV/HCV genotype 1 or 4-infected patients with a very high or a very low probability to respond to dual therapy with Peg-IFN and RBV. Furthermore, the predictive ability of models based on these factors should be analyzed in patients on direct acting antivirals.

Acknowledgements

Author contribution:

K.N.: Planning and conducting the study, collecting and interpreting data and drafting the manuscript.

A. Caruz: Planning and conducting the study, collecting and interpreting data.

A.R.-J.: Collecting and interpreting data.

P.B.: Collecting and interpreting data.

L.M.: Collecting and interpreting data.

L.M.R.: Genotyping, collecting and interpreting data.

R.H.: Collecting and interpreting data.

A.C.: Collecting and interpreting data.

V.S.: Planning and conducting the study, interpreting data and drafting the manuscript.

F.A.D.L.: Collecting and interpreting data and drafting the manuscript.

J.M.: Collecting and interpreting data and drafting the manuscript.

A.R.: Planning and conducting the study, interpreting data and drafting the manuscript.

J.A.P.: Planning and conducting the study, interpreting data and drafting the manuscript.

The work was supported in part by the Red de Investigación en SIDA (grant number RETIC RD06/006/RD12/0017), the Fundación Progreso y Salud, Consejería de Salud de la Junta de Andalucía (grant number PI-0247–2010, PI-0157–2011, PI-0430–2012 PI10/01232), the Fondo de Investigaciones Sanitarias (grant number PI10/01664), the Ministerio de Sanidad y Servicios Sociales (grant number EC11–304) and the Fundación para la Investigación y la Prevención del Sida en España (grant number 121004/10). K.N. is the recipient of a Sara Borrell postdoctoral perfection grant from the Instituto de Salud Carlos III (grant number SCO/523/2008). J.A.P. is the recipient of an intensification grant from the Instituto de Salud Carlos III (grant number Programa-I3SNS).

Conflicts of interest

Source of funding: D.M. has received consulting fees from ViiV Healthcare, Bristol-Myers Squibb, Abbott Pharmaceuticals, Merck Sharp & Dohme, Janssen-Cilag and Boehringer Ingelheim. V.S. has received consulting fees from Merck Sharp & Dohme and Boehringer Ingelheim and Janssen. He has received research support from Merck Sharp & Dohme, Janssen and Gilead and Boehringer Ingelheim, and received lecture fees from Gilead, ViiV Healthcare, Bristol-Myers Squibb, Merck Sharp & Dohme and Janssen. A.R. has received consulting fees from Bristol-Myers Squibb, Abbott, Gilead, Janssen Cilag, Merck Sharp &Dohme and Boehringer Ingelheim; has received research support from Abbott, GlaxoSmithKline, Roche, Bristol-Myers Squibb, Abbott and Boehringer Ingelheim; and has received lecture fees from GlaxoSmithKline, Abbott, Bristol-Myers Squibb, Merck Sharp & Dohme, Jansen Cilag, Gilead, Boehringer Ingelheim and Schering-Plough. J.A.P. has received consulting fees from GlaxoSmithKline, Bristol-Myers Squibb, Abbott Pharmaceuticals, Gilead, Merck Sharp & Dohme, Schering-Plough, Janssen and Boehringer Ingelheim. He has received research support from GlaxoSmithKline, Roche, Bristol-Myers Squibb, Schering-Plough, Abbott Pharmaceuticals, Gilead, Merck Sharp & Dohme, Janssen and Boehringer Ingelheim, and has received lecture fees from GlaxoSmithKline, Roche, Abbott Pharmaceuticals, Bristol-Myers Squibb, Gilead, Merck Sharp & Dohme, Janssen, Boehringer Ingelheim and Schering-Plough.

All other authors: None to declare.

