ABSTRACT

Various clinical and genetic factors affect response to antiviral treatment of chronic hepatitis C virus (HCV) infection. The IL28B single-nucleotide polymorphism (SNP) rs12979860 is associated with a sustained viral response (SVR), and the suppressor cytokine signaling 3 (SOCS3) gene is over-expressed in HCV-1b non-responders. The aim of this study was to look for correlations between genetic, clinical and viral factors implicated in response to antiviral treatment in chronic HCV infection. We evaluated 190 controls and 148 HCV-infected patients (102 HCV-1 and 46 HCV-2). Demographic, metabolic and histological features related to antiviral treatment were recorded. Univariate and multivariate analyses were used to identify correlations between the genetic and non-genetic factors examined and response to antiviral treatment. IL28B expression was higher in CC SNPs versus other alleles in controls (P=0.01) and this difference was associated with viral infection (HCV vs controls P=0.006), particularly in HCV-2 patients (P=0.003). SOCS3 and IL28B expression was correlated with controls (P=0.011), whereas there was an inverse correlation between the expression of the two genes in HCV patients and HCV-1b non-responders (P=0.014 and P=0.03, respectively). Multivariate analysis showed that the only independent SVR predictive factor was rapid virological response. The frequency of IL28B rs12979860 SNP alleles in controls (C allele=71.1% and T allele=28.9%) was comparable to that of the HCV population (C allele=66.6% and T allele=33.4%). Rapid virological response seems to be the only significant independent predictor of an SVR to antiviral treatment. The inverse correlation between SOCS3 and IL28B expression in genotype 1b non-responders suggests that SOCS3 may affect IL28B expression thereby influencing response to antiviral therapy.

Introduction

Hepatitis C virus (HCV)-related chronic hepatitis is a multi-factorial disease, and its clinical expression is conditioned by viral, genetic and metabolic co-factors. The progression of liver fibrosis and the natural history of the disease is principally related to: i) virus genotype; ii) apparent duration of the disease; iii) steatosis; iv) co-infection with hepatitis B virus and/or HIV; and v) chronic alcohol consumption. Response to dual therapy with pegylated interferon and ribavirin is variable because viral and host characteristics may determine whether or not patients achieve a sustained virological response. New treatment protocols including direct antiviral agents have been recently approved for use in HCV-1 subjects and promise to change the
response scenario in these patients. Nonetheless, some of the factors influencing the response to previous therapeutic regimens seem also to affect the new therapy schedules, and this is particularly true for IL28B alleles. Therefore, elucidation of the mechanisms whereby these factors influence therapeutic response might help to identify novel means with which to fight HCV infection.

We previously identified a link between the response to antiviral treatment of HCV-related liver disease and a variety of genes, some of which are up-regulated while others are down-regulated. In particular, we and others found that the suppressor cytokine signaling 3 (SOCS3) gene, which affects the insulin pathway, had a single-nucleotide polymorphism (SNP) that was correlated to response rates in HCV-1 patients and was overexpressed in non-responder patients with genotype 1b HCV (HCV-1) versus patients with genotype 2 HCV (HCV-2).

In 2009, three genome-wide association studies reported that several highly correlated common SNPs in the vicinity of three IFN-κ genes were highly predictive of response to pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy in patients with HCV-1. The first report came from Ge et al. who analyzed 1131 HCV-1 patients for predictors of response to 48 weeks of treatment with PEG-IFN and RBV. The rs12979860 SNP, which is upstream of the IL28B gene on chromosome 19, was strongly associated with a sustained virological response (SVR), among patients of European ancestry and African-American patients. The specific mechanisms whereby variations in IL28B SNPs affect HCV suppression remain unknown. Improved viral clearance could result from alterations in IL28B expression, mRNA splicing, half-life, or cytokine-receptor affinity or specificity.

The aim of the present study was to assess whether genetic, viral, and clinical factors interact in the regulation of response to antiviral therapy.

