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Serum 25(OH)D_3 levels affect treatment outcomes for telaprevir/peg-interferon/ribavirin combination therapy in genotype 1b chronic hepatitis C

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ABSTRACT

Background: Close relationships between chronic hepatitis C and vitamin D levels have been reported. For genotype 1b infection, the current standard of care is pegylated interferon/ribavirin therapy combined with a protease inhibitor. The present study analyzed the relationship between outcomes of triple therapy and serum 25(OH)D_3 levels.
Methods: Factors contributing to sustained virological response were investigated in 177 patients with chronic hepatitis C who received telaprevir-based triple therapy in this prospective study.
Results: The sustained virological response rate was 86.9% in patients with 25(OH)D_3 levels of >18 ng/ml; this was higher than the 66.7% in patients with 25(OH)D_3 levels of ≤18 ng/ml ($P=0.003$). 25(OH)D_3 levels and *IL28B* genotype were identified as significantly independent factors contributing to sustained virological response. The sustained virological response rate did not differ according to 25(OH)D_3 levels in patients with the *IL28B* major genotype. The sustained virological response rate was 64.9% in patients with the *IL28B* minor genotype and 25(OH)D_3 levels of >18 ng/ml, and was 38.5% in those with decreased 25(OH)D_3 levels ($P=0.045$).
Conclusions: In triple therapy, 25(OH)D_3 levels were an independent factor contributing to sustained virological response. Of particular note, the sustained virological response rate was significantly lower in patients with the *IL28B* minor genotype.

1. Introduction

Pegylated interferon (PEG-IFN)/ribavirin combination therapy, together with protease inhibitors such as telaprevir and boceprevir, is currently one of the standards of care for chronic infection with hepatitis C virus (HCV) genotype 1b [1–6]. The sustained virological response (SVR) rate has improved to about 70–80%. Several factors contributing to the SVR have been reported in PEG-IFN/ribavirin/telaprevir combination therapy. Baseline factors contributing to SVR are genotype of interleukin-28B gene (*IL28B*) [7–9] and prior treatment response [9,10], as well as other factors such as core amino acid 70 substitution [8], liver fibrosis [9] and alpha-fetoprotein [11]. HCV RNA dynamics, including rapid virological response (RVR) [9,10], and drug adherence [12] are also considered to be important on-treatment factors associated with SVR.

Factors contributing to SVR in PEG-IFN/ribavirin therapy include age, gender, low-density lipoprotein (LDL)-cholesterol, liver fibrosis, interferon sensitivity-determining region, and core amino acid 70 and 91 substitutions [13–19]. In addition, serum levels of 25(OH)D_3, representing serum vitamin D, are reported to be an important factor [20,21]. Serum vitamin D_3 plays an important role in immunoregulatory actions on chronic HCV infection. In recent years, several studies have reported the direct anti-HCV effects of

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vitamin D [22,23]. However, much remains unknown about the effects of vitamin D on PEG-IFN/ribavirin combined with protease inhibitors.

We conducted a prospective analysis to investigate the relationship between serum 25(OH)D_3 levels and the effectiveness of treatment with PEG-IFN/ribavirin/telaprevir, which is the current standard of care for patients with chronic HCV genotype 1b infection.

2. Patients and methods

2.1. Study design

Subjects were 177 consecutive patients who visited Nippon Medical School Chiba Hokusoh Hospital, Shinmatsudo Central General Hospital, Jikei University School of Medicine Katsusika Medical Center, Jikei University School of Medicine Kashiwa Hospital, and Nippon Medical School between December 2011 and December 2012, and who met the inclusion criteria, agreed to receive PEG-IFN/ribavirin/telaprevir combination therapy and agreed to participate in the study. The study protocols followed the ethical guidelines established in accordance with the 2008 Declaration of Helsinki and were approved by the Ethics Committee of each institution. All patients provided written informed consent.

The inclusion criteria were as follows: patient age between 18 and 75 years; high viral load (>5.0 log IU/ml) as determined by quantitative analysis with real-time polymerase chain reaction (PCR); infection with HCV genotype 1b; white blood cell (WBC) count >2000/mm^3; neutrophil count >1000/mm^3; platelet count >50,000/mm^3; and haemoglobin concentration >10.0 g/dl. Exclusion criteria were as follows: other liver diseases, including autoimmune hepatitis, primary biliary cirrhosis, and alcoholic disease; positive result for hepatitis B surface antigen and antibody to human immunodeficiency virus type-1; decompensated liver cirrhosis; liver failure; severe renal disorder; abnormal thyroid function; poorly controlled diabetes; poorly controlled hypertension; medication with Chinese herbal medicine; medical history of interstitial pneumonia; severe depression; and allergy to IFN, ribavirin or biological preparations such as vaccines. In a large number of patients enrolled in this study, liver biopsy was performed within 12 months of enrollment. The presence or absence of cirrhosis was established according to Metavir score. For the remaining patients who were not subjected to liver biopsy, the presence or absence of cirrhosis was determined by computed tomography findings.

In the present study, the analysis only included those who completed 24 weeks of treatment. The primary objective of this study was to analyze factors associated with SVR and the relationships between serum 25(OH)D_3 levels and SVR rate. Its secondary objective was to analyze factors associated with serum 25(OH)D_3 levels.

2.2. Treatment protocol

All patients received combination therapy with PEG-IFN α-2b (Peg-Intron®; MSD, Tokyo, Japan), ribavirin (Rebetol®; MSD) and telaprevir (Telavic®; Mitsubishi Tanabe Pharma, Osaka, Japan) for 12 weeks, followed by 12 weeks of PEG-IFN α-2b and ribavirin. Patients received a subcutaneous injection of PEG-IFN α-2b at a dose of 1.5 μg/kg/week and oral administration of ribavirin. Ribavirin dose was adjusted by body weight (600, 800 and 1000 mg/day for <60, 60–80, and >80 kg, respectively) based on the guidelines of the Ministry of Health, Labour and Welfare of Japan. Telaprevir at a dose of 750 mg was administered every 8 h after meals. Doses were appropriately reduced when an adverse event such as anaemia, skin rash or renal insufficiency occurred during treatment course.

2.3. Definition of virological response

Patients were divided into categories according to the Japan Society of Hepatology guidelines. When HCV RNA was undetectable at 4 weeks of treatment, patients were considered to have achieved rapid virological response (RVR). Patients who were negative for the virus at the completion of treatment were judged as having achieved end-of-treatment response (ETR). Patients were followed for 24 weeks after treatment completion. Patients who were negative for virus at 24 weeks after treatment completion were judged as having SVR. Patients who exhibited ETR but became positive for the virus at 24 weeks after completion of treatment were considered to have relapsed. Patients who were persistently positive for HCV RNA throughout the treatment period were considered as having non-response (NR). Among patients with NR, patients whose plasma HCV RNA levels decrease by 2 log IU/ml from baseline at treatment 12 weeks but never become undetectable were considered as having partial response. Patients who failed to suppress plasma HCV RNA by at least 2 log IU/ml from baseline after combination therapy were considered as having null response. Treatment was stopped for patients with HCV RNA >3 $\log_{10}$ IU/ml at week 4, detectable HCV RNA at week 12, or a >2 $\log_{10}$ IU/ml increase in HCV RNA levels from the lowest levels during therapy.

2.4. Laboratory tests

Peripheral blood examination, liver function tests and renal function tests were performed weekly until 12 weeks after treatment initiation and then monthly until 24 weeks after completion of treatment. Serum levels of 25(OH)D_3 represented those of vitamin D, because 25(OH)D_3 is stable in the blood circulation and comprises a major portion of vitamin D in the body. Serum 25(OH)D_3 levels were measured at baseline by double-antibody radioimmunoassay at a commercial laboratory (SRL Inc., Tokyo, Japan). HCV RNA levels were measured by the real-time PCR method (COBAS® AmpliPrep; Roche Diagnostics, Tokyo, Japan). Gene mutations in the core and nonstructural protein 5A (NS5A) regions of the HCV genome were measured by the direct sequencing method. Genomic DNA was extracted from whole blood using a DNA isolation kit on the MagNA Pure LC Instrument (Roche Diagnostics, Basel, Switzerland). Single nucleotide polymorphisms (SNPs) rs8099917 of *IL28B* were determined by real-time detection PCR using the TaqMan® SNP genotyping assay on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The rs8099917 genotype was classified into two categories: TT (major genotype) and non-TT (minor genotype: TG or GG).

2.5. Statistical analysis

Fisher's exact test was performed in order to compare SVR rates according to prior treatment response, *IL28B* genotype and serum 25(OH)D_3 levels. Logistic regression analysis for univariate comparison was performed to investigate whether each factor influenced SVR rates and high serum 25(OH)D_3 levels. Multiple logistic regression analysis was also performed to identify significant, independent factors that influenced serum 25(OH)D_3 levels. A receiver operating characteristic (ROC) curve was generated in order to analyze levels of serum 25(OH)D_3 that most reasonably predicted SVR. All statistical analyses were performed using IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan). The level of statistical significance was set at $P < 0.05$.

Table 1
Baseline characteristics of the 177 patients.

Factor	N = 177
Age (years)	60 (18–75)
Male gender	80 (45.2%)
Prior treatment response (naïve, relapse, partial, null response)	95 (53.7%)/56 (31.6%) /18 (10.2%)/8 (4.5%)
BMI	22.90 (15.94–37.81)
Leucocytes (per μl)	4700 (2200–11,100)
Haemoglobin (g/dl)	14.0 (11.1–17.5)
Platelets (10^3/μl)	16.6 (5.6–40.7)
AST (IU/l)	43 (13–215)
ALT (IU/l)	46 (13–305)
gamma-GT (IU/l)	38 (11–339)
Total bilirubin (mg/dl)	0.8 (0.3–2.3)
Albumin (g/dl)	4.1 (2.8–5.0)
LDL-cholesterol (mg/dl)	98 (21–194)
Serum creatinine (mg/dl)	0.66 (0.36–1.34)
Prothrombin Time (%)	95.5 (18.6–157.1)
Alpha-fetoprotein (ng/ml)	4.6 (1.4–625.7)
HCV RNA (log IU/ml)	6.6 (5.0–7.8)
ISDR wild type	104 (58.8%)
Core aa70 wild type	114 (64.4%)
Core aa91 wild type	117 (66.1%)
IL28B genotype (rs8099917) TT	114 (64.4%)
25 (OH)D_3 (ng/ml)	21 (7–61)
Fibrosis non-cirrhosis	159 (89.8%)

Categorical values are represented as the number of patients. Continuous variables are represented as median (range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL-cholesterol, low-density lipoprotein cholesterol; gamma-GT, gamma-glutamyltransferase; T-Bil, total bilirubin; ISDR, interferon sensitivity determining region; aa, amino acid; IL28B, interleukin 28B.

