Table 1: Profile and laboratory data at the start of telaprevir-based peginterferon plus ribavirin combination therapy in patients infected with hepatitis C virus genotype 1b.

Demographic data	
Number of patients	150
Gender (male/female)	82/68
Age (years)	58 (18–75)
Body weight (kg)	61.55 (41.2–115.8)
Body mass index (kg/m^2)	23.4 (17.3–37.8)
Absence or presence of cirrhosis* (noncirrhosis/cirrhosis)	121/29
Histological fibrosis of liver (F0/1/2/3/4/ND)	3/36/28/13/11/59
Genetic variations	
rs8099917 (TT/TG or GG)	115/35
Amino acid substitutions in the HCV genotype 1b	
Core amino acid substitution 70 (wild-type/mutant-type)**	97/53
Number of amino acid substitutions in ISDR (0-1/2≤)	114/36
Laboratory data	
HCV-RNA ($\log_{10}$IU/mL)	6.7 (5.0–7.8)
White blood cells (/μL)	5200 (2000–8700)
Hemoglobin (g/dL)	14.2 (11.0–17.5)
Platelets ($\times 10^4/\mu$L)	17.1 (7.0–35.3)
Aspartate aminotransferase (IU/L)	45 (13–221)
Alanine aminotransferase (IU/L)	49 (13–305)
Gamma-glutamyl-transpeptidase (IU/L)	40 (12–427)
Albumin (g/dL)	4.1 (3.3–4.7)
Fasting low density lipoprotein-cholesterol (mg/dL)	97.5 (21–194)
Fasting plasma glucose (mg/dL)	101 (74–210)
Homeostasis model assessment-insulin resistance	2.15 (0.68–13.45)
Alpha-fetoprotein (ng/mL)	4.5 (1–235)
Treatment	
Initial dose of PEG-IFN (μg/kg)	1.50 (0.94–1.94)
Initial dose of RBV (mg/kg)	11.5 (6.6–14.0)
Initial dose of TVR (1500/2250 mg)	59/91
Initial dose of TVR (mg/kg)	30.6 (16.7–51.1)

PEG-IFN: peginterferon; RBV: ribavirin; TVR: telaprevir; HCV: hepatitis C virus; ISDR: interferon-sensitivity determining region; ND: not detected.
*Determined by the liver biopsy METAVIR scores within 12 months of enrollment or by an aspartate aminotransferase to platelet ratio index (APRI) >1.5.
**"Wild-type" (arginine) or "mutant-type" (glutamine or histidine).
Data expressed as number of patients or median (range).

response" (NVR) reflected HCV RNA levels that never dropped below the detection level during therapy. We also defined rapid virological response as the absence of detectable HCV RNA 4 weeks after starting treatment.

The study protocol was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Institutional Review Boards of all participating sites. Written informed consent was acquired from each individual.

2.2. Genotyping and Quantification of HCV RNA. The HCV G1b genotype was defined according to the method previously reported by Ohno et al. [16]. The serum HCV RNA concentration was measured at the beginning of therapy and every 4 weeks until 24 weeks after the end of therapy, using the previously described RT-PCR method. The linear dynamic range of the assay was 1.2–7.8 Log_{10} IU/mL, and samples below the level of detection were considered HCV-negative.

2.3. Detection of aa Substitutions in Core aa 70 and the ISDR of HCV Genotype 1b. The substitutions in core aa 70 and in the ISDR were determined using a direct sequencing method. Briefly, RNA was extracted from the serum and, after reverse transcription, the substitution in aa 70 was determined according to the method previously reported by Akuta et al. [17]. The "wild-type" aa 70 in the core region is

arginine and a "mutant-type" involved a change to glutamine or histidine. Also, aa substitutions in the range of 2209–2248 in the NS5A (the ISDR) were determined using the method of Enomoto et al. [18]. The number of aa substitutions in ISDR was classified into "0-1" and 2 or more.

2.4. Genetic Variation Near the IL-28B Gene. Genomic DNA was extracted from whole blood using MagNA Pure LC and the DNA Isolation Kit (Roche Diagnostics). The rs8099917 single nucleotide polymorphism (SNP) near the IL-28B gene [19] was genotyped by RT-PCR using the TaqMan SNP Genotyping Assay and the 7500 Fast RT-PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotype was classified into 2 categories: TT (major genotype) and non-TT genotype (minor genotype, TG or GG).

2.5. Statistical Analysis. Pearson or Mantel-Haenszel chi-square test, Fisher's exact test, or Mann-Whitney test was used to compare frequencies in categorical data or differences in continuous data between groups, respectively. Possible variables contributing to RVR and SVR included baseline and on-treatment features. Variables that reached statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) in bivariate comparisons were subsequently entered into multivariate logistic regression analysis using forward and backward stepwise selection method to identify significantly independent factors associated with RVR and SVR. P values of <0.05 denoted the presence of a statistically significant difference. All statistical analyses were carried out using STATISTICA for Windows version 6 (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Virological Responses during Triple Therapy. Of the 150 patients, 116 (77.3%) had a RVR. The frequency of undetectable serum HCV RNA was 94.4% at the end of treatment. Of the 150 patients for whom a final virological response was evaluated (either SVR or non-SVR), 131 patients (87.3%) achieved SVR.

3.2. Factors Affecting RVR with the Triple Therapy. The frequency of rs8099917 genotype TT (major genotype) (81.0% versus 61.8%; $P = 0.0195$) was significantly higher in patients achieving RVR than in non-RVR patients. Pretreatment serum HCV RNA (6.60 (5.0–7.7) versus 6.95 (5.2–7.8) $\log_{10}$ IU/mL; $P = 0.0153$) was also significantly lower in patients achieving RVR than in non-RVR patients. Gender ($P = 0.0724$) and serum alpha-fetoprotein (AFP) ($P = 0.0655$) displayed marginal differences between the RVR and non-RVR patients. Achievement of RVR was not significantly related to the core aa 70 substitutions or to the number of ISDR aa substitutions. In the multivariate logistic regression analysis for elucidating the independent baseline predictive factors for RVR, rs8099917 genotype TT (odds ratio (OR), 0.308; 95% confidence interval (CI), 0.123–0.775; $P = 0.0116$) and pretreatment serum HCV RNA concentrations (OR, 0.293; 95% CI, 0.127–0.675; $P = 0.0037$) were identified (Table 2).

3.3. Factors Affecting SVR with the Triple Therapy. The frequency of rs8099917 genotype TT was higher in patients with SVR than non-SVR (85.5% versus 15.8%; $P = 1.33 \times 10^{-10}$). Serum gamma-glutamyl-transpeptidase concentration was significantly lower in SVR patients than in non-SVR individuals ($P = 0.0459$). Serum albumin ($P = 0.0968$) and fasting plasma glucose concentrations ($P = 0.0549$) demonstrated only marginal differences. The substitutions in core aa 70 and the number of substitutions in the ISDR did not predict SVR. In the multivariate logistic regression analysis for elucidating pretreatment independent predictive factors for SVR, rs8099917 genotype TT (OR, 0.071; 95% CI, 0.015–0.337; $P = 0.0007$) alone was identified. In addition, achievement of RVR (83.2% versus 36.8%; $P = 2.47 \times 10^{-5}$) after starting treatment was a significant predictor of SVR (Table 3).

3.4. Influence of the Substitution in Core aa 70 and the Substitution Number in ISDR on SVR in Patients with rs8099917 Genotype TT or Non-TT. Among the 150 patients, 115 had an rs8099917 TT genotype and 35 had a non-TT (TG or GG) genotype. Of the 115 patients who had genotype TT, 74 had "wild-type" amino acids, whereas 41 had "non-wild-type" aa in the core aa 70 of the HCV isolate. Of the 35 patients who had non-TT (TG or GG) genotype, 23 were "wild-type," whereas 12 had a "non-wild-type" core aa 70 genotype.

Similarly, among the 115 patients having genotype TT, 87 patients had a "0-1" number of amino acid substitutions, whereas 28 had "2≤" number of ISDR aa substitutions. In the 35 patients with a non-TT genotype, 27 patients had "0-1" and 8 had "2≤" number of ISDR aa substitutions.

The rates of SVR in these patients are illustrated in Figure 1. Neither the substitution in aa 70 in the HCV core region nor the number of aa substitutions in the ISDR impacted the prediction of SVR, regardless of the rs8099917 genotype.

3.5. Significance of RVR on Achieving SVR in Patients with an rs8099917 Non-TT (TG or GG) Genotype. In the 94 patients carrying an rs8099917 TT genotype and achieving RVR, the SVR rate was 98.9% (93/94), whereas it was 90.5% (19/21) in patients carrying genotype TT, but not achieving RVR. In contrast, the SVR rate was only 23.1% (3/13) in patients who had the rs8099917 genotype non-TT (TG or GG) without achieving RVR, whereas it was 72.7% (16/22) in patients achieving RVR ($P = 0.0125$) (Figure 1).

3.6. Safety and Characteristics of the Patients Who Did Not Achieve SVR. Of the 150 patients who had a defined final virological response, only 19 (12.7%) were classified as non-SVR: 3 (15.8%) "NVR"; 10 (52.6%) "relapse"; and 6 (31.6%) "breakthrough." Three patients were carrying genotype TT, whereas 16 had a non-TT genotype. Cessation of the therapy due to adverse effects occurred in only 2 patients who were

Table 2: Patient characteristics at the start of triple therapy for hepatitis C virus genotype 1b, according to achievement of a rapid virological response.