Materials and Methods

Patients

One hundred and forty-eight patients with chronic hepatitis C infection were included in the study (Table 1). All were of Caucasian origin and they were recruited from two Italian university hospitals (Hematology Division of the University of Naples Federico II, and Department of Internal Medicine of the Second University of Naples). The inclusion criteria were: elevated alanine aminotransferase (ALT) levels during the previous six months; HCV-Ab positivity; HCV-RNA; genotype 1 (HCV-1) and genotype 2-3 (HCV-2), liver biopsy; and no history of alcohol abuse. Exclusion criteria were: presence of histological and/or clinical signs of cirrhosis; HBV infection; alcohol abuse (>20 mg/day in women, and >30 mg/day in men in the five years before enrolment), evaluated according to Reid et al.; history of drug abuse; and a positive human immunodeficiency virus (HIV) test. Markers of hepatitis B virus and HIV infection were tested using commercially available enzyme-linked immunosorbent assays (Abbott Lab., Abbott Park, IL, USA).

Liver biopsies and histology

The hepatic percutaneous biopsies were performed with Surecut 17G needles, via the intercostal route using an echo-assisted method. Liver specimens were used for histological examination if they were at least 1.5-cm long and contained >5 portal spaces. The specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin, Red Sirius, Ubiquitin, Trichrome, and Prussian blue. Biopsies were evaluated with the Ishak score for necroinflammation grading and fibrosis staging and by the Brunt score for the presence and extent of steatosis by a skilled pathologist.

RNA preparation and hepatitis C virus-RNA determination

RNA preparation and qualitative determination of HCV-RNA were carried out as previously described. HCV-RNA was quantified according to the Cobas Monitor test (version 2; Roche Diagnostics, Milan, Italy).

Hepatitis C virus genotyping

HCV genotypes were determined by hybridization of serum polymerase chain reaction (PCR) products to type- and subtype-specific probes 1a, 1b, 2a, 2b and 3a, as previously described. All patients included in this study were genotype 1b (HCV-1) or 2-3 (HCV-2) infected (Table 1).

Antiviral therapy and classification of patients

All patients had received their first course of antiviral therapy and were treated for 48 weeks with PEG-IFN-α in combination with RBV, according to current consensus protocols (HCV guidelines of the European Association for the Study of the Liver, and of the American Association for the Study of Liver Diseases). The virological response to therapy was assessed via measurements of HCV-RNA at baseline, after 4 weeks [rapid virological response (RVR)], 12 weeks (early virological response), 24 weeks [genotype 2 end of treatment (EOT)] and 48 (genotype 1b EOT) weeks of treatment, and 24 weeks after treatment completion [sustained virological response (SVR)]. Based on the qualitative HCV-RNA results,
subjects were defined as either SVR patients (no detectable HCV-RNA after 24 and 48 weeks of treatment for genotype 2 and 1b respectively, nor 6 months thereafter), or non-responders (NR) (viral breakthrough, and virological non-response, with the continued presence of HCV-RNA at the end of treatment). Patients who relapsed at the end of treatment were not included in this study. According to the standard therapy protocols, treatment was discontinued in NR at week 12 of therapy, based on a positive quantitative HCV-RNA test.

**Healthy control population**

Our control population of 190 Italian subjects was selected from healthy blood donors coming from the blood bank of the University of Naples Federico II. Chronic hepatitis C infection was excluded in all of them by a negative anti-HCV assay (Abbott, Wiesbaden, Germany). No control subject had clinical or biochemical signs of acute HCV infection or of other forms of chronic hepatitis. The controls were age- and gender-matched to the HCV patients. The study was approved by the Ethics Committee of the University of Naples Federico II and the Second University of Naples, and patients gave their informed consent.

**Generation of cell lines from hepatitis C virus patients, RNA isolation and reverse transcription**

Epstein-Barr-virus-transformed lymphoblastoid cell lines (EBV-LCLs) were generated, as previously described, from the peripheral blood mononuclear cells of 140 patients and of 190 healthy individuals. Total RNA was extracted from EBV-LCLs using Trizol reagent (Life Technologies Corp., Carlsbad, CA, USA). Synthesis of cDNA from total RNA (2 μg) was performed using iScript cDNA synthesis kit (Biorad, Milan, Italy).

