

sons, those patients who do not achieve SVR need to be identified, so as to free them of unnecessary side effects and reduce costs, preferably before the start of the combination therapy.

Viral- and host-related factors are useful as predictors of treatment efficacy to 48-week IFN/ribavirin combination therapy. With regard to viral factors, amino acid (aa) substitutions at position 70 and/or 91 in the core region of HCV-1b are pretreatment predictors of virological response to combination therapy [1–4], and also affect clinical outcome, including hepatocarcinogenesis [5, 6]. Furthermore, the NS5A region of HCV-1b, including IFN-sensitivity-determining region (ISDR) [7, 8] and IFN/ribavirin resistance-determining region (IRDR) [9, 10], are also useful as pretreatment predictors of virological response to combination therapy [11, 12]. With regard to host factors, genetic variations near *IL28B* gene (rs8099917, rs12979860) on chromosome 19, which encodes IFN- λ -3, are pretreatment predictors of virological response to combination therapy in individuals infected with HCV-1 [13–16], and also affect clinical outcome, including spontaneous clearance of HCV [17]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of SVR to triple therapy of telaprevir/pegylated (PEG)-IFN/ribavirin in Japanese patients infected with HCV-1b [18]. However, to our knowledge, there are no previous reports of IFN/ribavirin combination therapy based on multivariate analysis to investigate pretreatment predictors, including all of aa substitutions in core aa 70/91 and NS5A-ISDR/IRDR, and genetic variation near *IL28B* gene.

The aim of the present study was to investigate predictive factors of treatment efficacy, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in Japanese adults infected with HCV-1b.

Patients and Methods

Study Population

A total of 1,249 HCV-1b-infected Japanese adult patients were consecutively recruited into the study protocol of combination therapy with IFN (PEG-IFN α -2b or IFN α -2b) plus ribavirin between December 2001 and January 2009 at Toranomon Hospital, Tokyo, Japan. Among these, 490 patients, who could complete a total of 48 weeks of combination therapy, were enrolled in this retrospective study, and fulfilled the following criteria: (1) negativity for hepatitis B surface antigen (HBsAg) in serum; (2) HCV-1b only 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) within the preceding 2 months of enrolment; (4) no hepatocellular carcinoma; (5) body weight >40 kg; (6) lack of coinfection with human immunodeficiency virus; (7) no previous treatment with antiviral or immunosuppressive agents within the preceding 3 months of enrolment; (8) none was an alcoholic; lifetime cumulative alcohol intake was <500 kg; (9) none had other forms of liver diseases, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, or autoimmune liver disease, and (10) none of the females was pregnant or breastfeeding.

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 their informed consent before participating in this trial.

The treatment efficacy was evaluated in terms of HCV-RNA negativity at the end of treatment (end-of-treatment response (ETR)) and 24 weeks after the completion of therapy (SVR), based on the COBAS TaqMan HCV test (Roche Diagnostics). SVR in patients who achieved ETR was defined as SVR after ETR. ETR, SVR, and SVR after ETR could be evaluated in 487 (99%), 448 (91%), and 321 (66%) of 490 patients, respectively.

422 (86%) patients received PEG-IFN α -2b at a median dose of 1.4 μ g/kg (range 0.7–1.9) subcutaneously each week plus oral ribavirin at a median dose of 11.1 mg/kg (range 3.7–15.1) daily for 48 weeks. The remaining 68 (14%) patients received 6 million units of IFN α -2b intramuscularly each day for 48 weeks (daily for the initial 2 weeks, followed by three times per week for 46 weeks), and oral ribavirin at a median dose of 11.3 mg/kg (range 6.8–13.4) daily for 48 weeks.

Table 1 summarizes the profiles and laboratory data of the 490 patients at the commencement of treatment. They included 310 males and 180 females aged 20–75 years (median 54).

Measurement of HCV RNA

The antiviral effects of treatment on HCV were assessed by measuring plasma HCV-RNA levels. In this study, HCV-RNA levels 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 aa Substitutions in Core, and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [19], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on the previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [1]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [7, 8] was determined, and the number of aa substitutions in ISDR was defined as wild-type (WT) (0, 1) or non-wild-type (non-WT) (≥ 2) in comparison with HCV-J. Furthermore, the sequence of 2,334–2,379 aa in the NS5A of HCV-1b (IRDR) reported by El-Shamy et al. [9, 10] was determined and then compared with the consensus sequence constructed on the previous study. In the present study, aa substitutions of the core region and NS5A-ISDR/IRDR of HCV-1b were analyzed by direct sequencing [10, 18].

Genetic Variation near *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 Invader assay, TaqMan assay, or direct sequencing as described previously [20, 21].

In this study, genetic variations near *IL28B* gene (rs8099917), reported as the pretreatment predictors of treatment efficacy in Japanese patients [14, 18], were investigated.

Statistical Analysis

Non-parametric tests (Mann-Whitney U test, χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Correlation analysis was evaluated by the Spearman rank correlation test. Uni- and multivariate logistic regression analyses were used to determine those factors that significantly contributed to ETR, SVR, and SVR after ETR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *p* values <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 uni- and multivariate analyses. Potential predictive factors associated with ETR, SVR, and SVR after ETR 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, γ -glutamyl transpeptidase (GGT), leukocyte count, hemoglobin, platelet count, level of viremia, α -fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, ribavirin dose/body weight, genetic variation near *IL28B* gene, and aa substitution in the core region, and NS5A-ISDR/IRRDR. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Response to Therapy

ETR was achieved by 372 of 487 (76%) patients, SVR by 244 of 448 (54%), and SVR after ETR by 244 of 321 (76%).

Number of aa Substitutions in NS5A-ISDR and NS5A-IRRDR

As a whole, 0, 1, and ≥ 2 aa substitutions in ISDR were found in 56% (227 of 406), 23% (95 of 406), and 21% (84 of 406) of patients, respectively. Thus, the percentage of patients with ≤ 1 aa substitution in ISDR (WT) was 79% (322 of 406). Furthermore, ≤ 3 , 4–5, and ≥ 6 aa substitutions in IRRDR were found in 36% (73 of 200), 34% (67 of 200), and 30% (60 of 200) of patients, respectively (fig. 1).

Table 1. Patient profile and laboratory data at commencement of the 48-week combination therapy of IFN + ribavirin in 490 patients infected with HCV-1b

<i>Demographic data</i>	
Number of patients	490
Male/female	310/180
Age, years	54 (20–75)
History of blood transfusion	169 (34%)
Family history of liver disease	96 (20%)
Body mass index, kg/m ²	22.6 (15.7–34.7)
<i>Laboratory data</i>	
Level of viremia, log IU/ml	6.4 (2.2–7.7)
Serum AST, IU/l	50 (16–296)
Serum ALT, IU/l	67 (12–836)
Serum albumin, g/dl	3.9 (3.1–4.7)
GGT, IU/l	44 (10–592)
Leukocyte count, n/mm ³	4,700 (1,200–10,900)
Hemoglobin, g/dl	14.4 (10.6–18.1)
Platelet count, $\times 10^4$ /mm ³	16.7 (6.4–37.5)
α -Fetoprotein, μ g/l	5 (1–459)
Total cholesterol, mg/dl	170 (96–284)
High-density lipoprotein cholesterol, mg/dl	46 (13–95)
Low-density lipoprotein cholesterol, mg/dl	100 (32–190)
Triglycerides, mg/dl	90 (33–416)
Uric acid, mg/dl	5.5 (2.3–9.4)
<i>Treatment</i>	
PEG-IFN α -2b/IFN α -2b	422/68
Ribavirin dose, mg/kg	11.2 (3.7–15.1)
<i>aa substitutions in the HCV-1b</i>	
Core aa 70, arginine/glutamine (histidine)	266/151
Core aa 91, leucine/methionine	246/169
ISDR of NS5A, 0/1/ ≥ 2	227/95/84
IRRDR of NS5A, $\leq 3/4-5/\geq 6$	73/67/60
<i>Genetic variation near IL28B gene</i>	
rs8099917 genotype, TT/TG/GG	150/65/4

Data represent number of patients with percentages in parentheses, or median (range) values.