	RVR	Non-RVR	RVR versus non-RVR (1: non-RVR/2: RVR) Univariate analysis *P* value	Multivariate analysis OR	95% CI	*P* value
Pretreatment factors						
Demographic data						
Number of patients	116	34				
Gender (male/female)	68/48	14/20	0.0724	0.462 (1: male/2: female)	0.200–1.065	0.0697
Age (years)	58 (18–75)	57 (29–74)	0.8152			
Body weight (kg)	61.8 (41.2–101.6)	60.1 (44.1–115.8)	0.2956			
Body mass index (kg/m^2)	23.4 (18.1–34.3)	23.4 (17.3–37.8)	0.6846			
Absence or presence of cirrhosis* (noncirrhosis/cirrhosis)	94/22	27/7	0.8331			
Histological fibrosis of liver (F0/1/2/3/4/ND)	2/26/24/11/8/45	1/10/4/2/3/14				
Genetic variations						
rs8099917 (TT/TG or GG)	94/22	21/13	0.0195	0.308 (1: TT/2: TG or GG)	0.123–0.775	0.0116
Amino acid substitutions in the HCV genotype 1b						
Core amino acid substitution 70** (wild-type/mutant-type)	74/42	23/11	0.6793			
Number of amino acid substitutions in ISDR (0-1/2≤)	85/31	29/5	0.1490			
Laboratory data						
HCV-RNA ($\log_{10}$IU/mL)	6.60 (5.0–7.7)	6.95 (5.2–7.8)	0.0153	0.293 (by 1.0 $\log_{10}$IU/mL)	0.127–0.675	0.0037
White blood cells (/μL)	5300 (2000–8700)	4790 (3290–7900)	0.2152			
Hemoglobin (g/dL)	14.1 (11.4–17.5)	14.45 (11.0–17.2)	0.5063			
Platelets (/μL)	16.9 (7.0–35.3)	17.35 (7.0–28.8)	0.8858			
Aspartate aminotransferase (IU/L)	42 (13–221)	51.5 (20–135)	0.2394			
Alanine aminotransferase (IU/L)	49 (13–305)	51 (25–169)	0.5716			
Gamma-glutamyl-transpeptidase (IU/L)	37 (12–427)	44.5 (12–359)	0.3205			
Albumin (g/dL)	4.1 (3.3–4.7)	4.0 (3.3–4.7)	0.5075			
Fasting low density lipoprotein-cholesterol (mg/dL)	99 (21–194)	87.5 (58–133)	0.1850			
Fasting plasma glucose (mg/dL)	101 (74–215)	97.5 (80–158)	0.4837			
Homeostasis model assessment-insulin resistance	1.73 (0.68–13.5)	2.24 (0.72–10.1)	0.3215			
Alpha-fetoprotein (ng/mL)	4.4 (1.4–136)	5.35 (1–235)	0.0655	0.978 (by 1.0 ng/mL)	0.886–1.085	0.6998

Table 2: Continued.

	RVR	Non-RVR	RVR versus non-RVR (1: non-RVR/2: RVR)			
			Univariate analysis	Multivariate analysis		
			P value	OR	95% CI	*P* value
Treatment						
Initial dose of PEG-IFN (μg/kg)	1.50 (1.07–1.82)	1.49 (0.94–1.94)	0.5824			
Initial dose of RBV (mg/kg)	11.3 (6.6–14.1)	11.8 (6.8–13.6)	0.2833			
Initial dose of TVR (1500/2250 mg)	44/72	15/19	0.5161			
Initial dose of TVR (mg/kg)	30.3 (16.7–51.1)	32.1 (19.4–47.1)	0.6470			
After starting treatment factors						
Adherence of PEG-IFN during 4 weeks (%)	100 (50–100)	100 (75–100)	0.4041			
Adherence of RBV during 4 weeks (%)	94.4 (40.4–100)	100 (75–100)	0.1486			
Adherence of TVR*** during 4 weeks (%)	83.3 (4.2–100)	84.7 (66.7–100)	0.7318			

PEG-IFN: peginterferon; RBV; ribavirin; TVR: telaprevir; HCV: hepatitis C virus; ISDR: interferon-sensitivity determining region; RVR: rapid virological response.

*Determined by the liver biopsy METAVIR scores within 12 months of enrollment or by an aspartate aminotransferase to platelet ratio index (APRI) >1.5.

**"Wild-type" (arginine) or "mutant-type" (glutamine or histidine).

***Calculated on the basis of 2250 mg/day.

Data expressed as number of patients or median (range).

Table 3: Background characteristics of hepatitis C virus genotype 1b patients, based on their achieving sustained virological response.

	SVR	Non-SVR	SVR versus non-SVR (1: non-SVR/2: SVR)			
			Univariate analysis	Multivariate analysis		
			P value	OR	95% CI	*P* value
Pretreatment factors						
Demographic data						
Number of patients	131	19				
Gender (male/female)	74/57	8/11	0.2392			
Age (years)	58 (18–75)	57 (40–68)	0.7495			
Body weight (kg)	61.1 (41.2–115.8)	62.5 (45–92.8)	0.8698			
Body mass index (kg/m^2)	23.4 (17.3–37.8)	23.3 (17.7–31.9)	0.8321			
Absence or presence of cirrhosis* (1: noncirrhosis/2: cirrhosis)	107/24	14/5	0.4095			
Histological fibrosis of liver (F0/1/2/3/4/ND)	3/32/25/11/9/51	0/4/3/2/2/8				
Genetic variations						
rs8099917 (TT/TG or GG)	112/19	3/16	1.33×10^{-10}	0.071 (1: TT/2: TG or GG)	0.015–0.337	7.38×10^{-4}
Amino acid substitutions in the HCV genotype 1b						
Core amino acid substitution 70** (wild-type/mutant-type)	85/46	12/7	0.8830			
Number of amino acid substitutions in ISDR (0-1/2≤)	100/31	14/5	0.8003			
Laboratory data						
HCV-RNA ($\log_{10}$IU/mL)	6.7 (5.0–7.8)	6.8 (5.8–7.6)	0.7495			
White blood cells (/μL)	5300 (2000–8700)	4590 (3290–7900)	0.1117			
Hemoglobin (g/dL)	14.2 (11.0–17.4)	13.6 (11.1–17.5)	0.2681			
Platelets ($\times 10^4/\mu$L)	17.2 (7.0–35.3)	16.5 (7.6–33.6)	0.9123			
Aspartate aminotransferase (IU/L)	43 (13–221)	60 (22–134)	0.1671			
Alanine aminotransferase (IU/L)	48 (13–305)	56 (20–161)	0.2868			
Gamma-glutamyl-transpeptidase (IU/L)	34.5 (12–272)	82 (14–427)	0.0459	0.973 (by 10 IU/L)	0.872–1.085	0.6134
Albumin (g/dL)	4.1 (3.3–4.7)	4.0 (3.3–4.7)	0.0968	8.639 (by 1.0 g/dL)	0.875–85.736	0.0617
Low density lipoprotein-cholesterol (mg/dL)	99 (51–194)	82 (58–128)	0.1140			
Fasting plasma glucose (mg/dL)	101 (74–210)	91 (80–116)	0.0549	1.621 (by 10 mg/dL)	0.853–3.082	0.1346
Homeostasis model assessment-Insulin Resistance	2.15 (0.68–13.5)	1.98 (0.79–12.6)	0.4320			
Alpha-fetoprotein (ng/mL)	4.5 (1–234.7)	12.5 (2.4–117.5)	0.3595			

Table 3: Continued.

	SVR	Non-SVR	SVR versus non-SVR (1: non-SVR/2: SVR)			
			Univariate analysis	Multivariate analysis		
			P value	OR	95% CI	*P* value
Treatment						
Initial dose of PEG-IFN (μg/kg)	1.50 (0.94–1.94)	1.48 (1.22–1.72)	0.5049			
Initial dose of RBV (mg/kg)	11.4 (6.6–14.0)	11.6 (6.8–13.3)	0.9482			
Initial dose of TVR (1500/2250 mg)	50/81	9/10	0.4429			
Initial dose of TVR (mg/kg)	30.1 (16.7–51.1)	32.2 (22.2–38.3)	0.8102			
On-treatment factors						
Treatment						
Adherence to PEG-IFN (%)	100 (12.5–100)	94.4 (8.3–100)	0.2302			
Adherence to RBV (%)	66.7 (25.0–100)	67.7 (7.7–100)	0.7006			
Adherence to TVR*** (%)	69.4 (25.0–100)	66.7 (15.5–100)	0.1669			
Early virological response						
Achievement of RVR (yes/no)	109/22	7/12	2.47×10^{-5}			

PEG-IFN: peginterferon; RBV: ribavirin; TVR: telaprevir; HCV: hepatitis C virus; ISDR: interferon-sensitivity determining region; RVR: rapid virological response.

*Determined by the liver biopsy METAVIR scores within 12 months of enrollment or by an aspartate aminotransferase to platelet ratio index (APRI) >1.5.

**"Wild-type" (arginine) or "mutant-type" (glutamine or histidine).

***Calculated on the basis of 2250 mg/day.

Data expressed as number of patients or median (range).