**Quantitative real-time polymerase chain reaction analysis**

Quantitative RT-PCR (qRT-PCR) using Power SYBR Green PCR Master Mix (Applied Biosystems, Branchberg, NJ, USA) was performed to measure *IL28*, *IL28A*, *IL28B* and *SOCS3* expression. Samples were amplified on an Applied Biosystems 7900HT Sequence Detection System using standard cycling conditions. The primers were designed by the Primer Express 2.1 program (Applied Biosystems). Two specific forward primers were designed to distinguish *IL28A* and *B* genes (Figure 1). β-actin was used as internal control. Relative gene expressions were calculated with the 2−ΔΔCt method.

**Table 1. Demographic characteristics of the study population.**

| Variable          | Patients no. 148 | Controls no. 190 | P       |
|-------------------|-----------------|-----------------|---------|
| Age, mean (±SD)   | 54.00 (±10.00)  | 53.50 (±9.70)   | 0.65    |
| Gender, %         |                 |                 |         |
| M: 50.60%         | M: 50.05%       | F: 49.80%       | 0.95    |
| BMI, mean (±SD)   | 26.5 (±4.29)    | 25.4 (±3.55)    | 0.014   |
| ALT, mean (±SD)   | 84.5 (±80.90)   | 30.6 (±5.40)    | <0.0001 |
| Diabetes, %       |                 |                 |         |
| Yes: 25.80%       | Yes: 0%         | <0.0001         |
| No: 74.20%        | No: 100%        |                 |
| Genotype, %       |                 |                 |         |
| 1b: 51.70%        | 1b: 51.05%      |                 |
| 2: 49.30%         | 2: 49.94%       |                 |
| Grading, mean (±SD) | 9.35 (±4.10)  | -               |         |
| Staging, mean (±SD) | 3.30 (±1.50)  | -               |         |

| Steatosis score*  |     |     |     |
|-------------------|-----|-----|-----|
| 0                 | 61.2%| -   | -   |
| 1                 | 19.7%| -   | -   |
| 2                 | 15.7%| -   | -   |
| 3                 | 11.0%| -   | -   |

| Antiviral response |     |     |     |
|-------------------|-----|-----|-----|
| SVR overall       | 60.7%| -   | -   |
| SVR G-1           | 47.8%| -   | -   |
| SVR G-2           | 74.4%| -   | -   |
| RVR overall       | 50.6%| -   | -   |
| RVR G-1           | 39.1%| -   | -   |
| RVR G-2           | 62.8%| -   | -   |

SD, standard deviation; BMI, body mass index; ALT, alanine aminotransferase; SVR, sustained viral response; RVR, rapid virological response. *Steatosis score assessed on liver specimens by Brunt scoring system as reported in Materials and Methods section.
where the ΔCt was calculated using the differences in the mean Ct between selected genes and the internal control (β-actin). Primer sequences are available upon request (achille.iolascon@unina.it).

**IL28 and SOCS3 genotyping**

Genomic DNA was prepared using the Wizard Genomic DNA purification kit (Promega Corp., Madison, WI, USA). Genotypes of IL28B-SNP (rs12979860 C/T) were determined by restriction fragment length polymorphism analysis using Hpy166I enzyme (New England Biolabs, Ipswich, MA, USA). A 429-bp fragment of the IL28B promoter region was amplified from genomic DNA by PCR in a 25 μL volume with Master Mix 2X (Promega). The oligonucleotide primers were designed from the sequence of the promoter region of human IL28B with the Primer 3 program (Primer3 v. 0.4.0, freeware online). Primer sequences are available upon request (achille.iolascon@unina.it). The restriction fragment length polymorphism assay was performed in 30 μL reaction volume containing the PCR product and the restriction enzyme. In the presence of the C allele, the enzyme cut its 429-bp PCR product into three bands of 322, 215 and 107 bp; whereas in the presence of the T allele, four bands of 292, 185, 107 and 30 bp were obtained. The restricted products were checked by DNA agarose gel electrophoresis. The genotypes of SOCS3-SNP (rs4969170 A/G) were determined with the TaqMan SNP Genotyping Assay (Applied Biosystems), as previously described.

