

Fig. 4. Viremia level and aa substitutions in core region/ISDR/IRRDR. **a** Concerning the number of substitutions in ISDR, viremia levels of patients with WT were significantly higher than those of patients with non-WT ($p < 0.001$). **b** Concerning the number of substitutions in IRRDR, viremia levels of patients with ≤ 5 aa substitutions were significantly higher levels than those of patients with ≥ 6 ($p = 0.027$). **c** Concerning the substitution of

core aa 70, viremia levels of patients with Arg70 were not significantly different from those of patients with Gln70 (His70). **d** Concerning the substitution of core aa 91, viremia levels of patients with Met91 were significantly higher than those of patients with Leu91 ($p = 0.028$). Thus, levels of viremia might be influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

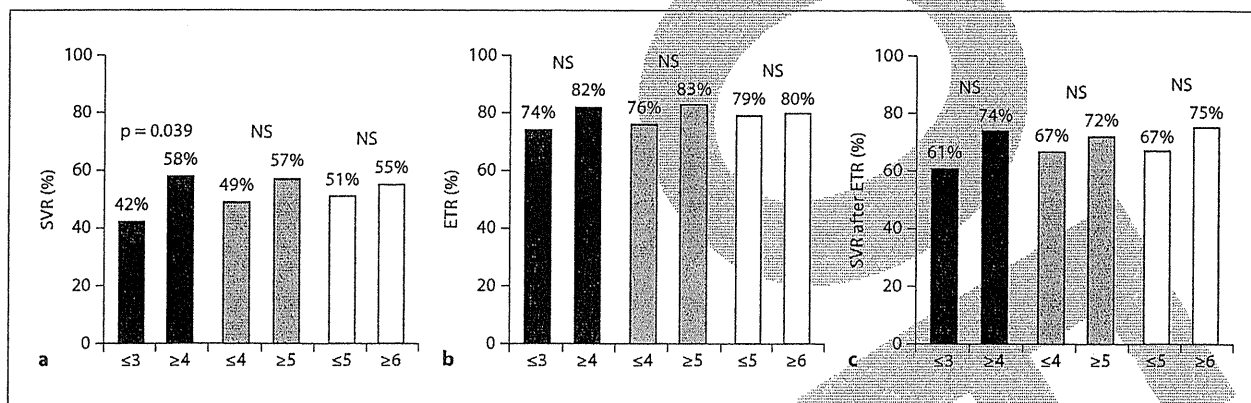


Fig. 5. Treatment response according to the number of aa substitutions in NS5A-IRRDR. **a** A significantly higher proportion of patients with ≥ 4 (58%) aa substitutions showed SVR compared to patients with ≤ 3 (42%) ($p = 0.039$), and it was useful as predictor

of SVR to categorize into two groups of ≤ 4 and ≥ 5 aa substitutions by univariate analysis. **b, c** ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

Fig. 6. SVR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

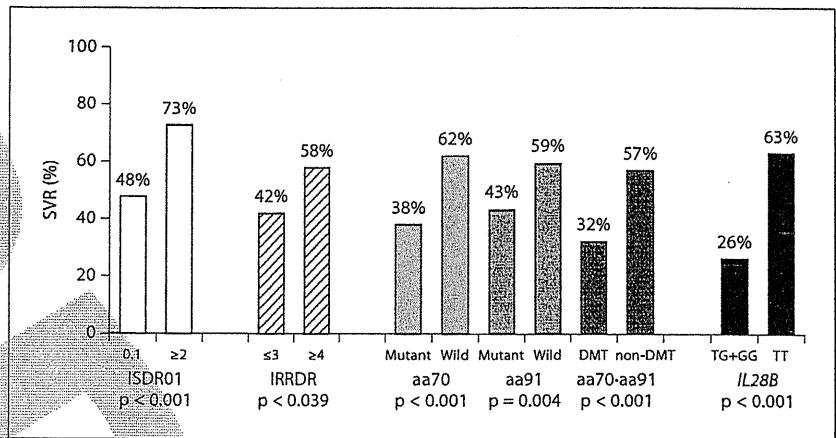


Fig. 7. ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

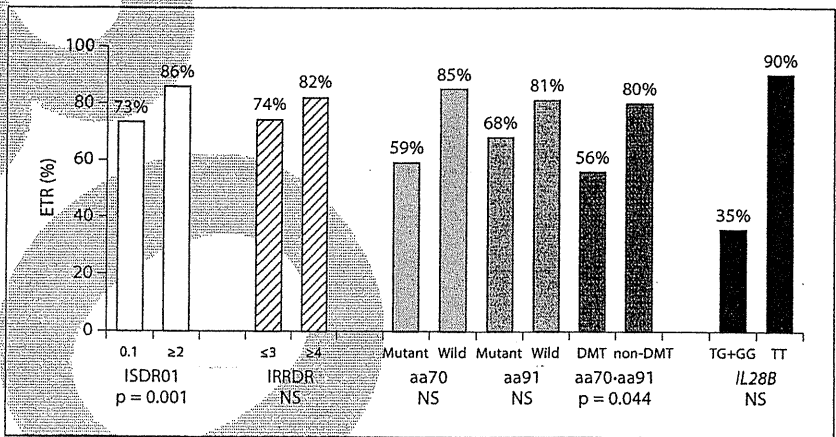


Table 2. Factors associated with SVR to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG	1	<0.001
	2: TT	16.7 (4.54–61.3)	
Gender	1: Female	1	<0.001
	2: Male	10.5 (3.47–32.3)	
ISDR of NS5A	1: WT	1	0.027
	2: Non-WT	5.68 (1.22–26.3)	