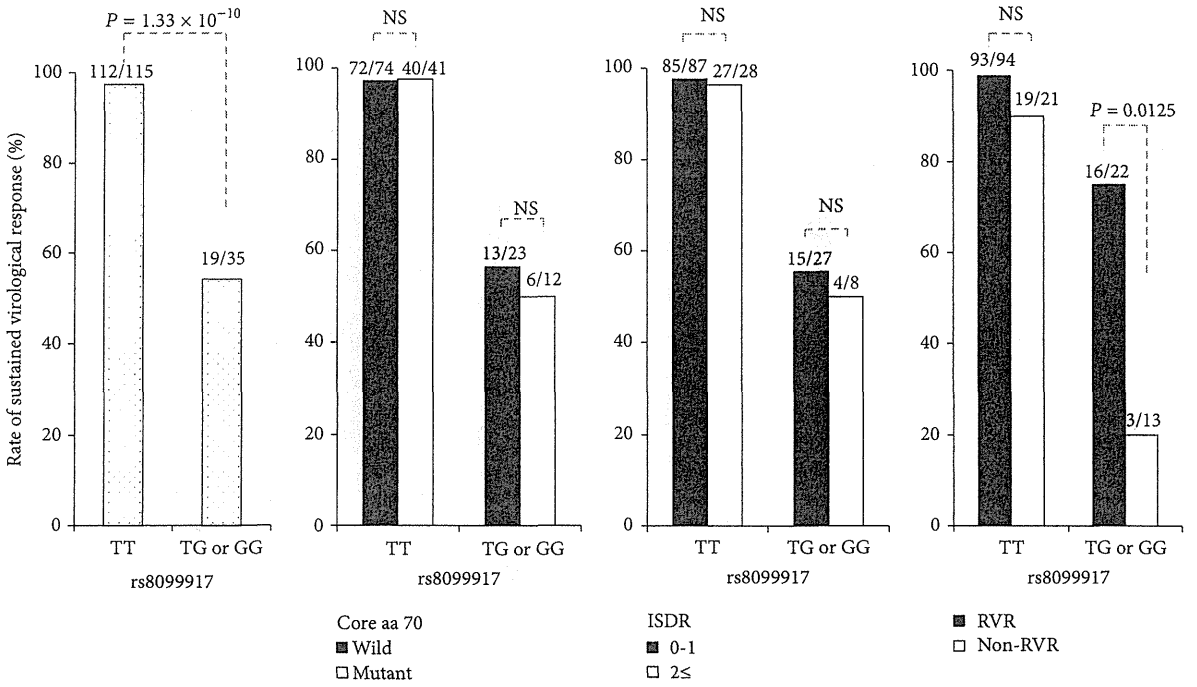

FIGURE 1: According to the genetic variation in rs8099917, near the IL28B gene, a significantly higher proportion of patients with the TT genotype showed a sustained virological response (SVR) than did patients with the TG or GG genotype. In contrast, based on the amino acid substitutions in the core region (amino acid 70) and interferon sensitivity-determining region (ISDR), there was no significant association between the SVR rate and these substitutions, irrespective of the rs8099917 genotype. Furthermore, the SVR rate was 98.9% in patients with the rs8099917 TT genotype who achieved rapid virological response (RVR), whereas the SVR rate was 23.1% among patients with the TG or GG rs8099917 genotype who did not achieve RVR.

carrying genotype TT; these patients discontinued the therapy at 2 weeks and at 10 weeks due to cerebral infarction and renal impairment, respectively. The remaining 1 patient who was carrying genotype TT finished the scheduled treatment, but the HCV RNA reappeared 4 weeks after the end of the therapy. These 3 genotype TT patients were classified as having relapses.

3.7. Comparison of SVR Rates, according to rs8099917 Genotype and the Existence of Cirrhosis, between Patients Aged ≤65 Years and Those Aged >65 Years. Among the 150 patients, 29 (19.3%) were aged >65 years. The frequency of the non-TT genotype (TG or GG) rs8099917 in the patients in both age groups was similar. However, the frequency of cirrhosis in patients aged over 65 years (9/29; 31.0%) was higher than that in the younger patients (20/121; 16.5%). The SVR rate in the older (aged >65 years) patients was similar to that in those aged ≤65 years, regardless of the rs8099917 genotype or the existence of cirrhosis (Figure 2). In 29 patients aged >65 years, the frequencies of the major side effect were as follows: anemia occurred in 28 (96.6%), elevation of serum uric acid in 20 (69.0%), skin rashes in 18 (62.1%), headache in 15 (51.7%), nausea in 11 (37.9%), and elevation of serum creatinine in 10 (34.4%). The frequencies were similar to those of the ≤65 years patients. Cessation of the therapy due to adverse effects occurred in only 1 (3.4%) patient; this patient discontinued the therapy at 10 weeks due to renal impairment (data not shown).

4. Discussion

Genetic variation near the IL28B gene and the substitutions in core aa 70 of HCV G1b have been suggested to be predictive of virological outcomes for triple therapy. However, in the present study of treatment-naïve patients, none of the viral factors (substitution of core aa70 or number of substitutions in the ISDR) or the host factors [12, 20], except for the rs8099917 genotype, were observed as predictors of 24-week triple therapy efficacy.

In the previous study, IL28 SNP genotype had a limited impact on SVR rates with triple therapy in treatment-experienced patients [21], and the strength of association between IL28B genotype and treatment outcome was attenuated in the triple therapy arms compared to the combination therapy arm [22].

In this study, the rs8099917 genotype displayed a striking influence on the outcome of triple therapy, along with "achievement of RVR." In the patients carrying the rs8099917 genotype TT, even if RVR was not achieved, the SVR rate was as high as 90%. In contrast, the SVR rate was only 23.1% for patients with the TG or GG genotype who did not achieve RVR but reached 72.7% when RVR was achieved. Therefore,

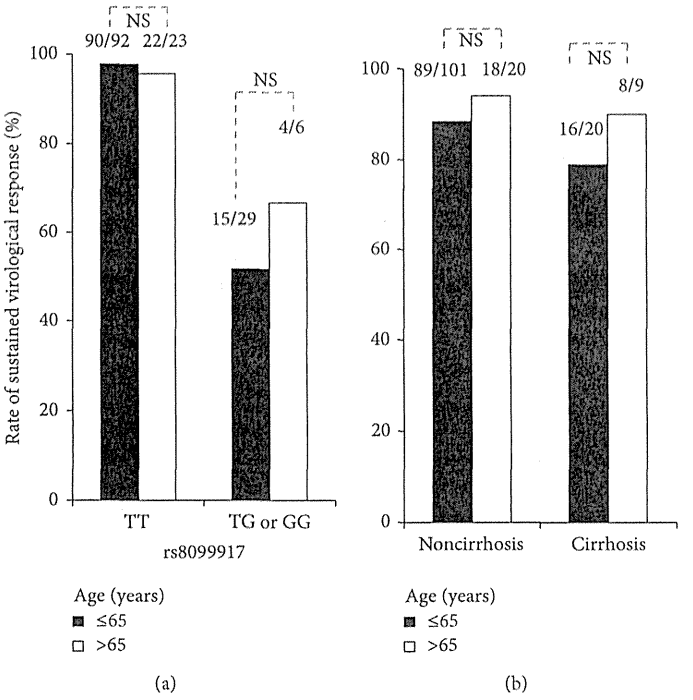

FIGURE 2: There was no significant relation between sustained virological response rate and age. The patients older than 65 years had hemoglobin levels similar to those aged ≤65 years.

"achievement of RVR" was particularly important for SVR in patients with a minor (non-TT) rs8099917 genotype. From these findings, rs8099917 genotyping should be examined in treatment-naïve patients and the patients carrying the TT genotype should be considered for triple therapy. On the other hand, in patients with a TG or GG genotype, the introduction of the triple therapy may be recommended, but extension of treatment period or cessation of the therapy should be considered if HCV RNA remains detectable following 4 weeks of therapy.

Substitutions in the core aa 70 and the number of substitutions in the ISDR of HCV G1b were reported to be important predictors of combination therapy efficacy, and these findings are gaining consensus [12, 23]. In previous reports on the triple therapy, the rs8099917 genotype and the presence of a substitution in the core aa 70 were repeatedly identified as predictors of SVR in patients with HCV G1b infection [8, 9]. However, our study on treatment-naïve patients revealed that core aa 70 substitutions do not predict the achievement of SVR. This discrepancy may be explained due to the differences in the study populations and the inclusion criteria of drop-out patients. Akuta et al. [9] reported that their study population contained both treatment-naïve patients and patients with a history of interferon-based therapy. In addition, 25% of their study population was treated with triple drugs for 12 weeks alone, without the follow-up PEG-IFN plus ribavirin combination therapy. Similarly, Chayama et al. [8] reported that their study population contained not only treatment-naïve patients but also combination therapy experienced patients. In addition, their study included patients who received a very short duration of the triple therapy. Therefore, therapy duration variability and the difference of study population from our study may have had an impact on their study outcomes.

All of our patients were in the intent-to-treat with the 24-week triple therapy and only 6 patients (4.0%) were disrupted. This may lead to our conclusion that substitutions in the core aa 70 did not impact treatment outcomes in treatment-naïve, HCV G1b-infected patients. However, the relatively small number of patients in our study may limit these conclusions; the results should be verified by a larger-scale study.