**Clinical and genetic characteristics**

For each patient we recorded: age, sex, body mass index (BMI), extent of liver fibrosis and steatosis, ALT, viral load and HCV genotype, presence of metabolic syndrome and/or diabetes, IL28, IL28A, IL28B and SOCS3 gene expression, and IL28B and SOCS3 SNPs. These data were analyzed as independent factors by both a univariate analysis and a binary logistic regression analysis vs SVR to antiviral treatment (defined as HCV RNA negativity 24 weeks after completion of antiviral treatment) and RVR (defined as HCV RNA negativity at week 4 of treatment).

**Statistical analysis**

A comparison of the genotype, haplotype and allele frequencies between the groups was performed using the chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the relative disease risk conferred by a specific allele, haplotype and genotype. Tests for deviation from Hardy-Weinberg equilibrium were assessed with the Pearson chi-square test. A Mann-Whitney test was used to compare differences in quantitative variables between two groups. The statistical dependence between IL28B and SOCS3 genes was established by Spearman’s ρ correlation coefficient. Univariate analysis of clinical characteristics was carried out by performing the Mann-Whitney U test to compare continuous variables, chi-square with Yates correction or the Fisher-exact test to compare categorical variables. Multivariate analyses were performed by carrying out a binary logistic regression. Statistical analyses were carried out with the Statistical Program for Social Sciences (SPSS®) ver.16.0 for Macintosh® (SPSS Inc., Chicago, IL, USA). A two-sided P value of less than 0.05 was considered statistically significant.

**Results**

**Clinical, demographical and genetic characteristics**

Table 1 shows the patients’ clinical, demographical and genetic features. There were no statistically significant differences between men and women in terms of patients’ characteristics. The control group (n=190) selected from a blood bank of healthy donors of Italian origin was matched for age and sex with the study population. Because of these selection criteria, patients differed from controls in terms of prevalence of diabetes (which was present only in patients and not in controls), ALT (which were reasonably normal in healthy subjects) and BMI (which was higher in patients compared to controls, as was previously described in HCV populations).

![Figure 1. Total IL28 gene expression.](image_url)
**IL28B** rs12979860 C/T frequencies in a control and hepatitis C virus population

We investigated the **IL28B** rs12979860 polymorphism in 148 HCV cases and in 190 healthy controls. Allele frequencies were defined and tested for Hardy-Weinberg equilibrium in controls (P=0.70) and cases (P=0.35). Allele frequencies in the control group (C: 0.71; T: 0.29) were similar to those reported on the 1000 Genomes website (http://browser.1000genomes.org/index.html) (C: 0.68; T: 0.32; P=0.86), and similar to those reported by Ge **et al.** and by Thomas **et al.** for European populations.11,12 However, unlike the two latter studies, allele frequencies did not differ between our healthy controls and HCV-infected patients (C: 0.71/0.67; T: 0.29/0.33; p<0.12) (Table 2). Conversely, we found an association between the C allele and antiviral response (OR=0.44 vs NR; 95% CI 0.23-0.83; P=0.017). However, when we analyzed allele frequencies in our HCV patients divided in HCV genotype, there was a difference, albeit not significant (P=0.65), in NR between HCV1 and HCV2 patients.

**IL28B** gene expression analysis in control and hepatitis C virus populations

We evaluated **IL28B** expression in EBV-LCLs from HCV-affected patients and from control subjects. Quantitative RT-PCR analyses showed that **IL28** expression was associated with viral infection as previously described.22 This association was more pronounced in HCV-2 than in HCV-1 patients (Figure 1). **IL28B** forms a cytokine gene cluster with two other closely related cytokine genes, **IL28A** and **IL29**, on a chromosome region mapped to 19q13.11 Since **IL28B** and **IL28A** transcripts share a high nucleotide sequence homology (98%), we performed a qRT-PCR analysis to selectively amplify the two specific transcripts in our HCV patients. We found that **IL28B** gene expression was higher in HCV-2 than in HCV-1 patients (P=0.00006, Mann-Whitney test). Conversely, **IL28A** gene expression did not differ significantly between the two genotypes (Figure 2).

**IL28B** and **SOCS3** gene expression correlation analysis in control and hepatitis C virus populations

We found a direct correlation in healthy subjects between **SOCS3** and **IL28B** gene expression (Spearman’s ρ 0.46, P=0.01). Differently, there was an in-

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Table 2. **IL28B**-SNP rs12979860 genotype and allele frequencies. Absolute allele and genotype frequencies in the control group and in the hepatitis C virus population from Italy.

| Alleles   | Controls (no. 190) | HCV (no. 148) | P     |
|-----------|--------------------|---------------|-------|
| CC        | 51.1%              | 42.6%         | ns    |
| CT        | 40.0%              | 48.0%         | ns    |
| TT        | 8.9%               | 9.5%          | ns    |

**Cumulative genotypes**

| C         | 71.1%              | 66.6%         | ns    |
| T         | 28.9%              | 33.4%         | ns    |

HCV, hepatitis C virus; ns, not significant.

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**Figure 2.** Gene expression analysis. **IL28** (**IL28A** and **IL28B**), **IL28A** and **IL28B** relative expression with respect to the reference gene, β-actin, is shown. The analysis was performed on 72 hepatitis C virus (HCV) patients (36 HCV-1 and 36 HCV-2). **IL28** expression was slightly higher, although the difference was not significant, in HCV-2 (0.07; 0.01-2.84) than in HCV-1 patients (0.06; 0.02-0.60) (P=0.63, Mann-Whitney test), while **IL28B** expression was significantly higher in HCV-2 (0.07; 0.001-0.73) than in HCV-1 patients (0.01; 0.001-0.03) (P=0.00006; Mann-Whitney test). **IL28A** exhibited an opposite trend (HCV-2: 0.03; 0.002-0.67 vs HCV-1: 0.05; 0.002-0.32) (P=0.55, Mann-Whitney test). Data are presented as median; min-max.
verse correlation (Spearman’s ρ –0.28, P=0.008) between SOCS3 and IL28B expression in HCV patients (Figure 3A). This inverse correlation was more pronounced in the NR subset of HCV-1 patients (Spearman’s ρ –0.42, P=0.03) (Figure 3B).

**Clinical and genetic factors of non-response**

To determine the relative importance of the clinical and genetic factors of response to treatment, we evaluated, at univariate and multivariate analysis, age, sex, genotype, viral load, histological grading and staging, steatosis score, BMI, ALT, diabetes, metabolic syndrome, RVR, SOCS3 and IL28B expression, and SOCS3-SNP and IL28B-SNP frequencies in our patients. Univariate analysis showed that the following factors were related to response to antiviral treatment: RVR (P=0.009), genotype (P=0.011), viral load (P=0.012), steatosis (P=0.009), ALT (P=0.004), diabetes (P=0.006), SOCS3 expression (P=0.005) and IL28B-SNP (P=0.004) (Table 3). At multivariate analysis (binary logistic regression) the only independent factor predicting response to antiviral treatment was RVR (P=0.015) (Table 4). We next carried out univariate and multivariate analyses to evaluate whether the above-indicated factors were able to predict RVR. At univariate analysis the factors related to RVR were: genotype 2 (P=0.03), absence of diabetes (P=0.044) and metabolic syndrome (P=0.026), and the C allele of IL28B-SNP (P=0.0004) (Table 4). At multivariate analysis the only factor predicting RVR was the IL28B-SNP C allele (P=0.001; OR 6.103; 95% CI 2.147-7.350) (Table 4).

**Discussion**

Recent years have seen a surge of interest in genetic factors that influence the response to antiviral therapy in HCV-infected patients. In fact, genome-wide association studies showed that several common SNPs in the vicinity of three IFN-κ genes predict response to antiviral therapy in HCV-1 patients. The three genes encode IFN-κ1 (IL29), IFN-κ2 (IL28A), and IFN-κ3 (IL28B). The same set of SNPs was subsequently associated with natural clearance of HCV. IL28B encodes IFN-κ3, a cytokine distantly related to type 1 (a and b) IFNs and the IL-10 family. The C allele at SNP rs12979860 has been associated with an SVR.
IL28B polymorphisms were performed in patients with HCV-1; more recently, the association between rs12979860 and response to PEG-IFN and RBV treatment was confirmed in genotype 2 and 3 patients.23,24 Our IL28B allele frequencies and response rates are similar to those previously reported. However, ours is the first study of patients from Southern Italy. Unlike the above-cited reports, in which there was a slight but significant higher prevalence of the C/C allele in the general population versus HCV patients, in our study the prevalence of IL28B alleles did not differ significantly between HCV patients and healthy subjects. This finding may be due to the exclusion criteria we used to select our control group. In fact, we excluded HCV-Ab-positive HCV-RNA-negative patients in whom C/C alleles may be more frequent given the tendency of these subjects to spontaneously clear HCV virus after infection.12 Fifty-two percent of our HCV patients with the C/C genotype had an SVR, compared with 75% of patients with genotype C/T and 58% with genotype T/T. In contrast to previous observations in North American patients with genotype 1 HCV,11,23 the SVR rate of genotype C/T patients in our population was intermediate between that of genotype C/C and T/T patients, suggesting that the presence of T/T affects treatment response.