3. Results

3.1. Backgrounds and virological response rates

Subject characteristics are shown in Table 1. Subjects consisted of 80 males and 97 females. Median patient age was 60 years (range, 18–75 years). There were 93 treatment-naïve patients, 56 relapsers, 18 partial responders and 18 null responders. Among the 177 patients, 141 (80.1%) had RVR after 4 weeks of triple therapy, 162 (92.0%) showed ETR, and 142 (80.7%) achieved SVR. Serum 25(OH)D_3 levels, calculated based on prior treatment response, were as follows: treatment-naïve, 22 ng/ml, relapsers: 20 ng/ml; partial responders, 19 ng/ml; and null responders, 18 ng/ml. The following adverse events were observed among the 177 patients: anaemia, 73.4%; skin rash, 41.8%; renal dysfunction, 29.9%; increased uric acid levels, 66.1%; psychiatric symptoms, 11.9%; and digestive symptoms, 18.1%. Serious adverse events requiring treatment discontinuation did not occur in any patient, and there were no cases that met the criteria for stopping treatment.

3.2. Factors contributing to achieving SVR

Univariate analysis identified the following factors contributing to SVR (Table 2): age ($P = 0.041$, odds ratio (OR) = 1.047, 95% confidence interval (CI) = 1.00–1.09); gender ($P = 0.043$, OR = 1.027, 95% CI = 1.03–5.15); white blood cell count ($P = 0.002$, OR = 1.001, 95% CI = 1.00–1.00); haemoglobin level ($P = 0.021$, OR = 1.382, 95% CI = 1.05–1.82); platelet count ($P = 0.020$, OR = 1.01,0 95% CI = 1.02–1.19); gamma-glutamyl transpeptidase (γ-GTP) ($P = 0.028$, OR = 1.006, 95% CI = 1.00–1.01); LDL-cholesterol ($P = 0.001$, OR = 1.026, 95% CI = 1.01–1.04); albumin ($P = 0.037$, OR = 3.239, 95% CI = 1.07–9.77); fibrosis ($P = 0.032$, OR = 3.088, 95% CI = 1.10–8.64), 25(OH)D_3 level ($P = 0.010$, OR = 1.087, 95% CI = 1.02–1.16); core amino acid 70 substitution ($P = 0.0001$, OR = 5.175, 95% CI = 2.28–11.74); core amino acid 91 substitution ($P = 0.021$, OR = 2.525, 95% CI = 1.15–5.53); *IL28B* genotype ($P = 2.22 \times 10^{-8}$, OR = 18.594, 95% CI = 6.68–51.78);and prior treatment response ($P = 0.0001$, OR = 36.815, 95% CI = 4.35–311.44) (Table 2).

Multivariate analysis identified 25(OH)D_3 levels ($P = 0.042$, OR = 1.089, 95% CI = 1.00–1.18) and *IL28B* genotype ($P = 1.38 \times 10^{-5}$, OR = 20.349, 95% CI = 5.23–79.16) as significantly independent factors contributing to SVR.

3.3. Relationship between serum 25(OH)D_3 levels and virological response rates

The cut-off value for serum 25(OH)D_3 levels that most efficiently predicted SVR was determined based on ROC curve analysis. The cut-off value for serum 25(OH)D_3 levels was 18 ng/ml (sensitivity = 0.746, specificity = 0.529, positive predictive value = 0.667, negative predictive value = 0.131, and area under the curve (AUC) = 0.658). Using a cut-off value of 18 ng/ml, SVR rates were 86.9% (106/122) in patients with serum 25(OH)D_3 levels of >18 ng/ml and 66.7% (36/54) in patients with serum 25(OH)D_3 levels of ≤18 ng/ml ($P = 0.003$; Fig. 1). Meanwhile, RVR and ETR rates were not significantly different between the two groups. Subsequently, variations in SVR rate between different serum 25(OH)D_3 levels in each prior treatment were examined. In treatment-naïve patients, SVR rates were 88.2% (60/68) in patients with serum 25(OH)D_3 levels of >18 ng/ml and 69.2% (18/26) in patients with serum 25(OH)D_3 levels of ≤18 ng/ml ($P = 0.062$). In relapsers, SVR rates were 94.9% (37/39) in patients with serum 25(OH)D_3 levels of >18 ng/ml and 88.2% (15/17) in patients with serum

Table 2
Univariate logistic regression analysis of baseline factors associated with sustained virological response.

Factors		Univariate		
		OR	95% CI	P value
Age	By 1year decrements	1.047	1.00–1.09	0.041
Gender	Male	2.301	1.03–5.16	0.043
Leukocytes (per μl)	By 1000 increments	1.001	1.00–1.00	0.002
Haemoglobin (g/dl)	By 1 g/dl increments	1.382	1.05–1.82	0.021
Platelets ($\times 10^3$/μl)	By 10 increments	1.010	1.02–1.19	0.020
gamma-GT (IU/ml)	By 10 decrements	1.006	1.00–1.01	0.028
LDL-cholesterol (mg/dl)	By 10 mg/dl increments	1.026	1.01–1.04	0.001
Albumin (g/dl)	By 0.1 g/dl increments	3.239	1.07–9.77	0.037
Fibrosis	Non-cirrhosis	3.088	1.10–8.69	0.032
25(OH)D_3 (ng/ml)	By 1 ng/ml increments	1.087	1.02–1.16	0.010
Core aa70 substitution	Wild-type	5.175	2.28–11.74	0.0001
Core aa91 substitution	Wild-type	2.525	1.15–5.53	0.021
IL28B genotype	TT	18.594	6.68–51.78	2.22×10^{-8}
Prior treatment response	Not null response	36.815	4.35–311.44	0.0001

gamma-GT, gamma-glutamyltransferase; LDL, low-density lipoprotein; aa, amino acid; IL28B, interleukin 28B.

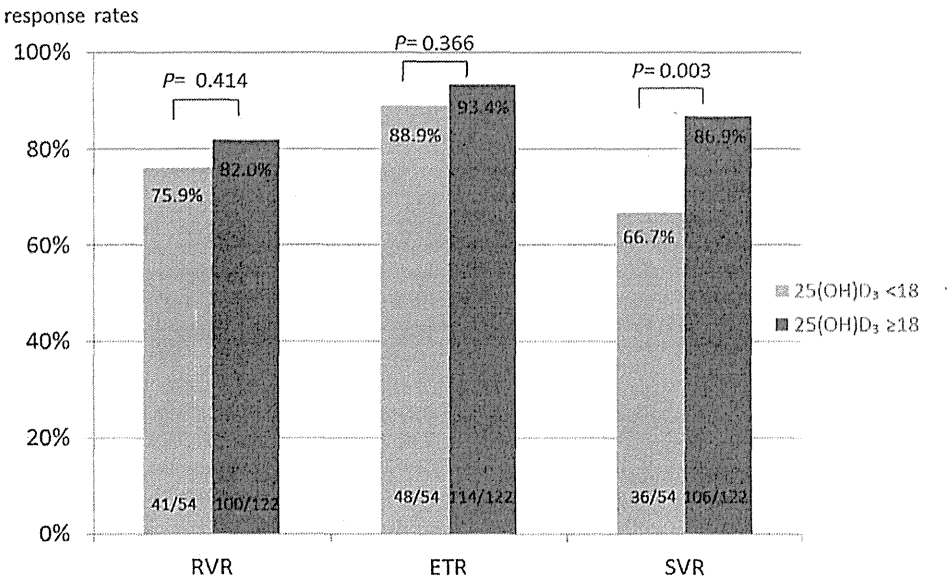

Fig. 1. Comparison of virological response rates according to serum 25(OH)D_3 levels. In each response category, patients were divided into two groups (<18 ng/ml *vs.* ≥18 ng/ml) based on the cut-off value of serum 25(OH)D_3 level. RVR, rapid virological response; ETR, end of treatment response; SVR, sustained virological response.

25(OH)D_3 levels of ≤18 ng/ml (P=0.577). In partial responders, SVR rates were 72.7% (8/11) in patients with serum 25(OH)D_3 levels of >18 ng/ml and 42.9% (3/7) in patients with serum 25(OH)D_3 levels of ≤18 ng/ml (P=0.332). In null responders, SVR rates were 25.0% (1/4) in patients with serum 25(OH)D_3 levels of >18 ng/ml and 0.0% (0/4) in patients with serum 25(OH)D_3 levels of ≤18 ng/ml (P=1.000).

3.4. Relationship between serum 25(OH)D_3 levels and IL28B genotype and SVR rates

Among patients with the *IL28B* major genotype TT, SVR rates were 96.5% (82/85) in those with serum 25(OH)D_3 levels of >18 ng/ml and 92.9% (26/28) in patients with serum 25(OH)D_3 levels of ≤18 ng/ml (P=0.596; Fig. 2). Among patients with *IL28B* minor genotype non-TT, SVR rates were 64.9% (24/37) in patients with serum 25(OH)D_3 levels of >18 ng/ml and 38.5% (10/26) in patients with serum 25(OH)D_3 levels of ≤18 ng/ml (P=0.045; Fig. 2).

3.5. Factors that influenced serum 25(OH)D_3 levels

Factors that influenced serum 25(OH)D_3 levels were also investigated. Univariate analysis showed that the following factors were significantly associated with high levels of 25(OH)D_3: gender (P=0.0001, OR=4.264, 95% CI=2.05–8.88); haemoglobin levels (P=0.001, OR=1.530, 95% CI=1.20–1.95); LDL-cholesterol (P=0.004, OR=1.019, 95% CI=1.01–1.03); serum creatinine

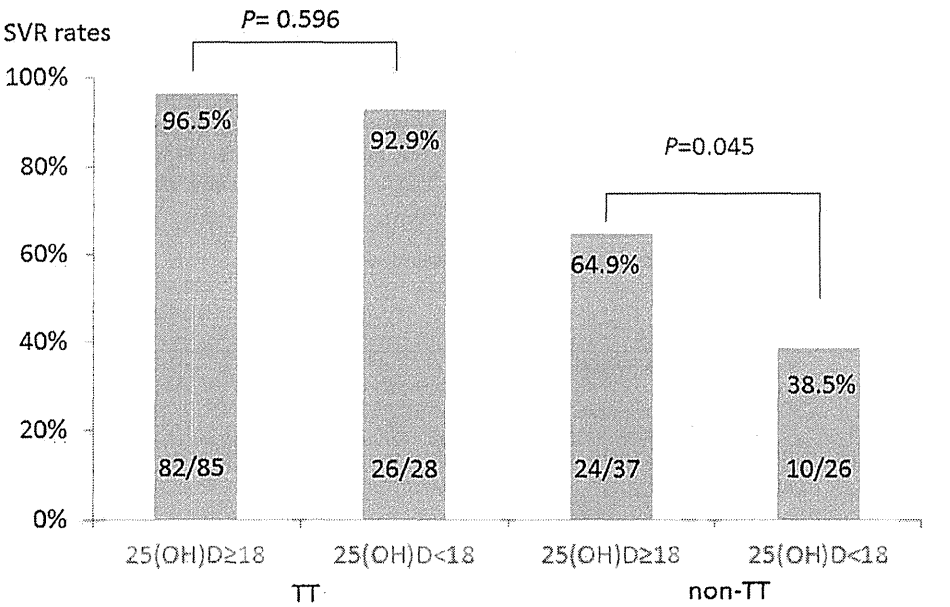

Fig. 2. Comparison of sustained virological rates according to IL28B genotype and serum 25(OH)D_3 level. The cut-off values of serum 25(OH)D_3 levels that predict SVR were determined from the ROC curve analysis and divided into two groups: ≥18 and <18 ng/ml. SVR, sustained virological response.

