‘Favourable’ *IL28B* polymorphisms are associated with a marked increase in baseline viral load in hepatitis C virus subtype 3a infection and do not predict a sustained virological response after 24 weeks of therapy

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**INTRODUCTION**

Recently, several large genome-wide association studies (Ge et al., 2009; Rauch et al., 2010) and candidate gene association studies (Labie & Gilgenkrantz, 2010; Mangia et al., 2010; McCarthy et al., 2010; Matsunaga et al., 2010; Mosbruger et al., 2010; Rallón et al., 2010; Thomas et al., 2009) have shown that single-nucleotide polymorphisms (SNPs) linked to the *IL28B* gene, which codes for the type III cytokine lambda interferon (IFN-λ), play a key role in determining the clinical outcome of hepatitis C virus (HCV) infection. These studies have shown that *IL28B*-linked genetic variation is associated with the outcome of primary infection (Rauch et al., 2010; Thomas et al., 2009) and the treatment of chronic infection with pegylated IFN-α (PEG-IFN-α) and ribavirin (Ge et al., 2009; Rauch et al., 2010; Suppiah et al., 2009; Tanaka et al., 2009), suggesting a common mechanistic pathway.

Several SNPs linked to *IL28B* and associated with clinical outcome have been identified to date, although as yet a specific causal variant has not been identified (Rauch et al., 2010). The two SNPs that have been evaluated most thoroughly are rs12979860 and rs8099917, which are in tight linkage (Hayes et al., 2011; Smith et al., 2011).

Whilst the association of *IL28B* genetic variants with clinical outcome appears robust for HCV genotype 1 infection and more recently genotype 4 infection (De Nicola et al., 2012), the data evaluating genotypes 2 and 3 are currently unclear. Although a small number of studies have assessed this, their interpretation is complicated by the fact that genotypes 2 and 3 have been grouped into single analyses (Lindh et al., 2011; Mangia et al., 2010; Rauch et al., 2010), with the large majority of treated patients infected with HCV genotype 2 (Mangia et al., 2010).
2010), or by use of variable treatment regimes (Mangia et al., 2010; Scherzer et al., 2011). Overall results from these studies are conflicting, showing no association with a sustained virological response (SVR) (defined as viral load undetectable 6 months after the end of treatment; Rauch et al., 2010), an association with an SVR particularly in those patients who did not have a rapid virological response (RVR) (defined as viral load undetectable 4 weeks after the start of treatment; Mangia et al., 2010) or an association with an RVR but not an SVR (Scherzer et al., 2011). The only large study to date that has evaluated IL28B SNPs in subtype 3a patients alone was performed in a Scandinavian patient population. However, in this study, the majority of patients received 14 weeks of therapy, significantly less than the 24 weeks that represents the current standard of care. This study showed that the IL28B polymorphisms were associated with an RVR but not with an SVR, and that, in subgroup analysis, IL28B polymorphisms may be used to predict relapse in RVR patients (Moghaddam et al., 2011).

The distribution of IL28B alleles worldwide is not uniform. The favourable IL28B alleles dominate in parts of Asia, whilst a more even distribution of alleles is found in the west (Ge et al., 2009; Thomas et al., 2009). This observation is thought to explain the racial differences observed in treatment outcome (Ge et al., 2009; Thomas et al., 2009). Within the UK, HCV subtype 3a infection is the dominant infecting viral subtype Reference (Health Protection Agency: Annual Report 2009) and, whilst overall RVR and SVR rates are higher than those observed in genotype 1 infection, viral relapse following treatment occurs in 30–40% of treated patients (Fried et al., 2002; Mangia et al., 2005; Manns et al., 2001). As such, clarifying the association between IL28B polymorphisms and treatment outcome of subtype 3a-infected patients within the UK is an important goal.