Our study indicates that IL28B expression is up-regulated in the presence of HCV. In fact, the relative IL28B 2^ΔΔCT expression was significantly higher in our HCV patients than in controls. Interestingly, this up-regulation was HCV genotype-specific; indeed IL28B expression was significantly higher in HCV-2 patients than in HCV-1 patients and controls. This result is strengthened by the fact that we analyzed specifically IL28B and not the entire IL28 gene. In fact, we found that IL28B but not IL28A was higher in HCV-2 than in HCV-1 patients, and this might reflect the higher SVR rates achieved by HCV-2 patients.

SOCS3 expression has been reported to be significantly higher in HCV-1 patients than in HCV-2 patients, and higher in NR non-SVR patients than in SVR responders.9,10,25,26 Moreover, like IL28B-SNP genotypes, also SOCS3-SNP genotypes are associated with different rates of antiviral response.9 SOCS proteins interfere with the binding of cytokine receptors and with intracellular molecules that act downstream.2,27 It has also been demonstrated that SOCS3 over-expression in human hepatoma HepG2 cells suppresses IFN-α-induced STAT activation and gene expression of the antiviral proteins 2′,5′-OAS and MxA, thereby resulting in down-regulation of the IFN-α pathway.28 On the other hand, IFN type 1 and type 3 (which are encoded by IL28B) stimulate similar pathways, namely, binding of their receptors results in phosphorylation of the JAK1 and Tyk2 kinases, activation of the transcription factor complex containing STAT1, STAT2, and IFN regulatory factor 9, and up-regulation of a similar set of IFN-stimulated genes.27,29 It is noteworthy that SOCS3 is an IFN-stimulated

| Variable                        | Mann-Whitney U | Wilcoxon W | Z    | 2 Tails Asint Sig. |
|---------------------------------|----------------|------------|------|--------------------|
| Age                             | 789.500        | 2167.500   | -0.618 | 0.536              |
| Gender                          | 931.500        | 2416.500   | -0.131 | 0.896              |
| Genotype                        | 682.000        | 1312.00    | -2.552 | 0.011              |
| Viral load                      | 670.000        | 2122.000   | -2.556 | 0.012              |
| Grading                         | 740.000        | 2171.000   | -1.616 | 0.106              |
| Staging                         | 851.000        | 2336.000   | -0.812 | 0.417              |
| Steatosis                       | 677.000        | 2162.000   | -2.597 | 0.009              |
| BMI                             | 764.000        | 2249.000   | -1.526 | 0.127              |
| ALT                             | 561.500        | 1939.50    | -2.849 | 0.004              |
| Diabetes                        | 674.000        | 2159.500   | -2.737 | 0.006              |
| Metabolic syndrome              | 813.500        | 2298.500   | -1.457 | 0.145              |
| SOCS3 expression                | 608.000        | 2093.000   | -2.831 | 0.005              |
| IL28B expression                | 598.000        | 1579.000   | -0.312 | 0.755              |
| SNP SOCS3                       | 781.000        | 2266.000   | -1.531 | 0.126              |
| SNP IL28B                       | 631.500        | 2062.000   | -2.808 | 0.005              |
| RVR                             | 519.000        | 1015.000   | -2.609 | 0.009              |

BMI, body mass index; ALT, alanine aminotransferase; RVR, rapid virological response.
gene, thereby providing a regulatory link between the two gene pathways (Figure 4).

