Interleukin-28B polymorphisms are associated with an early viral response in patients receiving hepatitis C therapy

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Abstract. Prediction of the efficacy of pegylated interferon (PEG-IFN) plus ribavirin (RBV) therapy against hepatitis C (HCV) infection is valuable for determining its applications. This study investigated the relationship between the early response of HCV to PEG-IFN/RBV therapy and the interleukin (IL)-28B genetic polymorphism in patients with HCV infection. The genotypes of *IL-28B* rs8099917 T>G single nucleotide polymorphism were determined in 144 patients with HCV infection. Among them, 59 were treated with PEG-IFN/RBV. The frequency of IL-28B TT homozygosity was 75.2% in patients with HCV serotype 1 and 84.6% in patients with serotype 2. Multivariate analysis showed that *IL-28B* TT homozygosity (P=0.014) and the platelets number (P=0.030) was associated with the early suppression of HCV-RNA at 12 weeks after the start of PEG-IFN/RBV therapy. The IL-28B polymorphism was a significant pretreatment predictor of the response to PEG-IFN/RBV therapy in patients with HCV infection.

Introduction

Hepatitis C virus (HCV) is the leading cause of chronic liver disease. It accounts for 70% of all chronic hepatitis cases, 40% of all cases of liver cirrhosis and 60% of hepatocellular carcinomas (HCC) (1,2). Since the successful eradication of HCV is associated with a reduced risk of liver cirrhosis and HCC (3,4), antiviral therapy plays a crucial role in the management of HCV-infected patients. Currently, pegylated interferon (PEG-IFN) plus ribavirin (RBV) is considered to be the most effective standard of care for chronic hepatitis C (5-7),

but the rate of sustained virological response (SVR; HCV RNA-negative for 24 weeks after the cessation of therapy) is approximately 50% in patients with HCV genotype 1, the most common genotype in many countries (5,6). Furthermore, 20-30% of HCV genotype 1 patients are non-responders to PEG-IFN/RBV therapy (3).

Prediction of the efficacy of PEG-IFN/RBV therapy is important for determining its applications, since PEG-IFN/ RBV carries potential serious side effects and is costly when used over a long period of time to achieve SVR. The outcome of interferon therapy has been reported to be associated with both viral and host factors. Representative viral factors are the HCV genotype, high HCV RNA titers (8), amino acid substitutions in the NS5A (9) and core region (10). The host factors include age, body mass index, insulin resistance and stage of liver fibrosis (4,11). Furthermore, it has recently been reported that a single nucleotide polymorphism (SNP) of the host gene interleukin (IL)-28B is significantly associated with the response to PEG-IFN/RBV therapy (12,13). A genome-wide association study of the genetic determinants of a therapeutic response in HCV-1 patients treated with PEG-IFN/RBV identified a SNP upstream of the IL-28B gene on chromosome 19, which was associated with an approximately 2-fold difference in SVR rates in patients of European, African-American or Hispanic ancestry (13). Non-responders were required to have been more than 80% compliant to both PEG-IFN/RBV dosing, and the ethnicity was defined by their genetic ancestry (13).

This study investigated the relationship between the early response of HCV to PEG-IFN/RBV therapy and the IL-28B genetic polymorphism in Japanese patients with HCV infection.

Patients and methods

Study populations. A total of 144 patients with chronic HCV infection (HCV serotype 1, n=105 and serotype 2, n=39), who were treated at the Hospital of the Shiga University of Medical Science, the Notogawa Hospital and the Social Insurance Shiga Hospital, were included in the study. Table I lists the demographic features of the subjects. Among these patients,

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Table I. Patient baseline characteristics.

Gender (male/female)	88/67	
Age (years)	59.8±11.2	(28-92)
ALT (IU/l)	83.3±115.4	(17-1,199)
γ-GTP (IU/l)	56.6±68.8	(7.8-638)
Platelet ($10^6/\mu$l)	15.6±5.40	(3.5-9.0)
HCV-RNA level ($\log_{10}$/ml)	6.1±0.64	(3.5-9.0)
HCV serotype 1/2	105/39	

Data are presented as the means ± SD (range).

59 were treated with PEG-IFN/RBV therapy. The patients received weekly injections of PEG-IFN at 1.5 μg/kg body weight, and the oral administration of ribavirin for 48 weeks. The amount of ribavirin was adjusted based on the patient's body weight (600 mg for <60 kg, 800 mg for 60-80 kg and 1,000 mg for >80 kg). Only patients with >75% compliance with the prescribed doses of PEG-IFN/RBV were included in this study. Informed consent was obtained from all patients. The ethics committee of each participating medical center approved this study.