Table 3
Univariate and multivariate logistic regression analyses of factors associated with increase in serum 25(OH)D_3 levels (>18 ng/ml) in the 177 patients with chronic hepatitis C.

Factors		Univariate			Multivariate		
		OR	95% CI	*P* value	OR	95% CI	*P* value
Gender	Male	4.264	2.05–8.88	0.0001	3.029	1.15–7.98	0.025
Haemoglobin (g/dl)	By 1.0 increments	1.530	1.20–1.95	0.001			
LDL-cholesterol (mg/dl)	By 10 increments	1.019	1.01–1.03	0.004	1.014	1.00–1.03	0.049
Serum creatinine (mg/ml)	By 0.1 increments	30.705	2.78–339.56	0.005			
IL28B genotype	TT	2.133	1.10–4.12	0.024			

LDL, low-density lipoprotein; IL28B, interleukin 28B. Multivariate analysis was performed with factors significantly associated with 25(OH)D_3 levels by univariate analysis.

($P = 0.005$, OR = 30.705, 95% CI = 2.78–339.56); and *IL28B* genotype ($P = 0.024$, OR = 2.133, 95% CI = 1.10–4.12) (Table 3). Multivariate analysis identified gender ($P = 0.025$, OR = 3.029, 95% CI = 1.15–7.98) and LDL-cholesterol ($P = 0.049$, OR = 1.014, 95% CI = 1.00–1.03) as significantly independent factors that influenced serum 25(OH)D_3 levels (Table 3).

4. Discussion

This study is the first to investigate the correlations between serum 25(OH)D_3 levels and treatment outcomes in PEG-IFN/ribavirin/telaprevir combination therapy for chronic infection with HCV genotype 1b. In this patient cohort, the SVR rate was 80.7% and the IL28B genotype was the strongest contributor to SVR. These findings were consistent with previous reports [7–9]. Moreover, the present study demonstrated that serum 25(OH)D_3 levels are an important independent contributor in certain patient sub-populations. 25(OH)D_3 circulates stably in the body and represent the highest serum concentration among the various forms of vitamin D_3. Vitamin D_3 is produced *via* two routes, dietary intake and synthesis from ultraviolet light in the skin, and is converted to 25(OH)D_3 by hydroxylation in the liver. Thereafter, 25(OH)D_3 binds to vitamin D-binding protein and is transported to the kidney, where the 1α position is hydroxylated to form 1α,25$(OH)_2D_3$ [24]. Levels of 25(OH)D_3 decrease in various disease conditions: ageing; obesity; liver failure; nephrotic syndrome; rickets; state of total gastrectomy [24,25]; and chronic hepatitis [26,27]. Vitamin D appears to play a key role in immunoregulatory mechanisms. There have been several studies on the expression of vitamin D receptors in macrophages, T-cells and B-cells [28], vitamin D-enhanced antigen presentation capacity in dendritic cells [29], and vitamin D-induced cytotoxic activity in natural killer (NK) cells [30]. In recent years, an *in vitro* study showed that 25(OH)D_3 has direct anti-HCV effects by suppressing the formation of infectious HCV particles [23]. Similarly, another *in vitro* study observed that vitamin D decreased HCV secretion in cell culture. IFN was reported to have a synergistic effect on reduced HCV secretion [22].

There have been numerous reports on serum 25(OH)D_3 levels with regard to PEG-IFN/ribavirin dual combination therapy for chronic hepatitis C. Most of these have shown a relationship between serum 25(OH)D_3 levels and PEG-IFN/ribavirin treatment outcomes. Bitetto et al. reported unfavourable responses to IFNα/ribavirin therapy following liver transplantation in patients with low vitamin D concentrations [31]. Vitamin D supplementation was suggested to improve antiviral treatment outcomes [31]. Petta et al. reported that serum 25(OH)D_3 levels were lower in chronic hepatitis C patients than in healthy volunteers, and that serum 25(OH)D_3 levels are linked to progression of hepatic fibrosis. Petta et al. also reported that low vitamin D levels were linked to low SVR rate in IFN-based therapy [27]. Bitetto et al. found that the cut-off value of serum 25(OH)D_3 levels (20 ng/ml) was a useful predictor of SVR to PEG-IFN/ribavirin in combination with *IL28B* rs12979860 genotype [20]. Meanwhile, Kitson et al. reported no relationship between serum 25(OH)D_3 levels and treatment effects in PEG-IFN/ribavirin therapy; in contrast to numerous studies that found 25(OH)D_3 serum levels to be low in patients achieving SVR [32]. We also reported that serum 25(OH)D_3 levels are lower in cirrhotic patients and that serum 25(OH)D_3 levels influence the outcome of peg-IFN/ribavirin dual combination therapy for HCV genotype 1b patients with compensated cirrhosis [33].

The present study had several limitations. First, the sample size was small and may be inadequate for analysis. The second limitation is that 25(OH)D_3 levels are affected by various factors, such as age and season; for example, serum 25(OH)D_3 levels are higher in summer and autumn than in winter and spring. Third, the present study did not measure SNPs associated with vitamin D. Hence, further research will be needed to address these issues. Although the presence of hepatic steatosis may have had an impact on the effects of treatment, this was difficult to assess because not all of the present patients underwent liver biopsy.

The present study identified high serum 25(OH)D_3 levels as an independent factor contributing to SVR in PEG-IFN/ribavirin/telaprevir triple combination therapy for chronic HCV genotype 1b infection. Of note, the SVR rate did not differ according to serum 25(OH)D_3 levels in patients with the *IL28B* major genotype. The prominently high SVR rate probably diminishes the importance of serum 25(OH)D_3 levels. Importantly, serum 25(OH)D_3 levels may make a significant contribution to the prediction of SVR in patients with the unfavourable *IL28B* minor genotype. As a promising future possibility, SVR can be predicted by measuring serum 25(OH)D_3 levels prior to treatment in next-generation protease inhibitors, such as simeprevir, combined with PEG-IFN/ribavirin [34,35]. Moreover, we previously reported the possibility that higher SVR rates can be achieved using add-on alfacalcidol, an active form of vitamin D, in PEG-IFN/ribavirin therapy for elderly patients with chronic hepatitis C, who may have low serum vitamin D levels [36]. Increased serum 25(OH)D_3 levels through vitamin D supplementation may improve SVR rate in patients with low serum 25(OH)D_3 levels or unfavourable factors, such as the *IL28B* minor genotype. It may also be of interest to confirm whether the prolonged administration of PEG-IFN/ribavirin enhances treatment effects in patients with IL28B minor genotype and low serum 25(OH)D_3 levels.

In conclusion, serum 25(OH)D_3 levels are an independent factor contributing significantly to SVR in the triple combination therapy with PEG-IFN/ribavirin/telaprevir for chronic HCV genotype 1b infection. Specifically, the 25(OH)D_3 index may be a useful predictor in patients with the *IL28B* minor genotype.

Conflict of interest
None declared.

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Effect of fluvastatin on 24-week telaprevir-based combination therapy for hepatitis C virus genotype 1b-infected chronic hepatitis C

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Objectives **The addition of fluvastatin significantly improves sustained virological response (SVR) in pegylated interferon and ribavirin (peg-IFN/RBV) combination therapy for patients infected with the hepatitis C virus. However, the add-on effect on telaprevir-based triple combination therapy remains unknown. The aim of this study was to investigate the effect of fluvastatin on telaprevir-based combination therapy by conducting a prospective, open-label, randomized, controlled trial.**

Patients and methods **Among 124 genotype 1b-infected chronic hepatitis C patients recruited, 116 eligible patients were allocated randomly to two study arms; they received 12 weeks of telaprevir/peg-IFN/RBV, followed by 12 weeks of peg-IFN/RBV with or without 24 weeks of fluvastatin (fluvastatin group and control group, respectively). Treatment outcomes and adverse effects were compared between the two groups.**

Results **There were 56 men and 60 women, median age 60 years (range, 28–71 years). Rapid virological response and end of treatment response rates were 87.9% (51/58) and 96.6% (56/58) in the control group and 75.9% (44/58) and 98.3% (57/58) in the fluvastatin group, respectively. SVR rates in the control group and the fluvastatin group were 84.5% (49/58) and 81.0% (47/58), respectively; there was no significant difference ($P=0.806$). Stratified analysis showed that no factors associated with the SVR rate were found between the two groups. No adverse events were associated with fluvastatin.**

Conclusion **In this trial, administration of fluvastatin with telaprevir/peg-IFN/RBV was a safe combination. However, fluvastatin had no add-on effect on 24-week telaprevir-based combination therapy for chronic hepatitis C genotype 1b-infected patients.** ***Eur J Gastroenterol Hepatol*** **00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Pegylated interferon and ribavirin (peg-IFN/RBV) therapy, in combination with protease inhibitors such as telaprevir or boceprevir, is currently the standard of care for genotype 1b chronic hepatitis C. The combination improves treatment outcomes, and sustained virological response (SVR) can be achieved in more than 70% of patients [1–3], especially in more than 80% of patients with the *interleukin 28B (IL28B)* major genotype [4–6]. At the same time, there are patients in whom SVR cannot be achieved; therefore, further development and modification of treatment drugs are needed.

Previously, we showed in a prospective, randomized, controlled trial that the SVR rate for genotype 1b chronic hepatitis C can be increased significantly by combining fluvastatin, a statin that is a hydroxymethylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitor, with peg-IFN/RBV therapy [7,8]. An add-on effect with fluvastatin has especially been observed in male patients and patients with the *IL28B* major genotype.