In our study, the RVR and SVR rates were 80.2% and 87.3%, respectively; these rates tend to be higher than those reported previously [4, 5]. One of the reasons for the higher SVR rate may be the comparatively high frequency (76.7%) of the TT genotype in our cohort. Another reason for higher SVR rate was the extremely low prevalence of drop-outs, owing to the aggressive management of adverse effects and careful adjustment of the dosage of triple drugs. The drop-out rate in the present study was only 1.3% (2 of 150 patients), as compared to 11.1–16.7% in other Japanese studies [7, 9, 24]. Although it is the small number of cases (only 9 of 150 patients), detailed search for aa substitutions in the HCV NS3 protease domain may be required for the care of patients who are classified as NVR or breakthrough, because the relationship between aa substitutions in this domain and resistance to NS3-4A protease inhibitors has been documented [25, 26].

In previous studies [4–10], the treatment of elderly patients with triple therapy is approached with caution and

the inclusion criteria for clinical trials are usually set to maximum age of 65 years. Although differences in efficacy and in the frequency and severity of side effects between the patients aged >60 and those aged ≤60 years have not been observed [24], the safety and the efficacy of the treatment in patients aged >65 years has not been adequately demonstrated. However, there is a need for an effective antiviral therapy for older patients in Japan because of the large numbers of older HCV G1 patients. Thus, we included a small number of patients aged >65 years in the present study. The SVR rate of older (>65 years) patients seemed to be similar to that in those aged ≤65 years and there was not a notable increase in the number of drop-outs. Our finding that adherence to the telaprevir or ribavirin dose schedule did not clearly affect the achievement of SVR suggests that continuation of the scheduled length of therapy may be far more important for achieving SVR than maintaining a particular drug dosage. Moreover, the efficacy of triple therapy for the minor rs8099917 genotype or the presence of cirrhosis was similar between patients aged >65 years and those aged ≤65 years. From these observations, we propose that patients aged >65 years can be treated with triple therapy if the drug dosages are adequately regulated.

5. Conclusions

In conclusion, for treatment-naïve, chronic HCV G1b-infected patients, the rs8099917 genotype was a significant factor predicting a successful virological effect of the triple therapy; a substitution in core aa 70 and the number of substitutions in the ISDR did not impact treatment outcomes. In addition, achievement of RVR after starting treatment was exceedingly important to accomplish SVR in patients carrying the rs8099917 non-TT genotype. Thus, the patient's rs8099917 genotype should be determined before starting triple therapy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Pretreatment prediction of the outcome of response-guided peginterferon-α and ribavirin therapy for chronic hepatitis C

Naohiko Masaki,* Masaya Sugiyama,* Noritomo Shimada,† Yasuhito Tanaka,‡ Makoto Nakamuta,§ Namiki Izumi,¶ Sumio Watanabe,** Akihito Tsubota,†† Masafumi Komatsu,‡‡ Tsutomu Masaki,§§ Nobuyuki Enomoto,¶¶ Masashi Yoneda,*** Kazumoto Murata,* Kiyoaki Ito,*,*** Kazuhiko Koike††† and Masashi Mizokami*

*The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, †Division of Gastroenterology and Hepatology, Shinmatsudo Central General Hospital, ††Institute of Clinical Medicine and Research, The Jikei University School of Medicine, Chiba, ‡Department of Virology, Nagoya City University Graduate School of Medical Sciences, Nagoya, §Department of Gastroenterology, NHO Kyushu Medical Center, Fukuoka, ¶Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, **Department of Gastroenterology, Juntendo University, †††Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, ‡‡Department of Gastroenterology, Akita City Hospital, Akita, §§Department of Gastroenterology, Kagawa Medical University School of Medicine, Kagawa, ¶¶First Department of Medicine, Yamanashi University School of Medicine, Yamanashi, and ***Division of Gastroenterology, Department of Internal Medicine, Aichi Medical University School of Medicine, Aichi, Japan

Key words
(TA) dinucleotide repeat, chronic hepatitis C, IL28B, response-guided therapy

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Correspondence
Naohiko Masaki, The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1 Kohnodai, Ichikawa, Chiba 272-8516, Japan.
Email: nmasaki@hospk.ncgm.go.jp

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Abstract

Background and Aim: The accuracy for predicting virological outcomes of peginterferon-α and ribavirin therapy in patients with chronic hepatitis C is limited to approximately 80%, even with *IL28B* genotyping. Our *in vitro* study revealed that the numbers of (TA) dinucleotide repeats [(TA)n] of rs72258881, which is located in the promoter region of *IL28B* gene, might regulate *IL28B* transcription. We aimed to evaluate the usefulness of these host factors for predicting virological outcomes of this therapy in response-guided clinical settings.

Methods: A nationwide, multi-center prospective study in Japan determined *IL28B* (rs8099917) genotype, (TA)n of rs72258881, and amino acid substitutions of hepatitis C virus and used these for multivariate analysis together with other parameters at pretreatment.

Results: After enrolling 215 patients with genotype 1 and high viral load from 23 hospitals between October 2009 and February 2011, intent-to-treat analysis identified 202 patients in whom the final virological outcomes could be determined. Non-virological response by non-TT genotype was predicted with 79.7% accuracy. When combined with the (TA)n, the incidences of virological response tended to be higher in the longer (TA)n group, regardless of rs8099917 genotype. Multivariate logistic regression analysis revealed that rs8099917 non-TT genotype ($P < 0.001$), shorter (TA)n ($P = 0.011$), mutation of amino acid 70 in the virus core region ($P = 0.029$), and lower levels of serum albumin ($P = 0.036$) were independently associated with non-virological response.

Conclusions: *IL28B* genotype and (TA)n of rs72258881 may independently affect virological outcomes of peginterferon-α and ribavirin as host factors, even in response-guided therapy.

Introduction

Globally as many as 150 millions of people are infected by hepatitis C virus (HCV) and every year approximately 350 000 patients die of HCV-related liver diseases, such as liver cirrhosis or hepatocellular carcinoma.[1] The standard care for the treatment of chronic hepatitis C has been PEGylated interferon-α (PEG-IFN-α) and ribavirin (P/R) with sustained virological response (SVR) rates as high as 50% achieved, even in difficult-to-treat combination of HCV genotype 1 and high viral load.[2] Recently introduced protease inhibitors, such as boceprevir[3,4] or telaprevir,[5,6] can improve the SVR rate as much as 70–80% in IFN-naïve patients. Furthermore, the era of interferon-free treatment with only-oral directly-acting antivirals has just arrived.[7–9] However, elderly HCV-infected patients are often unable to either tolerate or afford these new treatment regimens. In such circumstances, it is very

important to identify easy-to-treat patients prior to treatment. Factors that contribute to the success of drug-mediated eradication of HCV include host factors (such as age, gender, the extent of liver fibrosis, and insulin resistance[10,11]), viral factors (such as HCV genotype, viral load, and amino acid substitutions in core[12] and NS5A[13] regions), as well as drug-related factors (such as treatment regimens, adherence to these regimens, total drug doses, and the duration of treatment).[14] The most epoch-making discoveries in this field were that single nucleotide polymorphisms (SNPs) in the *IL28B* gene (rs8099917, rs12979860) can predict virological response (VR) to P/R.[15–19] However, all these findings were based primarily on retrospective studies with per protocol analysis. Furthermore, even if those factors are all concentrated, the accuracy with which therapeutic outcomes can be predicted still remains approximately 80%. We recently reported that the number of (TA) dinucleotide repeats [(TA)n] of rs72258881, which is located in the promoter region of *IL28B* gene, might regulate its transcription.[20] Here we report our efforts to verify the role of (TA)n of rs72258881, by conducting a prospective multicenter cohort study with intent-to-treat analysis, in Japanese patients infected with HCV genotype 1 who were treated by response-guided therapy (RGT) with P/R.

Methods

Study Design. From October 2009 to February 2011, 233 patients with chronic hepatitis C were prospectively enrolled from nationwide 23 hospitals in Japan (Trial Registration: UMIN-CTR000002580); however, 18 patients were considered to be ineligible and excluded from this study because of violation of the following entry criteria: (1) infection with HCV serotype 1[21] or genotype 1 (1a or 1b)[22] without co-infection with hepatitis B virus or human immunodeficiency virus; (2) pretreatment HCV RNA levels ≥ 5.0 $\log_{10}$ IU/mL, as determined using a quantitative real-time PCR method (COBAS AmpliPrep/COBAS TaqMan HCV test; Roche Molecular Systems, Pleasanton, CA, USA); (3) standard P/R therapy according to the American Association of the Study of the Liver Diseases (AASLD) guidelines.[23] Consequently, 215 patients met the entry criteria and were treated with weekly administration of PEG-IFN-α2a (Chugai Pharmaceutical, Tokyo, Japan) and daily administration of ribavirin (Chugai Pharmaceutical), or with weekly administration of PEG-IFN-α2b (MSD Co., Tokyo, Japan) and daily administration of ribavirin (MSD Co.). Whereas the dose of PEG-IFN-α2a was 180 μg, regardless of the patient's body weight, doses of PEG-IFN-α2b were adjusted based on the patient's body weight: respective weekly doses of PEG-IFN-α2b for patients < 45 kg, ≥ 45 kg, and < 60 kg; ≥ 60 kg and < 75 kg; ≥ 75 kg and < 90 kg; and ≥ 90 kg were given 60 μg, 80 μg, 100 μg, 120 μg, and 150 μg of PEG-IFN-α2b. Respective daily doses of ribavirin for patients < 60 kg, ≥ 60 kg and < 80 kg, and ≥ 80 kg were given 600 mg, 800 mg, and 1000 mg. Dose modifications of PEG-IFN-α or ribavirin, relating to adverse events, were based on the manufacturers' recommendations.

