2. Results

2.1. Patient Characteristics According to IL28B and ITPA Genotypes

First, we genotyped IL28B rs8099917, and ITPA rs1127354 and rs6051702 in 97 HCV-infected patients (Table 1). Sixty and 37 patients possessed IL28B rs8099917 major and minor genotypes, respectively. Seventy-four and 23 patients possessed ITPA rs1127354 major and minor genotypes, respectively, and 59 and 38 possessed ITPA rs6051702 major and minor genotypes, respectively.

Table 1. Background of study population at enrollment.

Study variables	Total (n = 97)
Age (years)	55.1 ± 10.8
Gender (male/female)	44/53
SNP genotype	
IL28B rs8099917 TT/TG/GG	60/35/2
ITPA rs1127354 CC/CA/AA	74/21/2
ITPA rs6051702 AA/AC/CC	59/32/6
Response to previous therapy	
Naïve/relapse/null response	67/17/13
HCV RNA (H/L)	95/2
HCV genotype (G1/G2)	81/16
AST (IU/L)	56.0 ± 49.4
ALT (IU/L)	67.9 ± 62.4
γGTP (IU/L)	53.5 ± 73.2
WBC (/mm^3)	5,410 ± 1,640
Hemoglobin (g/dL)	14.0 ± 1.1
Platelets (×10^4/mm^3)	17.5 ± 5.1
History of diabetes mellitus (+/−)	15/82
US (CLD/cirrhosis/unknown)	83/12/2
Treatment Response	
RVR (+/−/unknown)	14/82/1
EVR (+/−)	52/45
SVR (+/relapser/null/unknown)	40/27/22/8

H, high viral load (≥5 log IU/mL); L, low viral load (<5 log IU/mL); G1, genotype 1; G2, genotype 2; WBC, white blood cell count; US, ultrasound finding; CLD, chronic liver disease.

IL28B rs8099917 major-type patients included more interferon treatment-naïve patients than minor-type patients. Lower γGTP levels were seen in IL28B rs8099917 major-type patients (Table 2). ITPA rs1127354 major-type patients were older than ITPA rs1127354 minor-type patients and tended to be female-dominant in the present study (Table 2).

2.2. Treatment Response According to IL28B and ITPA Genotypes

Next, we compared the treatment response among patients according to IL28B and ITPA genotypes (Table 3). IL28B rs8099917 could predict SVR, as previously reported [4–9], while both ITPA genotypes did not in the present study. We reconfirmed that IL28B rs8099917 is one of the predictive values for treatment response in interferon-included regimens.

Table 2. Baseline characteristics of patients grouped according to *IL28B* and *ITPA* genetic variations.

Study variables	*IL28B rs8099917*			*ITPA rs1127354*			*ITPA rs6051702*		
	TT	TG/GG	*P-value*	CC	CA/AA	*P-value*	AA	AC/CC	*P-value*
No. of patients	60	37		74	23		59	38	
Age (years)	55.7 ± 11.2	54.7 ± 10.1	N.S.	56.8 ± 9.7	49.6 ± 12.2	0.0043	55.6 ± 11.3	54.4 ± 9.9	N.S.
Gender (male/female)	25/35	19/18	N.S.	29/45	15/8	0.0511	29/30	15/23	N.S.
Response to previous therapy (naïve/relapse/null response)	46/10/4	21/7/9	0.029	48/17/9	19/0/4	N.S.	40/12/7	27/5/6	N.S.
HCV RNA (H/L)	58/2	37/0	N.S.	73/1	22/1	N.S.	58/1	37/1	N.S.
HCV genotype (G1/G2)	49/11	32/5	N.S.	63/11	18/5	N.S.	48/11	33/5	N.S.
AST (IU/L)	53.3 ± 56.2	60.3 ± 36.0	N.S.	52.8 ± 31.9	66.2 ± 84.4	N.S.	51.6 ± 30.1	62.8 ± 69.5	N.S.
ALT (IU/L)	62.4 ± 65.3	76.9 ± 57.0	N.S.	62.3 ± 48.5	85.7 ± 93.5	N.S.	62.4 ± 47.5	76.4 ± 80.2	N.S.
γGTP (IU/L)	35.5 ± 34.5	82.8 ± 104	0.0016	55.1 ± 80.9	48.7 ± 40.1	N.S.	51.6 ± 72.1	56.5 ± 75.6	N.S.
WBC (/mm^3)	5580 ± 1820	5140 ± 1260	N.S.	5390 ± 1630	5470 ± 1680	N.S.	5570 ± 1690	5160 ± 1540	N.S.
Hb (g/dL)	13.9 ± 1.1	14.3 ± 1.1	N.S.	13.9 ± 1.0	14.3 ± 1.2	N.S.	14.0 ± 1.1	14.0 ± 1.1	N.S.
Platelets (×10^4/mm^3)	17.9 ± 5.3	16.8 ± 5.0	N.S.	17.4 ± 5.4	17.7 ± 4.3	N.S.	17.8 ± 5.4	17.0 ± 4.7	N.S.
History of diabetes mellitus (+/−)	9/51	7/30	N.S.	11/63	4/19	N.S.	8/51	7/31	N.S.
US (CLD/cirrhosis/unknown)	51/8/1	32/4/1	N.S.	62/10/2	21/2	N.S.	50/8/1	33/4/1	N.S.

H, high viral load (≥5 log IU/mL); L, low viral load (<5 log IU/mL); G1, genotype 1; G2, genotype 2; WBC, white blood cell count; US, ultrasound finding; CLD, chronic liver disease.

Table 3. Treatment response in patients grouped according to *IL28B* and *ITPA* genetic variations.