The correlation between ISDR and IRRDR was analyzed. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR (*r* = 0.308, *p* < 0.001) (fig. 2).

aa Substitutions in the Core Region and NS5A-ISDR/IRRDR

Concerning the substitution of core aa 70, the number of aa substitutions in ISDR of 256 patients with Arg70 (median 0) was not significantly different from that of 146 patients with Gln70 (His70) (median 0) (fig. 3a). Fur-

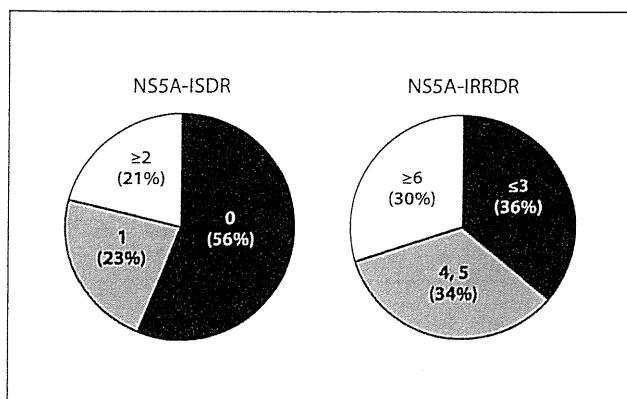


Fig. 1. The number of aa substitutions in NS5A-ISDR and NS5A-IRRDR. The percentage of patients with ≤ 1 aa substitution in ISDR (WT) was 79%.

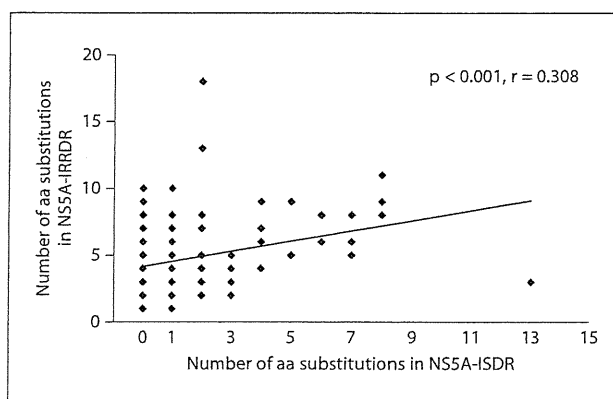


Fig. 2. Correlation between NS5A-ISDR and NS5A-IRRDR. There was a significant positive correlation between the number of aa substitutions in ISDR and that in IRRDR ($r = 0.308$, $p < 0.001$).

thermore, the number of aa substitutions in IRRDR of 123 patients with Arg70 (median 5) was also not significantly different from that of 77 patients with Gln70 (His70) (median 4) (fig. 3b).

Concerning the substitution of core aa 91, the number of aa substitutions in ISDR of 240 patients with Leu91 (median 1) was significantly higher than that of 161 patients with Met91 (median 0) ($p < 0.001$) (fig. 3c). Furthermore, the number of aa substitutions in IRRDR of 111 patients with Leu91 (median 5) was significantly higher than that of 89 patients with Met91 (median 3) ($p < 0.001$) (fig. 3d).

Viremia Level and aa Substitutions in Core Region/ISDR/IRRDR

Concerning the number of substitutions in ISDR, viremia levels of 321 patients with WT (median 6.5) were significantly higher than those of 84 patients with non-WT (median 5.7) ($p < 0.001$) (fig. 4a).

Concerning the number of substitutions in IRRDR, viremia levels of 140 patients with ≤ 5 substitutions (median 6.4) were significantly higher than those of 60 patients with ≥ 6 (median 6.1) ($p = 0.027$) (fig. 4b).

Concerning the substitution of core aa 70, viremia levels of 265 patients with Arg70 (median 6.4) were not significantly different from those of 151 patients with Gln70 (His70) (median 6.3) (fig. 4c).

Concerning the substitution of core aa 91, viremia levels of 169 patients with Met91 (median 6.5) were significantly higher than those of 245 patients with Leu91 (median 6.2) ($p = 0.028$) (fig. 4d).

Thus, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

Treatment Response according to the Number of aa Substitutions in IRRDR

Concerning the number of aa substitutions in IRRDR, a significantly higher proportion of patients with ≥ 4 aa substitutions (58%) showed SVR compared to patients with ≤ 3 (42%) ($p = 0.039$). In contrast, the SVR rate was not significantly different between patients with ≤ 4 (49%) and those with ≥ 5 (57%) aa substitutions. Likewise, the SVR rate was not significantly different between patients with ≤ 5 (51%) and those with ≥ 6 (55%) aa substitutions (fig. 5a).

The ETR rate was not significantly different between patients with ≤ 3 (74%) and those with ≥ 4 (82%) aa substitutions, nor between patients with ≤ 4 (76%) and those with ≥ 5 (83%). Likewise, the ETR rate was not significantly different between those with ≤ 5 (79%) and those with ≥ 6 (80%) aa substitutions (fig. 5b).

The SVR rate after ETR was not significantly different between patients with ≤ 3 (61%) and those with ≥ 4 (74%) aa substitutions, nor between patients with ≤ 4 (67%) and those with ≥ 5 (72%). Likewise, they were not significantly different between patients with ≤ 5 (67%) and those with ≥ 6 (75%) aa substitutions (fig. 5c).

Thus, it was useful as predictor of SVR to categorize into two groups of ≤ 4 and ≥ 5 aa substitutions by univariate analysis. However, the ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

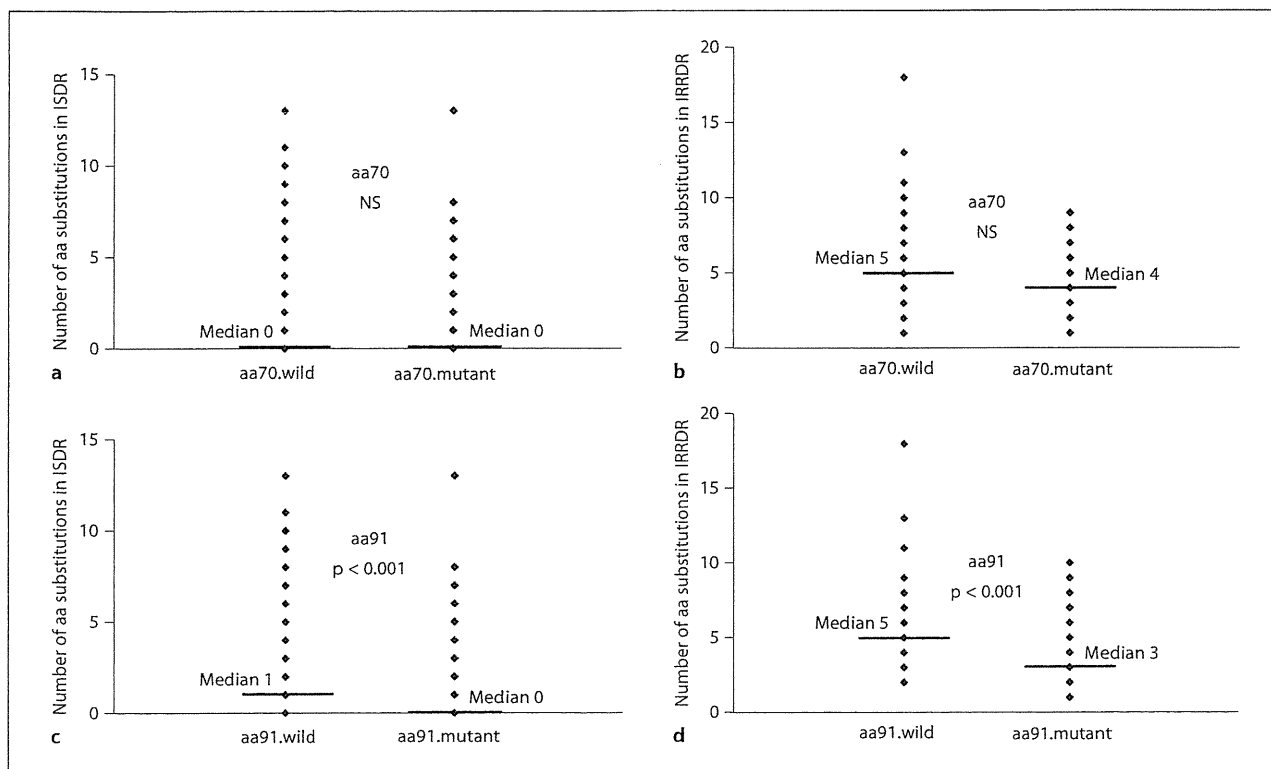


Fig. 3. aa substitutions in the core region and NS5A-ISDR/IRRDR. **a, b** Concerning the substitution of core aa 70, the number of aa substitutions in ISDR/IRRDR of patients with Arg70 was not significantly different from that of patients with Gln70 (His70). **c, d** Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91 ($p < 0.001$).