The treatment duration was determined based on RGT according to guidelines of AASLD[23] and the Japan Society of Hepatology (JSH).[24] Patients in whom serum HCV RNA had disappeared within 12 weeks after starting therapy received a 48-week treatment regimen. Patients in whom serum HCV RNA was still detectable at 12 weeks, but not at 24 weeks after starting therapy received a 72-week extended treatment regimen.

VR was defined as achieving SVR or transient virological response (TVR); whereas SVR was defined as undetectable HCV RNA in serum 24 weeks after the cessation of treatment, TVR was defined as undetectable HCV RNA at the cessation of treatment with reappearance of HCV RNA in serum thereafter. Non-virological response (NVR), which was defined as detectable viremia throughout the 24 weeks of P/R therapy, was classified as one of two categories. The first of these, "null responder", was defined as < 2 log-unit decline in the serum levels of HCV RNA from the pretreatment baseline value within the first 12 weeks and detectable viremia at 24 weeks after the start of P/R. The second, "partial responder", was defined as ≥ 2 log-unit decline in the serum levels of HCV RNA from the pretreatment baseline value within the first 12 weeks and detectable viremia at 24 weeks after the start of P/R. Patients whose treatment was withdrawn due either to the presence of serum HCV RNA after 24 weeks of therapy or to viral breakthrough (VBT) were also included in this study for intent-to-treat analysis. VBT was included in NVR, and was subclassified into "null responder" or "partial responder", according to the above criteria. Adherence to PEG-IFN-α and ribavirin up to 12 weeks after the start of P/R were calculated.

The study protocol (Fig. 1) complied with the Helsinki Declaration and was approved by the ethics committee of each participating institution. At the time of enrollment, written informed consent was obtained for the collection and storage of serum and peripheral blood.

DNA Extraction. Genomic DNA was extracted from the buffy coat fraction of patients' whole blood using a GENOMIX kit (Talent SRL; Trieste, Italy).

IL28B genotyping. We previously reported that the rs8099917 polymorphism is a better predictor of the response of P/R to chronic hepatitis C in Japanese patients than any other SNPs reported near the *IL28B* gene.[25] Therefore, the rs8099917 polymorphism was genotyped using the InvaderPlus assay (Third Wave Japan, Tokyo, Japan), which combines the polymerase chain reaction (PCR) and the invasive signal amplification reaction.[26,27] In this prospective cohort study, in order to meet the requirements of RGT, both the doctors and the patients were blinded to the results of *IL28B* genotyping until final determination of virological outcomes.

Detection of amino acid substitutions in core and NS5A regions of HCV. Amino acids 70 and 91 of the HCV core region and the amino acid sequence of interferon-sensitivity determining region (ISDR: residues 2209–2248 of the NS5A region) were determined by direct sequencing, as previously reported.[12,13]

TA repeat genotyping. To determine the genotype of the TA repeat polymorphism, we used GeneScan analysis to detect the fragment size of the fluorescently labeled PCR amplicon. This method requires the use of nested PCR to prevent amplification of *IL-29* region with high sequence similarity to regions within the *IL-28A* and *IL-28B* genes. The details of the nested PCR and

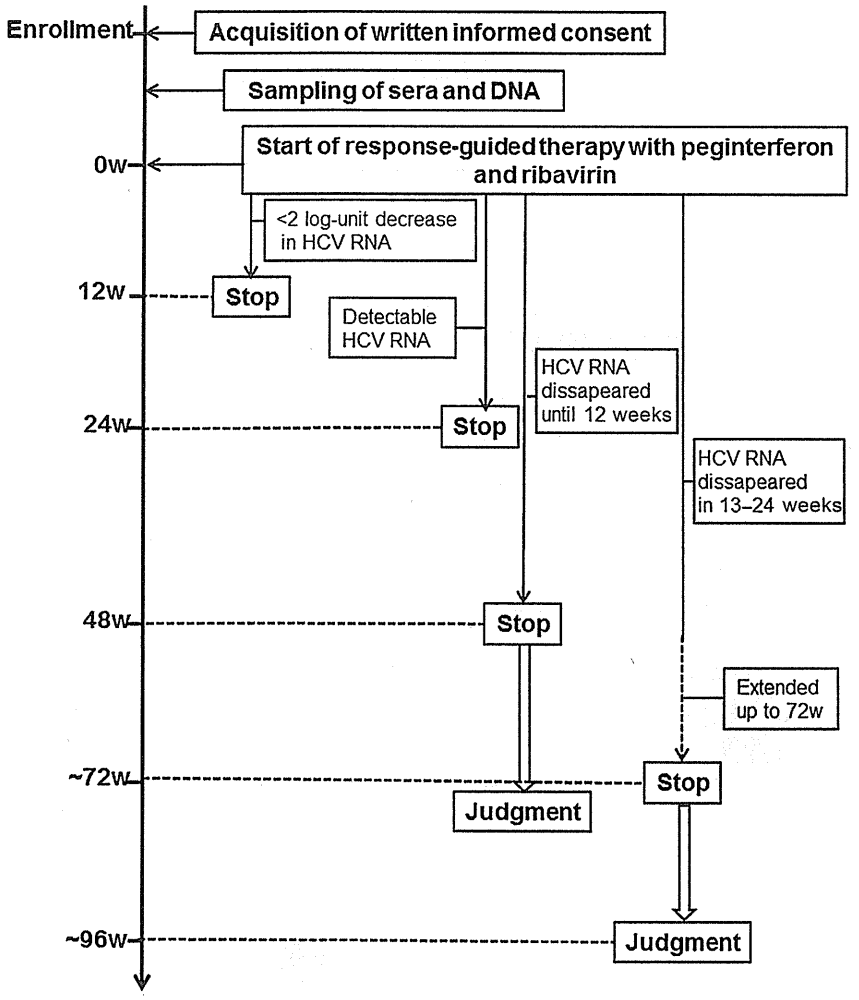

Figure 1 Study protocol for response-guided therapy. Durations of treatments were determined depending on the virological responses to peginterferon-α and ribavirin. If the serum hepatitis C virus (HCV) RNA became undetectable within 12 weeks (w), the treatment was stopped at 48 weeks. If the serum HCV RNA disappeared between 13 to 24 weeks after treatment, the duration was extended up to 72 weeks. Both cases were considered to meet the standard of care.

GeneScan analysis may be found in the online version at the publisher's web-site as Supporting Information Table S1. The repeat number was validated by capillary sequencing as described previously.[20]

Evaluation of liver fibrosis by a simple noninvasive index (FIB-4). The Fibrosis 4 (FIB-4) index that was used to evaluate liver fibrosis in each patient correlates well with hepatic fibrosis (as determined by liver biopsy) and requires only readily available clinical parameters for its determination.[28]

Statistical Analysis. Quantitative variables were expressed as the mean ± standard deviation, unless otherwise specified. Categorical variables were compared using Pearson's χ^2-test or Fisher's exact test. Continuous variables were compared using the Mann–Whitney *U*-test. Multivariate and simultaneous logistic regression analysis was performed to determine predictive factors for NVR, by using the variables which were found to be $P < 0.150$ by univariate analysis. In addition, a decision tree modeled these pretreatment factors to predict NVR. All *P* values were two-tailed, and $P < 0.05$ was considered statistically significant. Data analyses were performed using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA).

Results

Treatment profiles and virological outcomes. In this prospective study, 215 patients infected by HCV of serotype 1 or genotype 1 were eligible. The sub-genotypes of HCV were as follows: 1a ($n = 2$), 1b ($n = 208$), 1b + 2b ($n = 1$), and indeterminate ($n = 4$). By the end of November 2012, virological outcomes had been determined in 202 patients, except for 13 patients (6%) who were lost to follow-up. Whereas all of these patients were treated with P/R, 160 patients (74%) completed standard of care (SOC) treatment for at least 48 weeks, the remaining 55 patients had to withdraw from treatment owing to either serious adverse events (SAE) in 25 patients (12%), poor response in 24 patients (11%), or other unrelated causes in 6 patients (3%). The SAE or unrelated causes responsible for the termination of the treatment were described in the small inset of Figure 2.