In this study, we assessed IL28B genotype using SNP rs80999917 in a large cohort of patients infected with subtype 3a infection recruited from two centres in the UK in association with baseline viral load, biological response 4 weeks into the treatment and treatment outcome following PEG-IFN-α and ribavirin therapy. We also addressed the hypothesis that a favourable IL28B genotype is associated with viral clearance following 24 weeks of therapy with PEG-IFN-α and ribavirin.

RESULTS

Patient characteristics: age <40 and an RVR is associated with an SVR to therapy

The majority of patients were Caucasian (n=174), whilst the remainder were of Asian (n=26) or African (n=1) origin. There were no statistically significant differences in the allelic distribution between Caucasian and Asian patients in the cohort, and the ethnic distribution was similar in the Oxford and Nottingham cohorts (Oxford cohort: 84.8% Caucasian, 14.4% Asian and 0.8% African; Nottingham cohort: 88.7% Caucasian, 11.3% Asian and 0% African). Baseline patient characteristics and treatment outcome were assessed (Table 1). In our cohort, 66% of the patients were male. The median age was 46 (range 23–69 years). The proportion of patients infected through intravenous drug use was 63% in the Oxford cohort and 58.4% in the Nottingham cohort. The overall SVR rate in the HCV subtype 3a cohort was 68.4% in keeping with previously published studies (Fried et al., 2002; Manns et al., 2001). A non-SVR was observed in 31.6% of patients; of these, 6.9% were non-responders and 24.7% had a virological relapse after treatment. Pre-treatment liver biopsy was performed in 62% of the cohort; of these 13% had liver cirrhosis (Ishak stage 5 or 6).

Of the baseline characteristics examined, only age (<40 years) significantly correlated with an SVR (P=0.0008).

IL28B genetic status is not associated with an SVR

Host genotype was assessed, and patients were stratified into those with the TT allele (associated with an increase in the SVR in genotype 1 infection), and those with the GG or GT allele (associated with a non-SVR in genotype 1 infection). Overall, there was no statistically significant association between IL28B status and a SVR (Table 2): the SVR rate in those with the TT allele was 71%, whilst in those with the GG or GT alleles the SVR rate was 63%. The trend test P value for IL28B genotype in association with an SVR/non-SVR was non-significant at P=0.1419 [per allele; odds ratio (OR) 1.556].

Stratifying by ethnicity, we noted no differences in the rate of SVR among TT or GT allele carriers in the Caucasian or Asian population groups (P=0.8409 and P=0.1920, respectively).

IL28B genetic status is associated with an RVR but not an SVR to therapy

Next, we examined the relationship between IL28B genetic status and an RVR (Table 3) through the assessment of HCV viral load data 4 weeks into therapy in 109 patients. The majority of patients (65%) had an RVR, with viral loads undetectable at the 4-week time point. When stratified according to IL28B genetic status, an RVR was highly statistically significantly associated with IL28B genetic status: 79% of patients with the TT allele had an RVR, compared with only 41% of those with the GG or GT allele (P=0.0001). The trend test P value for IL28B genotype in association with an RVR/non-RVR was 0.0001 (per allele; OR 4.03).

In 122 patients, a 4-week viral load was assessed. In these, final treatment outcome data were available in 96 patients. As previously described, an RVR was significantly associated with an SVR to therapy (Table 4): of the 66 patients with an RVR, 89% achieved an SVR. In contrast, in the 30 patients without an RVR (non-RVR), an SVR was observed...
In only 36% of patients \((P=0.0001)\). In a multivariate analysis that included RVR data and patient baseline characteristics (Table 1), only the RVR remained significantly associated with an SVR, with an OR of 14.

**IL28B genetic status and association with an SVR in RVR/non-RVR patients**

As expected, a minority of patients had a non-RVR status (Mangia et al., 2005). In these patients, there was no statistically significant association of IL28B genetic status with an SVR. Of 19 non-RVR patients that carried the unfavourable GT or GG allele, 12 had a non-SVR, whilst seven had an SVR. Similarly, of 11 non-RVR patients with the favourable allele (TT), seven had a non-SVR and four had an SVR (Table 5).