Predictors of SVR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 15 parameters that correlate with SVR: gender (male sex; $p < 0.001$), age (< 55 years; $p < 0.001$), ribavirin dose (≥ 11.0 mg/kg; $p = 0.006$), AST (< 58 IU/l; $p = 0.039$), leukocyte count ($\geq 4,500/\text{mm}^3$; $p = 0.043$), hemoglobin (≥ 14.0 g/dl; $p = 0.001$), platelet count ($\geq 15.0 \times 10^4/\text{mm}^3$; $p < 0.001$), GGT (< 50 IU/l; $p = 0.028$), uric acid (≥ 5.5 mg/dl; $p = 0.005$), level of viremia (< 6.0 log IU/ml; $p < 0.001$), α -fetoprotein (< 10 $\mu\text{g/l}$; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), the number of aa substitutions in ISDR (non-WT; $p < 0.001$) and IRRDR (≥ 4 ; $p = 0.039$). Figure 6 shows the SVR 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 3 parameters that independently influenced

SVR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), gender (male sex; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.027$) (table 2).

Predictors of ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 14 parameters that correlated with ETR: gender (male sex; $p = 0.001$), age (< 55 years; $p = 0.004$), AST (< 39 IU/l; $p = 0.027$), hemoglobin (≥ 14.0 g/dl; $p = 0.035$), platelet count ($\geq 15.0 \times 10^4/\text{mm}^3$; $p < 0.001$), albumin (≥ 3.9 g/dl; $p = 0.014$), GGT (< 50 IU/l; $p < 0.001$), uric acid (≥ 5.5 mg/dl; $p = 0.003$), level of viremia (< 6.0 log IU/ml; $p = 0.001$), low-density lipoprotein cholesterol (≥ 85 mg/dl; $p = 0.004$), α -fetoprotein (< 10 $\mu\text{g/l}$; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.021$). Figure 7 shows the ETR rate according to aa

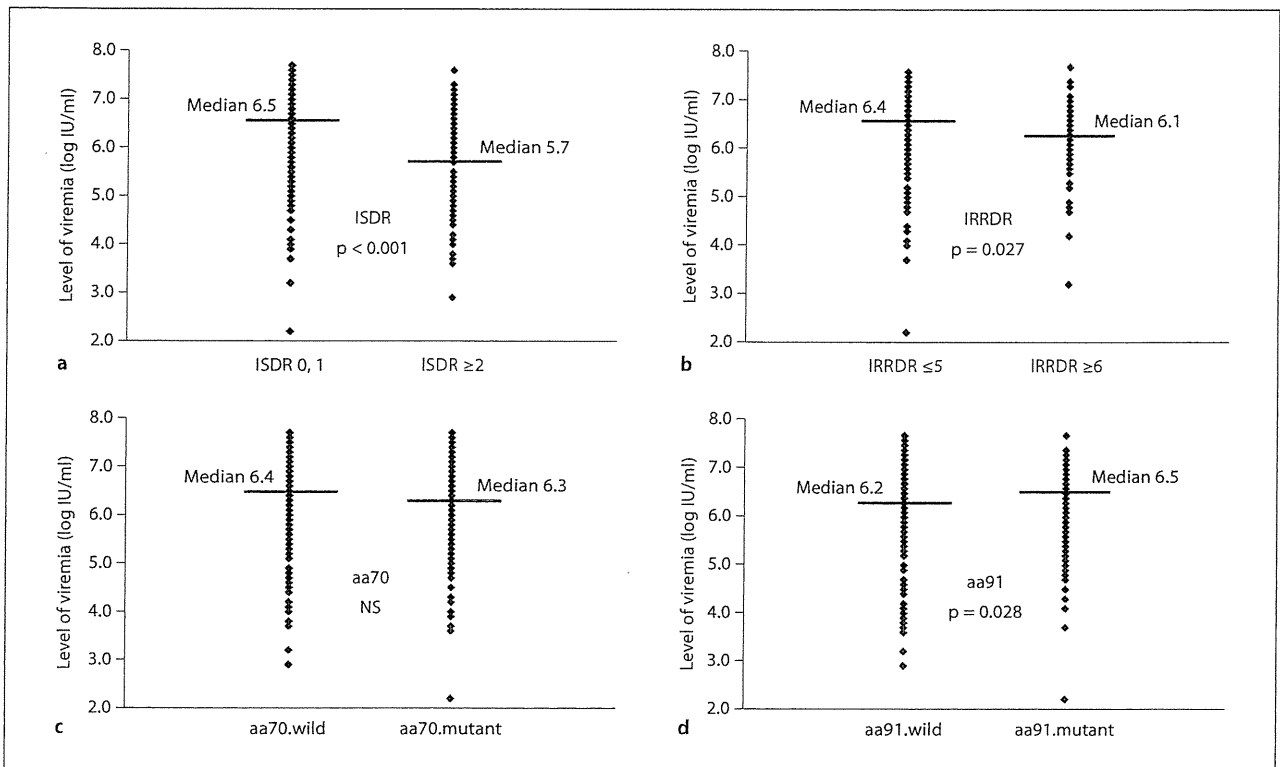


Fig. 4. Viremia level and aa substitutions in core region/ISDR/IRRDR. **a** Concerning the number of substitutions in ISDR, viremia levels of patients with WT were significantly higher than those of patients with non-WT ($p < 0.001$). **b** Concerning the number of substitutions in IRRDR, viremia levels 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 Leu91 ($p = 0.028$). Thus, levels of viremia might be influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

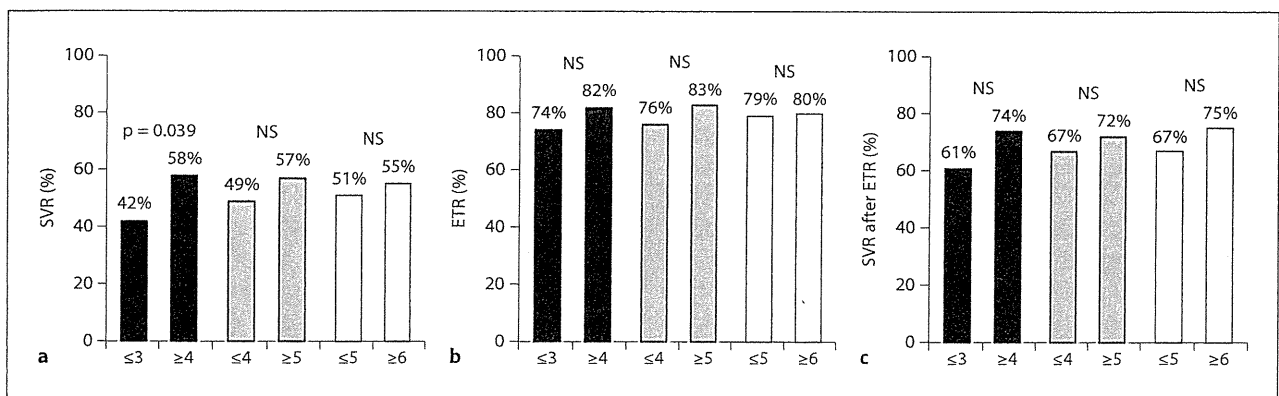


Fig. 5. Treatment response according to the number of aa substitutions in NS5A-IRRDR. **a** A significantly higher proportion of patients with ≥ 4 (58%) aa substitutions showed SVR compared to patients with ≤ 3 (42%) ($p = 0.039$), and it was useful as predictor

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.