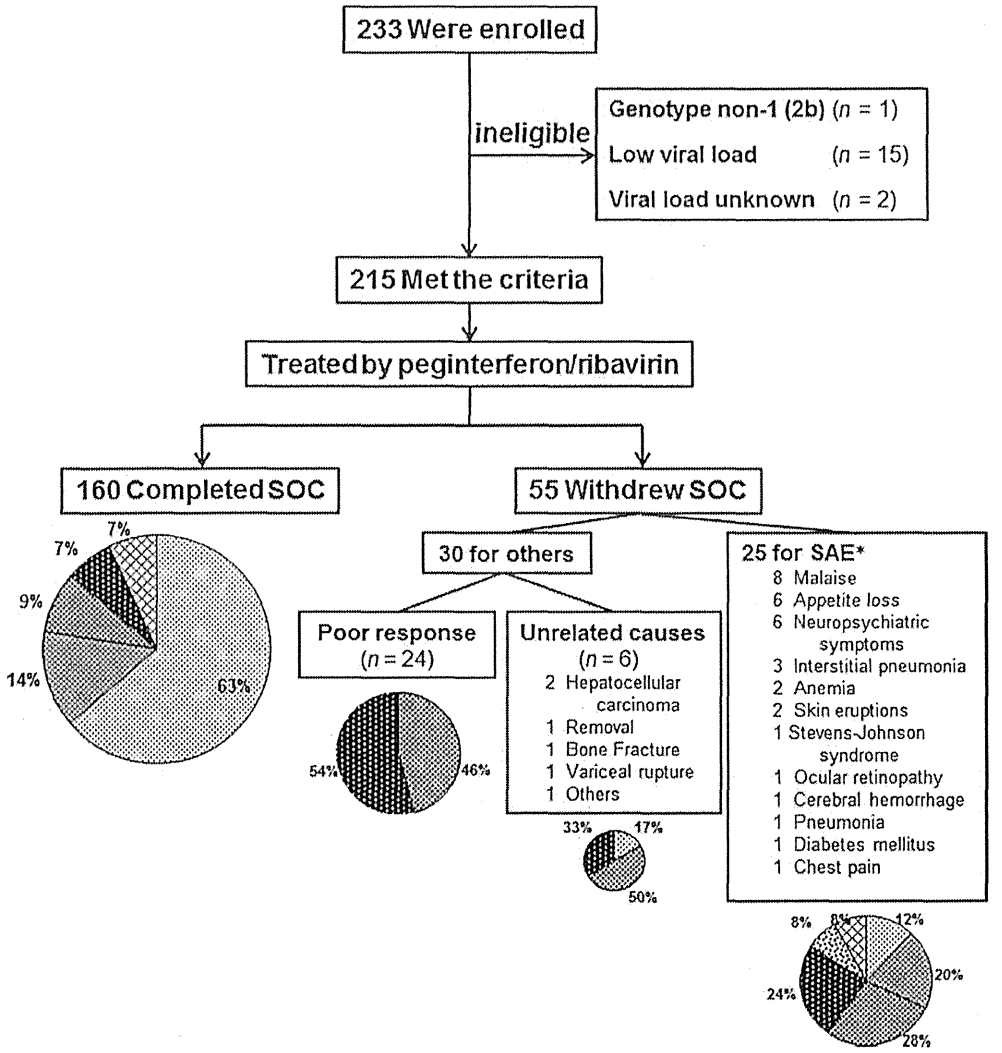

Figure 2 Enrollment and outcomes. Among 233 patients enrolled in this study, 18 patients were ineligible for the following reasons: genotype non-1 (2b) (n = 1); low viral load (n = 15); and unknown viral load (n = 2). Consequently, 215 patients met the entry criteria, and were treated with peginterferon and ribavirin. Among them, 160 patients completed standard of care (SOC). The remaining 55 patients were withdrawn from SOC, as detailed earlier. The virological outcomes with intent-to-treat analysis, as detailed in the Methods section, were shown as a pie chart for each group. *Serious adverse events were duplicated in some patients. RGT, response-guided therapy; SAE, serious adverse events; SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response; R, responder; f/u, follow-up. ⁘, SVR; ▩, TVR; ▩, Partial R (NVR); ■, Null R (NVR); ▩, undetemined (NVR); ◠, Lost-to-f/u.

As shown in Figure 2, if the patients completed at least 48 weeks of P/R (SOC) under conditions that observed the requirements of RGT, the SVR rate was as high as 63%, and the incidences of VR (the sum of SVR and TVR) was 77%. In the patients where the treatment was terminated for SAE or unrelated causes, the respective incidences of VR were reduced to 32% or 17%, respectively. In particular, treatment of 24 patients had to be terminated owing to the poor response to P/R, as defined by the requirements of RGT. This resulted in 11 "partial responders" (46%) and 13 "null responders" (54%). In addition, 9 cases developed viral breakthrough, and were subclassified into "partial responder": 6 of them completed SOC, while 3 stopped treatment because of the poor response to P/R (data not shown).

Patients' characteristics and IL28B genotypes. Whereas 154 individuals (71.6%) had the rs8099917 TT genotype (major-homo), 60 had the TG (hetero) genotype and 1 had the GG (minor-homo) genotype. The patients were classified into two groups, TT and non-TT (TG/GG), according to their rs8099917 genotypes, and their characteristics were compared. As shown in Table 1, lower levels of γ-GTP ($P < 0.001$), higher levels

Table 1 Comparisons of host and viral factors between *IL28B* TT and TG/GG genotypes

Factors	TT genotype (154)	TG/GG genotype (61)	*P* value
Age (years)	58 ± 11 (154)	58 ± 12 (61)	0.679
Gender (male/female)	88/66	31/30	0.448
Body weight (kg)	60.2 ± 11.2 (149)	58.7 ± 12.5 (60)	0.318
IFN naïve/experienced	123/31	47/14	0.711
PEG-IFN-α2a/-α2b	23/131	11/50	0.679
Albumin (g/dL)	4.1 ± 0.5 (153)	4.1 ± 0.4 (60)	0.721
AST (U/L)	56 ± 37 (154)	66 ± 49 (61)	0.332
ALT (U/L)	70 ± 52 (154)	77 ± 59 (61)	0.422
T.Bil (mg/dL)	0.86 ± 0.33 (150)	0.84 ± 0.37 (61)	0.658
ALP (U/L)	268 ± 93 (154)	254 ± 87 (61)	0.509
γ-GTP (U/L)	46 ± 43 (154)	78 ± 99 (61)	**< 0.001**
T.Chol (mg/dL)	172 ± 39 (151)	160 ± 29 (58)	**0.013**
LDL-C (mg/dL)	103 ± 28 (132)	84 ± 27 (57)	**< 0.001**
FBS (mg/dL)	101 ± 21 (130)	108 ± 29 (53)	0.076
IRI (μU/mL)	10.9 ± 9.5 (66)	15.5 ± 20.9 (26)	0.541
AFP (ng/mL)	14.4 ± 67.1 (141)	17.8 ± 26.2 (57)	**0.009**
HCV RNA (Log IU/mL)	6.5 ± 0.5 (154)	6.4 ± 0.6 (61)	0.243
WBC (/μL)	4917 ± 1367 (154)	4940 ± 1105 (61)	0.525
Hemoglobin (g/dL)	13.8 ± 1.3 (154)	13.7 ± 1.7 (61)	0.633
Platelets (x10^4/μL)	16.5 ± 5.9 (154)	16.9 ± 4.7 (61)	0.347
FIB-4 index	2.82 ± 1.77 (154)	2.80 ± 1.76 (61)	0.994
Core 70 amino acid (wild/mutant)	109/43	18/41	**< 0.001**
Core 91 amino acid (wild/mutant)	102/50	38/21	0.747
ISDR mutation (*n* = 0–1/2-)	126/25	50/7	0.523

Data are shown as mean ± standard deviation. Figures in parentheses are the numbers of data available in each factor. Significant *P* values are shown in bold.

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; HCV, hepatitis C virus; IRI, immune-reactive insulin; ISDR, interferon-sensitivity determining region; LDL-C, low-density lipoprotein cholesterol; T.Bil, total bilirubin; T.Chol, total cholesterol; WBC, white blood cell; γ-GTP, γ-glutamyl transpeptidase.

of total cholesterol (T.Chol; $P = 0.013$), higher levels of low-density lipoprotein cholesterol (LDL-C; $P < 0.001$), and lower levels of α-fetoprotein (AFP; $P = 0.009$) were significantly associated with TT genotype. The percentages of wild type of core 70 amino acid of patients with the TT genotype were significantly higher than those of patients with either TG or GG genotypes (71.7% *vs* 30.5%, $P < 0.001$).

Virological response, IL28B genotypes, and (TA)n of rs72258881. Intent-to-treat analysis of the entire cohort indicated SVR, TVR, and NVR rates of 49.3%, 12.6%, and 32.1%, respectively. As shown in Figure 3a, serum HCV RNA disappeared significantly earlier in patients with TT genotype than in those with either of the TG or GG genotype. Assessment of the usefulness of the rs8099917 non-TT genotype to predict NVR among 202 patients in whom the final virological outcome could be determined indicate a sensitivity of 63.8% (44/[44 + 25]); specificity of 88.0% (117/[117 + 16]); positive predictive value of 73.3% (44/[44 + 16]); negative predictive value of 82.4% (117/[25 + 117]), and an accuracy of 79.7% ([44 + 117]/202).

As shown in Figure 3b, the (TA)n of rs72258881 varied from 11 to 18, with the most frequent numbers of repeats being 12 ($n = 147$; 68.4%). Given that more than 12 repeats were found in 67 patients (31.2%), the cohort was divided into 2 × 2 groups, according to rs8099917 genotype (TT or non-TT) and (TA)n ($n = 11$–12 or $n = 13$–18), and the incidences of VR were calculated as [SVR + TVR]/[SVR + TVR + NVR]. As shown in Figure 3c, the incidences of VR tended to be higher in the group with longer (TA)n than in the group with shorter (TA)n, regardless of the rs8099917 genotype. In particular, in patients with the non-TT genotype, the longer (TA)n might increase VR more than the twofold, relative to that for patients with a shorter (TA)n [compare 18.4% in $(TA)_{11-12}$ *vs* 40.9% in $(TA)_{13-18}$, $P = 0.074$].