In the RVR patients, the rate of non-SVR in patients carrying the favourable allele was 7/48, compared with 0/18 in patients carrying the unfavourable alleles; this difference was not statistically significant (Table 5).

**IL28B genetic status in association with baseline viral load**

In 134 patients, HCV viral load was assessed in association with IL28B genetic status. In patients with the TT allele \((n=87)\), the median pre-treatment viral load was significantly higher than that seen in patients with the GT or GG allele \((n=47)\) [median viral load for the TT allele, 925,961 IU ml\(^{-1}\) (range 2200–21,116,965 IU ml\(^{-1}\)], and for the GT or GG allele, 260,284 IU ml\(^{-1}\) (range 740–7,560,000 IU ml\(^{-1}\)]; \(P=0.001\) (Fig. 1).

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**Table 1. Patient baseline characteristics in association with treatment outcome**

UK, unknown; NR, non-responder; REL, relapse.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total no. patients ((n=158, 100%))</th>
<th>SVR ((n=108, 68.4%))</th>
<th>Non-SVR ((NR+REL)) ((n=50, 31.6%))</th>
<th>NR ((n=11, 6.9%))</th>
<th>REL ((n=39, 24.7%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>35 (22)</td>
<td>32 (91)</td>
<td>3 (9)</td>
<td>0 (0)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>123 (78)</td>
<td>76 (61)</td>
<td>47 (38)</td>
<td>11 (23)</td>
<td>36 (76)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105 (66)</td>
<td>69 (66)</td>
<td>36 (34)</td>
<td>10 (27)</td>
<td>26 (73)</td>
</tr>
<tr>
<td>Female</td>
<td>53 (33)</td>
<td>39 (73)</td>
<td>14 (27)</td>
<td>1 (7)</td>
<td>13 (93)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>51 (32)</td>
<td>43 (84)</td>
<td>8 (15)</td>
<td>1 (12)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>25–30</td>
<td>44 (28)</td>
<td>34 (77)</td>
<td>10 (23)</td>
<td>3 (30)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>15 (9)</td>
<td>10 (66)</td>
<td>5 (33)</td>
<td>2 (40)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>UK ((n=48))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline viral load</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;400,000 IU ml(^{-1})</td>
<td>37 (23)</td>
<td>28 (75)</td>
<td>9 (25)</td>
<td>2 (23)</td>
<td>7 (77)</td>
</tr>
<tr>
<td>&gt;400,000 IU ml(^{-1})</td>
<td>60 (38)</td>
<td>44 (73)</td>
<td>16 (27)</td>
<td>6 (38)</td>
<td>10 (62)</td>
</tr>
<tr>
<td>UK ((n=62))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (Ishak score 0–4)</td>
<td>78 (49)</td>
<td>46 (59)</td>
<td>32 (41)</td>
<td>8 (25)</td>
<td>22 (75)</td>
</tr>
<tr>
<td>Yes (Ishak score 5–6)</td>
<td>20 (13)</td>
<td>11 (55)</td>
<td>9 (45)</td>
<td>1 (16)</td>
<td>8 (84)</td>
</tr>
<tr>
<td>UK ((n=60))</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*\(P=0.0008\) for SVR versus non-SVR.

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**Table 2. IL28B status in association with treatment outcome**

<table>
<thead>
<tr>
<th>IL28B SNP rs8099917</th>
<th>Total no. patients ((n=158, 100%))</th>
<th>SVR ((n=108, 68.4%))</th>
<th>Non-SVR ((NR+REL)) ((n=50, 31.6%))</th>
<th>NR ((n=11, 6.9%))</th>
<th>REL ((n=39, 24.7%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>101 (64)</td>
<td>72 (71)</td>
<td>29 (29)</td>
<td>5 (17)</td>
<td>24 (83)</td>
</tr>
<tr>
<td>GG or GT</td>
<td>57 (36)</td>
<td>36 (63)</td>
<td>21 (37)</td>
<td>6 (28)</td>
<td>15 (71)</td>
</tr>
<tr>
<td>GG</td>
<td>6 (4)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>0 (0)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>GT</td>
<td>51 (32)</td>
<td>33 (64)</td>
<td>18 (36)</td>
<td>6 (33)</td>
<td>12 (66)</td>
</tr>
</tbody>
</table>
IL28B genetic status and association with liver cirrhosis

Liver biopsy was performed in 128 patients. There was no statistically significant association of IL28B genetic status with liver fibrosis. Of 98 patients without cirrhosis, 67 (68%) carried the TT allele and 31 (32%) the GT or GG allele. Similarly, in the 30 cirrhotic patients, 24 (80%) carried the TT allele (Table 6).

DISCUSSION

Multiple large genome-wide association studies and candidate genetic studies have shown an association between host polymorphism linked to the IL28B gene and clinical outcome of HCV infection in both natural (Rauch et al., 2010; Thomas et al., 2009) and therapy-induced HCV clearance in genotype 1 infection (Ge et al., 2009; Rauch et al., 2010; Suppiah et al., 2009; Tanaka et al., 2009). As a result, IL28B genetic testing is now frequently used to guide management in patients with genotype 1 infection. However, the data in subtype 3a infections remain unclear; a small number of studies have addressed this but are most likely to eradicate infection and benefit from current treatment remains an important clinical issue that is particularly relevant in the UK.

Table 3. Association of RVR with IL28B genetic status and treatment outcome

<table>
<thead>
<tr>
<th>IL28B SNP rs8099917*</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. patients (n=122, 100 %)</td>
</tr>
<tr>
<td>TT</td>
<td>78 (64)</td>
</tr>
<tr>
<td>GG or GT</td>
<td>44 (36)</td>
</tr>
</tbody>
</table>

*P=0.0001 for RVR versus non-RVR.

Table 4. Association of RVR with SVR

<table>
<thead>
<tr>
<th>Four-week virological response*</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. patients (n=96, 100 %)</td>
</tr>
<tr>
<td>RVR</td>
<td>66 (68)</td>
</tr>
<tr>
<td>Non-RVR</td>
<td>30 (32)</td>
</tr>
</tbody>
</table>

*P=0.0001 for SVR versus non-SVR.
The TT and GT or GG alleles, respectively.

tv and the range is indicated by dots and squares for

IL28B is a clear predictor of an SVR, 

Fig. 1. IL28B status in association with baseline viral load. Pre-
treatment HCV viral load, shown in this plot in association with the
TT or the GT/GG allele, was significantly higher in patients with the
favourable allele (TT) than in patients carrying the unfavourable GT/
GG alleles [median viral load in IU ml \(^{-1}\) for the TT allele, 925 961
(range 2200–21 116 965) and for the GT or GG allele, 260 284
(range 740–7 560 000); \(P=0.001\)]. Horizontal bars represent the
median value, and the range is indicated by dots and squares for
the TT and GT or GG alleles, respectively.

Table 5. IL28B genetic status in association with SVR in non-
RVR and RVR patients

<table>
<thead>
<tr>
<th>IL28B SNP rs8099917</th>
<th>Non-SVR</th>
<th>SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-RVR patients (n=30)</td>
<td>TT</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>GG or GT</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>19/30</td>
</tr>
<tr>
<td>RVR patients (n=66)</td>
<td>TT</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>GG or GT</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7/66</td>
</tr>
</tbody>
</table>

strongest predictor (indeed the only predictor in multi-
variate analysis) of an SVR was an RVR. Approximately
two-thirds of subtype 3a patients had an RVR to treatment,
where this could be assessed, and 89% of these had an
SVR. In contrast, only 36% of patients who did not achieve
an RVR had an SVR. Both this study and that of
Moghaddam et al. (2011) showed that, although an RVR
is a clear predictor of an SVR, IL28B genetic status is
associated with an RVR but not an SVR in subtype 3a
infection. This observation is counter-intuitive but may
relate to the fact that the biological mechanism that clearly

Table 6. IL28B genetic status in association with liver cirrhosis

<table>
<thead>
<tr>
<th>IL28B SNP rs8099917</th>
<th>Non-cirrhotic ((n=98))</th>
<th>Cirrhotic ((n=30))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>67 (69%)</td>
<td>24 (80%)</td>
</tr>
<tr>
<td>GG or GT</td>
<td>31 (31%)</td>
<td>6 (20%)</td>
</tr>
</tbody>
</table>

links IL28B genetic status to early viral suppression, after 4
weeks of therapy, is distinct from the mechanism required
to completely eradicate the virus long term. However,
despite intense research efforts within the scientific
community, the genetic causal variant and the biological
role of IFN-\(\lambda\) that links IL28B polymorphisms to viral
control is unknown. Furthermore, little is known about the
mechanism by which PEG-IFN-\(\lambda\) plus ribavirin therapy
induces early and sustained viral clearance in subtype 3a
infections, or why viral relapse is common in patients with
subtype 3a infection after 24 weeks of therapy. Each of
these represents an important avenue of research that may
eventually identify discrete mechanisms involved in early
and sustained virus control.

We found no association of IL28B genetic status with liver
fibrosis at biopsy. However, a recent study that included
342 subtype 3a patients with liver biopsy data reported an
association of the favourable IL28B alleles with a more
rapid rate of liver fibrosis and liver inflammation (Bochud
et al., 2012). This finding was particularly pronounced for
subtype 3a infections. It was hypothesized that the unfavourable IL28B allele may be associated with an attenuated
adaptive response. There is currently no evidence to support
this hypothesis, but the authors argued that this could
account for both higher rates of viral clearance and also a
higher rate of disease progression with the favourable IL28B
alleles that occurs as the result of an intact antiviral response.
Proof of this hypothesis will require detailed functional
analysis of intrahepatic T-cell populations in association
with IL28B genetic status.

An additional finding was the association of the IL28B
genetic polymorphism that confers protection in genotype
1 infections (TT alleles) with a higher viral load at baseline
in subtype 3a infections. Similar findings have been
observed in genotype 1 infections. At first, this observation
appears counter-intuitive. However, the TT host genotype
is associated with spontaneous viral clearance following
primary infection and is known to protect patients from
persistent infection. Although speculative, it is plausible
that patients with the protective TT allele develop
persistent infection as a consequence of other deleterious
factors. These factors might include, for example, exposure
to viral strains with a high replicative capacity, a high HCV
burden at the time of primary infection or other hitherto-
unidentified host factors that predispose patients to per-
sistent infection. As such, those patients who develop
persistent HCV infection in spite of the protective TT allele
may be particularly prone to subsequent infection with a
high HCV viral load.
In conclusion, our findings suggest that, within the UK population, IL28B genetic polymorphisms are associated with the rapid clearance of virus from the peripheral blood during the first 4 weeks of treatment, but that the same polymorphisms are associated with a high baseline viral load and do not predict an SVR. Our data showed that virological relapse still commonly occurs in patients with an RVR and a favourable IL28B genetic make-up. These data suggest that, to predict virological relapse, alternative mechanisms and biomarkers should be explored in subtype 3a infections. These should include the detailed evaluation of viral genetic signatures, quasi-species make-up before treatment and after virological relapse, and host antiviral immunity in association with distinct clinical outcomes.

METHODS

Clinical cohort. In total, 201 patients from two well-characterized cohorts of patients with chronic HCV subtype 3a infection within the UK (from Oxford, n=112, and Nottingham, n=89) were recruited to the study and characterized for IL28B genetic status. Clinical data that form part of standard clinical care were collected prospectively between 2000 and 2011. Written informed consent and ethical approval were obtained for all patients for inclusion in the study, and blood sampling for genetic testing was performed. Patients who had not previously donated blood for research purposes were recalled for genetic testing when required, and their notes were reviewed to retrieve missing data where these had not been collected prospectively. Baseline viral load and liver fibrosis scores were not always available as these frequently do not form part of the clinical management of patients with subtype 3a infection.

Of the initial study cohort (201 patients), 158 patients who received a single course of PEG-IFN-α and ribavirin therapy were included in a final treatment outcome analysis (SVR vs non-SVR). Patients were included in the treatment outcome analysis if they received 24 weeks of treatment, or if they received a shorter duration of treatment and treatment was stopped due to a non-virological response. Patients were excluded from the final treatment outcome analysis if they received >24 weeks of treatment (n=13), the final treatment outcome was unknown (n=5), treatment was stopped early due to side effects (n=9) or patients received prior treatment with PEG-IFN-α/ribavirin (n=4) or amantadine (n=1). In seven patients, treatment was planned but did not commence; these patients could not be included in the treatment outcome analysis but were retained for assessment of HCV viral load and/or liver fibrosis in association with IL28B genetic status. Liver biopsy was performed in 128 patients before treatment. Liver fibrosis was graded according to the Ishak criteria (Ishak et al., 1995). Liver cirrhosis was graded as Ishak score 5–6. The mode of infection and ethnicity were also assessed. Patients known to be co-infected with hepatitis B virus or human immunodeficiency virus were excluded from the study.

Patient treatment. All patients were treatment naïve at baseline. Treatment in all patients consisted of standard doses of PEG-IFN-α2b (Pegasys, 180 µg weekly) or -α2b (ViraferonPeg, 1.5 µg kg⁻¹ week⁻¹) in combination with weight-based doses of ribavirin (800–1200 mg). Dose reduction was performed if clinically indicated. RVR was defined as HCV RNA undetectable by reverse transcription-PCR (RT-PCR) 4 weeks into treatment. SVR was defined as HCV RNA negativity 24 weeks after the end of treatment. Non-response was defined as detectable viral RNA by RT-PCR 12 weeks into treatment.

Clinical samples (viral load and HCV genotyping). HCV viral load was determined at baseline and 4 weeks into treatment in a subset of patients (n=134 and n=122, respectively). All viral load and genotype testing was performed in clinically accredited laboratories using commercially available assays and reported in standardized units (IU ml⁻¹).

IL28B SNP analysis: rs8099917. The IL28B SNP rs8099917 was genotyped using a commercially validated TaqMan SNP genotyping assay (assay ID_11710096_10; Applied Biosystems). DNA was extracted from whole blood (Gentra Puregene Blood kit; Qiagen). The DNA (1–20 ng ml⁻¹) was used for PCR in an LC480 PCR thermocycler (Roche). Each sample was run in triplicate using internal positive- and negative-control samples. The rs8099917 SNP typing was confirmed by direct hemi-nested PCR amplification using Roche HiFi Expand enzyme (Roche Diagnostics), and Sanger sequencing (ABI) with the following primers: 917_Ex_F, 5ʹ-CATACACATGGGAGTTAAAGTAAAGC-3ʹ, 917_Ex_R, 5ʹ-GCTGGCCCCAGGACCTTGCACTAG-3ʹ, and 917_In_R 5ʹ-CCTGGACCAACCACACTTCA-3ʹ. Amplification conditions for both first-round and nested amplifications were 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s.

Statistical analysis. The association of baseline characteristics with treatment outcome: IL28B genotype with SVR, RVR and ethnicity; and RVR with SVR was assessed using Fisher’s exact test (Graphpad Prism, version 5). In addition, the Cochran–Armitage test for trend was performed in the additive model assessing IL28B genotype in association with clinical outcome. The association of baseline viral load with IL28B genetic status was determined using a non-parametric Mann–Whitney test. Multivariate analysis of univariate variables was determined using logistic regression with a backward procedure using SPSS version 12.0 (SPSS Inc.). A value of p<0.05 was considered statistically significant.

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