Statins inhibit cholesterol synthesis by suppressing mevalonic acid synthesis from HMG-CoA by HMG-CoA reductase in the cholesterol biosynthesis pathway. Low-density lipoprotein (LDL)-cholesterol was reported to decrease 20–30% when fluvastatin was administered orally at 20–40 mg/day [9,10]. Statins also inhibit the synthesis of geranylgeranyl pyrophosphate from farnesyl pyrophosphate in the cholesterol synthesis pathway [11].

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Geranylgeranyl pyrophosphate binds to host protein and forms geranylgeranylated protein. Geranylgeranylated protein has been shown to play an important role in the replication of hepatitis C virus (HCV) RNA [12]. Thus, statins are considered to inhibit replication of HCV RNA by inhibiting the synthesis of geranylgeranyl pyrophosphate and the subsequent formation of geranylgeranylated protein [13]. Moreover, statins have been shown to inhibit HCV RNA replication *in vitro* [14,15].

Telaprevir is a strong inhibitor of cytochrome P450 3A (CYP3A). Consequently, when telaprevir is combined with drugs metabolized by CYP3A4, the plasma concentrations of those drugs increase, which may result in an increase in adverse events. Atorvastatin, lovastatin, and simvastatin are mainly metabolized by CYP3A4, and so their combination with telaprevir is contraindicated [16]. It has also been suggested that statin plasma concentrations of fluvastatin, pitavastatin, and pravastatin increase when combined with telaprevir. Careful monitoring for adverse events during treatment is recommended [16].

The add-on effect on telaprevir-based triple combination therapy remains unknown. On the basis of our previous finding that the SVR rate was improved by combining fluvastatin with peg-IFN/RBV therapy, we designed the present prospective, randomized, controlled study to examine the effect of fluvastatin on telaprevir-based combination therapy in patients with HCV genotype 1b. We also evaluated the safety of the fluvastatin add-on in peg-IFN/RBV/telaprevir therapy.

Patients and methods

Study design

This was an open-label, prospective, randomized, multicenter trial. Among 124 consecutive patients with genotype 1b, chronic hepatitis C who visited Nippon Medical School Chiba Hokusoh Hospital, Shinmatsudo General Central Hospital, Nippon Medical School, and Hakujikai Memorial Hospital between December 2011 and November 2012, 116 patients fulfilled the inclusion criteria. The remaining eight patients were excluded: six had thrombocytopenia and two did not provide written informed consent. Patients were eligible for enrollment if they fulfilled the following criteria: HCV RNA detectable in serum by real-time PCR; white blood cell count of more than 2000/mm^3; platelet count of more than 500 000/mm^3; and hemoglobin levels of more than 10 g/dl on laboratory testing before treatment initiation. The exclusion criteria were as follows: positive result for hepatitis B surface antigen and antibody to HIV-1, complications by other chronic liver diseases such as autoimmune hepatitis, primary biliary cirrhosis, or alcoholic hepatitis; decompensated liver cirrhosis; current development of hepatocellular carcinoma; severe renal disease; abnormal thyroid function; poorly controlled diabetes; poorly controlled hypertension; medication with Chinese herbal medicine; medical history of interstitial pneumonia; pregnancy or possibility of pregnancy; lactating; severe depression; medical history of allergy to biological preparations such as vaccine; medication with fibrates or statins; or medical history of allergy to interferon, ribavirin, telaprevir, or fluvastatin.

Using a random number table generated by a computer, the study patients were allocated randomly to either the group receiving peg-IFN/RBV/telaprevir without fluvastatin, provisionally designated as the control group, or the group receiving peg-IFN/RBV/telaprevir with fluvastatin, provisionally designated as the fluvastatin group.

The study protocol was formulated following ethical guidelines established in conformity with the 2004 Declaration of Helsinki after approval by the Ethics Committees of Nippon Medical School Chiba Hokusoh Hospital (No. 523029) and Shinmatsudo Central General Hospital. All patients provided written informed consent.

Treatment and definition of virological response

All patients received combination therapy with peg-IFNα2b (PEGINTRON; MSD, Tokyo, Japan), ribavirin (REBETOL; MSD), and telaprevir (TELAVIC; Mitsubishi Tanabe Pharma, Osaka, Japan) for 12 weeks, followed by 12 weeks of peg-IFNα2b and ribavirin. Peg-IFNα-2b was injected subcutaneously 1.5 μg/kg once weekly. The patients received oral administration of ribavirin. The dose of ribavirin was adjusted by body weight (600, 800, and 1000 mg/day for <60, 60–80, and >80 kg, respectively) on the basis of the guidelines of the Ministry of Health, Labor and Welfare of Japan. Telaprevir was administered at a dose of 750 mg every 8 h after meals. The doses were appropriately reduced when an adverse event such as anemia, skin rash, or renal insufficiency occurred during the course of treatment. Fluvastatin was administered orally at 30 mg/day for 24 weeks. When HCV RNA was undetectable at 4 and 12 weeks after the initiation of treatment, patients were considered to have achieved a rapid virological response (RVR) and a complete early virological response (cEVR), respectively. Patients who tested undetectable for HCV RNA at the time of treatment completion were considered to have achieved end of treatment response (ETR). The patients were followed for 24 weeks after treatment completion. Patients who were found undetectable for HCV RNA at 24 weeks after treatment completion were considered to have achieved an SVR. Patients who showed an ETR, but in whom the virus was detected at 24 weeks after completion of treatment were considered to have a relapse. Patients who failed to achieve HCV RNA negativity by the end of treatment were considered to have a nonvirological response.

Laboratory tests

Peripheral blood examination, liver function tests, and renal function tests were performed weekly until 24

weeks after treatment initiation and then monthly until 24 weeks after the completion of treatment. For biochemical tests before treatment initiation, data were obtained in the fasting state. HCV RNA levels were measured using real-time PCR (COBAS AmpliPrep; Roche Diagnostics, Tokyo, Japan). Gene mutations in the core amino acids 70 and 91, and NS5A regions [interferon sensitivity determining region (ISDR)] of the HCV genome were determined using the direct sequencing method. Core amino acid 70 was defined as the wild type (arginine) or the mutant type (glutamine or histidine), and core amino acid 91 was defined as the wild type (leucine) or the mutant type (methionine). Amino acid mutations in the ISDR were defined as wild type (0, 1) or mutant type (others). Genomic DNA was extracted from whole blood using a DNA isolation kit on the MagNA Pure LC Instrument (Roche Diagnostics, Basel, Switzerland). Single nucleotide polymorphisms (SNPs) at rs8099917, which is located in the locus adjacent to the *IL28B* gene on chromosome 19, were determined using real-time PCR using TaqMan SNP Genotyping Assays on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, California, USA). The rs8099917 genotype was classified into two categories: TT (major genotype) and non-TT (minor genotype: TG or GG). SNPs at rs1127354, which is located in the locus adjacent to the *inosine triphosphatase (ITPA)* gene, were genotyped by real-time detection PCR using the TaqMan SNP Genotyping Assay and the 7500 Fast Real-Time PCR System (Applied Biosystems). The rs1127354 genotype was classified into two categories: CC (major genotype) and non-CC (minor genotype: CA or AA).

Safety assessments

During the on-treatment period, all patients underwent chemical and hematologic assessment and were monitored for safety during every hospital visit, from the start of dosing to 4 weeks after the last dose of the study drug was administered. Furthermore, adverse events, including statin-induced rhabdomyolysis, were monitored carefully in consideration of possible interactions between telaprevir and fluvastatin.

Statistical analysis

The planned sample size was based on the assumption that the SVR rate would be 70% in the control group and 85% in the fluvastatin group, resulting in a necessary sample size of 240 patients with a two-sided significance level of 5% and statistical power of 80%. Fisher's exact test and the Mann–Whitney *U*-test were used for comparison of the baseline and on-treatment factors. Fisher's exact test was used for comparison of RVR, cEVR, ETR, and SVR rates between the groups. Statistical analysis was carried out using SPSS version 17.0 (IBM Japan, Tokyo, Japan). The level of significance was set at P less than 0.05.

Results

Patient characteristics

Of 124 patients with chronic hepatitis C who were screened as study candidates, 116 patients were eligible for this prospective, controlled study. There were 56 men and 60 women aged 28–71 years (median, 60 years). One hundred and twelve patients completed the therapy as scheduled and were subjected to analysis (intention-to-treat analysis). The remaining 4 patients (one with depression and one with anemia in the fluvastatin group; one with severe skin rash (grade 3); and one with severe appetite loss in the control group) discontinued therapy, but were included in the analysis. The control and fluvastatin groups each included 58 patients. No significant differences were found between the two groups with respect to patient background data such as sex, age, previous treatment response, peripheral blood counts, core amino acid 70 and 91 substitutions, *IL28B* genotype, and *ITPA* genotype (Table 1).

Virological response

The overall RVR, cEVR, ETR, and SVR rates were 81.9% (95/116), 97.4% (112/115), 97.4% (113/116), and 82.8% (96/116) in all 116 patients, respectively. The RVR, cEVR, ETR, and SVR rates in the control group were 87.9% (51/58), 96.5% (55/57), 96.6% (56/58), and 84.5% (49/58), respectively, whereas the rates in the fluvastatin group were 75.9% (44/58), 98.3% (57/58), 98.3% (57/58), and 81.0% (47/58), respectively. The differences in the RVR, cEVR, ETR, and SVR rates were not statistically significant between the control group and the fluvastatin group (Fig. 1). We next compared the SVR rate between the control and the fluvastatin groups, stratifying baseline factors such as sex and *IL28B* genotype. No factors associated with the SVR rate were found to show a statistically significant difference between the two groups (Fig. 2).