Factors associated with NVR. After excluding 13 patients who were lost to follow-up, we attempted to identify the variables that were associated with final virological outcome. As shown in Table 2, univariate analysis indicated that nine variables were significantly different or tended to be different between VR and NVR. NVR was associated with higher levels of serum AST, γ-GTP, and FIB-4, but with lower levels of T.Chol. Whereas the core 70-mino acid mutation was a viral factor related to NVR, the rs8099917 non-TT genotype and shorter (TA)n were host factors related to NVR. For nine variables for which univariate analysis indicated that the *P* value less than 0.15, multivariate logistic

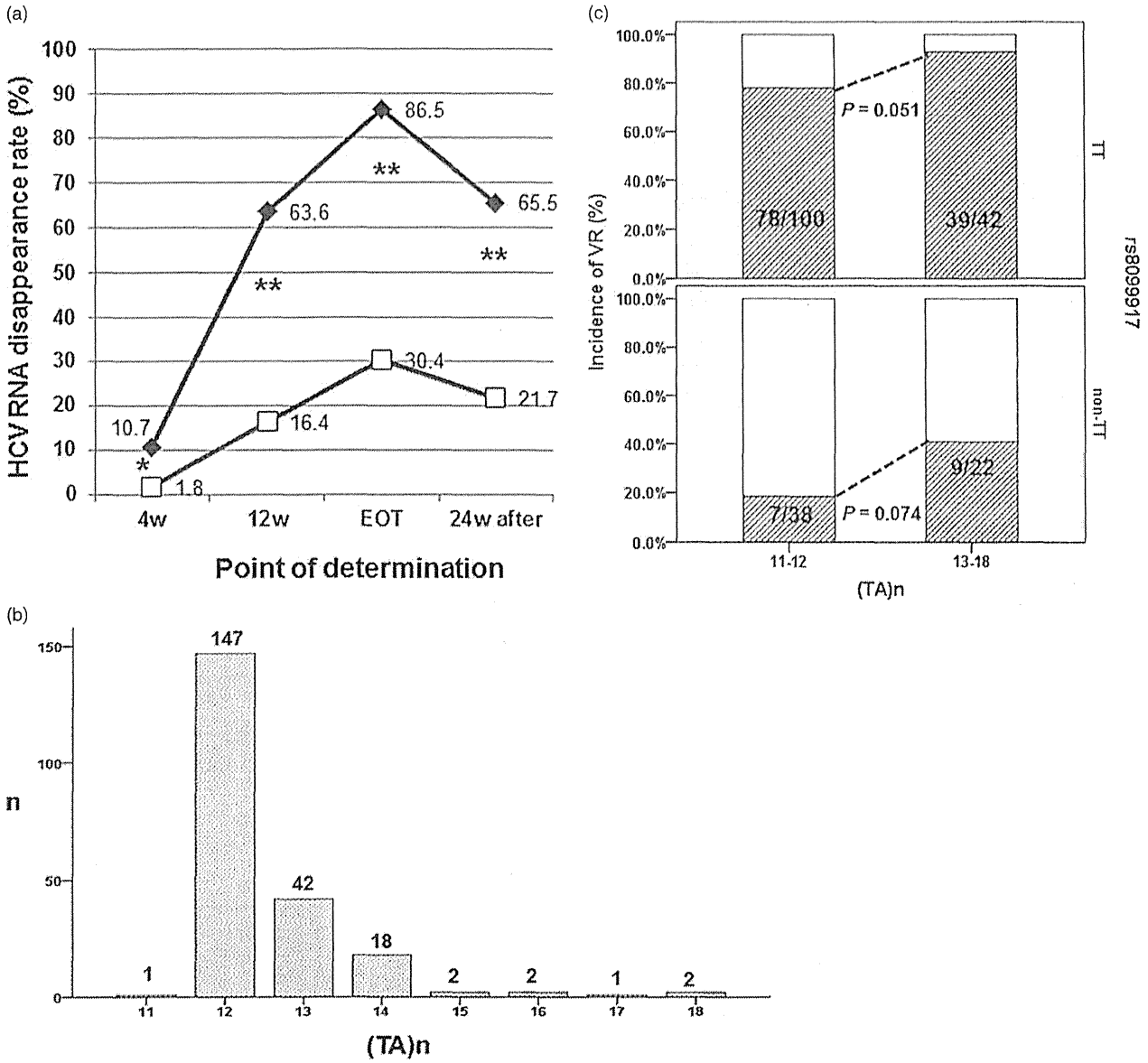

Figure 3 Genotype of rs8099917, (TA) dinucleotide repeat [(TA)n] of rs72258881 and virological response. (a) Hepatitis C virus (HCV) RNA disappearance rate. Serum HCV RNA disappeared significantly earlier in patients with TT genotype than in those with TG/GG genotypes. $*P < 0.05$, $**P < 0.001$. The abbreviation used was: EOT, end of treatment; HCV, hepatitis C virus; w, weeks. —◆—, TT genotype; —□—, TG/GG genotype. (b) Distributions of (TA)n in this cohort. The most frequent (TA)n was 12 (n = 147: 68.4%). In 67 patients (31.2%), the numbers were more than 12. (c) The incidences of virological response (VR) in four groups stratified by rs8099917 genotype and (TA)n. The longer (TA)n might favor virological responses to PEGylated interferon-α and ribavirin, regardless of the *IL28B* genotype.

regression analysis identified four variables for the prediction of NVR: HCV core 70 amino acid mutation, rs8099917 non-TT genotype, shorter (TA)n, and the lower levels of serum albumin. Finally, a decision tree temporarily modeled 22 pretreatment factors (see the legend to Fig. 4) to predict NVR. For this purpose, 13 patients who were lost to follow-up were excluded from the analysis, in order to avoid their influence on final decision. As shown in Figure 4, if the rs8099917 genotype was primarily selected as the predictive factor ($P < 0.001$, $\chi^2 = 57.647$), then the shorter (TA)n attributed to the NVR in patients with the TT genotype ($P = 0.023$, $\chi^2 = 5.166$). In cases with the shorter (TA)n (n = 11 or 12), the presence of the core 70 amino acid mutation in HCV might significantly increase the incidences of NVR ($P = 0.008$, $\chi^2 = 9.115$). On the other hand, in the patients with non-TT genotype, higher viral load was the second most powerful determinant of NVR ($P = 0.001$, $\chi^2 = 15.645$).

Table 2 Univariate and multivariate analyses of patients with chronic hepatitis C treated with pegylated interferon-α and ribavirin with respect to VR and NVR

Variable	Univariate analysis			Multivariate analysis			
	VR (133)	NVR (69)	*P* value	B	*P* value	Odds ratio	95% CI
Gender (Male/Female)	74/59	38/31	1.000				
Age (years)	58 ± 11 (133)	59 ± 11 (69)	0.973				
Body weight (kg)	59.9 ± 11.5 (130)	59.7 ± 11.6 (68)	0.992				
Hx. of IFN treatment (naïve/experienced)	108/25	52/17	0.363				
PEG-IFN-α2a/-α2b	18/115	12/57	0.533				
Albumin (g/dL)	4.2 ± 0.5 (133)	4.0 ± 0.4 (67)	0.148	0.118	**0.036**	1.125	1.008–1.256
AST (U/L)	52 ± 34 (133)	70 ± 50 (69)	**0.022**	−0.001	0.919	0.999	0.983–1.016
ALT (U/L)	66 ± 47 (133)	81 ± 64 (69)	0.244				
T.Bil (mg/dL)	0.88 ± 0.32 (129)	0.81 ± 0.35 (69)	0.085	0.102	0.083	1.108	0.987–1.243
ALP (U/L)	258 ± 80 (133)	273 ± 109 (69)	0.378				
γ-GTP (U/L)	45 ± 43 (133)	75 ± 95 (69)	**< 0.001**	−0.002	0.508	0.998	0.991–1.005
T.Chol (mg/dL)	172 ± 39 (130)	161 ± 32 (66)	**0.016**	0.004	0.556	1.004	0.991–1.017
HCV RNA (Log IU/mL)	6.5 ± 0.6 (133)	6.6 ± 0.5 (69)	0.384				
WBC (/μL)	4982 ± 1248 (133)	4784 ± 1271(69)	0.275				
Hemoglobin (g/dL)	13.9 ± 1.2 (133)	13.7 ± 1.8 (69)	0.308				
Platelets (× 10^4/μL)	17.0 ± 5.9 (133)	16.0 ± 5.0 (69)	0.377				
FIB-4 index	2.7 ± 1.7 (133)	3.1 ± 1.8 (69)	**0.035**	0.055	0.755	1.056	0.749–1.490
Core 70 amino acid (wild/mutant)	93/37	23/45	**< 0.001**	−0.914	**0.029**	0.401	0.177–0.910
Core 91 amino acid (wild/mutant)	92/38	42/26	0.205				
ISDR mutation (*n* = 0–1/2-)	108/20	59/8	0.528				
rs8099917 (TT/non-TT)	117/16	25/44	**< 0.001**	−2.735	**< 0.001**	0.065	0.025–0.171
(TA)n (*n* = 11–12/13–18)	85/48	53/16	0.079	−1.226	**0.011**	0.294	0.114–0.757
PEG-IFN adherence (%)	95.5 ± 10.3 (132)	89.7 ± 17.3 (64)	0.093				
Ribavirin adherence (%)	94.8 ± 10.9 (133)	90.3 ± 17.7 (64)	0.232				