References

1. Soriano V, Mocroft A, Rockstroh J, Ledergerber B, Knysz B, Chaplinskas S, *et al*. **Spontaneous viral clearance, viral load, and genotype distribution of hepatitis C virus (HCV) in HIV-infected patients with anti-HCV antibodies in Europe.** *J Infect Dis* 2008; **198**:1337–1344.
2. Mira JA, Rivero A, de Los Santos-Gil I, López-Cortés LF, Girón-González JA, Márquez M, *et al*. **Hepatitis C virus genotype 4 responds better to pegylated interferon with ribavirin than genotype 1 in HIV-infected patients.** *AIDS* 2012; **26**:1721–1724.
3. European AIDS Clinical Society. Guidelines version 6.1 November 2012. http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/EACS Guidelines-v6.1-English-Nov 2012.pdf. [Accessed 12 March 2012]
4. Sulkowski M, Sherman KE, Soriano V, Rockstroh JK, Dieterich DT, Girardó PM, *et al*. **Telaprevir in combination with peginterferon alfa-2a/ribavirin in HCV/HIV co-infected patients: SVR24 final study results**. *63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD 2012),* 9–13 November 2012, Boston, USA [Abstract 54].
5. Sulkowski M, Pol S, Cooper C, Fainboim H, Slim J, Rivero A, *et al.* **Boceprevir + pegylated interferon + ribavirin for the treatment of HCV/HIV co-infected patients: end-of-treatment (week 48) interim results**. In *Program and abstracts of the 19th Conference on Retroviruses and Opportunistic Infections*, 3–6 March 2012, Seattle, USA [Abstract 47].
6. Moreno A, Bárcena R, Quereda C, Pérez-Elías MJ, García-Hoz, Casado JL, *et al.* **Does HIV coinfection reduce initial safety or efficacy of boceprevir (BOC) or telaprevir (TPV)-based triple therapy in cirrhotic patients in the 'real-life'?** *63rd Annual Meeting of the American Association for the Study of Liver Disease*, 9–13 November 2012, Boston, US [Abstract 1789].
7. Berg T, Buggisch P, Hueppe D, Mauss S, Wedemeyer H, Decker-Burgard S. **Telaprevir-based triple-therapy in patients with chronic hepatitis C in Germany: a 12-week interim analysis of real-life data.** *J Int AIDS Soc* 2012; **15**:18424.
8. Rallón N, Naggie S, Benito JM, Medrano J, Restrepo C, Goldstein D, *et al*. **Association of a single nucleotidepolymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus co-infected patients.** *AIDS* 2010; **24**:F23–F29.
9. Pineda JA, Caruz A, Rivero R, Neukam K, Salas I, Camacho A, *et al*. **Prediction of response to pegylated interferon plus ribavirin by IL28B gene variation in patients coinfected with HIV and hepatitis C virus.** *Clin Infect Dis* 2010; **51**:788–795.