Drug adherence and drug interactions

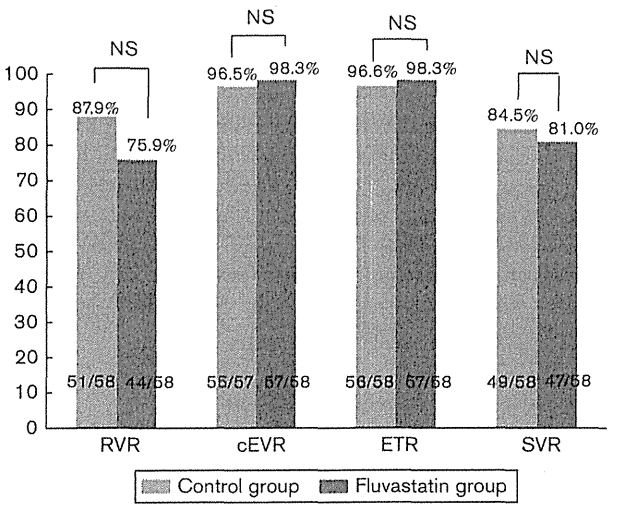
The effects of drug dosage in the two groups were also analyzed. The median dose of telaprevir was 130 500 mg (range, 42 000–189 000) in all patients, 126 000 mg (range, 66 000–189 000) in the control group, and 143 250 mg (range, 42 000–189 000) in the fluvastatin group. There was no statistically significant difference in the dose of telaprevir between the groups ($P = 0.152$). In investigating the dosage of PEG-IFNα2b, the median dose was found to be 1.51 μg/kg/week (range, 0.83–1.92) in all patients, 1.52 μg/kg/week (range, 0.83–1.92) in the control group, and 1.50 μg/kg/week (range, 1.07–1.88) in the fluvastatin group. There was no statistically significant difference between the groups ($P = 0.211$). Investigation of the dosage of ribavirin showed that the median dose was 7.89 mg/kg/day (range, 3.55–14.80) in all patients, 8.62 mg/kg/day (range, 4.01–12.21) in the control group, and 7.43 mg/kg/day (range, 3.55–14.80) in the

Table 1 Baseline characteristics and factors of the 116 patients who fulfilled the study criteria

Factors	Telaprevir/peg-IFN/RBV with fluvastatin ($n=58$)	Telaprevir/peg-IFN/RBV without fluvastatin ($n=58$)	P value
Age (years)	58 (30–71)	61 (28–70)	0.536
Sex (males/females, n)	30/28	26/32	0.577
BMI (kg/m^2)	22.96 (15.94–33.31)	22.15 (17.93–34.29)	0.573
Previous treatment			
Naive/relapse/NVR	34/18/6	23/22/13	0.131
White blood cells (/mm^3)	4880 (2820–8180)	4800 (2200–8200)	0.516
Hemoglobin (g/dl)	14.1 (10.9–17.2)	13.8 (10.7–16.8)	0.732
Platelets ($\times 10^3$/μl)	16.2 (6.9–33.6)	17.9 (7.0–16.8)	0.170
AST (IU/l)	43 (13–214)	40 (17–205)	0.657
ALT (IU/l)	50 (16–273)	41 (14–291)	0.577
γ-GTP (IU/l)	47 (12–296)	39 (11–339)	0.349
Total bilirubin (mg/dl)	0.6 (0.3–1.8)	0.8 (0.3–1.3)	0.072
Uric acid (mg/dl)	5.4 (2.8–7.9)	5.3 (2.4–9.4)	0.196
Serum creatinine (mg/dl)	0.67 (0.32–1.01)	0.64 (0.38–1.18)	0.208
Glucose (mg/dl)	105 (80–169)	95 (74–210)	0.196
LDL cholesterol (mg/dl)	94 (21–155)	104 (39–189)	0.122
HCV RNA (log IU/ml)	6.7 (5.1–7.7)	6.6 (5.0–7.6)	0.344
α-Fetoprotein (ng/ml)	4.6 (1.1–625.7)	5.1 (2.0–90.1)	0.803
ISDR mutation 0, 1/ ≥ 2	43/15	50/8	0.162
Core aa 70 wild/mutant	41/17	36/22	0.432
Core aa 91 wild/mutant	42/16	35/23	0.238
IL28B (rs8099917) TT/non-TT	36/22	35/23	1.000
ITPA (rs1127354) CC/non-CC	50/8	50/8	1.000

Categorical variables are given as number. Continuous variables are given as median (range).
Aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity determining region; ITPA, inosine triphosphatase; LDL cholesterol, low-density lipoprotein cholesterol; NVR, nonvirological response; peg-IFN, pegylated interferon; RBV, ribavirin.

Fig. 1



Comparison of the virological response rates between the fluvastatin group and the control group. cEVR, complete early virological response; ETR, end of treatment response; RVR, rapid virological response; SVR, sustained virological response.

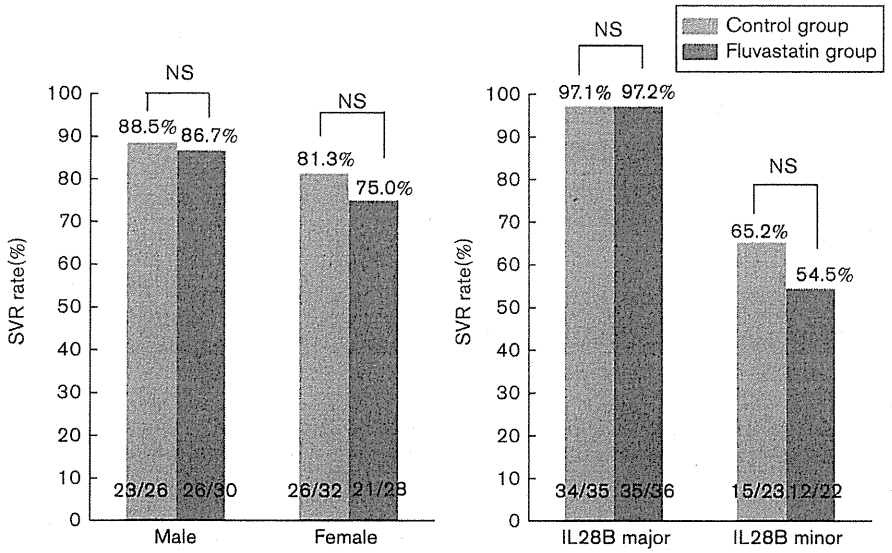
fluvastatin group. The dose was significantly lower in the fluvastatin group ($P = 0.048$).

Next, the drug interactions between telaprevir and fluvastatin were investigated. As the plasma concentrations of fluvastatin and telaprevir could not be measured, the changes in LDL cholesterol in the fluvastatin group were investigated. In the present study, LDL cholesterol in the fluvastatin combination group was 97 mg/dl (range, 21–189) at the start of treatment and 76 mg/dl (range, 25–129) during treatment. The decrease rate was 24.7%, comparable with the normal administration of fluvastatin alone (20–30%) [9,10].

Adverse events

A summary of adverse events is shown in Table 2. Adverse events occurred in many of the study patients. Adverse events such as severe anemia, mild anemia, skin rash and eruption, renal disorders, increase in serum uric acid, gastrointestinal disorders including nausea and appetite loss, and psychiatric disorders including insomnia and depression were similar between the two groups. Anemia was the most common clinical adverse event in both groups. Anemia was classified as severe anemia (hemoglobin levels of less than 8.5 g/dl) and mild anemia (hemoglobin levels of less than 10.0 g/dl). All of the patients with mild and severe anemia required dose reductions in RBV or telaprevir. Only one patient in the fluvastatin group discontinued the study because of severe anemia, and after the completion of treatment, the patient developed a relapse. Increase in serum uric acid was well controlled with the administration of allopurinol or febuxostat. One patient in the control group stopped the study at week 7 because of severe skin rash (grade 3); nevertheless, the patient achieved an SVR. No adverse events, such as rhabdomyolysis, were associated with fluvastatin. There were no deaths in the study.

Fig. 2



Comparison of the sustained virological response rates between the fluvastatin group and the control group according to sex or IL28B genotype. SVR, sustained virological response.

Table 2 Incidence of adverse events according to the treatment group

Adverse events	Fluvastatin group ($n=58$) [n (%)]	Control group ($n=58$) [n (%)]
Mild anemia	43 (74.1)	42 (72.4)
Severe anemia	26 (44.8)	20 (34.5)
Renal disorders	14 (24.1)	19 (32.8)
Skin rash and eruption	26 (44.8)	23 (40.0)
Gastrointestinal disorders	20 (34.5)	16 (27.6)
Psychiatric disorders	7 (12.1)	11 (19.0)
Serum uric acid increased	38 (65.5)	40 (69.0)
Discontinuation of treatment	2 (<0.1)	2 (<0.1)

Mild anemia was classified as hemoglobin levels <10 g/dl. Severe anemia was classified as hemoglobin levels <8.5 g/dl. Renal disorders were defined as serum creatinine concentration of 1.5 or more times above normal. Gastrointestinal disorders included nausea, diarrhea, and loss of appetite. Psychiatric disorders included insomnia and depression. Increasing serum uric acid was defined as uric acid levels > 8.5 mg/dl. Skin rash included all grades.

Discussion

The present study is the first prospective, randomized trial to be conducted on the use of a statin combined with peg-IFN/RBV/telaprevir therapy for genotype 1b chronic hepatitis C. Previously, we reported that combining fluvastatin with peg-IFN/RBV therapy for chronic hepatitis C with genotype 1b, high viral load reduced the post-treatment relapse rate, and, as a result, increased the SVR rate [7,8]. Kohjima *et al.* [17] and Bader *et al.* [18] also reported the effects of an add-on statin to peg-IFN/RBV in chronic hepatitis C. The effect of peg-IFN/RBV therapy combined with a statin was subsequently investigated in a meta-analysis, and favorable results were shown [19]. However, to date, there have been no reports on how a statin affects peg-IFN/RBV therapy in combination with a protease inhibitor such as telaprevir, which is the mainstream treatment for chronic hepatitis C with genotype 1b.

The present study found no add-on effect from the combination of fluvastatin with peg-IFN/RBV/telaprevir therapy for HCV genotype 1b-infected chronic hepatitis C. Several reasons may be considered for this result. In our previously reported trial of fluvastatin combined with peg-IFN/RBV therapy, the patients in whom an add-on effect of fluvastatin was observed included male patients, patients with pretreatment relapse, patients with the *IL28B* major genotype, and patients with core amino acid 70 wild type. In other words, an add-on effect of fluvastatin was considered to occur in patients who tended to respond to peg-IFN/RBV therapy [7]. However, it has been reported that there is a high probability (> 80%) that *IL28B* major genotype patients in particular, who receive peg-IFN/RBV therapy combined with telaprevir, will achieve an SVR [4–6]. Improving on this very high response rate is considered difficult. The next reason for finding no add-on effect from the combination of fluvastatin with peg-IFN/RBV/telaprevir therapy relates to reports that ETR can be achieved in many *IL28B* minor genotype patients [1,4,5,20]. Similar results were obtained in the present study. In a previous report, the results of a trial of fluvastatin combined with peg-IFN/RBV therapy showed suppression of the relapse rate after treatment completion [8]. Thus, in the present study, we expected that the relapse rate might have been

suppressed after ETR in the *IL28B* minor genotype patients; unfortunately, no such suppression was observed. As a result, in patients with the *IL28B* major genotype, for whom a statin add-on effect is expected, the treatment outcome is not improved as a sufficient effect is already obtained with peg-IFN/RBV/telaprevir without fluvastatin. In the *IL28B* minor genotype patients, in whom the effect of peg-IFN/RBV therapy combined with telaprevir is insufficient, it may be concluded that essentially no statin add-on effect can be expected.