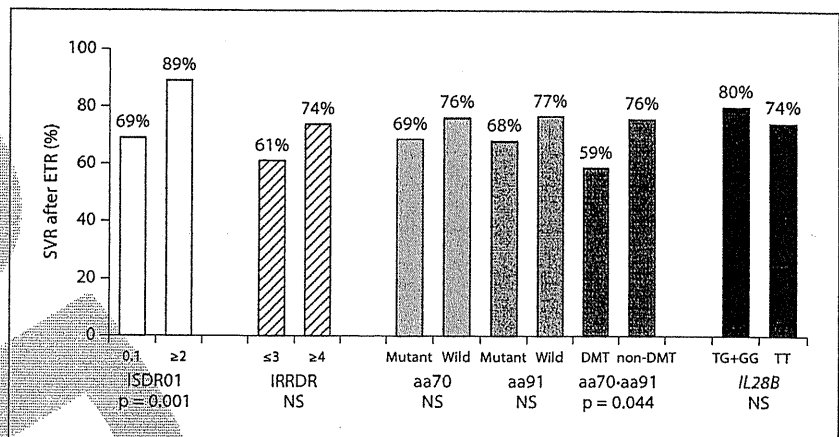
Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Factors associated with ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG	1	<0.001
	2: TT	18.2 (6.29–52.6)	
Level of viremia log IU/ml	1: ≥6.0	1	0.001
	2: <6.0	9.20 (2.59–32.6)	
Core aa 70	1: Gln70 (His70)	1	0.004
	2: Arg70	4.68 (1.65–13.3)	
Serum albumin g/dl	1: <3.9	1	0.030
	2: ≥3.9	3.08 (1.11–8.47)	

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Fig. 8. SVR after ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.



substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 4 parameters that independently influenced ETR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), level of viremia ($< 6.0 \log \text{ IU/ml}$; $p = 0.001$), substitution of aa 70 (Arg70; $p = 0.004$), and albumin ($\geq 3.9 \text{ g/dl}$; $p = 0.030$) (table 3).

Predictors of SVR after ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 11 parameters that influenced SVR after ETR: gender (male sex; $p < 0.001$), age (< 55 years; $p < 0.001$), ribavirin dose ($\geq 11.0 \text{ mg/kg}$; $p = 0.025$), leukocyte count ($\geq 4,500/\text{mm}^3$; $p = 0.033$), hemoglobin ($\geq 14.0 \text{ g/dl}$; $p = 0.025$), platelet count ($\geq 15.0 \times 10^4/\text{mm}^3$; $p = 0.001$), level of viremia ($< 6.0 \log \text{ IU/ml}$; $p = 0.020$), total cholesterol ($< 170 \text{ mg/dl}$; $p = 0.017$), α -fetoprotein ($< 10 \mu\text{g/l}$; $p = 0.004$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.044$), and the number of aa substitutions in ISDR (non-WT; $p = 0.001$). Figure 8 shows the SVR after ETR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 6 parameters that independently influenced the SVR after ETR: gender (male sex; $p < 0.001$), ribavirin dose ($\geq 11.0 \text{ mg/kg}$; $p = 0.002$), the number of aa substitutions in ISDR (non-WT; $p = 0.012$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.023$), platelet count ($\geq 15.0 \times 10^4/\text{mm}^3$; $p = 0.033$), and α -fetoprotein ($< 10 \mu\text{g/l}$; $p = 0.042$) (table 4).

Comparison of Factors Associated with Treatment Efficacy Identified by Multivariate Analysis

Table 5 shows the variables that achieved statistical significance on multivariate logistic regression for each evaluation of treatment efficacy. Rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core region was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. Level of viremia was an important predictor of ETR. Thus, genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia) were important predictors of treatment efficacy. Furthermore, gender, α -fetoprotein, albumin, and platelet count were also identified as other important predictors of treatment efficacy, in addition to genetic variation near *IL28B* and viral factors.

Discussion

Using multivariate analysis, the present study identified viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene) that influenced treatment efficacy to 48-week IFN/ribavirin combination therapy, which is in agreement with recent findings [22, 23]. Identification of these viral and host factors before the start of IFN/ribavirin combination therapy should help to select better therapeutic regimens, including triple therapy of telaprevir/PEG-IFN/ribavirin [24–26], for those patients who are less likely to achieve SVR.

According to the number of substitutions in ISDR, a previous report showed that levels of viremia were sig-

Table 4. Factors associated with SVR in patients who achieved ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
Gender	1: Female 2: Male	1 4.27 (2.15–8.55)	<0.001
Ribavirin dose, mg/kg	1: <11.0 2: ≥11.0	1 2.95 (1.48–5.86)	0.002
ISDR of NS5A	1: WT 2: Non-WT	1 4.00 (1.35–11.8)	0.012
Core aa 70 and 91	1: Gln70 (His70) and Met91 2: Arg70 and/or Leu91	1 2.96 (1.16–7.52)	0.023
Platelet count × 10 ⁹ /mm ³	1: <15.0 2: ≥15.0	1 2.19 (1.07–4.50)	0.033
α-Fetoprotein μg/l	1: ≥10 2: <10	1 2.66 (1.04–6.80)	0.042

Only variables that achieved statistical significance (p < 0.05) on multivariate logistic regression are shown.

Table 5. Comparison of factors associated with efficacy of 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	ETR response (at 48 weeks)	SVR after ETR response	SVR
<i>IL28B</i>	rs8099917 p < 0.001, 18.2 (6.29–52.6) ^a		rs8099917 p < 0.001, 16.7 (4.54–61.3) ^a
Virus	Core aa 70 p = 0.004, 4.68 (1.65–13.3) ^a Level of viremia p = 0.001, 9.20 (2.59–32.6) ^a	Core aa 70 and 91 p = 0.023, 2.96 (1.16–7.52) ^a ISDR p = 0.012, 4.00 (1.35–11.8) ^a	ISDR p = 0.027, 5.68 (1.22–26.3) ^a
Others	Albumin p = 0.030, 3.08 (1.11–8.47) ^a	α-Fetoprotein p = 0.042, 2.66 (1.04–6.80) ^a Platelet count p = 0.033, 2.19 (1.07–4.50) ^a Gender p < 0.001, 4.27 (2.15–8.55) ^a Ribavirin dose p = 0.002, 2.95 (1.48–5.86) ^a	Gender p < 0.001, 10.5 (3.47–32.3) ^a

Only variables that achieved statistical significance (p < 0.05) on multivariate logistic regression are shown.
^a OR (95% CI).

nificantly lower in patients with non-WT of ISDR than in those with WT [8]. The present study indicated that substitution of IRRDR and core aa 91, in addition to substitution of ISDR, also significantly influenced levels of viremia. Furthermore, there was a significant positive correlation between the number of aa substitutions in

ISDR and those in IRRDR, and the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. To our knowledge, this is the first report of the relationship between viremia levels and aa substitutions in core region/ISDR/IRRDR. This result might be interpreted to mean

that core aa 91/ISDR/IRRDR might be associated with viremia levels involved in resistance to combination therapy. Further studies that examine the functional impact of aa substitutions to combination therapy should be conducted to confirm the above finding.

The present results showed that α -fetoprotein, albumin, platelet count, and gender were predictors of virological response to IFN/ribavirin combination therapy. Previous data indicated that absence of advanced liver fibrosis was a positive predictor of SVR to IFN monotherapy and IFN/ribavirin combination therapy [2, 3, 13, 27–29], and that advanced liver fibrosis was usually associated with higher levels of α -fetoprotein, and lower levels of albumin and platelet count [1, 3, 30–32]. Furthermore, gender is also a predictor of treatment response to IFN/ribavirin combination therapy [2, 3, 14]. In the present study based on a large number of patients, histopathological changes in the liver and gender were identified as independent predictors of virological response, in addition to genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia).

In a previous study, multivariate analysis identified core region, gender, and stage of liver fibrosis as parameters that independently influenced the SVR of patients who achieved early virological response, but ISDR was not entered into uni- and multivariate analysis [3]. To our knowledge, the present study based on multivariate analysis is the first report to identify ISDR as pretreatment

predictor of SVR after ETR to combination therapy. Interestingly, ISDR was not a predictor of ETR, but was a significant predictor of SVR to combination therapy. Thus, the underlying mechanisms of failure to develop SVR in those patients who achieve HCV-RNA negativity remain unclear. Further studies that examine the impact of aa substitutions of ISDR to combination therapy should be conducted to confirm the above finding.

One limitation of the present study was that aa substitutions in areas other than the core region and NS5A-ISDR/IRRDR of the HCV genome were not examined. Other limitations were differences in host factors including race [24, 33, 34] and differences in viral factors, such as the distribution of HCV-1a or -1b, and geographic diversities of HCV-1b [35]. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of 48-week IFN/ribavirin combination therapy, and further understanding of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

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Amino Acid Substitution in HCV Core Region and Genetic Variation near the *IL28B* Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment

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Key Words

Hepatitis C virus · Core region · *IL28B* · Telaprevir · Peginterferon · Ribavirin · Viral dynamics

Abstract

Objectives: Genetic variation near the *IL28B* gene and substitution of aa 70 and 91 in the core region of HCV-1b are useful as predictors of treatment efficacy to telaprevir/pegylated interferon (PEG-IFN)/ribavirin, but its impact on viral dynamics is not clear. **Methods:** This study investigated predictive factors of viral dynamics during 12- or 24-week regimen of triple therapy in 80 Japanese adults infected with HCV-1b. **Results:** After 24 h of commencement of treatment, the proportion of patients with Arg70 and Leu91 substitutions in the core region who showed ≥ 3.0 log drop in HCV RNA level was significantly higher than that of patients with Gln70 (His70) and/or Met91. At 8 and 12 weeks, HCV RNA loss rate of patients with rs8099917 genotype TT near *IL28B* gene was significantly higher than that of patients with non-TT.

Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥ 3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and SVR (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and SVR. **Conclusions:** This study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy.

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Introduction

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 kIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis

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C [3]. Such a background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads [4, 5]. Recently, a new strategy was introduced in the treatment of chronic HCV infection by means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [6]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity [7, 8]. Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin could achieve sustained virological response rates of 35–60 and 61–69% in patients infected with HCV-1, respectively [9, 10]. Furthermore, a recent study (PROVE3) also showed that the 24- and 48-week regimen of triple therapy could achieve sustained virological response rates of 51 and 53% in HCV-1 infected patients in whom initial PEG-IFN/ribavirin treatment failed, respectively [11].

Amino acid (aa) substitutions at positions 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy [12–14], and also affect clinical outcome, including hepatocarcinogenesis [15, 16]. Furthermore, genetic variations near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 as host-related factor, which encodes IFN- λ -3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1 [17–20], and also affect clinical outcome, including spontaneous clearance of HCV [21]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of sustained virological response to triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b [22]. However, it is not clear at this stage whether genetic variation near the *IL28B* gene and aa substitution of the core region can be used before therapy to predict viral dynamics during triple therapy.

The present study included 80 patients with HCV-1b and high viral loads, who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict viral dynamics during treatment, including viral-(aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near *IL28B* gene).

Patients and Methods

Study Population

Between May 2008 and September 2009, 81 patients infected with HCV were recruited to this study at the Department of Hepatology in Toranomon Hospital in metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN and ribavirin] (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

Eighty of the 81 patients met the following inclusion and exclusion criteria: (1) Diagnosis of chronic hepatitis C. (2) HCV-1b confirmed by sequence analysis. (3) HCV RNA levels of ≥ 5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20–65 years. (6) Body weight ≥ 35 kg and ≤ 120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of ≤ 50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of ≥ 110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of ≥ 12 g/dl, neutrophil count $\geq 1,500/\text{mm}^3$, and platelet count of $\geq 100,000/\text{mm}^3$ at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. In this study, all of the 80 patients were evaluated for the pretreatment predictors for viral dynamics during triple therapy, and 77 of the 80 patients were followed up for at least 24 weeks after the completion of treatment. The treatment efficacy was evaluated by 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 or 500 mg three times a day at an 8-hour (q8) interval after the meal. PEG-IFN α -2b (PEG-Intron; Schering Plough, Kenilworth, N.J., USA) was injected subcutaneously at a median dose of 1.5 $\mu\text{g}/\text{kg}$ (range 1.3–2.0 $\mu\text{g}/\text{kg}$) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose 600–1,000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below $1,500/\text{mm}^3$, neutro-

Table 1. Profile and laboratory data at commencement of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b