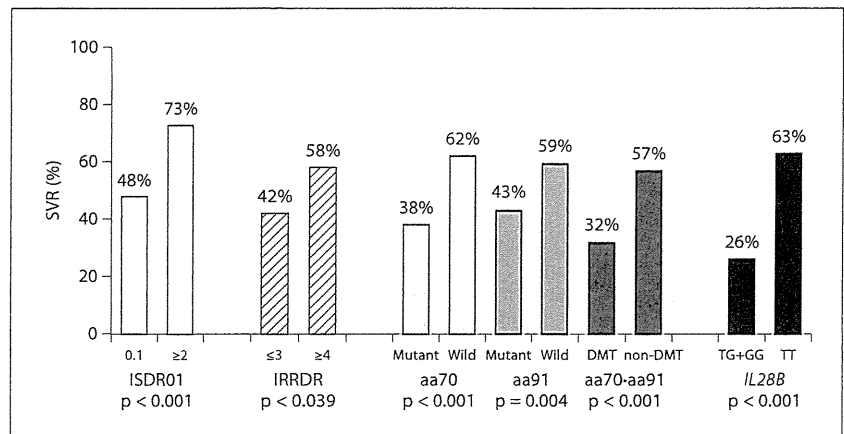


Fig. 6. SVR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

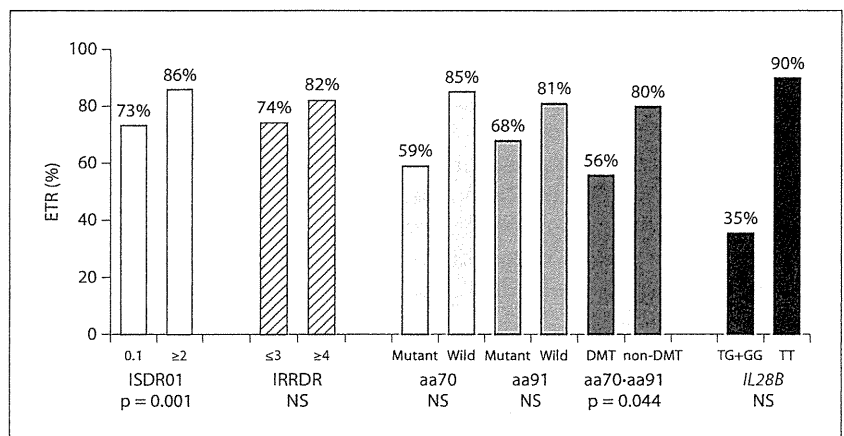


Fig. 7. ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

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	
	2: TT	16.7 (4.54–61.3)	<0.001
Gender	1: Female	1	
	2: Male	10.5 (3.47–32.3)	<0.001
ISDR of NS5A	1: WT	1	
	2: Non-WT	5.68 (1.22–26.3)	0.027

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	
	2: TT	18.2 (6.29–52.6)	<0.001
Level of viremia log IU/ml	1: ≥ 6.0	1	
	2: < 6.0	9.20 (2.59–32.6)	0.001
Core aa 70	1: Gln70 (His70)	1	
	2: Arg70	4.68 (1.65–13.3)	0.004
Serum albumin g/dl	1: < 3.9	1	
	2: ≥ 3.9	3.08 (1.11–8.47)	0.030

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

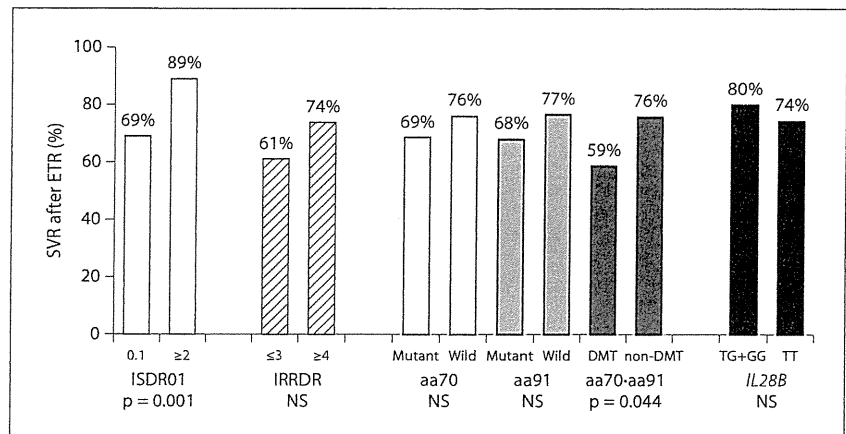


Fig. 8. SVR after ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

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	1	<0.001
	2: Male	4.27 (2.15–8.55)	
Ribavirin dose, mg/kg	1: <11.0	1	0.002
	2: ≥11.0	2.95 (1.48–5.86)	
ISDR of NS5A	1: WT	1	0.012
	2: Non-WT	4.00 (1.35–11.8)	
Core aa 70 and 91	1: Gln70 (His70) and Met91	1	0.023
	2: Arg70 and/or Leu91	2.96 (1.16–7.52)	
Platelet count × 10 ⁴ /mm ³	1: <15.0	1	0.033
	2: ≥15.0	2.19 (1.07–4.50)	
α-Fetoprotein μg/l	1: ≥10	1	0.042
	2: <10	2.66 (1.04–6.80)	

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	Core aa 70 and 91 p = 0.023, 2.96 (1.16–7.52) ^a	
	Level of viremia p = 0.001, 9.20 (2.59–32.6) ^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	Gender p < 0.001, 10.5 (3.47–32.3) ^a
		Ribavirin dose p = 0.002, 2.95 (1.48–5.86) ^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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Influence of *ITPA* Polymorphisms on Decreases of Hemoglobin During Treatment with Pegylated Interferon, Ribavirin, and Telaprevir

Fumitaka Suzuki,¹ Yoshiyuki Suzuki,¹ Norio Akuta,¹ Hitomi Sezaki,¹ Miharuru Hirakawa,¹ Yusuke Kawamura,¹ Tetsuya Hosaka,¹ Masahiro Kobayashi,¹ Satoshi Saito,¹ Yasuji Arase,¹ Kenji Ikeda,¹ Mariko Kobayashi,² Kazuaki Chayama,³ Naoyuki Kamatani,⁴ Yusuke Nakamura,⁵ Yuzo Miyakawa,⁶ and Hiromitsu Kumada¹

Polymorphisms of the inosine triphosphatase (*ITPA*) gene influence anemia during pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy, but their effects during triple therapy with PEG-IFN, RBV, and telaprevir are not known. Triple therapy for 12 weeks, followed by PEG-IFN and RBV for 12 weeks, was given to 49 patients with RBV-sensitive (CC at rs1127354) and 12 with RBV-resistant (CA/AA) *ITPA* genotypes who had been infected with hepatitis C virus (HCV) of genotype 1. Decreases in hemoglobin levels were greater in patients with CC than CA/AA genotypes at week 2 (-1.63 ± 0.92 vs. -0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (-3.5 ± 1.1 vs. -2.2 ± 0.96 , $P = 0.001$), as well as at the end of treatment (-2.9 ± 1.1 vs. -2.0 ± 0.86 , $P = 0.013$). Risk factors for hemoglobin <11.0 g/dL at week 4 were female gender, age >50 years, body mass index (BMI) <23, and CC at rs1127354 by multivariate analysis. RBV dose during the first 12 weeks was smaller in patients with CC than CA/AA genotypes ($52 \pm 14\%$ vs. $65 \pm 21\%$ of the target dose, $P = 0.039$), but the total RBV dose was no different between them ($49 \pm 17\%$ and $54 \pm 18\%$ of the target, $P = 0.531$). Sustained virological response (SVR) was achieved in 70% and 64% of them, respectively ($P = 0.724$). **Conclusion:** *ITPA* polymorphism influences hemoglobin levels during triple therapy, particularly during the first 12 weeks while telaprevir is given. With careful monitoring of anemia and prompt adjustment of RBV dose, SVR can be achieved comparably frequently between patients with CC and CA/AA genotypes. (HEPATOLOGY 2011;53:415-421)

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IL28B, interleukin 28B; *ITPA*, inosine triphosphatase; PEG-IFN, pegylated interferon; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response.

From the ¹Department of Hepatology, Toranomon Hospital, Tokyo, Japan; ²Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan; ³Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan; ⁴Laboratory for Statistics, RIKEN Center for Genomic Medicine, Yokohama, Japan; ⁵Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; ⁶Miyakawa Memorial Research Foundation, Tokyo, Japan.

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Address reprint requests to: Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. E-mail: fmitakas@toranomon.gr.jp; fax: +81-44-860-1623.

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Worldwide, 123 million people are estimated to have been infected with hepatitis C virus (HCV),¹ and $\approx 30\%$ of them develop fatal liver disease such as cirrhosis and hepatocellular carcinoma.^{2,3} Currently, the standard of care therapy for patients infected with HCV is pegylated interferon (PEG-IFN) and ribavirin (RBV) for 48 weeks.⁴⁻⁶ However, the combined treatment can induce a sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after treatment completion, in at most 50% of patients infected with HCV-1, the genotype most prevalent and least responsive to IFN-based therapies.