It has also been reported previously that the SVR rate improved with the combination of pitavastatin and eicosapentaenoic acid (EPA) added to peg-IFN/RBV therapy for genotype 1b chronic hepatitis C, high viral load [17]. In particular, a significant improvement in the SVR rate was reported in patients with the IL28B minor genotype, which is resistant to treatment. Points that differed from our study [7] were that the statin formulation was different and EPA was used. A reported advantage of using EPA is that the expression of LDL receptors, which is strengthened with pitavastatin, is suppressed with the use of EPA [17]. This phenomenon is very important as one route of HCV infection of cells occurs through LDL receptors. The above indicates that the SVR rate in difficult-to-treat patients, such as patients with the *IL28B* minor genotype, may be improved with combination therapy consisting of a statin and EPA together with peg-IFN/RBV therapy plus telaprevir. It will be interesting to observed whether these add-on therapies to the combination of the next-generation protease inhibitor and peg-IFN/RBV [21,22], which may be available for clinical use in the future, will contribute toward improving the SVR rate.

With the use of statins, there is a concern of drug–drug interactions with protease inhibitors. Simvastatin, lovastatin, and atorvastatin, like telaprevir, are metabolized mainly by CYP3A4. Consequently, these statins are contraindicated for use with telaprevir [16]. Meanwhile, although the fluvastatin administered in the present study is metabolized by several enzymes, including CYP2C9, CYP3A4, and CYP2C8, it is metabolized mainly by CYP2C9 [23]. It is also reported that fluvastatin is metabolized by CYP3A4 at a high concentration (200 μmol/l) and by CYP2C9 at a low concentration (up to 0.2 μmol/l) [24]. In fact, the maximum blood concentration when fluvastatin is administered orally at 20–40 mg/day is about 0.35 μmol/l, close to the low concentration (up to 0.2 μmol/l). In the present study, the telaprevir dosage and frequency of adverse events were comparable between the fluvastatin and the nonfluvastatin groups. However, as the blood concentration of telaprevir was not measured, it is not known whether there was actually no drug–drug interaction. The LDL-cholesterol-decreasing effect of fluvastatin in this study was 24.7%, equivalent to that when fluvastatin is administered alone. Considering the above, there does not seem to be an increase in adverse events with the combined use of fluvastatin.

This study had several limitations. First, the sample size was small and may have been inadequate for analysis. The target number of cases was 240, but an interim analysis indicated a high possibility that no statin add-on effect was obtained, and entry was discontinued. In fact, mathematical analysis also showed that the SVR rate was not higher in the fluvastatin group than in the nonfluvastatin group. The second limitation is that the dosage of fluvastatin was not investigated. Future investigation will be needed to establish appropriate dosages.

Previously reported factors contributing toward SVR in triple therapy with telaprevir are previous treatment response, HCV core amino acid 70 substitution, *IL28B* genotype, and the presence of RVR [4,5,6,25]. These factors include items equivalent to those in the present study. The only independent baseline factor contributing toward SVR in our study was found to be the *IL28B* genotype. The SVR rate was very good at 97.2% (69/71) in patients with the *IL28B* major genotype, but was inadequate at 60.0% (27/45) in patients with the *IL28B* minor genotype. Although the presence or absence of RVR is an on-treatment factor, it was identified as an independent factor. It was shown that, in patients for whom *IL28B* status was unknown before treatment, the presence or absence of RVR was a very important factor in the acquisition of SVR.

In conclusion, administration of fluvastatin with telaprevir/peg-IFN/RBV was a safe combination in this trial. However, this prospective, open-label, randomized, controlled trial showed that fluvastatin had no add-on effect on telaprevir-based triple combination therapy for chronic hepatitis C patients with HCV genotype 1b.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

A 48-week telaprevir-based triple combination therapy improves sustained virological response rate in previous non-responders to peginterferon and ribavirin with genotype 1b chronic hepatitis C: A multicenter study

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Aim: The sustained virological response (SVR) rate of non-responders to peginterferon and ribavirin therapy (PR) is low for 24-week telaprevir-based triple combination therapy (T12PR24), compared to that of treatment-naïve patients or previous-treatment relapsers. This study investigated which characteristics of non-responders were associated with a better SVR rate to 48-week therapy (T12PR48).

Methods: A total of 103 Japanese non-responders with genotype 1b chronic hepatitis C received telaprevir-based therapy. Among them, 81 patients (50 partial and 31 null responders) received T12PR24 and 22 (seven partial and 15 null responders) who agreed to the extended therapy received T12PR48.

Results: Multivariate logistic regression analysis for SVR identified the interleukin-28B (*IL28B*) rs8099917 TT genotype ($P = 0.0005$, odds ratio [OR] = 10.38), extended rapid virological response ($P = 0.0008$, OR = 7.02), T12PR48 regimen ($P = 0.0016$, OR = 9.31) and previous partial responders ($P = 0.0022$, OR = 5.89). Among partial responders, the SVR rate did not differ significantly between T12PR48 (85.7%) and T12PR24 (70.0%). Among null responders, the SVR rate was significantly higher with T12PR48 than T12PR24 (66.7% vs 22.6%, $P = 0.0037$). Among patients with the *IL28B* non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (68.8% vs 37.7%, $P = 0.0288$). Moreover, among null responders with the non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (66.7% vs 9.1%, $P = 0.0009$).

Conclusion: T12PR48 improves the SVR rate in null responders, patients with the non-TT genotype, and null responders with a non-TT genotype.

Key words: 48-week therapy, chronic hepatitis C, non-responders, telaprevir

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INTRODUCTION

IN 2011, THE two first-generation direct-acting antiviral agents (DAA), telaprevir (TVR) and boceprevir, were approved for the treatment of chronic hepatitis C (CHC) patients with hepatitis C virus (HCV) genotype 1 in several countries. Triple combination therapy with

TVR or boceprevir, peginterferon-α and ribavirin is the current standard of care for genotype 1 CHC patients.[1] TVR, a non-structural (NS)3/4A serine protease inhibitor, was approved in Japan and has been available since November 2011. At present, the treatment of CHC has entered a new era with the introduction of potent DAA.

Peginterferon-α and ribavirin combination therapy (PR) has been the standard of care for CHC patients infected with HCV genotype 1 over the last 10 years. However, only 40–53% of patients achieve sustained virological response (SVR) even when including an extended 72-week therapy.[2–8] Among Japanese CHC patients treated with PR, approximately 25–31% were non-responders, which was defined as serum HCV RNA never disappearing during PR.[5–8]

Meanwhile, in treatment-naïve genotype 1 CHC patients, TVR-based triple combination therapy for a shortened period of 24 weeks (i.e. telaprevir, peginterferon-α and ribavirin for 12 weeks followed by an additional 12 weeks PR; T12PR24) is reported to remarkably improve the SVR rate compared to PR alone.[9–11] In treatment-experienced patients, the outcomes of TVR-based therapy depend on their previous response to interferon-based therapy.[12–17] In patients with previous relapses, T12PR24 dramatically improved the SVR rate in clinical trials.[12–15,17–19] In Japan, the SVR rate of previous relapsers treated with T12PR24 was very high with more than approximately 90% in clinical practice.[20–24] On the other hand, in non-responders including partial and null responders to previous PR, the SVR rate with T12PR24 was only approximately 40%.[12,16,21–25] In particular, previous studies in Japan showed that the SVR rate of null responders treated with T12PR24 was extremely low at less than 20%.[16,22–25] Even when treated with a 48-week regimen of triple combination therapy with or without a 4-week PR lead-in regimen (pooled T12PR48), the SVR rate was lower in null responders than partial responders.[13] Based on these results, T12PR48 is recommended for non-responders to previous PR in the USA, Canada and the EU. However, in Japan, generally, T12PR24 is recommended because clinical trials were performed only in the T12PR24 regimen. Therefore, it is unclear whether T12PR48 improves the SVR rate of Japanese non-responders to PR compared to T12PR24.

Accordingly, this study investigated which characteristics of non-responders to previous PR are associated with the improvement of SVR rate with extended T12PR48.

METHODS

Patients

BETWEEN DECEMBER 2011 and March 2013, 456 consecutive Japanese genotype 1b-infected CHC patients received TVR-based triple combination therapy at the study hospitals. Among all patients, 103 non-responders to PR were enrolled in this multicenter study.

The inclusion criteria were as follows: (i) diagnosis of CHC; (ii) persistently positive sera for HCV RNA for more than 6 months determined by quantitative real-time polymerase chain reaction (PCR) method (COBAS AmpliPrep/COBAS TaqMan HCV Test; Roche Diagnostics, Tokyo, Japan); (iii) HCV genotype 1b confirmed by sequence analysis; (iv) non-responders to previous PR in whom HCV RNA never disappeared during PR after 24 weeks of therapy; (v) aged 18–75 years; and (vi) bodyweight of more than 35 kg at the time of entry into the study. The exclusion criteria were as follows: (i) decompensated cirrhosis; (ii) positive for hepatitis B surface antigen or antibodies against HIV; (iii) previous or current development of hepatocellular carcinoma; (iv) coexistence of other liver diseases such as autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and alcoholic liver disease; (v) renal disease or creatinine clearance of 50 mL/min or less at baseline; (vi) hemoglobin level of less than 12 g/dL, white blood cell count of less than 2000/μL, neutrophil count of less than 1500/μL and platelet count of less than 8.0×10^4/μL at baseline; (vii) depression, schizophrenia or history thereof, or history of suicide attempts; and (viii) pregnancy in progress or planned for either partner during the study period. Liver biopsy was performed in 80 of 103 (77.7%) patients within 12 months of enrollment. The presence or absence of cirrhosis was established according to the METAVIR score.[26] For the remaining 23 patients, the presence or absence of cirrhosis was determined by ultrasonography and/or computed tomography findings.

The patients were divided into two categories according to the Japan Society of Hepatology guidelines.[27] Partial responders were defined as having a decrease in HCV RNA of 2 $\log_{10}$ IU/mL or more from baseline at treatment week 12 but detectable at treatment week 24, and null responders were defined as having a decrease in HCV RNA of less than 2 $\log_{10}$ IU/mL at treatment week 12.

