IL28B SNP rs12979860 Is a Critical Predictor for On-Treatment and Sustained Virologic Response in Patients with Hepatitis C Virus Genotype-1 Infection

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Abstract

Background: Single nucleotide polymorphisms (SNPs) of interleukin-28B (IL28B) have received considerable interest for their association with sustained virological response (SVR) when treating patients of genotype-1 hepatitis C virus (GT1-HCV) chronic infection with pegylated interferon and ribavirin (PegIFN/RBV). This study was to investigate the predictive power of IL28B SNPs for on-treatment responses and SVR in treatment-naïve patients with GT1-HCV chronic infection.

Methodology/Principal Findings: We analyzed ten SNPs of IL28B in 191 treatment-naïve patients with GT1-HCV chronic infection who received PegIFN/RBV. In these patients, rapid virological response (RVR), early virological response (EVR) and end of treatment response (ETR), CC genotype of rs12979860 (10.52; 3.45–32.04; <0.001) was the predictor. Similarly, for end of treatment response (ETR), CC genotype of rs12979860 (15.42; 4.62–51.18; <0.001) was the only predictor. For patients with RVR, only low baseline viral load (3.90; 1.57–9.68; 0.003) could predict the SVR. For patients without RVR, only rs12979860 (4.60; 1.13–18.65; 0.033) was the predictor for SVR.

Conclusions/Significance: rs12979860 is the critical predictor for RVR, EVR, ETR and SVR in treatment-naïve patients of GT1-HCV chronic infection. Furthermore, this SNP is the only predictor for SVR in patients without RVR. These results have provided evidence that rs12979860 is the ideal IL28B SNP for genetic testing in treating patients of GT1-HCV chronic infection.

Introduction

A combination therapy of pegylated interferon-alpha and ribavirin (PegIFN/RBV) is a well-accepted standard of care for patients with chronic hepatitis C (CHC) [1]. Current strategy for CHC treatment is to individualize the treatment duration guided by genotype and on-treatment viral response [1,2]. With these recommended therapies, a sustained virological response (SVR) rate could reach 42–79% among HCV genotype 1 and 76–95% among HCV genotype 2/3 [2]. Interestingly, better response rates were found in Asian patients, with SVR rates around 61–79% for HCV genotype 1 patients [3,4,5,6] and 80–95% for HCV genotype 2/3 patients [7,8,9]. As for the response-guided approach, rapid virological response (RVR) is regarded as an important predictor for SVR [5,10,11]. It is also a guide for shortening treatment duration from 48 weeks to 24 weeks for HCV genotype 1 with low viral load [4,5,12,13] and from 24 weeks to 12–16 weeks for genotype 2/3 chronic infection [10,14,15]. In addition, early virological response (EVR) is an important parameter for the decision to terminate or continue treatment because patients without EVR could hardly achieve SVR [16]. On the other hand, undetectable virus at the end of treatment is coined as an end-of-treatment response (ETR). Though an ETR does not accurately predict whether an SVR will be achieved, it is necessary for SVR to occur [1].

Host genetic factors on the treatment efficacy for chronic hepatitis C were proposed a long time ago due to the ethnic differences in the treatment outcome. Recent genome-wide associated
studies have explored this issue and demonstrated strong evidence that single nucleotide polymorphisms (SNPs) of Interleukin-28B (IL28B) were significantly correlated with SVR when patients were treated with PegIFN/RBV [17,18,19,20,21]. Notably, the frequency of advantageous C allele of rs12979860 of IL28B was reported highest in Asians and lowest in African-Americans. In addition, the prevalence rates of cc genotype of rs12979860 paralleled with the SVR in each population [17]. Furthermore, these genotypes of IL28B also correlated with the spontaneous clearance of hepatitis C virus [18] and with viral responses during treatment [21]. Because of its significant impact on the treatment outcome, a genetic testing for the genotype of SNP of IL28B before deciding on treatment strategies has been proposed [22,23]. However, several other SNPs of IL28B were also found to be highly associated with SVR, like rs8099917 [20], rs12980275 [19], and others [17,18,19,20]. Nevertheless, which SNP would be the most influential on SVR was still undetermined.

We investigated these issues and tried to increase our understanding of the predictive ability of 10 SNPs of IL28B for RVR, EVR and SVR in a cohort of patients with GT1-HCV chronic infection treated with PegIFN/RBV from a large medical center.

Materials and Methods

Patients

We analyzed a prospective cohort of 213 consecutive adult Taiwanese treatment-naïve patients with chronic hepatitis C virus genotype 1 who visited HCV team of Department of Gastroenterology and Hepatology, Linkou Medical Center, Chang Gung Memorial Hospital, and received 24 weeks of combination therapy with PegIFN/RBV between February 2002 and December 2008, and who agreed to provide blood samples for the human genome study. Patients with decompensated liver disease, hepatoma, co-infection with hepatitis B virus or with human immunodeficiency virus, with apparent autoimmune hepatitis and alcoholic liver disease were excluded from this cohort. All patients included in the study had received liver biopsies that were evaluated by one pathologist using the Metavir scoring system. HCV genotype was determined by a genotype specific probe based assay in the 5’ untranslated region (LiPA; Innogenetics, Ghent, Belgium). In these 213 patients, we excluded 22 patients who did not fit the 80/80/80 adherence rule (less than 80% of total PEG-IFN or total RBV doses or less than 80% of the total duration of therapy), with a final case number of 191 (Fig. 1).

Definitions of response to treatment were undetectable serum HCV-RNA levels 24 weeks after cessation of treatment as SVR, undetectable serum HCV-RNA levels at 4 week after starting treatment as RVR, at least two-log10 reduction of viral load at week 12 after starting treatment as EVR, and undetectable virus at the end of treatment as ETR.

The HCV-RNA levels in this study were measured using a commercial quantitative polymerase chain reaction (PCR) assay VERSANT HCV RNA 3.0. Assay (HCV 3.0 bDNA assay, Bayer Diagnostics, Berkeley, Calif., lower limit of detection: 5.2 × 10^3 IU/ml) or COBAS TaqMan HCV Test (TaqMan HCV; Roche Molecular Systems Inc., Branchburg, N.J., lower limit of detection: 15 IU/ml). If non-detection of HCV-RNA by VERSANT HCV RNA 3.0. Assay, it would be further tested by COBAS® AMPLICOR HCV Test, v2.0 (CA V2.0, Roche Diagnostic Systems, lower limit of detection: 50 IU/ml).

Genomic DNA extraction and IL28B Genotyping

Anti-coagulated peripheral blood was obtained from HCV patients. Genomic DNA was isolated from EDTA anti-coagulated peripheral blood using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN) as previously described[24]. The oligonucleotide sequences flanking ten IL28B polymorphisms were designed as primers for Taqman allelic discrimination. The allele specific primers were labeled with a fluorescent dye (FAM and VIC) and used in the PCR reaction. Aliquots of the PCR product were genotyped with allele specific probe of SNPs using real time PCR (ABI). Ten SNPs of IL28B including rs12979860, rs11881222, rs4803219, rs8099917, rs12980275, rs8105790, rs7248668, rs10853728, rs8103142 and rs28416813 were chosen according to previous reports [17,18,19,20].

Figure 1. Flow diagram illustrating the selection of subjects for analysis and their treatment outcomes.

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**Ethics Statements**

All patients in this study had provided written informed consent. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committees of Chang Gung Memorial Hospital.

**Statistical analysis**

Linkage disequilibrium (LD) between marker loci was assessed and haplotype blocks were constructed using Haploview 4.1. Chi-square tests and Fisher’s exact probability tests were used as appropriate to compare the categorical variables of the groups. Continuous variables were expressed as means and standard deviations (SDs) and compared using Mann-Whitney U test or Student's t-test. Univariate and multivariate logistic regression analyses for predictors of sustained virological response were conducted using patients’ demographic, clinical variables and IL28B SNPs. The clinical variables included gender, age, viral load of HCV-RNA, Metavir fibrosis stage, body mass index (BMI), Glycohemoglobin (HbA1c), albumin, aspartate transaminase, alanine transaminase, bilirubin, gamma-glutamyl transpeptidase and alkaline phosphatase.

The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. Breslow-Day test and Cochran’s-Mantel-Haenszel test were used to estimate the homogeneity of odds ratios (ORs) between stratified groups. All P values less than 0.05 by the two-tailed test were considered statistically significant. Variables that achieved a statistical significance less than 0.10 in the univariate analysis were entered into multivariate logistic regression analysis to identify the significant independent predictive factors. All statistical analyses were performed with statistical software SPSS for Windows (version 16, SPSS. Inc., Chicago, IL, USA).

**Results**

**Table 1. Baseline characteristics of genotype I chronic hepatitis C patients treated with PegIFN/RBV and sustained virological response.**

| Variable                  | Total (N = 191) | Non-SVR (N = 60) | SVR (N = 131) | P value |
|---------------------------|-----------------|------------------|---------------|---------|
| Sex, n (%)                |                 |                  |               |         |
| male                      | 123 (64.4)      | 26 (43.3)        | 97 (74.4)     | 0.131   |
| female                    | 68 (35.6)       | 34 (56.7)        | 64 (49.6)     |         |
| Baseline Viral Load (mean ± SD) | ≤0.4 × 10^5 IU/ml | 91 (47.6)       | 16 (26.7)     | 0.001   |
| Fibrosis Stage, n (%)     |                 |                  |               | 0.021   |
| F0-2                      | 103 (53.9)      | 25 (41.7)        | 78 (59.5)     |         |
| F3-4                      | 88 (46.1)       | 35 (58.3)        | 53 (40.5)     |         |
| Age, y (mean ± SD)        |                 |                  |               | 0.063   |
| BMI, kg/m² (mean ± SD)    | 24.5 ± 3.3      | 25.2 ± 3.1       | 24.2 ± 3.4    | 0.049   |
| HbA1c, % (mean ± SD)      | 5.6 ± 1.0       | 5.7 ± 1.1        | 5.6 ± 1.0     | 0.443   |
| ALB, g/dl (mean ± SD)     | 4.5 ± 0.4       | 4.4 ± 0.4        | 4.5 ± 0.3     | 0.507   |
| AST, IU/L (mean ± SD)     | 92.0 ± 51.6     | 89.6 ± 20.0      | 93.1 ± 51.5   | 0.532   |
| ALT, IU/L (mean ± SD)     | 149.1 ± 107.2   | 129.4 ± 1.2      | 158.2 ± 116.3 | 0.068   |
| GGT, IU/L (mean ± SD)     | 42.9 ± 48.5     | 51.9 ± 60.0      | 38.8 ± 41.9   | 0.034   |
| Bilirubin, mg/dl (mean ± SD) | 0.9 ± 0.3    | 1.0 ± 0.3        | 0.9 ± 0.3     | 0.213   |
| RVR, n (%)                | 133 (69.6)      | 28 (46.7)        | 105 (80.2)    | <0.001  |
| EVR, n (%)                | 183 (95.8)      | 54 (90.0)        | 129 (98.5)    | 0.007   |

**Table 1**. Baseline characteristics of genotype I chronic hepatitis C patients treated with PegIFN/RBV and sustained virological response.

**rs12979860 CC genotype is the most powerful predictor among SNPs of IL28B for RVR, EVR, ETR and SVR in chronic GT1 HCV infected patients treated with PegIFN/RBV**

Demographic characteristics of 191 CHC genotype-1 (GT1) patients enrolled in this prospective cohort were shown in Table 1. Among these patients, 133 (69.63%) achieved RVR, 183 (95.81%) achieved EVR and 131 (68.39%) achieved SVR (Figure 1). From the result of statistic analysis, the factors favoring SVR were low baseline viral load (HCV-RNA <0.4 × 10^5 IU/mL), less fibrosis stage (Metavir fibrosis score F0–F2), low body mass index (BMI), lower gamma-glutamyl transferase (GGT), RVR and EVR (Table 1). Ten SNPs of IL28B genetic variations were genotyped in each patient. Two loci, rs1103142 and rs28416813, were excluded from this analysis due to significant deviations from Hardy-Weinberg equilibrium in genotype and allele distributions. In addition, one haplotype block consisting of 3 SNPs, rs11881222, rs403219 and rs12979860, was identified due to strong evidence of linkage disequilibrium (Figure 2) and rs12979860 was chosen to represent this haplotype. In all the six SNPs under analysis, five SNPs were significantly associated with SVR except rs10853728 (Figure 3).

We then examined the predictive ability of each SNP and other clinical baseline parameters for SVR by logistic regression (Table 2). Interestingly, only the rs12972860 CC genotype, but not other SNPs, together with younger age and low baseline viral load (<0.4 × 10^5 IU/mL) became the significant predictors for SVR by the multivariate analysis (Table 2). However, TT genotype of rs8099917 was also known as another important predictor for SVR [19,20,25,26,27,28,29]. We reasoned that the impact of rs12979860 was too strong to mask the effect of genotype of rs8099917 on SVR. Therefore, we omitted the data of rs12979860 and re-analyzed. As shown in Table 3, without the factor of rs12979860, the TT genotype of rs8099917, together with younger age and low baseline viral load emerged as the significant predictors for SVR by the multivariate analysis. As for the RVR, it is also the same SNP, rs12972860, and baseline viral load could predict RVR by multivariate analyses (Table 4). For the EVR and ETR, only rs12979860 was the predictor but not the baseline viral load (Table 5 and 6).

**rs12979860 CC genotype had a significant impact on the SVR irrespective to baseline viral load**

As shown in Table 2, baseline viral load and IL28B genotypes are the two important predictors of the SVR when treated with PegIFN/RBV. Because IL28B is one of the cytokines that play an important role in anti-viral immunity, we then analyzed the relationship between these SNPs and the baseline viral load and stages of liver fibrosis. As show in Table 7, none of these SNPs correlated with the baseline viral load and fibrosis stage. We also studied the influence of the IL28B genotype on the SVR in patients with either high baseline viral load or low baseline viral load. Because rs12979860 is the single statistically significant
Figure 2. Pairwise linkage disequilibrium (LD) patterns for eight polymorphisms through IL28B regions (chromosome 19, nucleotide positions 44,426,620–44,436,990).
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IL28B rs12979860 Predict Response in HCV Hepatitis

Figure 3. IL28B genetic association with sustained virological response in patients with GT1-HCV infection treated with PegIFN/ RBV. Percentages of patients of different genotypes with SVR in eight different SNPs groups were shown. Numbers of patients were also shown below each genotype.
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**Table 2. Factors predicted SVR to Peg-IFN/RBV treatment in genotype I chronic hepatitis C patients by univariate and multivariate Logistic regression analysis.**

| Baseline Predictors | Odds Ratio | 95% CI | p value |
|---------------------|------------|--------|---------|
| **Univariable**     |            |        |         |
| Age (years old)     | 0.97       | 0.95–1.00 | 0.064 |
| Baseline Viral Load | 3.68       | 1.89–7.19 | <0.001 |
| Fibrosis stage (Metavir) | 2.06 | 1.11–3.83 | 0.002 |
| BMI (Kg/m²)         | 0.92       | 0.83–1.00 | 0.060 |
| ALT (IU/L)          | 1.00       | 0.99–1.01 | 0.090 |
| GGT (IU/L)          | 1.00       | 0.99–1.00 | 0.109 |
| rs12979860          | 4.90       | 1.84–13.03 | 0.001 |
| rs8099917           | 4.25       | 1.66–10.92 | 0.003 |
| rs12980275          | 3.84       | 1.48–9.99 | 0.006 |
| rs8105790           | 4.25       | 1.66–10.92 | 0.003 |
| rs7248668           | 3.84       | 1.48–9.99 | 0.006 |
| rs10853728          | 1.41       | 0.75–2.66 | 0.285 |
| **Multivariable**   |            |        |         |
| Age (years old)     | 0.96       | 0.93–0.99 | 0.012 |
| Baseline Viral Load |            |        |         |
| ≥0.4 × 10⁶ IU/ml    | 1          |        |         |
| <0.4 × 10⁶ IU/ml    | 4.65       | 2.23–9.66 | <0.001 |
| rs12979860          |            |        |         |
| CT/TT genotype      | 1          |        |         |
| CC genotype         | 7.74       | 2.55–23.53 | <0.001 |