Recently, Fellay et al.⁷ reported that polymorphisms of the inosine triphosphatase (*ITPA*) gene in chromosome 20 (20p13) influence RBV-induced anemia in a genome-wide association study (GWAS). Single nucleotide polymorphism (SNP) at rs1127354 for proline-to-threonine substitution (P32T) in the second of eight

exons in the *ITPA* gene, as well as that at rs7270101 in the second intron, affects the expression of *ITPA*.⁸⁻¹¹ Patients infected with HCV-1 carrying the CC genotype at rs1127354 are more prone to develop anemia than those with CA/AA genotypes during the combination therapy, and the decrease in hemoglobin is greater in patients with the AA than AC/CC genotypes at rs7270101.⁷ Their observations have been extended to many patients in a large-scale trial with pegIFN- α -2a on Caucasian and African Americans,¹² as well as in the Japanese receiving PEG-IFN- α -2b and RBV who were infected with HCV-1.¹³

For improving SVR in HCV-1 patients, protease inhibitors have been added to the standard treatment with PEG-IFN and RBV, and increased SVR by $\approx 20\%$.¹⁴⁻¹⁶ However, such a gain in efficacy is not without trade-offs, represented by aggravation of anemia. Early decreases in hemoglobin levels during the triple therapy reach 4 g/dL, and they exceed ≈ 3.0 g/dL in the standard treatment.^{14,15} Because there have been no reports focusing on the influence of *ITPA* genotypes on anemia developing in patients during triple therapy, hemoglobin levels were followed in 61 Japanese patients with HCV-1 who had received it. The results were correlated with polymorphisms at rs1127354 in the *ITPA* gene because the Japanese are monoallelic at rs7270101 and have the AA genotype exclusively.¹¹

Patients and Methods

Study Cohort. This retrospective cohort study was performed in 61 patients with chronic hepatitis C who met the following inclusion and exclusion criteria. Inclusion criteria were: (1) diagnosed with chronic hepatitis C; (2) HCV-1 confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels ≥ 5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (4) Japanese aged from 20 to 65 years at the entry; and (5) body weight between ≥ 40 kg and ≤ 120 kg at the time of registration. Exclusion criteria were: (1) decompensated liver cirrhosis; (2) hepatitis B surface antigen in serum; (3) hepatocellular carcinoma or its history; (4) autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis C; (5) chronic renal disease or creatinine clearance ≤ 50 mL/min at the baseline; (6) hemoglobin ≤ 12 g/dL, neutrophil $\leq 1,500/\text{mm}^3$ or platelet $\leq 100,000/\text{mm}^3$ at baseline.

Of the 61 patients, 44 (72%) had received IFN-based treatment before. Relapse occurred in 29 (47%) and the remaining 15 (25%) did not respond (null-

responders). All patients gave consent for analysis of SNPs in *ITPA* and interleukin 28 (*IL28B*) genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Toranomon Hospital. Written informed consent was obtained from each patient.

Triple Treatment with PEG-IFN- α -2b, RBV, and Telaprevir. Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan), 750 mg, was administered 3 times a day at an 8-hour (q8) interval after each meal. Pegylated-IFN- α -2b (PEG-Intron, Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose of 1.5 $\mu\text{g}/\text{kg}$ (range: 1.32-1.71 $\mu\text{g}/\text{kg}$) once a week. RBV (Rebetol, Schering Plough) 200-600 mg was administered after breakfast and dinner. The RBV dose was adjusted by body weight: 600 mg for ≤ 60 kg; 800 mg for >60 kg $\approx \leq 80$ kg; and 1,000 mg for ≥ 80 kg. The triple therapy with PEG-IFN- α -2b, RBV, and telaprevir was continued for 12 weeks, and then switched to PEG-IFN- α -2b and RBV for an additional 12 weeks. It was withdrawn when hemoglobin levels decreased < 8.5 g/dL. After the therapy was completed or discontinued, patients were followed for 24 weeks for SVR.

The RBV dose was cut by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1,000 mg) when hemoglobin decreased < 12 g/dL, and by another 200 mg when it was below < 10 g/dL. In addition, RBV was reduced by 200 mg in patients with hemoglobin < 13 g/dL at baseline and those in whom it decreased by 1 g/dL to < 13 g/dL within a week. PEG-IFN dose was reduced by one-half when the leukocyte count decreased $< 1,500/\text{mm}^3$, neutrophil count $< 750/\text{mm}^3$, or platelet count $< 80 \times 10^3/\text{mm}^3$; PEG-IFN was withdrawn when they decreased $< 1,000/\text{mm}^3$, $500/\text{mm}^3$, or $50 \times 10^3/\text{mm}^3$, respectively.

The triple therapy was withdrawn or stopped temporarily when hemoglobin decreased < 8.5 g/dL. In patients in whom hemoglobin increased ≥ 8.5 g/dL within 2 weeks after the withdrawal, treatment was resumed with PEG-IFN and RBV 200 mg. A reduction of telaprevir (MP-424) dose was not permitted. It was discontinued when severe side effects appeared, whereas PEG-IFN and RBV were continued. Growth factors were not used for elevating hemoglobin levels.

Determination of *ITPA* Genotypes. *ITPA* (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described.^{17,18}

Statistical Analyses. Continuous variables between groups were compared by the Mann-Whitney test (*U* test), and discontinuous variables by the chi-square test

Table 1. Baseline Characteristics of the 61 Patients Infected with HCV-1 Who Received Triple Therapy with Pegylated-Interferon, Ribavirin, and Telaprevir

	Total	<i>ITPA</i> Genotypes at rs1127354	
		CC	CA + AA
Demographic data			
Number	61	49	12
Sex (male/female)	34/27	28/21	6/6
Age (years)	56 (23-65)	55 (23-65)	58 (28-62)
Body weight (kg)	61.5 (41.0-92.9)	61.5 (41.0-92.9)	62.1 (44.4-81.1)
Body mass index (kg/m ²)	22.6 (17.6-32.4)	22.2 (17.6-32.4)	22.9 (17.8-26.5)
Genotypes of the <i>IL28B</i> gene			
rs8099917 (for 59 patients) (TT/TG + GG)	33/26	27/21	6/7
rs12979860 (for 57 patients) (CC/CT + TT)	30/27	36/22	4/5
Laboratory data			
Hemoglobin (g/dL)	14.4 (12.5-16.6)	14.4 (12.5-16.6)	14.2 (12.8-16.3)
Platelets (x 10 ⁴ /mm ³)	17.8 (9.1-33.8)	17.7 (9.1-33.8)	19.5 (13.1-31.6)
Albumin (g/dL)	3.9 (3.2-4.6)	3.9 (3.2-4.6)	3.9 (3.5-4.1)
Alanine aminotransferase (U/L)	39 (12-175)	41 (12-175)	28 (17-57)
Aspartate aminotransferase (U/L)	32 (15-137)	35 (15-137)	28 (20-35)
HCV RNA (log IU/mL)	6.7 (5.1-7.6)	6.8 (5.7-7.6)	6.6 (5.1-7.5)
HCV genotype 1a/1b	1/60	1/48	0/12
Previous IFN-based treatment			
Treatment naïve	17	12 (24%)	5 (42%)
Relapsed	29	23 (47%)	6 (50%)
Null response	15	14 (29%)	1 (8%)

Data are median values (range) or n.

and Fisher's exact test. Kaplan-Meier analysis and the log-rank test were applied to estimate and compare decreases of RBV dose between groups. Factors evaluated for influence on hemoglobin decrease by univariate analysis were: sex; age; body mass index (BMI); body weight; hemoglobin levels; initial PEG-IFN and RBV doses; amino acid substitutions in the HCV core protein; number of amino acid substitutions in the interferon sensitivity determining region; and *IL28B* polymorphisms (at rs8099917 and rs12979860). Factors associated with a decrease in hemoglobin levels ($P < 0.10$) were assessed by multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence interval (CI) was determined. All analyses were performed using SPSS software (SPSS II v. 11.0, Chicago, IL), and a P -value < 0.05 was considered significant.

Results

Triple Therapy in Patients with HCV-1 Infection. Baseline characteristics of the 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 in the *ITPA* gene are compared in Table 1. They all were infected with HCV-1. There were no significant differences between them, except that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were higher in patients with CC than

CA/AA genotypes ($P = 0.041$ and $P = 0.008$, respectively). Overall, *IL28B* genotypes resistant to PEG-IFN and RBV, TT/TG at rs8099917, and CC/CT at rs12979860 were rather frequent, and possessed by 44% and 47%, respectively, of the patients. This was due to inclusion of 15 nonresponders to previous IFN-based therapies, corresponding to 25% of the 61 patients studied, most of whom (14/15 [93%]) possessed IFN-resistant genotypes (TT/TG and CC/CT). Six of them had low hemoglobin levels (< 13 g/dL) at baseline and were started with an RBV dose decreased by 200 mg; they included five with CC and one with CA genotypes of the *ITPA* gene.

Modification of RBV Dose During Triple Therapy. RBV dose was reduced by ≥ 200 mg in all 61 patients studied during triple therapy because hemoglobin had decreased < 12.0 g/dL in them. During the first 12 weeks of therapy while telaprevir was given, the proportion of patients receiving the full RBV dose differed between those with CC and CA/AA genotypes (Fig. 1). RBV dose reduction was started earlier in the 49 patients with CC than the 12 with CA/AA genotypes (2.6 ± 1.3 vs. 4.8 ± 3.1 weeks after the start, respectively, $P = 0.010$). Thus, during the first 12 weeks with telaprevir the RBV dose was smaller in patients with CC than CA/AA genotypes ($52 \pm 14\%$ vs. $65 \pm 21\%$ of the target dose, $P = 0.039$). During the next 12

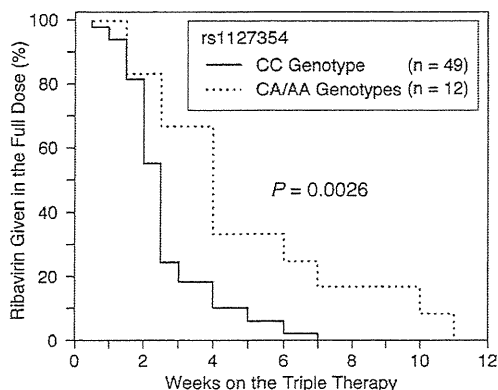


Fig. 1. Patients who received the full ribavirin dose during 12 weeks on triple therapy. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared.

weeks without telaprevir, in contrast, the RBV dose was somewhat larger in patients with CC than CA/AA genotypes ($47 \pm 24\%$ vs. $43 \pm 20\%$, $P = 0.649$). The total RBV dose during 24 weeks on therapy was comparable between the 49 patients with CC and the 12 with CA/AA genotypes ($49 \pm 17\%$ vs. $54 \pm 18\%$, $P = 0.531$). In patients with the CC genotype, the RBV dose was no different between those who achieved SVR and those who did not ($50 \pm 18\%$ vs. $47 \pm 13\%$, $P = 0.728$). The RBV dose did not differ either in patients with CA/AA genotypes with and without SVR ($57 \pm 17\%$ vs. $48 \pm 20\%$, $P = 0.368$).

The total dose of PEG-IFN was comparable among 49 patients with CC and 12 with CA/AA genotypes ($87 \pm 23\%$ vs. $86 \pm 20\%$ of the target, $P = 0.488$). The total telaprevir dose was no different either between them ($87 \pm 27\%$ vs. $71 \pm 36\%$ of the target, $P = 0.098$). Telaprevir was discontinued in 10 of the 49 (20%) patients with CC and 5 of the 12 (42%) with CA/AA genotypes ($P = 0.147$).

Decreases in Hemoglobin Levels During Triple Therapy. Figure 2 compares decreases in hemoglobin levels between 49 patients with CC and 12 with CA/AA genotypes of the *ITPA* gene. Data of six patients were omitted because the triple therapy was withdrawn 4–10 weeks after the start, including five with CC and one with CA genotype. Hemoglobin decreased more in patients with CC than CA/AA genotypes at week 2 (-1.63 ± 0.92 vs. -0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (-3.5 ± 1.1 vs. -2.2 ± 0.96 , $P = 0.001$). During week 8 through 12, hemoglobin reached the nadir of approximately -4 g/dL both in patients with CC and CA/AA genotypes. Thereafter, differences in hemoglobin decrease started to widen between patients with CC and CA/AA genotypes and

were significant at week 20 (-3.0 ± 1.2 vs. -2.4 ± 0.88 g/dL, $P = 0.048$) and week 24 (-2.9 ± 1.1 vs. -2.0 ± 0.85 g/dL, $P = 0.013$).

SVR was achieved by 35 (71%) of the 49 patients with CC and 8 (67%) of the 12 with CA/AA genotypes ($P = 0.736$). Hemoglobin levels did not differ between them 24 weeks after the completion of triple therapy (-0.57 ± 1.1 vs. -0.17 ± 0.87 g/dL, $P = 0.271$). Of the 32 patients with TT genotype of the *IL28B* gene at rs8099917, 30 (94%) gained SVR, more frequently than 10 of the 26 (38%) with TG/GG genotypes ($P < 0.001$). Likewise, 29 of the 30 (97%) patients with CC genotype at rs12979860 achieved SVR, more frequently than 11 of the 27 (41%) with CT/TT genotypes ($P < 0.001$).

Factors Influencing Decreases in Hemoglobin Levels. Hemoglobin decreased <11 g/dL at week 4 during the triple therapy in 27 of the 61 (44%) patients. Factors for hemoglobin <11.0 g/dL were female gender, age >50 years, body weight <60 kg, BMI <23 , and baseline hemoglobin <15 g/dL, as well as the CC genotype of the *ITPA* gene, in the univariate analysis (Table 2). Of them, female gender, age >50 years, BMI <23 , and the CC genotype remained significant in the multivariate analysis. Hemoglobin levels lowered <8.5 g/dL during the triple therapy in 13 of the 61 (21%) patients. Factors for hemoglobin <8.5 g/dL were female gender, age >60 years, body weight <60 kg, BMI <23 , and baseline hemoglobin <14 g/dL in the univariate analysis (Table 3). Of

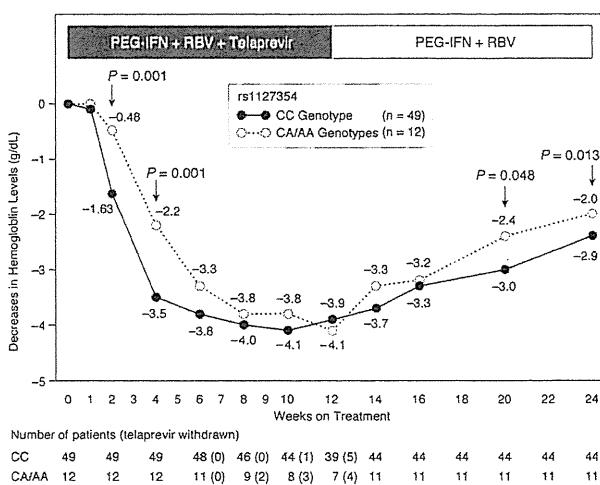


Fig. 2. Decreases in hemoglobin levels during triple therapy with telaprevir, PEG-IFN, and RBV. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared. Patients evaluated at each timepoint are indicated below, with the number of patients in whom telaprevir was withdrawn (PEG-IFN and RBV continued) in parentheses.

Table 2. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Low Hemoglobin Levels (< 11.0 g/dL) at Week 4 of Triple Therapy

Parameter	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	14.3 (4.1-50.0)	< 0.001	29.41 (3.8-250.0)	0.001
Age (> 50 years)	4.3 (1.0-17.5)	0.030	7.3 (1.1-47.6)	0.039
Body weight (< 60 kg)	11.5 (3.4-38.2)	< 0.001		
Body mass index (< 23)	8.4 (2.6-27.1)	< 0.001	17.2 (2.6-112.0)	0.003
Hemoglobin (< 15g/dL)	14.2 (3.5-57.4)	< 0.001		
<i>ITPA</i> gene (CC genotype)		0.062	36.8 (2.5-550.2)	0.009

Abbreviations: OR, odds ratio; CI, confidence level.

them, only age and body weight remained significant in the multivariate analysis.

Discussion

Anemia is a substantial risk in the standard of care therapy with PEG-IFN and RBV.⁴⁻⁶ Triphosphorylated RBV accumulates in erythrocytes of patients who receive RBV, increasingly with RBV dose and duration, and causes oxidative damage to erythrocyte membranes toward extravascular hemolysis by the reticuloendothelial system.^{19,20} Inosine triphosphate accumulates also in erythrocytes of individuals who have mutations in the *ITPA* gene, and results in benign red-cell enzymopathy.⁸ The expression of *ITPA* is genetically controlled and reduced in individuals who have point mutations in the *ITPA* gene.⁸⁻¹¹ As another achievement of GWAS in hepatology,²¹ in the wake of polymorphisms of the *IL28B* gene that influence the response to PEG-IFN and RBV,²²⁻²⁴ polymorphisms in the *ITPA* gene has been reported to influence anemia caused by RBV.⁷ How inosine triphosphate protects erythrocytes from hemolysis caused by RBV needs to be sorted out by *in vivo* and *in vitro* experiments. Inosine triphosphate may prohibit the accumulation of RBV in erythrocytes, or rather, it might act directly toward prohibition of hemolysis.