Treatments

Telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administrated every 8 h after meals

(q8h, 500 or 750 mg) or every 12 h after meals (q12h, 750 or 1125 mg). The initial daily dose of TVR (1500 or 2250 mg/day) and administration intervals (q8h or q12h) were determined by each attending physician according to age, sex, bodyweight and hemoglobin level. Peginterferon-α-2b (PEG-Intron; MSD, Tokyo, Japan) was injected s.c. at a median dose of 1.5 μg/kg per week. Ribavirin (Rebetol; MSD) dose was adjusted according to bodyweight (600, 800 and 1000 mg for <60, ≥60 to <80, and ≥80 kg, respectively). In patients with hemoglobin level of less than 13 g/dL at the start of therapy, ribavirin dose was reduced by 200 mg in accordance with the general consensus statements.[28] Triple therapy was administrated for 12 weeks, followed by an additional 12 weeks of peginterferon-α-2b and ribavirin combination therapy (T12PR24) or 36 weeks of peginterferon-α-2b and ribavirin (T12PR48) in patients who agreed to the extended therapy. The administration of each drug was appropriately reduced or withdrawn if a serious adverse event occurred or was suspected to be developing during the course of treatment. Treatment was stopped for patients with HCV RNA of more than 3 $\log_{10}$ IU/mL at week 4, detectable HCV RNA at week 12 or a more than 2 $\log_{10}$ IU/mL increase in HCV RNA levels from the lowest level during therapy irrespective of adverse events because of the low likelihood of achieving an SVR and high likelihood of developing antiviral resistance.

Definitions of outcomes

Virological response was analyzed on an intent-to-treat basis. The successful end-point of treatment was SVR for patients showing undetectable HCV RNA for 24 weeks after treatment cessation. Relapse was defined as when HCV RNA levels became undetectable by the end-of-treatment but became positive during the follow-up period. Viral breakthrough (VBT) was defined as when HCV RNA became undetectable during the treatment period but then became positive before the end of the treatment period. Non-response was defined as when HCV RNA was detectable throughout the treatment period. Extended rapid virological response (eRVR) was defined as undetectable HCV RNA at both weeks 4 and 12 after starting treatment.

All patients provided written informed consent. The study protocol conformed to ethics guidelines established in adherence with the 2008 Declaration of Helsinki and was approved by the ethics committee of each participating institution.

Measurement of HCV RNA and amino acid substitution in the core and NS5A regions of HCV genotype 1b

Hepatitis C virus genotype was determined by direct sequencing followed by phylogenic analysis of the NS5B region.[29] The antiviral effects of therapy on HCV were assessed by measuring serum HCV RNA levels during treatment at least once every 4 weeks before, during and after therapy. HCV RNA levels were determined using the COBAS AmpliPrep/CABAS TaqMan HCV Test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/mL, and undetectable samples were defined as negative.

Core amino acid substitution at position 70 was determined as described previously.[30] Core amino acid substitution at position 70 was defined as wild type (i.e. arginine) or mutant (i.e. glutamine or histidine). In addition, substitutions at amino acids 2290–2248 of the NS5A region (interferon-sensitivity determining region) were determined as described previously.[31] Amino acid substitutions in this region were defined as wild type (0 or 1) or non-wild type (≥2).

Single-nucleotide polymorphism genotyping

Genomic DNA was extracted from whole blood using the MagNA Pure LC and a DNA Isolation Kit (Roche Diagnostics). The genetic polymorphism near the interleukin-28B (*IL28B*) gene,[32,33] rs8099917, was genotyped by real-time PCR using the TaqMan SNP Genotyping Assays and the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified as TT (major genotype) or non-TT (minor genotype: TG or GG).

Statistical analysis

Continuous variables are expressed as means and standard deviations. Continuous data were analyzed using the non-parametric Mann–Whitney *U*-test. Categorical data were analyzed using the χ^2-test with a Yates correction or Fisher's exact test. Univariate and multivariate logistic regression analyses were performed to identify factors that significantly contributed to SVR. Odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values for statistical tests were two-tailed. The levels of significance and marginal significance were set at $P < 0.05$ and $P < 0.15$, respectively. Variables showing statistical or marginal significance in univariate analysis were entered into multivariate logistic regression analyses to identify significant independent predictive factors of SVR. All statistical analyses

were performed using SPSS version 17.0 (IBM-SPSS, Chicago, IL, USA). In this study, the adherence to each drug was excluded for the difference of treatment duration and the stopping rules.

RESULTS

Patient characteristics and treatment outcomes

PATIENT CHARACTERISTICS ARE summarized in Table 1. Of the 103 patients, 57 (55.3%) and 46 (44.7%) were partial and null responders, respectively. Partial responders had significantly higher platelet counts than null responders ($P = 0.0126$). α-Fetoprotein levels were significantly lower in partial responders than null responders ($P = 0.0202$). No other baseline factors differed significantly between groups.

Regarding treatment outcomes of TVR-based triple combination therapy, 58 patients (56.3%) achieved SVR, 23 (22.3%) showed relapse, 16 (15.5%) showed VBT and six (5.8%) showed non-response. Patients were stratified according to previous treatment response and regimen. Among the 50 partial responders treated with T12PR24, 35 (70.0%) achieved SVR, 12 (24.0%) showed relapse and three (6.0%) showed VBT. Among the seven partial responders treated with T12PR48, six (85.7%) achieved SVR and one (14.3%) showed VBT. Among all partial responders, the SVR rate was slightly higher with T12PR48 than T12PR24 (6/7 [85.7%] vs 35/50 patients [70.0%]), though not statistically significant ($P = 0.6763$) (Fig. 1).

Among the 31 null responders treated with T12PR24, seven patients (22.6%) achieved SVR, seven (22.6%) showed relapse, 11 (35.5%) showed VBT and six (19.4%) showed non-response. Among the remaining 15 patients treated with T12PR48, 10 (66.7%) achieved SVR, four (26.7%) showed relapse and one (6.7%) showed VBT. Among all null responders, the SVR rate was significantly higher with T12PR48 than T12PR24 (10/15 [66.7%] vs 7/31 patients [22.6%], $P = 0.0037$) (Fig. 1).

Besides treatment regimens, the SVR rate was significantly higher in partial responders than null responders (41/57 [71.9%] vs 17/46 patients [37.0%], $P = 0.0004$).

Predictive factors associated with SVR

The results of the univariate and multivariate analyses are shown in Table 2. In univariate analysis, the following

Table 1 Characteristics of patients

Variables	Partial responders	Null responders	*P*-value
No. of patients	57	46	
Sex (male/female) (male %)	37/20 (64.9%)	21/25 (45.7%)	0.0501
Age (years)	59.2 ± 8.0	56.9 ± 9.8	0.2575
Bodyweight (kg)	64.0 ± 12.4	62.5 ± 12.8	0.3740
Body mass index (kg/m^2)	23.6 ± 3.1	23.5 ± 3.3	0.6072
Absence or presence of cirrhosis (non-cirrhosis/cirrhosis) (cirrhosis %)	40/17 (29.8%)	27/19 (41.3%)	0.2245
rs 8099917 (TT/TG/GG) (TT %)	22/34/1 (38.6%)	12/32/2 (26.1%)	0.3353
White blood cells (/μL)	4835 ± 1461	4698 ± 1396	0.5886
Hemoglobin (g/dL)	14.1 ± 1.5	14.0 ± 1.5	0.6566
Platelets (×10^4/μL)	17.6 ± 6.3	14.8 ± 5.6	0.0126
Aspartate aminotransferase (IU/L)	53 ± 32	61 ± 29	0.0632
Alanine aminotransferase (IU/L)	58 ± 38	66 ± 41	0.1748
γ-Glutamyltransferase (IU/L)	70 ± 67	86 ± 83	0.1006
α-Fetoprotein (ng/mL)	16.2 ± 34.7	20.6 ± 28.6	0.0202
HCV RNA (log 10 IU/mL)	6.1 ± 1.2	6.6 ± 0.6	0.0513
Core amino acid substitution 70 (wild type/mutant type)	31/26 (54.4%)	18/28 (39.1%)	0.1233
ISDR of NS5A (wild type/non-wild type)	49/8 (86.0%)	43/3 (93.5%)	0.3647
Initial dose of peginterferon-α-2b (μg /kg)	1.5 ± 0.2	1.5 ± 0.2	0.2235
Initial dose of ribavirin (mg/kg)	11.0 ± 1.9	11.0 ± 1.9	0.7933
Initial daily dose of telaprevir (2250/1500 mg)	30/27	22/24	0.6277
Administration intervals of telaprevir (q8h/q12q)	30/27	29/17	0.2882

Data are expressed as numbers or means ± standard deviations.
HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NS, non-structural.

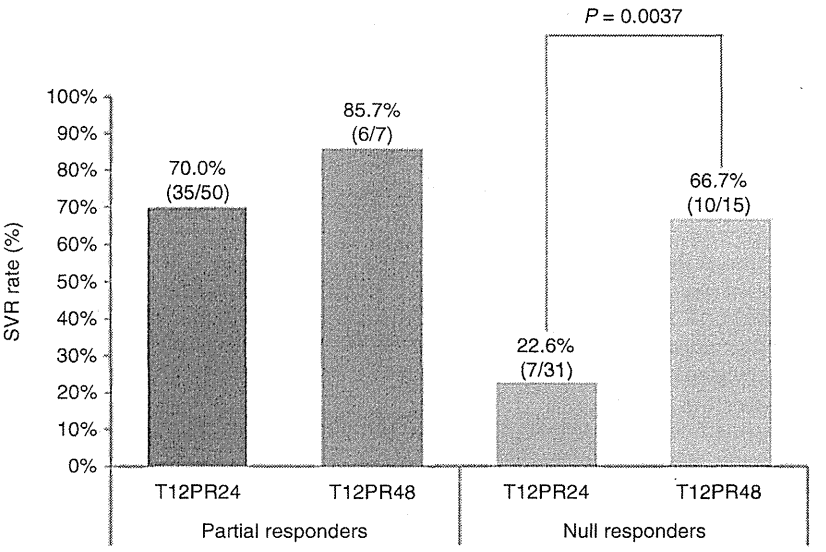

Figure 1 SVR rate according to response to previous peginterferon and ribavirin combination therapy and treatment regimen. SVR, sustained virological response; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination therapy.

factors were significantly associated with SVR: previous partial responders ($P = 0.0005$); *IL28B* TT genotype ($P = 0.0015$); wild-type core amino acid substitution at position 70 ($P = 0.0118$); eRVR ($P = 0.0002$); and higher white blood cell count ($P = 0.0418$), platelet count ($P = 0.0132$) and lower aspartate aminotransferase ($P = 0.0126$). Furthermore, in univariate analysis, the following factors were marginally associated with SVR: absence of cirrhosis ($P = 0.1220$); T12PR48 regimen ($P = 0.0858$); higher hemoglobin level ($P = 0.0813$), initial dose of peginterferon-α-2b ($P = 0.1237$) and initial daily dose of TVR ($P = 0.1411$); and lower alanine aminotransferase ($P = 0.1418$), γ-glutamyltransferase ($P = 0.0954$) and HCV RNA levels ($P = 0.0803$). The abovementioned variables were entered into the multivariate logistic regression analysis, and the following four independent factors were identified: *IL28B* TT genotype ($P = 0.0005$, OR = 10.38, 95% CI = 2.78–38.84), eRVR ($P = 0.0008$, OR = 7.02, 95% CI = 2.25–21.97), T12PR48 regimen ($P = 0.0016$, OR = 9.31, 95% CI = 2.32–37.38) and previous partial responders ($P = 0.0022$, OR = 5.89, 95% CI = 1.89–13.31) (Table 2).