Data are shown as mean ± standard deviation. Figures in parentheses are the numbers of data available in each variable. Multivariate and simultaneous logistic regression analysis was performed to determine predictive factors for NVR, by using nine variables which were found to be $P < 0.150$ by univariate analysis (albumin, AST, T.Bil, γ-GTP, T.Chol, fib-4 index, Core 70 amino acid, rs8099917, (TA)n). In addition, PEG-IFN adherence and ribavirin adherence were excluded from this analysis, since these two variables could not be available at pretreatment. The corresponding references in categorical variables were as follows: wild (Core 70 amino acid); TT (rs8099917); *n* = 13–18 [(TA)n]. Significant *P* values are shown in bold. The calculated values for serum albumin and T.Bil by multivariate logistic regression analysis correspond to those per 0.1 of increase.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCV, hepatitis C virus; Hx., history; ISDR, interferon-sensitivity determining region; NVR, non-virological response; PEG-IFN, pegylated interferon; T.Bil, total bilirubin; T.Chol, total cholesterol; VR, virological response; WBC, white blood cell; γGTP, γ-glutamyl transpeptidase.

Discussion

This is the first prospective evaluation of the usefulness of the rs8099917 SNP of the *IL28B* gene to predict virological outcome of RGT with P/R in the patients with chronic hepatitis C. Given that RGT has been accepted by AASLD,[2,23] European Association for the Study of the Liver (EASL),[29] and JSH[24] as the standard interferon-based treatment, it is often challenging to perform conventional per-protocol analysis in real clinical settings. With intent-to-treat analysis, the rates of SVR, TVR, and NVR were 49.3%, 12.6%, and 32.1%, respectively. The SVR rate in our cohort was as much as 10% higher than that in a report based on non-RGT with intent-to-treat analysis.[30] This might substantiate the value of RGT for the treatment of chronic hepatitis C. Differences between the TT genotype and non-TT genotype of rs8099917 in several background features are of interest. For instance, serum levels of γ-GTP and AFP were higher, while those of T.Chol and LDL-C were lower in patients with the non-TT genotype, compared with those with the TT genotype. Especially for the association of γ-GTP and LDL-C levels with *IL28B* genotypes, quite similar results were recently reported for HALT-C study[31] and our retrospective study,[32] respectively. However, the precise mechanisms by which *IL28B* genotypes affect levels of γ-GTP and LDL-C have yet to be elucidated. The current study confirmed that the core 70 amino acid mutation is more frequently associated with the non-TT genotype than with TT genotype.[33] The rs8099917 genotype could clearly differentiate between the effects of P/R on the disappearance of serum HCV RNA, which ultimately leads to SVR rate at 65.5% and 21.7%, in the TT and non-TT genotypes, respectively. However, given the less than 80% accuracy in predicting NVR in non-TT genotypes, there is likely a fair level of discrepancy between predicted and actual virological outcome. We have recently reported that the (TA) dinucleotide repeat of rs72258881, which lies in the promoter region of *IL28B* gene, can affect the transcriptional activity in a (TA)n length-dependent manner *in vitro*.[20] The current study has indicated that in clinical settings, increases in (TA)n tended to increase the incidences of VR in patients regardless of rs8099917 genotypes.

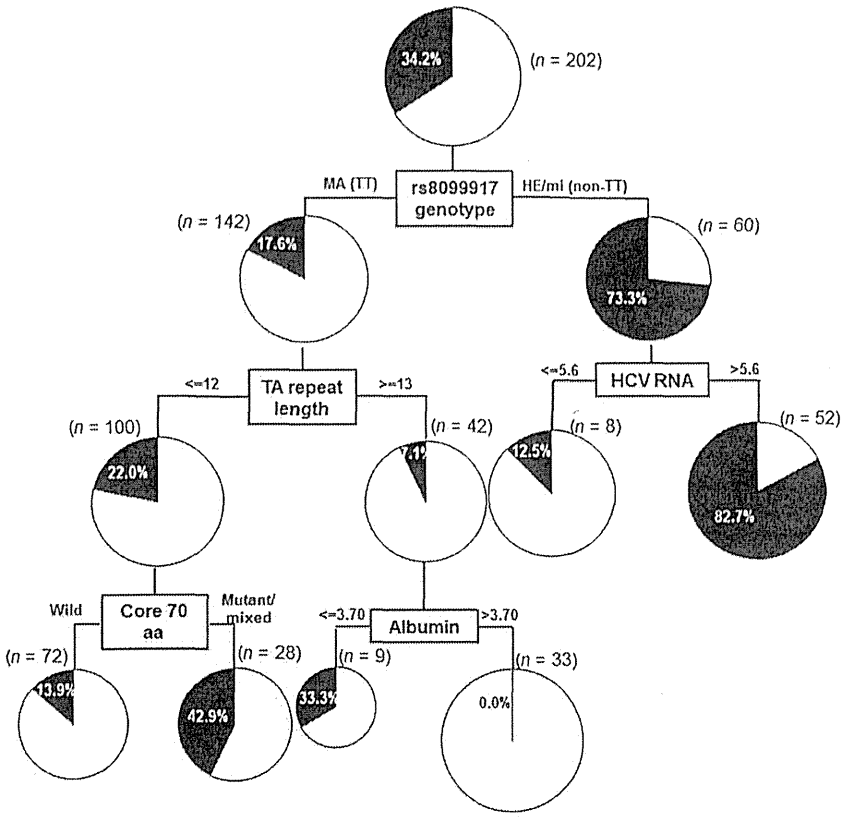

Figure 4 Decision tree analysis for the virological outcomes. Boxes indicate the factors used for splitting and the cut-off value for the split. Pie charts indicate the rate of non-virological response for each group of patients after splitting. A total of 202 patients were included in this analysis, after excluding 13 patients who were lost to follow-up, in order to avoid the influence on final decision. Among 22 pretreatment factors (gender, prior history of interferon, pegylated interferon regimen, age, body weight; serum albumin, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase, total cholesterol; white blood cell, hemoglobin, platelets; FIB-4; serum levels of hepatitis C virus (HCV) RNA (reverse transcription–polymerase chain reaction), core 70/91 amino acid mutation, interferon-sensitivity determining region mutation; rs8099917 genotype, TA repeat length) tested for their abilities to predict non-virological responses, determinations of (TA)n of rs72258881 and/or the HCV core 70 amino acid substitution were useful, especially for patients with the TT genotype. In the patients with a non-TT genotype, HCV viral load was the second most important determinant of virological response. MA, major-homo; HE, hetero; mi, minor-homo; aa, amino acid. The units used to measure levels of albumin and HCV RNA were g/dL and Log IU/mL, respectively.

Although the current study did not directly assess the expression of *IL28B* gene at the mRNA or protein levels, further investigation of mechanistic basis of the length-dependent effects of (TA)n on VR might help to elucidate how the responsiveness of patients to P/R is regulated by their levels of *IL28B* expression.

Univariate analysis indicated that NVR was associated with significantly higher levels of serum AST, γ-GTP, and FIB-4, together with lower levels of T.Chol, than for VR. These findings are consistent with those of our previous retrospective study that involved per-protocol analysis.[32] Regarding the viral factor, the core 70 amino acid mutation was significantly correlated with NVR, as reported previously.[12] Multivariate logistic regression analysis revealed that rs8099917 non-TT genotype, shorter (TA)n, core 70 amino acid mutation, and the lower levels of serum albumin were independently associated with NVR, in this prospective cohort with RGT. A decision tree analysis might demonstrate more clearly the clinical implications to measure simultaneously these host and viral factors at pretreatment.

There are several limitations to this study. First, the prospective study design prompted us to evaluate the virological response by performing intent-to-treat analysis, rather than strict per-protocol analysis. Especially, in patients for whom treatment needed to be terminated prematurely owing to SAE or other unrelated causes, virological outcomes were worse than expected. Nonetheless, the results of this study appear to reflect the real clinical settings used for the treatment of chronic hepatitis C. Another limitation was that the cohort contained fewer patients with non-TT genotype of rs8099917 than those with TT genotype. This might explain the unexpected observation that decision tree analysis did not select (TA)n as a predictive factor in the group with non-TT genotype. External validation is needed to establish whether the results of our prospective study could apply more generally. However, a

requirement for such studies is that the indications of current and future direct-acting antiviral agents for chronic hepatitis C should be further clarified in clinical settings.

In conclusion, the *IL28B* genotype and (TA)n of rs72258881 may independently affect the virological outcomes of RGT with P/R for chronic hepatitis C. At a minimum, when considering P/R-based regimens for chronic hepatitis C, pretreatment determinations of both genotypes as well as the core 70 amino acid mutation of HCV are promising cost-effective tools to predict VR.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Methods of TA repeat genotyping.