10. Bronowicki JP, Hézode C, Bengtsson L, Pol S, Bourlière M, Serfaty L, *et al*. **100% SVR in IL28B SNP rs12979860 C/C patients treated with 12 weeks of telaprevir, peginterferon and ribavirin in the PROVE-2 trial**. *47th Annual Meeting of the European Association for the Study of the Liver*, 18–22 April 2012, Barcelona, Spain [Abstract 1094].
11. Jacobson IM, Catlett I, Maroellin 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**:1369.
12. Poordad F, Bronowicki JP, Gordon SC, Zeuzem S, Jacobson IM, Sulkowski MS, *et al*. **Factors that predict response of patients with hepatitis C virus infection to boceprevir.** *Gastroenterology* 2012; **143**:608–618.
13. Pineda JA, Caruz A, Di Lello FA, Camacho A, Mesa P, Neukam K, *et al*. **Low-density lipoprotein receptor genotyping enhances the predictive value of IL28B genotype for the response to pegylated interferon plus ribavirin in HIV/hepatitis C virus-coinfected patients with genotype 1 or 4.** *AIDS* 2011; **25**:1415–1420.
14. Neukam K, Almeida C, Caruz A, Rivero-Juárez A, Rallón N, Di Lello FA, *et al*. **A model to predict the response to therapy against hepatitis C virus (HCV) including low-density lipoprotein receptor genotype in HIV/HCV-coinfected patients.** *J Antimicrob Chemother* 2013; **68**:915–921.
15. Beinhardt S, Aberle JH, Strasser M, Dulic-Lakovic E, Maieron A, Kreil A, *et al*. **Serum level of IP-10 increases predictive value of IL28B polymorphisms for spontaneous clearance of acute HCV infection.** *Gastroenterology* 2012; **142**:78–85.
16. Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, *et al*. **HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy.** *Gut* 2011; **60**:261–267.
17. Mosbruger TL, Duggal P, Goedert JJ, Kirk GD, Hoots WK, Tobler LH, *et al*. **Large-scale candidate gene analysis of spontaneous clearance of hepatitis C virus.** *J Infect Dis* 2012; **201**:1371–1380.
18. Agundez JA, Garcia-Martin E, Devesa MJ, Carballo M, Martinez C, Lee-Brunner A, *et al*. **Polymorphism of the TLR4 gene reduces the risk of hepatitis C virus-induced hepatocellular carcinoma.** *Oncology* 2012; **82**:35–40.
19. Danilovic DL, Mendes-Correa MC, Lima EU, Zambrini H, Barros K, Marui S. **Correlations of CTLA-4 gene polymorphisms and hepatitis C chronic infection.** *Liver Int* 2012; **32**:803–808.
20. Brenner DA. **Molecular pathogenesis of liver fibrosis.** *Trans Am Clin Climatol Assoc* 2009; **120**:361–368.
21. Gewaltig J, Mangasser-Stephan K, Gartung C, Biesterfeld S, Gressner AM. **Association of polymorphisms of the transforming growth factor-beta1 gene with the rate of progression of HCV-induced liver fibrosis.** *Clin Chim Acta* 2002; **316**:83–94.
22. Lee S, Varano J, Flexman JP, Cheng W, Watson MW, Rossi E, *et al*. **Decreased IP-10 and elevated TGFbeta1 levels are associated with viral clearance following therapy in patients with hepatitis C virus.** *Dis Markers* 2010; **28**:273–280.
23. Nattermann J, Vogel M, Nischalke HD, Danta M, Ahlenstiel G, Michalk M, *et al*. **The transforming growth factor-beta high-producer genotype is associated with response to hepatitis C virus-specific therapy in HIV-positive patients with acute hepatitis C.** *AIDS* 2008; **22**:1287–1292.
24. Abbas Z, Moatter T, Hussainy A, Jafri W. **Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3.** *World J Gastroenterol* 2005; **11**: 6656–6661.
25. Marek B, Kajdaniuk D, Mazurek U, Janczewska-Kazek E, Kos-Kudla B, Strzalka B, *et al*. **TGF-beta1 mRNA expression in liver biopsy specimens and TGF-beta1 serum levels in patients with chronic hepatitis C before and after antiviral therapy.** *J Clin Pharm Ther* 2005; **30**:271–277.
26. Barrett S, Collins M, Kenny C, Ryan E, Keane CO, Crowe J. **Polymorphisms in tumour necrosis factor-alpha, transforming growth factor-beta, interleukin-10, interleukin-6, interferon-gamma, and outcome of hepatitis C virus infection.** *J Med Virol* 2003; **71**:212–218.
27. Harfouch S, Guiguet M, Valantin MA, Samri A, Ouazene Z, Slama L, *et al*. **Lack of TGF-β production by hepatitis C virus-specific T cells during HCV acute phase is associated with HCV clearance in HIV coinfection.** *J Hepatol* 2012; **56**:1259–1268.
28. Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, *et al*. **A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C.** *Hepatology* 2007; **46**:297–306.
29. Deen PM, Verdijk MA, Knoers NV, Wieringa B, Monnens LA, van Os CH, van Oost BA. **Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine.** *Science* 1994; **264**:92–95.
30. Li W, Zhang Y, Bouley R, Chen Y, Matsuzaki T, Nunes P, *et al*. **Simvastatin enhances aquaporin-2 surface expression and urinary concentration in vasopressin-deficient Brattleboro rats through modulation of Rho GTPase.** *Am J Physiol Renal Physiol* 2011; **301**:F309–318.
31. Maillard P, Walic M, Meuleman P, Roohvand F, Huby T, Le Goff W, *et al*. **Lipoprotein lipase inhibits hepatitis C virus (HCV) infection by blocking virus cell entry.** *PLoS One* 2011; **6**: e26637.
32. Sheridan DA, Price DA, Schmid ML, Toms GL, Donaldson P, Neely D, Bassendine MF. **Apolipoprotein B-associated cholesterol is a determinant of treatment outcome in patients with chronic hepatitis C virus infection receiving antiviral agents interferon-alpha and ribavirin.** *Aliment Pharmacol Ther* 2009; **29**:1282–1290.
33. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, *et al*. **Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia.** *J Hepatol* 2006; **44**:97–103.
34. Kamal SM, El Kamary SS, Shardell MD, Hashem M, Ahmed IN, Muhammadi M, *et al*. **Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: the role of rapid and early virologic response.** *Hepatology* 2007; **46**:1732–1740.
35. Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, *et al*. **Boceprevir for untreated chronic HCV genotype 1 infection.** *N Engl J Med* 2011; **364**:1195–1206.
36. Sherman KE, Flamm SL, Afdahl NH, Nelson DR, Sulkowski MS, Everson GT, *et al*. **Response-guided combination treatment for hepatitis C virus infection.** *N Engl J Med* 2011; **365**:1014–1024.
37. Neukam K, Barreiro P, Rivero-Juárez A, Caruz A, Mira JA, Camacho A, *et al*. **Pegylated interferon plus ribavirin is suboptimal in IL28B CC carriers without rapid response.** *J Infect* 2013; **67**:59–64.
38. Rivero-Juarez A, Camacho A, Caruz A, Neukam K, Gonzalez R, Di Lello FA, *et al*. **LDLr genotype modifies the impact of IL28B on HCV viral kinetics after the first weeks of treatment with PEG-IFN/RBV in HIV/HCV patients.** *AIDS* 2012; **26**:1009–1015.
39. Neukam K, Rivero-Juárez A, Caruz A, Labarga P, Márquez M, Real LM, *et al*. **Variations in proprotein convertase subtilisin/kexin type 9 (PCSK9) gene are associated with response to therapy against hepatitis C virus genotype 3 infection**. *20th Conference on Retroviruses and Opportunistic Infections*, 3–6 March 2013, Atlanta, USA [Abstract 686].
40. Clausen LN, Astvad K, Ladelund S, Larsen MV, Schønning K, Benfield T. **Hepatitis C viral load, genotype 3 and interleukin-28B CC genotype predict mortality in HIV and hepatitis C-coinfected individuals.** *AIDS* 2012; **26**:1509–1516.
41. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, *et al*. **Genetic variation in IL28B and spontaneous clearance of hepatitis C virus.** *Nature* 2009; **461**:798–801.
42. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, *et al*. **Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study**. *Gastroenterology* 2010; **138**:1338–1345, 1345.e1–1345.e7.
43. Chu TW, Kulkarni R, Gane EJ, Roberts SK, Stedman C, Angus PW, *et al*. **Effect of IL28B genotype on early viral kinetics during interferon-free treatment of patients with chronic hepatitis C.** *Gastroenterology* 2012; **142**:790–795.
44. Zeuzem S, Soriano V, Asselah T, Bronowicki JP, Lohse AW, Mullhaupt B, *et al*. **SVR4 and SVR12 with an interferon-free regimen of BI 201335 and BI 207127, +/− ribavirin, in treatment-naïve patients with chronic genotype-1 HCV infection: interim results of SOUND-C2**. *47th Annual Meeting of the European Association for the Study of the Liver*, 18–22 April 2012, Barcelona, Spain [Abstract 101].