In the present study, 61 patients infected with HCV-1 received triple therapy with PEG-IFN, RBV, and telaprevir in the first 12 weeks followed by PEG-IFN and RBV in the second 12 weeks. Then the RBV dose and hemoglobin were compared between patients with CC and CA/AA genotypes in the *ITPA* gene. Two polymorphisms in the *ITPA* gene, in close linkage disequilibrium with an r^2 value of 0.65,⁷ have been recognized in Caucasians (rs1127354 and rs7270107); the respective CA/AA and AC/CC genotypes decrease the activity of inosine triphosphatase and protect against anemia induced by RBV.^{7,12} Because the Japanese are monoallelic at rs7270107 and possess the AA

genotype exclusively,^{11,25} only polymorphisms at rs1127354 were examined.

Of the 61 patients, 49 possessed the RBV-sensitive CC genotype and the remaining 12 had RBV-resistant CA/AA genotypes. Hemoglobin levels decreased both in patients with CC and CA/AA genotypes. They lowered ≈ 4 g/dL during weeks 8-12 on the triple therapy with telaprevir, and increased thereafter (Fig. 2). Between the two groups of patients, differences in hemoglobin decrease were greatest at week 4 (1.3 g/dL), as in the standard treatment with PEG-IFN and RBV.^{7,12,13}

When anemia and other side effects occurred, doses of RBV, PEG-IFN, and telaprevir were modified. Of the 61 patients studied, 27 (44%) were women and most of them were in old age. Beyond 50 years of age, women are less responsive than men to the standard treatment with PEG-IFN and RBV, probably because estrogens with an antifibrotic potential decrease after menopause.²⁶ Stringent precautions had to be taken, therefore, by reducing the RBV dose in the patients in whom hemoglobin levels decreased <12 g/dL, rather than the conventional threshold of <10 g/dL.

Reductions of RBV dose due to anemia in patients who receive PEG-IFN and RBV are influenced by *ITPA* polymorphisms.¹² Also, in patients who had received the triple therapy the RBV dose had to be reduced more in

Table 3. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Very Low Hemoglobin Levels (<8.5 g/dL) During Triple Therapy

Parameter	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	6.1 (1.5-25.1)	0.007		
Age (>60 years)	6.8 (1.8-26.0)	0.004	10.1 (1.9-53.9)	0.007
Body weight (<60 kg)	23.8 (2.9-200.0)	<0.001	33.3 (3.4-333.3)	0.003
Body mass index (<23)	14.1 (1.7-125.0)	0.001		
Hemoglobin (<14 g/dL)	4.3 (1.2-15.6)	0.023		

Abbreviations: OR, odds ratio; CI, confidence level.

patients with CC than CA/AA genotypes during the first 12 weeks while they received telaprevir ($52 \pm 14\%$ vs. $65 \pm 21\%$ of the target dose, $P = 0.039$). During the second 12 weeks off telaprevir, the RBV dose was somewhat greater in patients with CC than CA/AA genotypes ($47 \pm 24\%$ vs. $43 \pm 20\%$, $P = 0.649$). Thus, the total RBV dose during 24 weeks of therapy was comparable between patients with CC and CA/AA genotypes ($51 \pm 15\%$ and $57 \pm 18\%$, $P = 0.724$). Likewise, the total dose of PEG-IFN ($87 \pm 23\%$ vs. $86 \pm 20\%$ of the target, $P = 0.806$), as well as that of telaprevir ($87 \pm 27\%$ vs. $71 \pm 36\%$ of the target, $P = 0.098$), was no different between patients with CC and CA/AA genotypes. SVR was achieved comparably frequently in them (71% vs. 67% , $P = 0.736$).

Decreases in hemoglobin levels during the first 12 week were similar between the current triple therapy cohort and previous patients receiving PEG-IFN and RBV.^{12,13} The conservative hemoglobin levels chosen for RBV dose reduction may be a possible confounding factor on the impact of *ITPA* variants in anemia, which would have been greater should the RBV dose not be reduced in patients with RBV-sensitive CC genotypes.

ITPA polymorphisms at rs1127354 were associated with RBV-induced anemia in Japanese patients, without involvement of those at rs7270107 reported in Caucasian and African-American patients.¹³ Thus, *ITPA* polymorphisms at rs1127354 would play a major role in protecting patients from RBV-induced anemia. CC/CA genotypes at rs1127354 occurs in 6% of the Caucasian population, much less often in the Oriental population, at 16%.^{25,27} Although AC/CC genotypes at rs7270107 occurs in 13% of Caucasians, they do not exist in Orientals.^{11,25} Obviously, different polymorphisms need to be examined in patients of distinct ethnicities when the influence on RBV-induced anemia is to be evaluated.

In confirmation of our previous report,²⁸ the triple therapy achieved SVR more frequently in patients with CC than CT/TT genotypes of *IL28* at rs12979860 (96% vs. 41% , $P < 0.001$). About two-thirds of studied patients accomplished SVR with the triple treatment, although one-fourth of them were nonresponders to previous IFN-based treatments; they are known to respond poorly to repeated treatments. This would lend further support to the efficacy of triple therapy being higher than treatment with pegylated IFN and RBV.

There are strong points in this study. First, *ITPA* polymorphisms influence RBV-induced anemia in the triple therapy. Second, polymorphisms at rs1127350, without involvement of those at rs7270107, protect against RBV-induced anemia. Third, the triple therapy can be applied with high efficacy by careful monitoring of hemoglobin

and prompt modification of RBV dose. There are weak points in this study as well. First, it was a retrospective cohort study conducted in a small size of patients, especially those with CA/AA genotypes at rs1127350, and included null-responders to previous IFN-based therapies; the real impact of *ITPA* polymorphisms on RBV-induced anemia may have been obscured. Second, the study was conducted in Japanese patients, and the results may or may not be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, the results presented herein will promote future studies in which the influence of the *ITPA* polymorphism on RBV-induced anemia will be pursued in larger scale and on patients of various ethnicities around the world.

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Efficacy and Safety of Combination Therapy of Natural Human Interferon Beta and Ribavirin in Chronic Hepatitis C patients

Yasuji Arase, Yoshiyuki Suzuki, Fumitaka Suzuki, Naoki Matsumoto, Norio Akuta, Norihiro Imai, Yuya Seko, Hitomi Sezaki, Yusuke Kawamura, Masahiro Kobayashi, Tetsuya Hosaka, Satoshi Saito, Kenji Ikeda, Mariko Kobayashi and Hiromitsu Kumada

Abstract

Objective The aim of this study was to evaluate the efficacy and safety of combination therapy of natural human interferon-beta and ribavirin for patients for whom prior interferon therapy was discontinued due to depression induced by interferon-alpha.

Methods Inclusion criteria were as follows; 1) HCV-genotype 1b, 2) serum HCV RNA level of ≥ 100 KIU/mL, 3) stopping the prior interferon-alpha monotherapy or combination therapy of interferon-alpha and ribavirin due to the appearance of depression. A total of 14 were enrolled in this prospective cohort study. The treatment period of combination therapy was 48 weeks. Depression states, reflected by Beck depression inventories and Hamilton depression rating scale, were assessed during combination therapy. Nonparametric procedures were employed for the analysis of background features of the patients with sustained virological response (SVR) and without SVR. A p value of <0.05 was considered to indicate a significant difference.

Results Five of 14 patients (37.5%) had SVR by the intention to treat analysis. The SVR rate in patients who showed negative HCV RNA at 12 and 24 weeks after the initiation of combination therapy was 100% (4/4) and 83.3% (5/6), respectively. All of the patients continued the combination therapy owing to disappearance of severely adverse events contained the exacerbation of depression. Combination therapy did not yield a statistical difference in Beck depression inventories and Hamilton depression rating scale.

Conclusion The combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

Key words: chronic hepatitis C, depression, natural interferon-beta, ribavirin, HCV genotype 1b

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Introduction

The combination therapy of peginterferon-alpha and ribavirin has been widely recommended as a first choice for chronic hepatitis C patients with high virus-load (1-5). However, one big problem of the combination therapy is the treatment-related side effect (6, 7). In particular, physicians in charge tend to avoid the combination therapy of peginterferon-alpha and ribavirin for chronic hepatitis C pa-

tients with depression or interferon (IFN)-reduced depression.

IFN-beta-related side effects are mild and few compared to therapy of IFN-alpha (6-8). In particular, IFN-beta-induced mental disorders are mild compared to those induced by IFN-alpha (9). Moreover, IFN-beta could be given to elderly patients aged ≥ 70 years because of the mild side effects (10). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with genotype 1b and a high virus load (11, 12). The combination therapy of IFN-

beta and ribavirin has the possibility to show the strong effect for hepatitis C virus (HCV) and mild side effects originating from the treatment (13-15). We have reported that the combination of IFN-beta plus ribavirin therapy is effective and safety for HCV patients with high virus load and depressive state (14). However, the previous study was retrospective and a prospective study is necessary to evaluate the efficacy and safety of combination therapy of IFN-beta and ribavirin for HCV patients with high virus load and depressive state.

Thus, in the present study, we performed a prospective study to examine the efficacy and safety of combination therapy of IFN-beta and ribavirin in HCV genotype 1b patients who had stopped the IFN therapy due to depression induced by IFN-alpha. At the same time, depression states, reflected by Beck depression inventories (BDI) and Hamilton depression rating scale (Ham-D), were assessed during combination therapy (16, 17).

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of ≥ 100 KIU/mL before treatment; 3) stopping of IFN-alpha therapy due to depression appearance during the prior IFN-alpha treatment; 4) Ham-D of < 18 ; 5) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 6) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 7) white blood cell (WBC) $> 2,000/\text{mm}^3$, platelet count $> 80,000/\text{mm}^3$, and bilirubin < 2.0 mg/mL; follow up for > 6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy of IFN-beta and ribavirin as well as the potential adverse reactions to each patient and informed consent was obtained from each patient. This study was approved by the Human Ethics Review Committee of Toranomon Hospital.

From December 2007 to May 2008, 14 HCV patients were enrolled in this prospective cohort study at the study hospital. A sustained virological response (SVR) was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (18).

Laboratory investigation

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80°C until analysis. Using these blood samples, HCV-RNA level

before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (19). Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (18). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (20). The core protein of HCV-1b was determined by the previous report (21). Next, the genetic variations near the IL28B gene (rs8099917), reported as the pre-treatment predictors of treatment efficacy and clinical outcome, were investigated (22-26). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Clinical evaluation and biochemical and hematological tests were performed at 1, 2, and 4 weeks in the first month after the initiation of combination therapy. After that, these evaluations were done at monthly intervals. The patients were followed by both physicians of hepatology and psychiatry.

Combination therapy of IFN-beta and ribavirin

Treatment was provided for 48 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) by six times a week for 4 weeks, followed by three times a week for 44 weeks. The total dose was 936MU. Ribavirin (Rebetol, MSD KK., Tokyo, Japan) was given at the dose prescribed based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for ≤ 60 kg, 800 mg for > 60 kg and ≤ 80 kg, and 1,000 mg for > 80 kg).

Evaluation of the psychic state

The psychiatrist in charge evaluated the scores of BDI and Ham-D prospectively. BDI shows the subjective symptom of the depressive patients and Ham-D shows the objective evaluation by the psychiatrist. Scores on the BDI were divided the following; severe, 29-63; moderate, 20-28; mild, 14-19; and minimal, 0-13. Scores on the Ham-D were divided the following; very severe, > 23 ; severe, 19-22; moderate, 14-18; mild, 8-13; and normal ≤ 7 (27).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher's exact test. The following variables were evaluated as prognostic factors: sex, age, BDI score, Ham-D score, a HCV RNA level, IL28B (genetic variation in rs8099917), variation of HCV-core, biochemical factors (AST, ALT, gamma glutamyltransferase, total cholesterol), white blood cell (WBC), hemoglobin, platelet count, HCV RNA 4, 12, 24 week after the initiation of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of < 0.05 was considered to indicate a significant difference.

Table 1. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR *

	Total	SVR (n=5)	Non-SVR (n=9)	p value [†]
Age (years old)	62.1 ± 4.3	62.4 ± 4.2	61.9 ± 4.6	0.797
Sex (male/female)	6/8	2/3	4/5	0.898
Previous IFN therapy (combination/monotherapy)	8/6	3/2	5/4	0.898
Duration of previous IFN therapy (week)	11.9 ± 7.8	11.6 ± 10.2	12.0 ± 7.1	0.699
HCV-RNA (KIU/mL)	2588± 1455	2228± 1807	2788± 1296	0.759
Core aa70 (Wild/Mutant)	6/8	3/2	3/6	0.438
BDI score	11.9 ± 10.3	12.2 ± 14.2	11.7 ± 8.4	0.518
Ham-D score	3.5 ± 4.1	3.6 ± 5.5	3.4 ± 3.5	0.606
IL28B (genetic variation in rs8099917, genotype TT/TGorGG)	7/7	5/0	2/7	0.042
AST (IU/L)	50 ± 24	46 ± 37	52 ± 17	0.112
ALT (IU/L)	68 ± 33	60 ± 35	72 ± 32	0.518
GGT (IU/L)	55 ± 59	25 ± 5	72 ± 69	0.813
Total cholesterol (mg/dL)	175 ± 30	166 ± 35	179 ± 28	0.298
White blood cell(10 ³ /mm ³)	4.39 ± 1.24	4.16 ± 1.02	4.52 ± 1.39	0.898
Hemoglobin (g/dL)	14.1 ± 1.1	14.2 ± 1.5	14.0 ± 0.9	0.898
Platelet(10 ⁴ /mm ³)	15.8 ± 4.8	19.9 ± 2.4	13.5 ± 4.1	0.019
HCV RNA (+/-) 4W	11/3	2/3	9/0	0.083
HCV RNA (+/-) 12W	10/4	1/4	9/0	0.012
HCV RNA (+/-) 24W	8/6	0/5	8/1	0.004

Data are number of patients (percentage) or mean ± standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDI, Beck depression inventories; GGT, gamma-glutamyltransferase; Ham-D, Hamilton depression rating; HCV, hepatitis C virus;

*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks.

[†]Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher' exact test.

Result

Clinical characteristics of the patients

A total of 14 patients treated with IFN-beta +ribavirin were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =62.1 years, male/female =6/8, and HCV-RNA =2,588±1,455 KIU/mL. Patients were classified into two groups according to the difference of response: SVR (n=5), Non-SVR (n=9).

Efficacy of treatment

Five of 14 patients (37.5%) had SVR by the intention to treat analysis. Table 1 shows the differences in the clinical background between patients with SVR and those without SVR. The negativity rate of HCV RNA 12 weeks after the initiation of combination therapy was 80% (4/5) in SVR group and 0%(0/9) in Non-SVR group (p=0.012). The negativity rate of HCV RNA 24 weeks after the initiation of combination therapy was 100% (5/5) in SVR group and 11.1% (1/9) in Non-SVR group (p=0.004). Next, the platelet count in SVR group was significantly higher than that in Non-SVR group.

On the IL28B (genetic variation in rs8099917), all seven

patients with TG or GG at IL28B showed non-SVR. On the other hand, five of the seven patients with TT at IL28B showed SVR. The TT at IL28B that is associated with SVR was statistically significant in the present study (p=0.042).

Safety and tolerance of combination therapy

Of the 14 patients treated with IFN-beta + ribavirin included in this study, four patients necessitated a reduced dose of ribavirin due to the appearance of hemoglobin level <10 g/dL and two patients needed a reduced dose of IFN-beta due to WBC count of <2,000/mm³. Three patients had dipstick proteinuria of +1 at 4 week after the initiation of combination therapy. This proteinuria continued during combination therapy. However, no patient discontinued combination therapy because of treatment related adverse events related to exacerbation of depression. Fig. 1 shows the changes of BDI scores in 14 patients treated with IFN-beta + ribavirin. BDI scores during combination therapy were lower than that at the initiation time of treatment. Fig. 2 shows the changes of Ham-D scores in 14 patients. There was no statistically significant difference in changes of Ham-D scores during combination therapy compared to that at the initiation time of treatment.

Regarding the prescription of antidepressant and anti-anxiety drugs, antidepressants, such as sulpiride, and amitriptyline hydrochloride, were given to three patients at the