Serotyping. Serotyping was performed by an enzyme immunoassay-based Murex assay (Murex Diagnostics Inc., Norcross, GA, USA).

Genotyping. The samples were genotyped for *IL-28B* rs8099917 using the TaqMan® SNP Assay (Applied Biosystems Inc., Foster City, CA, USA) as previously described (14). Homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having the IL-28B minor allele, whereas homozygosity for the major sequence (TT) was defined as having the IL-28B major allele. The HCV RNA levels were analyzed using the TaqMan RT-PCR test. The measurement ranges of these assays were 1.2-7.8 log IU.

Statistical analysis. Hardy-Weinberg equilibrium (HWE) analysis was performed in these subjects by comparing the detected distribution of allele frequencies with the theoretical distribution estimated from the SNP allelic frequencies. P>0.05 (Chi-square statistics) was considered to indicate equilibrium. The categorical variables were presented as

frequencies and percentages when required. The continuous variables were reported as the means ± SD (range). The viral kinetics were assessed using the Chi-square test. Multivariate logistic regression analysis with stepwise forward selection was performed with a criterion of P<0.05 for the inclusion or removal of variables. All statistical analyses used the Ekuseru-toukei 2008 (Social Survey Research Information Co., Ltd., Tokyo, Japan) software with P<0.05 considered to be statistically significant.

Results

The genotype frequencies of the IL-28B polymorphisms in patients with HCV serotypes 1 and 2 are shown in Table II. The frequency of the IL-28B major allele, defined as homozygosity for the major sequence (TT), in HCV serotype 1 patients (75.2%) was lower than that in serotype 2 patients (84.6%) (not significant). The frequency of the rs8099917 T allele was 87.1% in patients with serotype 1, and 92.3% in patients with serotype 2. These data corroborate those of a recent report of the Japanese population described by Ochi *et al* (5).

The patients with HCV genotype 1 were stratified according to their IL-28B allele type, and the early responses of HCV-RNA at 4, 8 and 12 weeks after the start of PEG-IFN/RBV therapy were analyzed (Fig. 1). The rate of HCV-RNA positivity was significantly lower at 12 weeks in patients with the IL-28B major allele than in patients with the IL-28B minor allele. This finding suggests that IL-28B has a great impact on the early virological response to therapy. Using multivariate analysis, the major allele of IL-28B (P=0.014) and the number of platelets (P=0.030) were associated with negative HCV-RNA at 12 weeks (Table III). No significant difference was detected between the effects of PEG-IFNα-2a and 2b (Fig. 2).

Discussion

Twenty to thirty percent of patients infected with HCV genotype 1 are non-responders to PEG-IFN/RBV therapy. Furthermore, PEG-IFN/RBV therapy may be associated with considerable toxicity. Therefore, a pre-treatment prediction of the patient response to therapy is clinically valuable. However, there have been no reliable baseline predictors for the response to anti-viral therapy. This study demonstrated that the IL-28B polymorphism was an over-

Table II. Genotype distribution of IL-28B SNP rs8099917.

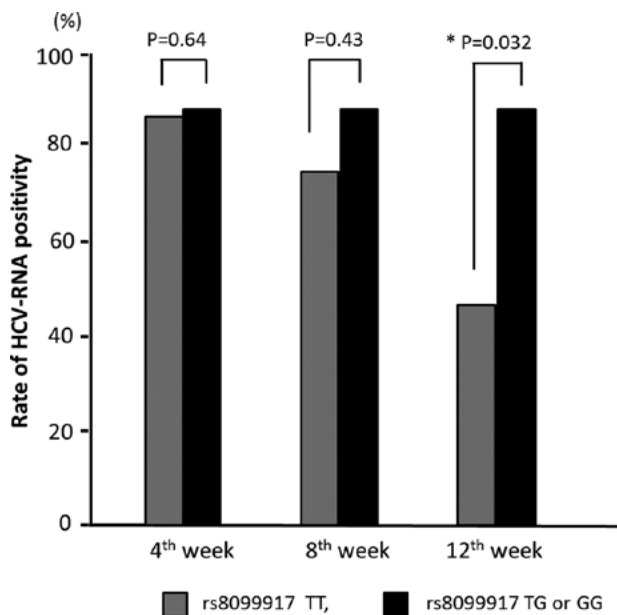|	rs8099917				
	TT	TG	GG	T (%)	G (%)
HCV serotype 1 (n)	79 (75.2%)	25 (23.8%)	1 (0.95%)	87.1	12.9
HCV serotype 2 (n)	33 (84.6%)	6 (15.4%)	0 (0.0%)	92.3	7.7
Total (n)	112 (77.8%)	31 (21.5%)	1 (0.69%)	89.7	10.3

Figure 1. Early viral response of the HCV genotype 1 to pegylated interferon (PEG-IFN) plus ribavirin (RBV). Patients of HCV genotype 1 were stratified according to their IL28B allele type, and the early responses of HCV-RNA at 4, 8 and 12 weeks after the start of PEG-IFN/RBV therapy were assessed.