UV: univariate logistic regression analysis. MV: multivariate logistic regression analysis. OR: odds ratio; CI: confidence interval. Fibrosis stage: Metavir scoring system. doi:10.1371/journal.pone.0018322.t002

*IL28B* predictor of SVR as shown previously, we focused on this locus in the groups of either high or low baseline viral load. As shown in Table 8, the genotype of rs12979860 was significantly associated with the SVR in both groups of high baseline viral load or low baseline viral load. Furthermore, the odds ratios between these two groups had no difference (Odds ratio, low viral load vs. high viral load: 6.37 vs. 5.99, p = 0.956, by Cochran’s and Mantel-Haenszel statistics). Therefore, in addition to the findings that this SNP had no relationship with baseline viral load, the impact of the SNP on SVR remained similar either in the group of high baseline viral load or low baseline viral load.

**Table 4. Factors predicted RVR to Peg-IFN/RBV treatment in genotype I chronic hepatitis C patients by univariate and multivariate Logistic regression analysis.**

| Baseline Predictors | Odds Ratio | 95% CI | p value |
|---------------------|------------|--------|---------|
| **Univariable**     |            |        |         |
| Baseline Viral Load | 2.42       | 1.27–4.62 | 0.007 |
| HbA1c (%)           | 0.74       | 0.55–0.99 | 0.046 |
| rs12979860          | 8.93       | 3.07–26.02 | <0.001 |
| rs8099917           | 4.51       | 1.76–11.61 | 0.002 |
| rs12980275          | 6.74       | 2.44–18.60 | <0.001 |
| rs8105790           | 4.51       | 1.76–11.61 | 0.002 |
| rs7248668           | 6.74       | 2.44–18.60 | <0.001 |
| rs10853728          | 1.53       | 0.81–2.89 | 0.192 |
| **Multivariable**   |            |        |         |
| Baseline Viral Load | ≥0.4 × 10⁶ IU/ml | 1     |         |
| <0.4 × 10⁶ IU/ml    | 2.83       | 1.40–5.73 | 0.004 |
| rs12979860          |            |        |         |
| CT/TT genotype      | 1          |        |         |
| CC genotype         | 10.52      | 3.45–32.04 | <0.001 |

rs12979860 CC genotype could not predict SVR in patients with RVR but could in patients without RVR

RVR is an important predictor for SVR and a useful guide in treating patients with chronic hepatitis C [1,2]. In the present study, for patients with RVR, SVR was achieved in 79.0% of the instances, significantly higher than patients without RVR (SVR: 44.83%, P <0.001). We evaluated the influence of these SNPs on the SVR in these two groups of patients. In patients with RVR, only low baseline viral load was a significant predictor for SVR but not any SNPs of *IL28B* or other clinical parameters. On the contrary, in patients without RVR, only CC genotype of rs12979860 could predict the SVR but not other clinical parameters including baseline viral load (Table 10). Taken together, we identified rs12979860, one of the 10 SNPs of *IL28B*, to be the most powerful predictor for RVR, EVR, ETR and SVR in GT1 HCV infected patients treated with PegIFN/RBV. In addition, rs12979860 genotype could predict the SVR in patients without RVR but could not in patients with RVR.

**Discussion**

Four seminal papers had recently revealed a significant impact of *IL28B* polymorphisms on the treatment outcome of patients with chronic hepatitis C [17,18,19,20]. Subsequently, several investigators also published similar observations in different populations different loci of SNPs of *IL28B* [21,25,26,27,28,29,30,31,32,33,34,35]. Herein, we extended these observations and demonstrated that rs12972860 is the most powerful SNP predictor for RVR, EVR, ETR and SVR in patients of CHC genotype 1 treated with PegIFN/RBV. Furthermore, we also demonstrated that the SNP could only predict the SVR in patients without RVR but could not predict the SVR in patients with RVR.
genotype and allele distributions. One of these two loci, rs105142, was a nonsynonymous SNP, within the \textit{IL28B} gene that encodes a lysine to arginine substitution at position 70 (K70R). Another locus, rs28416813, was a G to C substitution at 37 base pairs upstream of the translation initiation site[23]. In addition, we also found one haplotype block and we chose rs12979860 to represent this haplotype because this locus was frequently reported in previous literatures [17,18,29,30,31,32,33,34,35]. In total, 5 SNPs were significantly correlated with the SVR except one SNP, rs10853728, which was not. This SNP, rs10853728, had been reported to be associated with SVR in Japanese population with GT1 HCV infection [19] but was not associated with RVR and SVR in Taiwanese patients with GT2/3 HCV infection [36].

However, we found the genotype of rs12979860, but not other SNPs, together with age and viral load became the predictors of SVR. rs12979860 is the frequently mentioned SNP of IL28B that encodes a lysine to arginine substitution at position 70 (K70R). Another locus, rs28416813, was a G to C substitution at 37 base pairs upstream of the translation initiation site[23]. In our analysis, rs8099917 became the predictor for SVR only after omitting the data of rs12979860. Therefore, though several SNPs of IL28B had been claimed to be important for predicting SVR, it is clear the rs12979860 is the key predictor among these SNPs for SVR in GT1 HCV infected patients.

Concerning the relationship between baseline viral load and SNPs of \textit{IL28B}, the results from previous reports were not consistent. Some reports claimed the baseline viral load was correlated with the genotype of the SNP but others did not [17,28,32,34,37]. Our data showed no relationship between baseline viral load and the SNPs. In addition, we also demonstrated that in both groups of high viral load and low viral load, SNP of \textit{IL28B} could predict the SVR with similar predictive ability. Therefore, in our cohort, SNP of \textit{IL28B} was only related to the treatment outcome but was not related to the viral load.

A customized therapy for chronic hepatitis C based on genotype and treatment viral response is the current treatment strategy [1,2]. RVR is a useful predictor of SVR in treatment of chronic hepatitis C with either conventional IFN or PegIFN plus RBV therapies [5,10,11,38]. As for the predictor of RVR, previous reports have shown baseline viral load was the single predictor [38], or together with younger age, lower body weight and absence of advanced fibrosis as predictors [5,13,39]. In the present study, we found baseline viral load was the only clinical parameter as the predictor for RVR. As for the SNPs of IL28B, it is again the rs12979860, but not other SNPs, that was the predictor for RVR. On the other hand, RVR was also an important negative response guider for treatment[16]. In previous reports, only the baseline viral load could predict the EVR [40]. In the present analysis, only rs12979860 but not other SNPs nor other clinical parameters, including baseline viral load, was the predictor for EVR. These results again emphasized the significant impact of this SNP to the treatment responses. Taken together, only the genotype of rs12979860 is the predictor for RVR, EVR, ETR and SVR. These results are consistent with the idea that SNPs of \textit{IL28B} is related to the immune responses after interferon-plus RBV treatment [41].

Another interesting observation in our study was the different impact of the CC genotype of rs12979860 on the SVR in patients with or without RVR. In patients with RVR, only baseline viral load but not the genotype of rs12979860 could predict the SVR. This is an interesting observation. The possible explanation is the CC genotype of rs12979860 led to the RVR with odds ratio of 10.52. As a result, the majority of patients with RVR were CC genotypes. Therefore, the CC genotype lost their predictive ability for SVR in this patient group with high prevalence of CC genotype. On the contrary, in patients without RVR, only the genotype of rs12979860 but not the baseline viral load could predict the SVR. This group of patients without RVR was with lower prevalence of CC genotype. Therefore, the CC genotype could continue its influence and therefore predict the SVR in this group of patients. This observation was quite similar to the recent report about genotype 2/3 HCV infected patients who received treatment that the genotype of rs12979860 is a single predictor for SVR in patients without RVR [35]. Since the discovery of the strong association between SNPs of \textit{IL28B} and treatment outcome of chronic hepatitis C, a

| Table 5. Factors predicted EVR to Peg-IFN/RBV treatment in genotype I chronic hepatitis C patients by univariate and multivariate logistic regression analysis. |
| Baseline Predictors | Odds Ratio | 95% CI | p value |
|---------------------|------------|--------|--------|
| Univariable         |            |        |        |
| HbA1c (%)           | 0.63       | 0.41–0.97 | 0.034 |
| GGT (IU/L)          | 0.98       | 0.98–0.99 | 0.002 |
| rs12979860          | 36.21      | 6.68–196.38 | <0.001 |
| rs8099917           | 9.77       | 2.24–42.60 | 0.002 |
| rs12980275          | 18.67      | 4.06–85.84 | <0.001 |
| rs8105790           | 9.77       | 2.24–42.60 | 0.002 |
| rs7248668           | 36.21      | 6.68–196.38 | <0.001 |
| rs10853728          | 1.95       | 0.47–8.07  | 0.356 |
| Multivariable       | rs12979860 |        |        |
| CT/TT genotype      | 1          |        |        |
| CC genotype         | 36.21      | 6.68–196.38 | <0.001 |
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| Table 6. Factors predicted ETR to Peg-IFN/RBV treatment in genotype I chronic hepatitis C patients by univariate and multivariate logistic regression analysis. |
| Baseline Predictors | Odds Ratio | 95% CI | p value |
|---------------------|------------|--------|--------|
| Univariable         |            |        |        |
| Sex                 | 2.53       | 0.90–7.13 | 0.079 |
| BMI (kg/m^2)        | 0.88       | 0.77–1.02 | 0.081 |
| GGT (IU/L)          | 0.99       | 0.98–0.99 | 0.018 |
| rs12979860          | 13.58      | 4.34–42.56 | <0.001 |
| rs8099917           | 8.94       | 2.89–27.65 | <0.001 |
| rs12980275          | 6.90       | 2.19–21.79 | 0.001 |
| rs8105790           | 8.94       | 2.89–27.65 | <0.001 |
| rs7248668           | 13.58      | 4.34–42.56 | <0.001 |
| rs10853728          | 2.66       | 0.94–7.51  | 0.064 |
| Multivariable       |            |        |        |
| Sex                 |            |        |        |
| female              | 1          |        |        |
| male                | 3.12       | 0.98–9.95 | 0.055 |
| rs12979860          |            |        |        |
| CT/TT genotype      | 1          |        |        |
| CC genotype         | 15.42      | 4.64–51.18 | <0.001 |
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consideration of personalized approach for treatment of chronic hepatitis C based on these SNPs had been proposed [22]. As suggested by Dr. Afdhal et al, although IL28B genotyping is highly predictive of SVR in HCV GT1 infected patients, its predictive power at the individual patient level is far from absolute. Therefore, IL28B genotyping should not be the sole factor in deciding on a treatment strategy [45]. In addition, shortening of treatment duration based on these SNPs together with other clinical parameter is possible but there are insufficient data to make recommendations [45]. However, the predictive ability of IL28B genotypes is strong enough. Therefore, it is reasonable to recommend a pre-treatment genotyping for SVR prediction. Based on our results described previously, we suggested genotyping for rs12979860 rather than other SNPs of IL28B would be a suitable genetic testing before starting treatment for patients with GT1-HCV chronic infection.

On the other hand, though a strong association between IL28B polymorphism and SVR had been repeated reported, the underlying mechanisms are still unclear. Recent in-vitro report had shown that IL28B could inhibit HCV replication in a dose- and time-dependent manner and through the JAK-STAT pathway [42]. Consequently, it had been found that IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C [43]. Furthermore, serum IL-28A/B levels were significantly higher in patients with chronic hepatitis C with good allele of IL28B genotype [44]. All these evidences indicate both direct antiviral effect and immune-mediated effect of IL28B could be affected by these polymorphisms. However, detailed mechanistic understanding needs further investigation.

The limitation of this study was the retrospective nature of the analysis. However, it is common to all the papers published on this issue. The other limitation was that we had excluded the patients who didn’t adhere to the 80/80/80 principle in order to investigate the real effects of SNPs on the treatment response. However, this exclusion would possibly ignore the possibilities that some patients without 80/80/80 adherence were due to the effect of these SNPs though till now there was no such report. Prospective controlled studies would be necessary to evaluate the influence of different SNPs of IL28B gene, especially rs12979869 and rs8077717, on treatment outcome of patients with chronic hepatitis C.

In conclusion, our data demonstrated that the genotype of rs12979860 is the most powerful predictor for RVR, EVR, ETR and SVR among ten SNPs of IL28B in patients with GT1 HCV infection treated with PegIFN/RBV. Furthermore, we also demonstrated different impact of baseline viral load and genotype of rs12979860 on SVR in patients with or without RVR. Based on these results, we suggested that the genotyping of rs12979860 is the suitable target for genetic testing before treatment for patients with GT1-HCV chronic infection.

### Table 7. Influences of SNPs of IL28B on the baseline viral load and liver fibrosis.

| Genotypes of SNPs | Baseline Viral load (IU/ml) | Fibrosis stage (Metavir) |
|-------------------|----------------------------|-------------------------|
|                   | <0.4 × 10⁶ | ≥0.4 × 10⁶ | P value | F0-F2 | F3-F4 | P value |
| rs12979860        |            |            |         |       |       |         |
| CC                | 81         | 90         | 0.824   | 90    | 81    | 0.348   |
| CT/TT             | 10         | 10         | 13      | 7     |       |         |
| rs8099917         |            |            | 0.998   | 88    | 82    | 0.088   |
| GG+GT             | 81         | 89         |         | 88    | 82    |         |
| TT                | 10         | 11         | 15      | 6     |       |         |
| rs12980275        |            |            | 0.818   | 90    | 81    | 0.294   |
| AA                | 82         | 89         |         | 90    | 81    |         |
| AG                | 9          | 11         | 13      | 7     |       |         |
| rs8105790         |            |            | 1.000   | 88    | 82    | 0.088   |
| TT                | 10         | 11         |         | 88    | 82    |         |
| CC/CT             | 10         | 11         | 15      | 6     |       |         |
| rs7248668         |            |            | 0.637   | 91    | 80    | 0.565   |
| GG                | 80         | 91         |         | 91    | 80    |         |
| AA+AG             | 11         | 9          | 12      | 8     |       |         |
| rs10853728        |            |            | 0.880   | 64    | 61    | 0.298   |
| CC                | 59         | 66         |         | 64    | 61    |         |
| CT/TT             | 32         | 34         |         | 39    | 27    |         |

### Table 8. Influence of genotypes of rs12979860 on SVR in high and low baseline viral load.

| Baseline Viral load | rs12979860 | Non-SVR N (%) | SVR N (%) | OR (95% CI) | Homogeneity of OR a |
|---------------------|------------|---------------|-----------|-------------|---------------------|
| Low viral load (<0.4 × 10⁶ IU/ml) | CC | 11 (13.6) | 70 (86.4) | 6.37 (1.6–25.6) | p = 0.013 p = 0.956 |
|                     | CT/TT      | 5 (50.0) | 5 (50.0) |            |         |
| High viral load (≥0.4 × 10⁶ IU/ml) | CC | 36 (40.0) | 54 (60.0) | 5.99 (1.2–30.3) | p = 0.020 |
|                     | CT/TT      | 8 (80.0) | 2 (20.0) |            |         |

aCochrans and Mantel-Haenszel statistics.

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