In this scenario, we evaluated the relative expression of the two genes in HCV patients in relation to viral genotypes and response to antiviral treatment. We found a direct correlation between SOCS3 and IL28B expression in control subjects and an inverse correlation in HCV patients (Figure 3A) particularly in NR HCV-1 patients (Figure 3B). Thus, it is conceivable that an interaction between SOCS3 and IL28B results in inhibition of IL28B by SOCS3. More interestingly, the SOCS3-induced reduction of IL28B expression may reduce the IL28B-mediated immune response to HCV. This is in line with our previous observation of higher SOCS3 expression in NR HCV-1 patients.30 These findings suggest that SOCS3 and

Table 4. Multivariate analysis vs non-responders in hepatitis C virus patients.

| Variable | Sig.   | OR    | 95.0% CI | Inf | Sup |
|----------|--------|-------|----------|-----|-----|
| RVR      | 0.015  | 0.258 | 0.086    | 0.771 |
| Genotype | 0.207  | 1.745 | 0.877    | 4.954 |
| Viral load| 0.120  | 1.755 | 0.658    | 4.339 |
| Steatosis| 0.205  | 1.022 | 0.988    | 1.058 |
| ALT      | 0.158  | 1.005 | 0.998    | 1.011 |
| Diabetes | 0.232  | 1.788 | 0.689    | 4.639 |
| SOCS3    | 0.136  | 1.850 | 0.825    | 4.150 |
| SNP IL28B| 0.327  | 1.535 | 0.652    | 3.614 |

CI, confidence interval; OR, odds ratio; RVR, rapid virological response; ALT, alanine aminotransferase.

Figure 4. The IL28B gene produces the IL28B protein, also known as interferon (IFN)-λ, that binds its receptor. The IL28 receptor consists of two subunits (IL28r-α and IL10r-β) similar to IFNAR, the interferon α and β receptor. The two receptors activate the same post-receptor cascade triggered by the Jak-STAT interaction that leads to the assembly of the STAT1-STAT2-IRF9 complex. This complex goes to the nucleus where it activates the interferon stimulated genes (ISG), a complex of genes that produce a variety of proteins known as interferon stimulated responding elements (ISRE). The ISRE are the effectors of the antiviral response to endogenous and exogenous IFNs. SOCS3 is an ISG that produces the type 3 suppressor of cytokine signaling that down-regulates a variety of cytokines by interfering with their post-receptor intracellular signal transduction. In particular, SOCS3 interferes with the Jak-STAT mechanism thereby leading to down-regulation of IL28B and IFN-α and -β antiviral effects. The core protein of genotype 1 HCV induces over-expression of the SOCS3 gene and protein, resulting in non-response to antiviral therapy.
**IL28B** expression and their relative SNP alleles are important factors of response to antiviral treatment.

To verify this hypothesis in the clinical setting, we evaluated the clinical factors known to affect antiviral treatment in HCV patients together with **SOCS3** and **IL28B** SNPs and expression. Univariate analysis revealed that **SOCS3** expression and **IL28B**-SNP, RVR, HCV genotypes, ALT, steatosis grade and diabetes affected SVR rates. The only factor that was independently related to SVR at multivariate analysis was RVR.

We next asked what factors were related to an RVR. The results of the multivariate analysis show that allele C of **IL28B**-SNP predicts an RVR, and indeed there is an ongoing debate about whether RVR or **IL28B**-SNP is more predictive of an SVR. Viewed from another angle, we could say that genotype TT of **IL28B**-SNP (or lack of allele C) predicts the absence of an RVR. In this sense, one could say that **IL28B** genotyping pre-treatment could serve to identify easy-to-treat and difficult-to-treat patient whose treatment protocols could be tailored without waiting for an RVR, which is unlikely to occur.

**Conclusions**

This study is the first to report the prevalence of **IL28B** alleles in a population of Southern Italy. The **IL28B** genic expression was higher in genotype HCV-2 patients, which could explain the better response to antiviral therapy in genotype 2 compared with genotype 1 patients. In non-responder patients we identified an interaction between **SOCS3** and **IL28B**, which indicates an interplay between these two cytokines that could regulate the intracellular JAK-STAT pathway, and thereby negatively affect the response to therapy. Based on our data, the routine assessment of **IL28B** may be useful to discriminate easy from difficult-to-treat patients. RVR remains the only independent predictive factor of an SVR.

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