SVR rate stratified by treatment regimen and *IL28B* genotype

Patients were subsequently stratified according to previous treatment response and treatment regimen. Among partial responders treated with T12PR24, the SVR rate was significantly higher in patients with the TT genotype than those with the non-TT genotype (17/19 [89.5%] vs 18/31 patients [58.1%], $P = 0.0262$). On the other hand, among those treated with T12PR48, the SVR rate did not differ significantly with respect to the *IL28B* genotype (3/3 [100%] vs 3/4 patients [75%], $P = 1.0000$). Among those with the non-TT genotype, the SVR rate was higher in T12PR48 than in T12PR24 (3/4 [75%] vs 18/31 patients [58.1%]) though not statistically significant ($P = 0.6350$).

Among null responders treated with T12PR24, the SVR rate was significantly higher in patients with the TT genotype than those with the non-TT genotype (5/9 [55.6%] vs 2/22 patients [9.1%], $P = 0.0118$). On the other hand, among those treated with T12PR48, the SVR rate was not significantly different between patients with the TT and non-TT genotype (2/3 [66.7%] vs 8/12 patients [66.7%], $P = 1.0000$). Among patients with the non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (8/12 [66.7%] vs 2/22 patients [9.1%], $P = 0.0009$) (Fig. 2).

Apart from previous treatment response, there was no significant difference in the SVR between patients with an *IL28B* TT genotype who received T12PR48 and T12PR24 (5/6 [83.3%] vs 22/28 patients [78.6%], $P = 1.0000$). In contrast, among patients with a non-TT genotype, the SVR rate was significantly higher with T12PR48 than T12PR24 (11/16 [68.8%] vs 20/53 patients [37.7%], $P = 0.0288$).

Table 2 Factors associated with sustained virological response

Variable	Univariate			Multivariate		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Host-related factor						
Sex male vs female	1.46	0.66–3.20	0.3495			
Age (years)	0.99	0.95–1.03	0.6050			
Bodyweight (kg)	1.01	0.98–1.04	0.5460			
Body mass index (kg/m^2)	1.01	0.89–1.14	0.8802			
Cirrhosis absence vs presence	1.92	0.84–4.38	0.1220			
Partial responders vs null responders	4.37	1.90–10.04	0.0005	5.89	1.89–13.31	0.0022
rs8099917 TT vs non-TT	4.73	1.82–12.31	0.0015	10.38	2.78–38.84	0.0005
White blood cells (/μL)	1.00	1.00–1.00	0.0418			
Hemoglobin (g/dL)	1.27	0.97–1.66	0.0813			
Platelets (×10^4/μL)	1.09	1.02–1.17	0.0132			
Aspartate aminotransferase (IU/L)	0.98	0.97–1.00	0.0126			
Alanine aminotransferase (IU/L)	0.99	0.98–1.00	0.1418			
γ-Glutamyltransferase (IU/L)	1.00	0.99–1.00	0.0954			
α-Fetoprotein (ng/mL)	0.99	0.98–1.01	0.2280			
Virus-related factor						
HCV RNA ($\log_{10}$ IU/mL)	0.67	0.42–1.05	0.0803			
Core amino acid substitution 70 wild type vs mutant type	2.83	1.26–6.37	0.0118			
ISDR of NS5A non-wild type vs wild type	2.24	0.56–8.98	0.2551			
Treatment-response factor						
eRVR, + vs –	5.18	2.21–12.13	0.0002	7.02	2.25–21.97	0.0008
Treatment-related factor						
Initial dose of peginterferon-α-2b (μg/kg)	5.47	0.63–47.68	0.1237			
Initial dose of ribavirin (mg/kg)	1.13	0.92–1.39	0.2507			
Initial daily dose of telaprevir (2250/1500 mg)	1.81	0.82–3.97	0.1411			
Administration intervals of telaprevir (q8h/q12q)	0.70	0.32–1.54	0.3727			
T12PR48 vs T12PR24	2.48	0.88–6.97	0.0858	9.31	2.32–37.38	0.0016

CI, confidence interval; eRVR, extended rapid virological response; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NS, non-structural; OR, odds ratio; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin therapy; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin therapy.

SVR rate stratified by eRVR according to treatment regimen and *IL28B* genotype

The SVR rates of the patients described above and depicted in Figure 2 were further analyzed after stratification by eRVR. Among partial responders with the *IL28B* TT genotype treated with T12PR24, there was no significant difference in the SVR rate between patients who achieved eRVR and those who did not (13/14 [92.9%] vs 4/5 patients [80.0%], $P = 0.4678$). Among patients with the non-TT genotype treated with T12PR24, patients who achieved eRVR tended to have a higher SVR rate than those who did not achieve eRVR, although the difference was not significant (16/24 [66.7%] vs 2/7 patients [28.6%], $P = 0.0994$). Among the patients with the TT genotype treated with T12PR48, all three patients who achieved eRVR exhibited SVR. Meanwhile, among patients with the non-TT genotype treated with T12PR48, the SVR rate did not differ significantly between those who achieved eRVR and those who did not (1/1 [100%] vs 2/3 patients [66.7%], $P = 1.0000$) (Fig. 3).

In addition, among null responders with the TT genotype treated with T12PR24, the SVR rate tended to be higher in patients who achieved eRVR than those who did not (3/3 [100%] vs 2/6 patients [33.3%], $P = 0.1667$). Among patients with the non-TT genotype treated with T12PR24, the SVR rate tended to be higher in patients who achieved eRVR than those who did not (2/8 [25.0%] vs 0/14 patients [0%], $P = 0.1212$). Among those with the TT genotype treated with T12PR48, two of three (66.7%) patients who did not

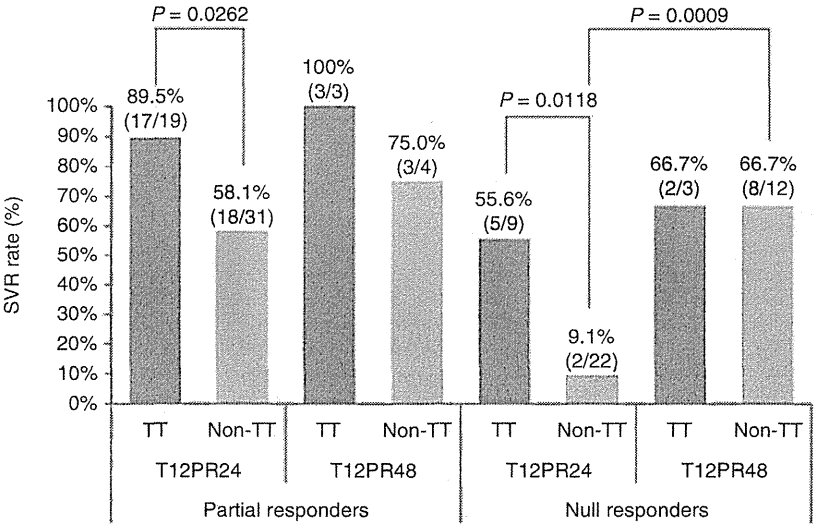

Figure 2 SVR rate stratified by treatment regimen and *IL28B* (rs8099917) genotype. IL, interleukin; SVR, sustained virological response; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy.

achieve eRVR achieved SVR. Among patients with the non-TT genotype treated with T12PR48, the SVR rate tended to be higher in patients who achieved eRVR than those who did not (6/8 [75.0%] vs 2/4 patients [50.0%], $P = 0.5475$) (Fig. 4).

DISCUSSION

THIS STUDY IS the first report indicating that T12PR48 regimen results in a significantly higher SVR rate for non-responders to previous PR than T12PR24 in Japan. Several reports showed that the SVR rate with T12PR24 for previous non-responders to PR was low, ranging 27–46%.[12,16,21–25] Furthermore, there was a remarkable difference in the SVR rate with T12PR24 between previous partial and null responders in Japan.[16,22–25] The REALIZE study revealed that the SVR rate of partial responders (56.7%) was superior to that of null responders (31.3%) even if null responders were treated with pooled T12PR48 (including T12PR48 and lead-in T12PR48).[13] Muir *et al.* reported the SVR rates in null responders with HCV genotype 1b treated with T12PR24 and T12PR48 were 12.5% (1/8) and 60% (6/10), respectively. Moreover, the SVR rate was 71.4% (5/7) in partial responders with genotype 1b treated with response-guided therapy, namely, patients who

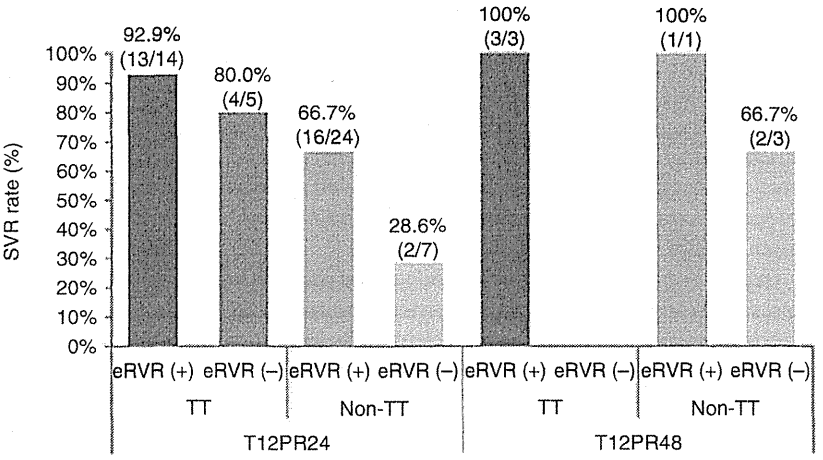

Figure 3 SVR rate in partial responders stratified by eRVR according to treatment regimen and *IL28B* genotype. eRVR, extended rapid virological response; IL, interleukin; SVR, sustained virological response; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy.