IL28B polymorphism genotyping as predictor of rapid virologic response during interferon plus ribavirin treatment in hepatitis C virus genotype 1 patients

Chiara Rosso, Maria Lorena Abate, Alessia Ciancio, Silvia Strona, Gian Paolo Caviglia, Antonella Olivero, Giovanni Antonio Touscoz, Mario Rizzetto, Rinaldo Pellicano, Antonina Smedile

Abstract

AIM: To clarify the association of interleukin-28B (IL28B) single nucleotide polymorphisms (SNPs) with hepatitis C virus (HCV) viremia changes for assessment of interferon (IFN) response.

METHODS: A cohort of 118 Caucasian treatment-naïve HCV-G1 infected patients, treated with pegylated-IFN alpha 2a or 2b associated with ribavirin (53 responders, 65 non-responders) during the period 2010-2012, were genotyped for IL28B SNPs rs12979860 C>T and rs8099917 T>G. Genotyping was performed by real-time allelic discrimination assay. Serum HCV RNA levels were assayed at 2, 4, 12, 24 and 48 wk during therapy. Correlation between IL28B genotypes and serum HCV RNA kinetics was investigated. Multivariable logistic regression analysis was performed to identify predictors of null-response.

RESULTS: Twenty-six out of 118 patients (22%) had no HCV RNA decline ≥ 1 log IU/mL at therapy week 4 (null-responders). IL28B genotype was rs8099917 (G*)/rs1297860(**) in 21/26 (80%) of null-responder patients. Using multivariate analysis, it was shown that the presence of the rs8099917 G allele was the best predictor of null-response (OR = 7.9, 95%CI: 1.99-31.18). The presence of at least one favorable genotype showed a positive predictive value of above 90% for HCV RNA reduction ≥ log at week 4. Analysis of the HCV RNA kinetics during 12 wk of therapy in patients with IL28B rs12979860 heterozygosis (n = 73), according to their rs8099917 status, showed that the viremia reduction was significantly different in patients carrying the rs8099917 G allele compared to those with favorable homozygosis.

CONCLUSION: Our findings emphasize the association of the IL28B rs8099917 G allele with HCV. Genotyping for both IL28B SNPs is useful in clinical practice for thorough patient risk stratification based on IFN responsiveness.

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Key words: Hepatitis C virus-G1; Interleukin-28B rs12979860; Interleukin-28B rs8099917; Interferon sensitivity; Triple therapy

Core tip: This work provides more insights into the advantage of interleukin-28B rs12979860 and rs8099917 genotyping for therapy management in hepatitis C virus-G1 infected patients. The relevance of this ap-
proach is cost-effective at the time of decisions regarding triple therapy. We observed that in rs12979860 heterozygous patients, carriage of the rs8099917 G allele correlated with lack of viremia decrease early during treatment, when it is critical to assess for interferon sensitivity.


INTRODUCTION

For years, the standard of care for hepatitis C virus genotype 1 (HCV-G1) infected patients consisted of 48 wk of combination therapy with pegylated-interferon α and ribavirin (PEG-IFN/RBV)[11]. This treatment regimen underwent a major change after the introduction of direct antiviral agents (DAAs). DAAs directly inhibit specific steps in the HCV life cycle, for example, the two currently approved molecules, boceprevir and telaprevir, target NS3/4A serine protease (protease inhibitors, PIs)[8]. Triple therapy with PIs improves on-treatment kinetics and increases sustained virological response (SVR) rate with decreased duration of therapy in both naïve and treatment-experienced HCV-G1 patients[3-6], but raises concerns regarding the generation of resistant viral variants and significant side effects[3]. Furthermore, PIs are expensive and are not yet available in many countries[8].

With PEG-IFN/RBV therapy, pre-treatment patient features, such as viral genotype 1, older age, high baseline viral load and the degree of fibrosis, markedly affect the likelihood of attaining a SVR and assist physicians in patient management[8]. A major breakthrough in the study of baseline predictors of dual therapy response has been the finding of two single nucleotide polymorphisms (SNPs) rs12979860 and rs8099917, located near the interleukin-28B (IL28B) gene encoding for IFN-λ-3, which display a strong association with SVR, mainly in HCV-G1 infected patients[10-14]. Subjects with favorable IL28B genotypes (such as CC for rs12979860 or TT for rs8099917) have a twofold improvement in SVR rates compared to patients carrying the risk allele for both SNPs. These genetic variants also affect viral kinetics on-therapy and during spontaneous viral clearance[15,16]. On-therapy viremia changes are even more informative: achievement of rapid viral response (RVR), defined as undetectable serum HCV RNA at treatment week 4, is considered the strongest predictor of SVR, and the lack of early viral response, defined as undetectable serum HCV RNA at week 12, has been correlated with treatment failure[13].

Patients who fail PEG-IFN/RBV therapy (non-responders, NR) are distinguished as follows: partial-responder (par-R) if HCV RNA declines > 2 log IU/mL early on treatment but is still positive at week 12; null-responder (null-R) when HCV RNA reduction is less than 1 log at week 4 on treatment, and relapser (Rel) when HCV RNA is undetectable at end of therapy but becomes positive during follow-up period[17]. Data from randomized controlled trials for PIs in pre-treated HCV-G1 patients clearly show that the pattern of response to dual therapy strongly affects the probability of achieving SVR after triple therapy, with a progressive increase in SVR rates from NR (31%-37%) to par-R (52%-57%) and to Rel (75%-86%)[18-20].

Although scientific communities and governmental health care organizations recommend triple therapy in HCV-G1 patients[19-21], the use of PIs in clinical practice needs optimization, especially in distinguishing subjects who can still benefit from PEG-IFN/RBV therapy or those who need triple therapy or should wait for new, more potent drugs. In a recent multicentric study, including 1045 HCV-G1 treatment-naïve patients, it has been shown that a consistent subset of this cohort, identified by features such as IL28B genotype, could benefit from conventional dual therapy leading to a reduction in adverse effects and economic costs[22-23].

The aim of this study was to retrospectively investigate the association of IL28B SNPs with viremia changes at week 4 in a cohort of treatment-naïve HCV-G1 infected patients during combination therapy and to evaluate the advantage of typing for both SNPs for the identification of IFN-sensitive patients.

MATERIALS AND METHODS

One-hundred and eighteen patients (M/F 62/56, median age 49 years, interquartile range 16) with HCV-G1 infection undergoing antiviral therapy at the Gastroenterology and Hepatology Division of Molinette Hospital, Turin, Italy, during the period 2010-2012, were retrospectively included in this analysis. Inclusion criteria were: (1) diagnosis of chronic hepatitis C (CHC) G1; (2) serum HCV RNA positive; (3) Caucasian ethnicity; (4) no co-infection with hepatitis B virus, hepatitis delta virus and human immunodeficiency virus; and (5) naïve to HCV treatment. Patients were treated with standard doses of PEG-IFN-α-2a or -2b associated with RBV. Ribavirin dosage was based on weight: patients less than 75 kg received 800 mg and those more than 75 kg received 1000-1200 mg. Therapy duration was 48 wk or less according to specific protocol. antiviral therapy was ended earlier in patients with severe side effects[24]. The stage of liver fibrosis was described according to the METAVIR score. Significant fibrosis was defined as F3-F4. The AST-to-platelet ratio index (APRI) was calculated according to the formula proposed by Wai et al[25]. The research was approved by the institutional ethics committee and was performed according to the 1979 Declaration of Helsinki. All patients
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Table 1. Demographic and clinical features of 118 hepatitis C virus genotype 1 infected patients n (%)  

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>49 (16)</td>
</tr>
<tr>
<td>Male gender</td>
<td>52.5%</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5 (3.6)</td>
</tr>
<tr>
<td>AST, IU/mL</td>
<td>82 (71)</td>
</tr>
<tr>
<td>ALT, IU/mL</td>
<td>55 (48)</td>
</tr>
<tr>
<td>gGT, IU/mL</td>
<td>46 (57)</td>
</tr>
<tr>
<td>Platelets, 10³/L</td>
<td>182 (90)</td>
</tr>
<tr>
<td>APRI index</td>
<td>0.4 (0.6)</td>
</tr>
<tr>
<td>Basal HCV RNA, log IU/mL</td>
<td>6 (1.1)</td>
</tr>
<tr>
<td>Fibrosis (stiffness)</td>
<td>6.7 (19)</td>
</tr>
<tr>
<td>Fibrosis Metavir</td>
<td></td>
</tr>
<tr>
<td>F0-F1-F2</td>
<td>20 (25)</td>
</tr>
<tr>
<td>F3-F4</td>
<td>59 (75)</td>
</tr>
<tr>
<td>IL28B SNP rs8099917</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>59 (50)</td>
</tr>
<tr>
<td>TG</td>
<td>56 (47)</td>
</tr>
<tr>
<td>GG</td>
<td>3 (3)</td>
</tr>
<tr>
<td>IL28B SNP rs12979860</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>30 (25)</td>
</tr>
<tr>
<td>CT</td>
<td>73 (62)</td>
</tr>
<tr>
<td>TT</td>
<td>15 (13)</td>
</tr>
<tr>
<td>HCV RNA decline &lt; 1 log: IU/mL w4</td>
<td></td>
</tr>
<tr>
<td>rs8099917_G*/rs12979860_**</td>
<td>26 (22)</td>
</tr>
<tr>
<td>rs8099917_TT/rs12979860_T*</td>
<td>21 (81)</td>
</tr>
<tr>
<td>rs8099917_TT/rs12979860_CC</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Therapy outcome</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>53 (55)</td>
</tr>
<tr>
<td>NR</td>
<td>65 (45)</td>
</tr>
</tbody>
</table>

Data are reported as median (interquartile range) and frequencies (%). IL: Interleukin; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; gGT: Gamma-glutamyl transpeptidase; IL28B: Interleukin-28B; SNP: Single nucleotide polymorphism; w4: Treatment week 4; R: Responder; NR: Non-responder.

Table 1. Demographic and clinical features of 118 hepatitis C virus genotype 1 infected patients n (%)  

Signed an informed consent for genetic testing.

Serum HCV RNA levels were assayed at 2, 4, 12, 24 and 48 wk during therapy, using real-time polymerase chain reaction (PCR) assay with a limit of detection of 15 IU/mL (CAP/CTM HCV 2.0, Roche Molecular Diagnostics, Pleasanton, CA). Patients were monitored for at least 6 months after the end of treatment in order to assess therapy outcome. Genomic DNA was isolated from 350 μL of blood sample using the EZ1 DNA Blood kit (Qiagen GmbH, Hilden, Germany). Genotyping for IL28B SNP rs12979860 and rs8099917 was performed by real-time allelic discrimination assay (TaqMan SNP Genotyping Assay, Applied Biosystems, Foster City, CA) using TaqMan SNP Genotyping Master Mix (Applied Biosystems) on a CFX96 Real-time PCR instrument (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis

Continuous variables are summarized as median ± interquartile range, and categorical variables as frequency and percentage. Comparisons between groups were performed using a Mann-Whitney U-test for non-normal continuous variables. For categorical data, the Fisher exact test was used. P < 0.05 (two-sided) was considered statistically significant. Stepwise logistic regression analysis was performed to identify predictors of null-R. For the regression model, the IL28B SNPs were evaluated according to the presence of the risk allele rs favorable homozygosis. All calculations were performed using SPSS software version 20.0 (IBM SPSS Statistics for Windows, Chicago, IL).

RESULTS

A total of 118 Caucasian HCV-G1 infected patients were included in the study. Demographics and clinical characteristics of the patient cohorts are reported in Table 1. IL28B typing showed that 59 of them (50%) carried genotype TG/GG at rs8099917 while 88 (75%) had genotype CT/TT at rs12979860.

Twenty-six patients out of 118 (22%) did not achieve an HCV RNA drop ≥ 1 log at week 4 (null-R) and 21/26 (81%) carried the rs8099917 G allele. All of them were NR to therapy at week 12. Univariate analysis revealed that baseline factors which were significantly associated with null-R were higher levels of AST, ALT, gGT (P = 0.032, 0.002, 0.001 respectively), lower platelet levels (P = 0.019), higher APRI index (P = 0.033) and the carriage of the rs8099917 G allele (P < 0.001). After logistic regression analysis, rs8099917 G allele carriage was determined to be the best predictor of null-R (OR = 7.9, 95%CI: 1.99-31.18, Table 2).

The predictive value analysis of single and combined IL28B SNPs for achieving HCV RNA drop ≥ 1 log after 4 wk of treatment shows that the presence of at least one favorable genotype yields a positive predictive value (PPV) above 90%, with the combination of both SNPs showing a mild improvement (likelihood ratio = 3.82), Table 3.

We analyzed HCV RNA kinetics during 12 wk of therapy in patients with IL28B rs12979860 CT heterozygosis (n = 73), according to their rs8099917 status. The reduction in viral load during treatment was significantly different in patients carrying the rs8099917 G allele compared to those with favorable homozygosis (P < 0.01). The decrease in serum HCV RNA levels at week 2, 4 and 12 was 2.1, 3.5 and 6.2 log10 in patients with the rs8099917 TT compared to 0.9, 1.55 and 3.7 log10 respectively, in those with the unfavorable TG/GG genotypes (P < 0.001), Figure 1.

DISCUSSION

The major implication for recommending triple rather than dual therapy for HCV-G1 patients is that higher numbers of patients may achieve a RVR and most likely respond to treatment. Considering the enthusiasm in the run to embrace new therapies, it should not be forgotten that an appreciable number (about 20%) of HCV-G1 patients (mainly those experiencing RVR) are able to eliminate the virus with conventional dual therapies[22]. The treatment risk/benefit ratio is mainly bound to the...
The SVR rates tended to be higher in patients with CC inhibitory nucleoside (in this case, telaprevir and boceprevir) and deleobuvir (NS5B polymerase inhibitor), which may be a result of the combination of two SNPs, rs12979860 and rs8099917, located 8 and 3 kb upstream of the IL28B gene, respectively. The strongest association with SVR to dual therapy, especially in HCV-G1 infected patients, was shown with the TT genotype of rs8099917 and the CT and TT genotypes of rs12979860. In a French multicentric cohort, treatment week 4 null-R was rs8099917 (G/*), and 21 of 26 (80%) patients showed this genotype, including 13 of 15 (87%) patients with a null genotype for both SNPs. Our data show that IL28B rs8099917 G allele carriage is the most significant baseline feature associated with lack of IFN sensitivity at week 4, in treatment-naïve HCV-G1 infected patients treated with PEG-IFN/RBV, independently of rs12979860T allele carriage. Evaluation of the PPV for the HCV RNA decrease ≥ 1 log at treatment week 4 according to each single SNP shows no difference between them, with a mild likelihood ratio of 1.99-3.18 for rs12979860 and 0.98-0.99 for rs8099917, but in our cohort, all null-R become NR at week 12 and may be considered as interferon insensitive.

The IL28B locus (coding for IFN-γ3) is pivotal to the pathogenesis of HCV chronic infection[15-17]. Two SNPs, rs8099917 T/G and rs12979860 C/T, located 8 and 3 kb upstream of the IL28B gene, respectively, showed the strongest association with SVR to dual therapy, especially in HCV-G1 infected patients. Both SNPs are in linkage disequilibrium but allele frequencies may be quite different among different ethnic groups. In addition, it has been reported that IL28B genetic variants affect HCV RNA decline early on therapy[15-17].

The mechanism by which the IL28B genetic variants influence the efficacy of dual treatment remains unknown and the need to genotype for both SNPs in the clinical setting, prior to therapy, remains questionable. In the majority of studies, only one SNP, either rs12979860 or rs8099917, was assayed according to prevailing ethnic background, thus explaining why there is higher spontaneous or therapy-induced clearance of HCV infection, and why incidence and severity of side effects, which are significantly increased with triple therapy and occur mostly in the main candidates for treatment, i.e., patients with advanced fibrosis or cirrhosis[30]. In a multicentric French trial, including about 500 cirrhotic treatment-experienced patients treated with triple therapy (CUPIC cohort), 40% showed serious adverse events, with high rates of discontinuation, and there were 1.7% and 0.5% cases of mortality in the groups treated with telaprevir and boceprevir, respectively[27]. Moreover, both PIs are metabolized through cytochromes P450 3A4 and 3A5. With regard to this, the risk of drug-drug interactions is a concern in clinical practice[28,29].

In the era of DAAs, IL28B testing offers the minimal additional information that could influence the clinician’s decision[27]. Nevertheless, triple therapy is not recommended in all patients and the evaluation of pre-treatment factors such as the IL28B SNPs still holds significance to establish the therapeutic schedule. Moreover, in a recent review, Matsuraya et al[30] reported that IL28B polymorphisms may affect viral kinetics even in the context of IFN-free regimens. In a phase 2, randomized, open-label trial of faldaprevir (NS3/4A protease inhibitor) and deleobuvir (NS5B polymerase inhibitor), the SVR rates tended to be higher in patients with CC at rs12979860 than in those with non-CC. This suggests that innate immunity may still be important and confirms the importance of IL28B genotyping in the context of new and future therapy regimens[34].

Our data show that IL28B rs8099917 G allele carriage is the most significant baseline feature associated with lack of IFN sensitivity at week 4, in treatment-naïve HCV-G1 infected patients treated with PEG-IFN/RBV, independently of rs12979860T allele carriage. Evaluation of the PPV for the HCV RNA decrease ≥ 1 log at treatment week 4 according to each single SNP shows no difference between them, with a mild likelihood ratio of 1.99-3.18 for rs12979860 and 0.98-0.99 for rs8099917, but in our cohort, all null-R become NR at week 12 and may be considered as interferon insensitive.

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genotyping for this SNP is informative enough. Moreover, in the European cohort of 910 G1 CHC patients analyzed by Suppiah et al[31], IL28B rs8099917 TG/GG variants were associated with lack of treatment-induced clearance. Halfon et al[32] reported that analysis of a single IL28B SNP was sufficient to predict treatment failure in patients with HCV. Furthermore, Lazarevic et al[33] comparing 3 IL28B SNPs, observed that the rs12979860 CC genotype was a better predictor of therapy success in a cohort of 106 HCV patients.

Our study confirms that IL28B alleles impact on viral kinetics during treatment in patients with HCV-G1 infection. In order to investigate the usefulness of typing for both SNPs, we analyzed HCV RNA kinetics during 12 wk of therapy in the sub-group of patients with rs12979860 heterozygosis with respect to rs8099917 genotypes. We observed a significant difference for HCV RNA decline in the absence of rs8099917 G allele carriage. These observations highlight the association of the IL28B rs8099917 G allele with the lack of HCV RNA reduction > 1 log at treatment week 4 in HCV-G1 patients treated with PEG-IFN/RBV therapy. Thus, genotyping for both IL28B SNPs is advantageous in clinical practice for patient risk stratification at therapy week 4, a key time-point for assessment of interferon responsiveness before the addition of PIs. The results of this study provide a strong rationale for the use of IL28B single nucleotide polymorphism (SNPs) testing to personalize antiviral therapy.

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8 Gellad ZF, Reed SD, Muir AJ. Economic evaluation of direct-acting antiviral therapy in chronic hepatitis C. *Antivir Ther* 2012; 17: 1189-1199 [PMID: 23186646 DOI: 10.3851/IMP2430]


13 Yee HS, Chang MF, Pocha C, Lim J, Ross D, Morgan TR, Monto A. Update on the management and treatment of hepatitis C virus infection: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center Program and the National Hepatitis C Program Office. *Am J Gastroenterol* 2012; 107: 669-89; quiz 690 [PMID: 22525303 DOI: 10.1038/ajg.2012.48]


19 Hézode C, Fontaine H, Dorival C, Larrey D, Zoulim F, Canva V, de Led inghen V, Poyran T, Samuel D, Bourlière
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