Figure 2. Comparison of the effects of PEG-INFα-2a and 2b on HCV positivity. Data were analyzed by the Kaplan-Meier method. There were no significant differences between the effects of the two PEG-IFNα formulations.

Table III. Multivariate analyses of factors associated with HCV-RNA-negativity at week 12.

	Odds ratio	95% CI	P-value
rs8099917 TT	66.2	2.320-999.9	0.014[a]
Gender	0.36	0.043-3.21	0.220
Age	1.07	0.990-1.18	0.220
BMI	0.72	0.430-1.21	0.210
γ-GTP	1.00	0.970-1.03	0.910
Platelets	1.33	1.030-2.12	0.030[a]

CI, confidence interval. BMI, body mass index. [a]Significant difference.

whelming predictor of an early viral response to PEG-IFN/RBV therapy. IL-28B encodes a protein known as IFNλ 3, which is thought to suppress the replication of various viruses, including HCV (15). The results of the present study may provide a rationale for developing diagnostic testing and a therapeutic approach for targeting IL-28B in chronic hepatitis C in the future.

A polymorphism upstream of the IL-28B gene has been reported to be strongly associated with a favorable response in HCV-1 patients (13). In this study, we confirmed the clinical relevance of this genetic discovery. The polymorphism was significantly associated with early viral clearance at week 12. The key marker for an improved treatment response was the IL-28B major allele (TT type). The major allele type was associated with improved early viral suppression, such that by week 12, the reduction in viral positivity was doubled in patients with the TT allele as compared to the non-TT IL-28B type HCV-1 patients. Genotyping of IL-28B prior to PEG-IFN/
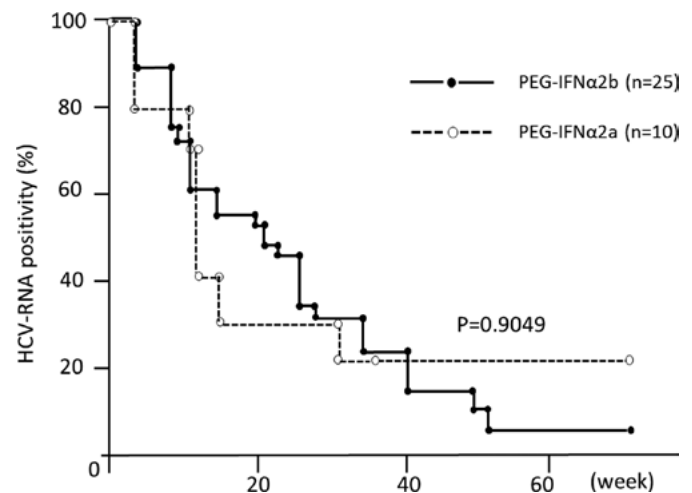
RBV therapy will aid clinicians and patients in PEG-IFN/RBV therapy management. Those patients who have the major allele (TT type) IL-28B have a greater likelihood of achieving early viral reduction, and should be considered ideal candidates. By contrast, patients with the minor allele (TG or GG type) are unlikely to achieve an early response to therapy, and need excellent compliance to the doses of PEG-IFN/RBV.

It has been reported that 20% of individuals infected with HCV are obese, and that the obesity in these individuals is associated with steatosis and the progression of fibrosis (4,11,16). These factors are shown to be associated with a nonresponse to treatment with PEG-IFN/RBV (16). However, we did not identify the BMI as one of the significant factors for a poor early viral response. On the other hand, we detected a significant association of platelet number. Since platelet number decreases in accordance with the progression of liver fibrosis (11), this suggests that PEG-IFN/RBV is more effective for patients with better histological changes of the liver.

Recent direct comparative trials, retrospective and meta-analysis studies have demonstrated that treatment with PEG-IFNα-2a is a significant and independent contributor to SVR in patients infected with genotype 1 (17), as compared to treatment with PEG-IFNα-2b, although the largest head-to-head trial (IDEAL study) failed to detect a significant difference in SVR rates between these two peg-IFNα formulations (18). Our observations also demonstrated that there are no differences in the early viral response between these two PEG-IFNα subtypes.

In conclusion, an IL-28B genotype is the strongest baseline predictor of an early viral response to PEG-IFN/RBV therapy in HCV-1 patients. It is likely that IL-28B SNP genotyping may become part of the clinical assessment before standard antiviral therapy in individuals chronically infected with HCV-1.

Acknowledgements

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