<i>Demographic data</i>	
Number of patients	80
Sex, M/F	43/37
Age, years*	55 (23–65)
History of blood transfusion	24 (20.0%)
Family history of liver disease	13 (16.3%)
Body mass index*	22.5 (13.2–32.4)
<i>Laboratory data*</i>	
Level of viremia, log IU/ml	6.8 (5.1–7.6)
Serum aspartate aminotransferase, IU/l	34 (15–118)
Serum alanine aminotransferase, IU/l	42 (12–175)
Serum albumin, g/dl	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase, IU/l	36 (9–229)
Leukocyte count, per mm ³	4,800 (2,800–8,100)
Hemoglobin, g/dl	14.3 (11.7–16.8)
Platelet count, × 10 ⁴ /mm ³	17.3 (9.5–33.8)
α-Fetoprotein, μg/l	4 (2–39)
Total cholesterol, mg/dl	180 (112–276)
Fasting plasma glucose, mg/dl	92 (64–125)
<i>Treatment</i>	
PEG-IFNα-2b dose, μg/kg*	1.5 (1.3–2.0)
Ribavirin dose, mg/kg*	11.5 (7.2–18.4)
Telaprevir dose, 1,500/2,250 mg/day	10/70
Treatment regimen (T12PR12 group/T12PR24 group)	20/60
<i>Amino acid substitutions in the HCV-1b</i>	
Core aa 70, arginine/glutamine (histidine)	47/33
Core aa 91, leucine/methionine	43/37
ISDR of NS5A, wild-type/non-wild-type	76/4
<i>Genetic variation near IL28B gene</i>	
rs8099917 genotype, TT/TG/GG/ND	46/30/2/2
rs12979860 genotype, CC/CT/TT/ND	43/31/2/4
<i>Past history of IFN therapy</i>	
Treatment naïve	27
Relapsers to previous treatment	33
Nonresponders to previous treatment	20
Data are numbers and percentages of patients, except those denoted by *, which represent the median (range) values. ND = Not determined.	

phil count below 750/mm³ or platelet count below 80,000/mm³; PEG-IFN was discontinued when these counts decreased below 1,000/mm³, 500/mm³ or 50,000/mm³, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800 to 600 and 1,000 to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the

discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 80 patients at the commencement of treatment. They included 43 males and 37 females, aged 23–65 years (median 55 years).

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. Furthermore, to investigate the pretreatment predictors for viral dynamics, HCV RNA levels during treatment were evaluated at 7 time points; 24 h, 1, 2, 4, 6, 8 and 12 weeks after the commencement of treatment. HCV RNA levels during treatment were evaluated in 80 (100%), 80 (100%), 80 (100%), 79 (98.8%), 75 (93.8%), 74 (92.5%), and 69 (86.3%) of the 80 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as loss of HCV RNA. Especially, falls in HCV RNA levels at 24 h relative to baseline were investigated as very early dynamics.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [23], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 80 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [12]. The sequence of 2209–2248 aa in the NS5A of HCV-1b (IFN sensitivity-determining region; ISDR) reported by Enomoto et al. [24] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non-wild-type (≥2). In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing [22].

Genetic Variation near IL28B Gene

Samples for genomewide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described previously [25, 26].

In this study, genetic variations near *IL28B* gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome [17–22], were investigated.

Statistical Analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to viral dynamics and sustained virological response. The ORs and 95%CI were also calculated. All p values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into

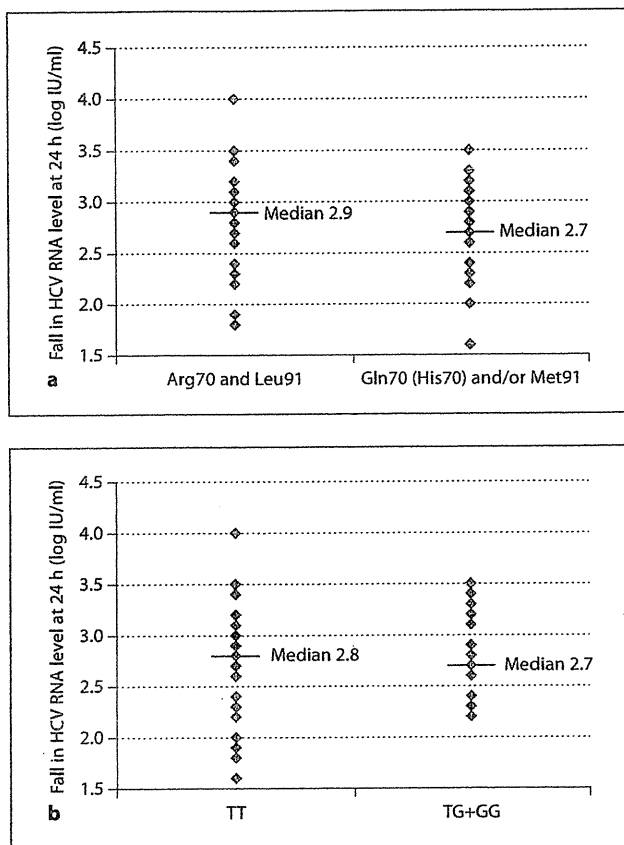


Fig. 1. a Very early dynamics according to amino acid substitutions in core region. After 24 h of commencement of the triple therapy, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml). **b** Very early dynamics according to genetic variation near the *IL28B* gene. After 24 h of commencement of the triple therapy, the decline of HCV RNA level of patients with rs8099917 genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml).

multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The potential pretreatment factors associated with treatment efficacy included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γ GTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, α -fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body

weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the *IL28B* gene, and amino acid substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Virological Response to Therapy and Loss of HCV RNA during Treatment

Sustained virological response was achieved by 63.6% (49 of 77 patients). The disappearance rate of HCV RNA during treatment was 0% (0 of 80), 1.3% (1 of 80), 33.8% (27 of 80), 81.0% (64 of 79), 90.7% (68 of 75), 94.6% (70 of 74), and 89.9% (62 of 69) at 24 hours, 1, 2, 4, 6, 8, and 12 weeks, respectively.

*Very Early Dynamics according to Amino Acid Substitutions in Core Region and Genetic Variation near the *IL28B* Gene*

After 24 h of commencement of the triple therapy, the proportion of patients with Arg70 and Leu91 substitutions who showed ≥ 3.0 log drop in HCV RNA level (45.2%; 14 of 31 patients) was significantly higher than that of patients with Gln70 (His70) and/or Met91 (14.3%; 7 of 49) ($p = 0.004$). Thus, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml) (fig. 1a).

After 24 h of commencement of treatment, the proportion of patients with rs8099917 genotype TT who showed ≥ 3.0 log drop in HCV RNA level (30.4%; 14 of 46 patients) was not significantly different from that of patients with genotype TG and GG (21.9%; 7 of 32). Thus, the decline of HCV RNA level of patients with genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml) (fig. 1b).

Hence, the fall in HCV RNA level at 24 h was influenced by aa substitution patterns in the core region, but was independent of genetic variation near *IL28B* gene.

*Rates of Loss of HCV RNA according to Amino Acid Substitutions in Core Region and Genetic Variation near the *IL28B* Gene*

According to the substitution of core aa 70 and 91, the rate of HCV RNA loss of patients with Arg70 and Leu91 was not significantly different from that of patients with

Gln70 (His70) and/or Met91 at each time point (1, 2, 4, 6, 8 and 12 weeks).

According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT (fig. 2).

Predictive Factors Associated with ≥ 3.0 log Fall in HCV RNA Level at 24 Hours

Univariate analysis identified two parameters that correlated with ≥ 3.0 log fall in HCV RNA level at 24 h significantly: substitution of aa 70 and 91 (Arg70 and Leu91; OR 4.94, $p = 0.003$) and body mass index (≥ 25.0 ; OR 3.92, $p = 0.022$). Two factors were identified by multivariate analysis as independent parameters that either significantly ($p < 0.05$) or marginally ($p < 0.10$) influenced ≥ 3.0 log fall in HCV RNA level at 24 h [Arg70 and Leu91 (OR 3.99, $p = 0.015$) and body mass index ≥ 25.0 (OR 3.24, $p = 0.061$)] (table 2).

Predictive Factors Associated with Loss of HCV RNA at 2, 4 and 12 Weeks

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 2 weeks significantly: platelet count ($\geq 15.0 \times 10^4/\text{mm}^3$; OR 6.99, $p = 0.014$) and level of viremia (< 7.0 log IU/ml; OR 3.13, $p = 0.045$). One factor was identified by multivariate analysis as independent parameter that either significantly or marginally influenced loss of HCV RNA at 2 weeks (platelet count $\geq 15.0 \times 10^4/\text{mm}^3$; OR 6.99, $p = 0.014$) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 4 weeks significantly: history of blood transfusion (absence; OR 5.71, $p = 0.006$) and body mass index (≥ 20.0 ; OR 4.29, $p = 0.019$). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 4 weeks (history of blood transfusion: absence; OR 4.29, $p = 0.026$, and body mass index ≥ 20.0 ; OR 3.47, $p = 0.069$) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 12 weeks significantly: sex (male; OR 9.52, $p = 0.043$) and genetic variation in rs8099917 (genotype TT; OR 9.00, $p = 0.048$). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 12 weeks (male sex; OR 11.0, $p = 0.036$, and rs8099917 genotype TT; OR 10.3, $p = 0.042$) (table 2).

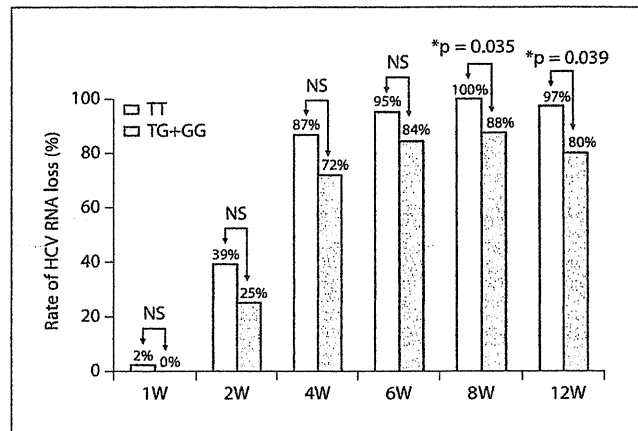


Fig. 2. Rates of loss of HCV RNA according to genetic variation near the *IL28B* gene. According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT.

Predictive Factors Associated with Sustained Virological Response

Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 3.51, $p = 0.011$), and genetic variation in rs8099917 (genotype TT; OR 11.1, $p < 0.001$) and rs12979860 (genotype CC; OR 10.2, $p < 0.001$). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced sustained virological response (rs8099917 genotype TT; OR 9.94, $p < 0.001$, and Arg70; OR 3.15, $p = 0.055$) (table 2).

Comparison of Factors Associated with Each Treatment Efficacy Identified by Multivariate Analysis

Table 3 shows independent parameters that either significantly or marginally influenced multivariate logistic regression for each evaluation of treatment efficacy. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥ 3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and sustained virological response (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and sustained virological response. Thus, genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy.

Table 2. Factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b, identified by univariate and multivariate analysis

Factor	Category	Univariate logistic regression		Multivariate logistic regression		
		OR (95% CI)	p	OR (95% CI)	p	
A ≥ 3.0 log fall in HCV RNA at 24 h	Substitution of aa 70 and 91	1: Gln70 (His70) and/or Met91	1		1	
		2: Arg70 and Leu91	4.94 (1.70–14.4)	0.003	3.99 (1.31–12.2)	0.015
	Body mass index	1: <25.0	1		1	
		2: ≥ 25.0	3.92 (1.22–12.6)	0.022	3.24 (0.95–11.1)	0.061
B HCV RNA loss at 2 weeks	Platelet count, $\times 10^4/\text{mm}^3$	1: <15.0	1		1	
		2: ≥ 15.0	6.99 (1.49–32.8)	0.014	6.99 (1.49–32.8)	0.014
	Level of viremia, log IU/ml	1: ≥ 7.0	1		–	–
		2: <7.0	3.13 (1.02–9.52)	0.045	–	–
C HCV RNA loss at 4 weeks	History of blood transfusion	1: presence	1		1	
		2: absence	5.71 (1.66–19.6)	0.006	4.29 (1.86–15.6)	0.026
	Body mass index	1: <20.0	1		1	
		2: ≥ 20.0	4.29 (1.26–14.5)	0.019	3.47 (0.91–13.3)	0.069
D HCV RNA loss at 12 weeks	Sex	1: female	1		1	
		2: male	9.52 (1.08–83.3)	0.043	11.0 (1.16–100)	0.036
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	9.00 (1.02–79.5)	0.048	10.3 (1.08–98.0)	0.042
E Sustained virological response	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	11.1 (3.68–33.5)	<0.001	9.94 (3.05–32.4)	<0.001
	Substitution of aa 70	1: Gln70 (His70)	1		1	
		2: Arg70	3.51 (1.33–9.26)	0.011	3.15 (0.97–10.2)	0.055
rs12979860 genotype	1: CT+TT	1		–	–	
		2: CC	10.2 (3.33–3.13)	<0.001	–	–

Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors.

The other significant predictors of HCV RNA loss were platelet count ($\geq 15.0 \times 10^4/\text{mm}^3$) at 2 weeks, history of blood transfusion (absence) at 4 weeks, and sex (male) at 12 weeks.

Discussion

Thompson et al. [27] reported that genetic variation near *IL28B* gene was also associated with increased on-treatment and sustained virological response and effectively predicted treatment outcome in treatment-naive HCV-1 patients treated with PEG-IFN plus ribavirin. However, HCV RNA loss at 4 weeks (rapid virological

response) was a strong predictor of sustained virological response regardless of genetic variation near the *IL28B* gene. This phenomenon probably explains why it might be important to identify the pretreatment factors that could predict viral dynamics during treatment. The present study is the first to identify the pretreatment factors that could predict viral dynamics during triple therapy in patients infected with HCV-1. These results should be interpreted with caution since races other than Japanese and the patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies including patients of other races and with HCV-1a to explore whether genetic variation near *IL28B* gene and aa substitution

Table 3. Comparison of factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b identified by multivariate analysis

Factor	≥3.0 log fall in HCV RNA (at 24 h)	HCV RNA loss (at 2 weeks)	HCV RNA loss (at 4 weeks)	HCV RNA loss (at 12 weeks)	Sustained virological response
Core aa 70 and 91	Arg70 and Leu91 p = 0.015 3.99 (1.31–12.2)*				Arg70 p = 0.055 3.15 (0.97–10.2)*
<i>IL28B</i> rs8099917				genotype TT p = 0.042 10.3 (1.08–98.0)*	genotype TT p < 0.001 9.94 (3.05–32.4)*
Others	body mass index p = 0.061 3.24 (0.95–11.1)*	platelet count p = 0.014 6.99 (1.49–32.8)*	body mass index p = 0.069 3.47 (0.91–13.3)* history of blood transfusion p = 0.026 4.29 (1.86–15.6)*	sex p = 0.036 11.0 (1.16–100)*	

Only variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on multivariate logistic regression are shown. * OR (95% CI).

of core region also affect viral dynamics during triple therapy.

Two studies showed that aa substitution of the core region and genetic variation near *IL28B* gene affected viral dynamics during treatment, and sustained virological response to 48-week PEG-IFN plus ribavirin therapy in patients infected with HCV-1 [27, 28]. Furthermore, a recent report also showed that aa substitutions of core region might be used to predict very early dynamics (within 48 h) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin [29]. In the present study, multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥ 3.0 log fall in HCV RNA level at 24 hours (i.e. viral dynamics of very early phase) and sustained virological response, and rs8099917 as a predictor of HCV RNA loss at 12 weeks (i.e. viral dynamics of later phase) and sustained virological response. This study is the first to report that genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy, and probably explains why the combination of these independent factors is very useful as pretreatment predictors of sustained virological response by triple therapy [22]. The underlying mechanisms of the different viral dynamics to treatment are still unclear, and further studies based on a larger number of patients are necessary to investigate the present results.

Previous data indicated that absence of advanced liver fibrosis and male gender were positive predictors of virological response to 48-week PEG-IFN plus ribavirin therapy [13, 28]. The present study also showed that higher levels of platelet count at 2 weeks, as a surrogate marker of milder liver fibrosis, and male gender at 12 weeks were significant positive predictors of HCV RNA loss during triple therapy. The other positive predictors were absence of history of blood transfusion at 4 weeks and higher levels of body mass index at 24 h and 4 weeks, but the underlying mechanisms are still unclear. Thus, this report identified the pretreatment factors that could predict viral dynamics during triple therapy, but this study, based on a small number of patients, might provide misleading results (e.g. possible type error). Further studies of a larger number of patients are required to explore predictors, including viral- and host-related factors.

The limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR) [30], were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation [31], were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both in vivo and in vitro studies [32, 33]. Thus, viral factors before and during triple therapy should be investigated in

future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, this study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple therapy, and further under-

standing of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

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Amino Acid Substitution in Hepatitis C Virus Core Region and Genetic Variation Near the Interleukin 28B Gene Predict Viral Response to Telaprevir with Peginterferon and Ribavirin

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Genetic variation near the IL28B gene and substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the predictive factors of sustained virological response to a 12-week or 24-week regimen of triple therapy in 72 of 81 Japanese adults infected with HCV genotype 1. Overall, sustained virological response and end-of-treatment response were achieved by 61% and 89%, respectively. Especially, the sustained virological response was achieved by 45% and 67% in the 12- and 24-week regimens, respectively. Multivariate analysis identified rs8099917 near the IL28B gene (genotype TT) and substitution at aa 70 (Arg70) as significant determinants of sustained virological response. Prediction of response to therapy based on a combination of these factors had high sensitivity, specificity, and positive and negative predictive values. The efficacy of triple therapy was high in the patients with genotype TT, who accomplished sustained virological response (84%), irrespective of substitution of core aa 70. In the patients having genotype non-TT, those of Arg70 gained high sustained virological response (50%), and sustained virological response (12%) was the worst in patients who possessed both genotype non-TT and Gln70(His70). **Conclusion:** This study identified genetic variation near the IL28B gene and aa substitution of the core region as predictors of sustained virological response to a triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV genotype 1b. (HEPATOLOGY 2010;52:421-429)

Abbreviations: aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ GTP, gamma-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; NPV, negative predictive value; PEG-IFN, pegylated interferon; PPV, positive predictive value

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Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).^{1,2} At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 KIU/mL) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.³ Such background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads.^{4,5} Recently, a new strategy was introduced in the treatment of chronic HCV infection by

means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection.⁶ Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity.^{7,8} Specifically, HCV RNA is suppressed below the limits of detection in the blood in almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin.⁹ However, treatment-resistant patients who do not achieve sustained virological response by the triple therapy have been reported.⁹⁻¹¹ The underlying mechanism of the response to the treatment is still not clear.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy,¹²⁻¹⁴ and also affect clinical outcome, including hepatocarcinogenesis.^{15,16} Furthermore, a recent report showed that aa substitutions in the core region can also be used before therapy to predict very early dynamics (within 48 hours) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin.¹⁷ However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict sustained virological response to triple therapy.

Recent reports showed that genetic variations near the IL28B gene (rs8099917, rs12979860) on chromosome 19 is a host-related factor, which encodes IFN- λ -3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,¹⁸⁻²¹ and also affect clinical outcome, including spontaneous clearance of HCV.²² However, it is not clear at this stage whether genetic variation near the IL28B gene can be used before therapy to predict sustained virological response to triple therapy.

The present study included 81 patients with HCV-1b and high viral loads who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict sustained virological response, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near the IL28B gene).

Patients and Methods

Study Population. Between May 2008 and September 2009, 81 patients infected with HCV were

recruited for this study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy (telaprevir [MP-424], PEG-IFN, and ribavirin) (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

All of 81 patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C. (2) HCV-1 confirmed by sequence analysis. (3) HCV RNA levels of ≥ 5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20-65 years. (6) Body weight ≥ 35 kg and ≤ 120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of ≤ 50 mL/minute at baseline, diabetes requiring treatment or fasting glucose level of ≥ 110 mg/dL, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of ≥ 12 g/dL, neutrophil count $\geq 1500/\text{mm}^3$, and platelet count of $\geq 100,000/\text{mm}^3$ at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. Furthermore, 72 of 81 patients were followed for at least 24 weeks after the completion of triple therapy. The treatment efficacy was evaluated by HCV-RNA negative at the end of treatment (end-of-treatment response) and 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 mg or 500 mg

Table 1. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1

Demographic data	
Number of patients	81
Sex (M/F)	44 / 37
Age (years)*	55 (23-65)
History of blood transfusion	24 (29.6%)
Family history of liver disease	13 (16.0%)
Body mass index (kg/m ²)*	22.5 (13.2-32.4)
Laboratory data*	
HCV genotype (1a/ 1b)	1/80
Level of viremia (log IU/mL)	6.7 (5.1-7.6)
Serum aspartate aminotransferase (IU/L)	34 (15-137)
Serum alanine aminotransferase (IU/L)	42 (12-175)
Serum albumin (g/dL)	3.9 (3.2-4.6)
Gamma-glutamyl transpeptidase (IU/L)	36 (9-229)
Leukocyte count (/mm ³)	4,800 (2,800-8,100)
Hemoglobin (g/dL)	14.3 (11.7-16.8)
Platelet count (× 10 ⁴ /mm ³)	17.1 (9.1-33.8)
Alpha-fetoprotein (μ g/L)	4 (2-39)
Total cholesterol (mg/dL)	180 (110-276)
Fasting plasma glucose (mg/dL)	92 (64-125)
Treatment	
PEG-IFNα-2b dose (μ g/kg)*	1.5 (1.3-2.0)
Ribavirin dose (mg/kg)*	11.7 (7.2-18.4)
Telaprevir dose (1,500 / 2,250 mg/day)	10/71
Treatment regimen (T12PR12 group / T12PR24 group)	20/61
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine / glutamine [histidine] / ND)	47/33/1
Core aa 91 (leucine / methionine / ND)	43/37/1
ISDR of NS5A (wild-type / non wild-type / ND)	76/4/1
Genetic variation near IL28B gene	
rs8099917 genotype (TT / TG / GG / ND)	42/30/2/7
rs 12979860 genotype (CC / CT / TT / ND)	42/32/2/5
Past history of IFN therapy	
Treatment-naive / Relapsers to previous treatment / nonresponders to previous treatment	27/33/21

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values. ND, not determined.

three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose 1.5 μg/kg (range: 1.3-2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200-600 mg twice a day after breakfast and dinner (daily dose: 600-1000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1500/mm³, neutrophil count below 750/mm³, or platelet count below 80,000/mm³; PEG-IFN was discontinued when these counts decreased below 1000/mm³, 500/mm³ or 50,000/mm³, respectively. When hemoglobin decreased to <10 g/dL, the daily dose of ribavirin was reduced from 600 to 400 mg, from 800 to 600 mg

and 1000 mg to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dL. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 81 patients at the commencement of treatment. They included 44 males and 37 females, ages 23 to 65 years (median, 55 years).

Measurement of HCV RNA. The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2-7.8 log IU/mL, and the undetectable samples were defined as negative.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b. In the present study, aa substitutions of the core region and NS5A-ISDR (IFN-sensitivity determining region) of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by polymerase chain reaction (PCR) using the following primers: (1) Nucleotide sequences of the core region: The first-round PCR was performed with CE1 (sense, 5'-GTC TGC GGA ACC GGT GAG TA-3', nucleotides: 134-153) and CE2 (antisense, 5'-GAC GTG GCG TCG TAT TGT CG-3', nucleotides: 1096-1115) primers, and the second-round PCR with CC9 (sense, 5'-ACT GCT AGC CGA GTA GTG TT-3', nucleotides: 234-253) and CE6 (antisense, 5'-GGA GCA GTC GTT CGT GAC AT-3', nucleotides: 934-953) primers. (2) Nucleotide sequences of NS5A-ISDR: The first-round PCR was performed with ISDR1 (sense, 5'-ATG CCC ATG CCA GGT TCC AG-3', nucleotides: 6662-6681) and ISDR2 (antisense, 5'-AGC TCC GCC AAG GCA GAA GA-3', nucleotides: 7350-7369) primers, and the second-round PCR with ISDR3 (sense, 5'-ACC GGA TGT GGC AGT GCT CA-3', nucleotides: 6824-6843) and ISDR4 (antisense, 5'-GTA ATC CGG GCG TGC CCA TA-3', nucleotides: 7189-7208) primers. ([1,2]; nested PCR.) All samples were initially denatured at 95°C for 2 minutes. The 35 cycles of

amplification were set as follows: denaturation for 30 seconds at 95°C, annealing of primers for 30 seconds at 55°C, and extension for 1 minute at 72°C with an additional 7 minutes for extension. Then 1 μ L of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo).

With the use of HCV-J (Access. No. D90208) as a reference,²³ the sequence of 1-191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 81 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91).¹² The sequence of 2209-2248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al.²⁴ was determined and the numbers of aa substitutions in ISDR were defined as wildtype (0, 1) or nonwildtype (≥ 2).

Genetic Variation Near the IL28B Gene. Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described.^{25,26}

In this study, genetic variations near the IL28B gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome,¹⁸⁻²² were investigated.

Statistical Analysis. Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to sustained virological response. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P* values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance (*P* < 0.05) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The

potential pretreatment factors associated with sustained virological response included the following variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γ GTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the IL28B gene, and aa substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using SPSS (Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of predictors of the response to therapy.

Results

Virological Response to Therapy. Sustained virological response was achieved by 44 of 72 (61.1%) patients. In all, 64 of 72 (88.9%) patients were considered end-of-treatment response. According to treatment regimen, sustained virological response were achieved by 45.0% (9 of 20 patients) and 67.3% (35 of 52 patients), in the T12PR12 group and the T12PR24 group, respectively. Of eight patients who could not achieve end-of-treatment response, six (75.0%) patients resulted in reevaluation of viral loads regardless of HCV-RNA temporary negative, and the other two patients (25.0%) did not achieve HCV-RNA negative during treatment.

Especially in the T12PR24 group, according to the past history of treatment, sustained virological response were achieved by 76.4% (13 of 17 patients), 86.4% (19 of 22 patients), and 23.1% (3 of 13 patients), in treatment-naïve, relapsers to previous treatment, and nonresponders to previous treatment, respectively.

Sustained Virological Response According to Amino Acid Substitutions in Core and NS5A Regions. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions (74.4%) showed sustained virological response than that of patients who showed Gln70(His70) (41.4%) (Fig. 1, *P* = 0.007). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 (65.0%) and Met91 (56.3%) (Fig. 1). Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype

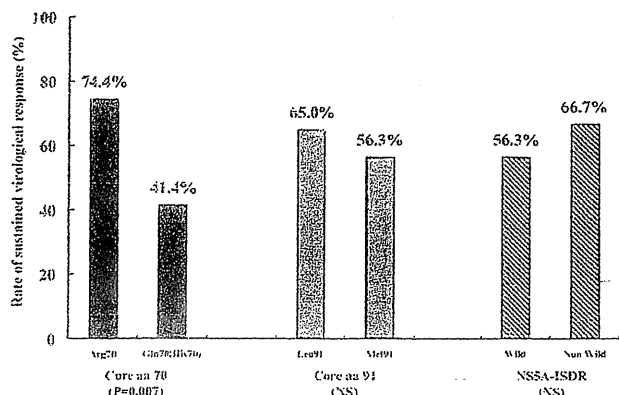


Fig. 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) ($P = 0.007$). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 and Met91. Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype and nonwildtype.

(56.3%) and nonwildtype (66.7%) (Fig. 1). Thus, sustained virological response was influenced by the substitution of core aa 70.

Sustained Virological Response According to Genetic Variation Near the IL28B Gene. According to the genetic variation in rs8099917, sustained virological response was achieved by 83.8% (31 of 37 patients), 29.6% (8 of 27 patients), and 0% (0 of 2 patients) in patients with genotype TT, TG, and GG, respectively. Thus, a significantly higher proportion of patients with genotype TT (83.8%) showed sustained virological response than that of patients who showed genotype non-TT (27.6%) (Fig. 2, $P < 0.001$) (Table 2).

According to the genetic variation in rs12979860, sustained virological response was achieved by 83.8% (31 of 37 patients), 34.5% (10 of 29 patients), and 0% (0 of 2 patients) in patients with genotype CC, CT, and TT, respectively. Thus, a significantly higher proportion of patients with genotype CC (83.8%) showed sustained virological response than that of patients who showed genotype non-CC (32.3%) (Fig. 2, $P < 0.001$) (Table 2).

Predictive Factors Associated with Sustained Virological Response. Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 4.12, $P = 0.007$), genetic variation in rs8099917 (genotype TT; OR 13.6, $P < 0.001$), and rs12979860 (genotype CC; OR 10.8, $P < 0.001$). Two factors were identified by multivariate analysis as independent

parameters that significantly influenced sustained virological response (rs8099917 genotype TT; OR 10.6, $P < 0.001$; and Arg70; OR 3.69, $P = 0.040$) (Table 3).

Assessment of Amino Acid Substitutions in Core Region and Genetic Variation Near the IL28B Gene as Predictors of Sustained Virological Response. The ability to predict sustained virological response by substitution of core aa 70 and rs8099917 genotype near the IL28B gene was evaluated. The sustained virological response rates of patients with a combination of Arg70 or rs8099917 genotype TT were defined as PPV (prediction of sustained virological response). The nonsustained virological response rates of patients with a combination of Gln70(His70) or rs8099917 genotype non-TT were defined as NPV (prediction of nonsustained virological response).

In patients with rs8099917 genotype TT, the sensitivity, specificity, PPV, and NPV for sustained virological response were 79.5, 77.8, 83.8, and 72.4%, respectively. Thus, genotype TT has high sensitivity, specificity, and PPV for prediction of sustained virological response. In patients with Arg70 the sensitivity, specificity, PPV, and NPV were 76.9, 63.0, 75.0, and 65.4%, respectively. Thus, Arg70 has high sensitivity and PPV in predicting sustained virological response. Furthermore, when both predictors were used the sensitivity, specificity, PPV, and NPV were 61.5, 85.2, 85.7, and 60.5%, respectively. When one or more of the two predictors were used the sensitivity, specificity, PPV, and NPV were 94.9, 55.6, 75.5, and 88.2%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response (Table 4).

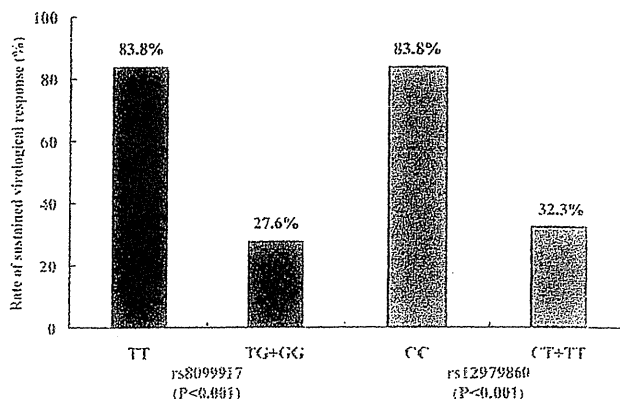


Fig. 2. According to the genetic variation in rs8099917 or rs12979860 near the IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively ($P < 0.001$ or $P < 0.001$, respectively).