Study variables	*IL28B rs8099917*			*ITPA rs1127354*			*ITPA rs6051702*		
	TT	TG/GG	*P-value*	CC	CA/AA	*P-value*	AA	AC/CC	*P-value*
No. of patients	60	37		74	23		59	38	
RVR (+/−/unknown)	12/47/1	2/35/0	0.085	10/63/1	4/19/0	N.S.	10/49/0	4/33/1	N.S.
EVR (+/−)	43/17	9/28	0.000014	36/38	16/17	N.S.	31/28	21/17	N.S.
SVR (+/Relapser/Null/unknown)	29/6/18/7	11/16/9/1	0.042	28/17/22/7	12/5/5/1	N.S.	27/13/16/3	13/9/11/5	N.S.

RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response.

2.3. Ribavirin-Induced Anemia According to IL28B and ITPA Genotypes

Next, we examined ribavirin-induced anemia among patients according to IL28B and ITPA genotypes (Figure 1). IL28B rs8099917 did not influence ribavirin-induced anemia (Figure 1A–D), nor did ITPA rs6051702 (Figure 1I-1L). ITPA rs1127354 major type led to significantly greater ribavirin-induced anemia than ITPA rs1127354 minor type in Japanese patients during peginterferon plus ribavirin treatment (Figure 1E-1H).

Figure 1. Ribavirin-induced reduction of hemoglobin according to IL28B and ITPA genotypes. (**A**)–(**D**), IL28B rs8099917; (**E**)–(**H**), ITPA rs1127354; (**I**)–(**L**), ITPA rs6051702. (**A**), (**E**) and (**I**) show the changes of hemoglobin (Hb) between days 0 and 14, (**B**), (**F**) and (**J**) between days 0 and 28, (**C**), (**G**) and (**K**) between days 0 and 54, and (**D**), (**H**) and (**L**) between days 0 and 84.

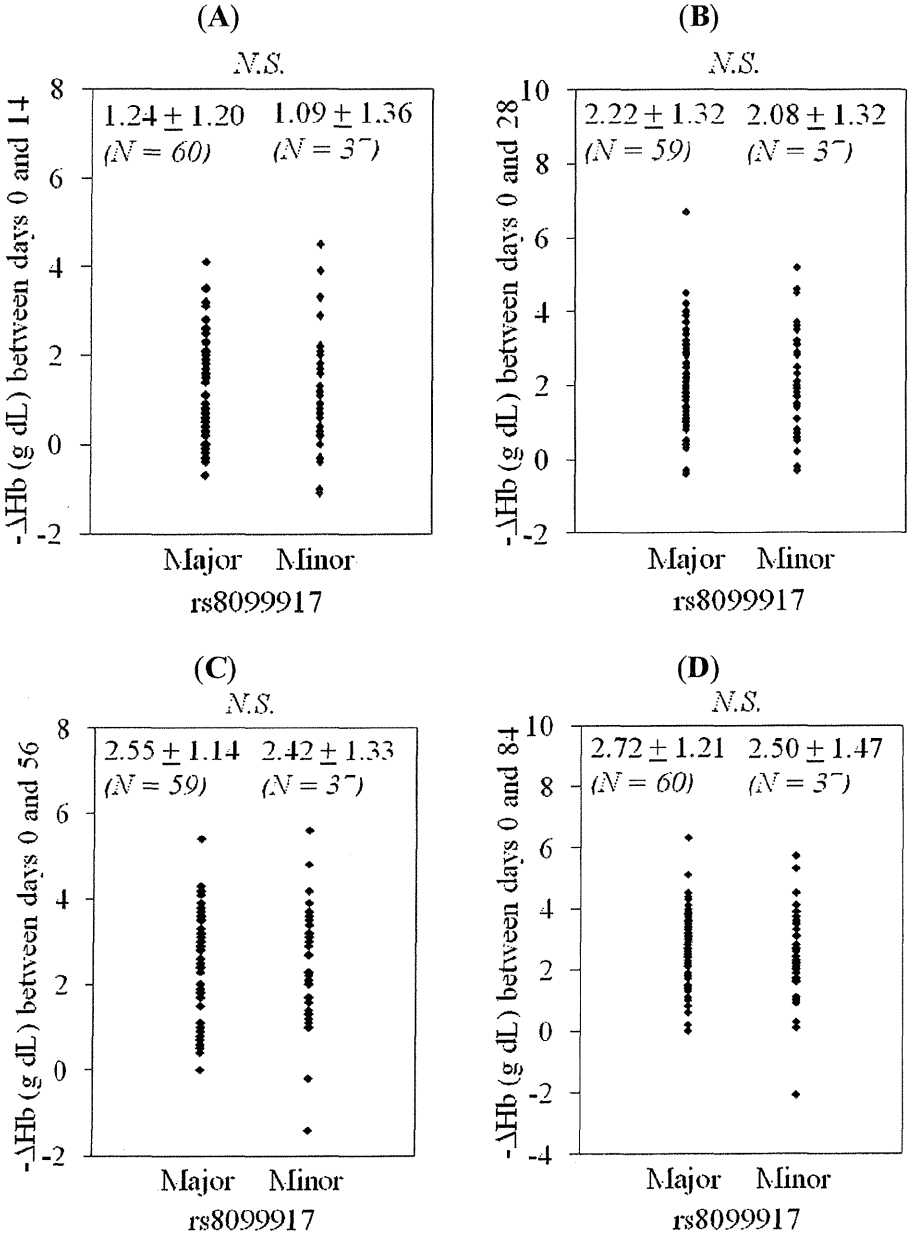


Figure 1. *Cont.*

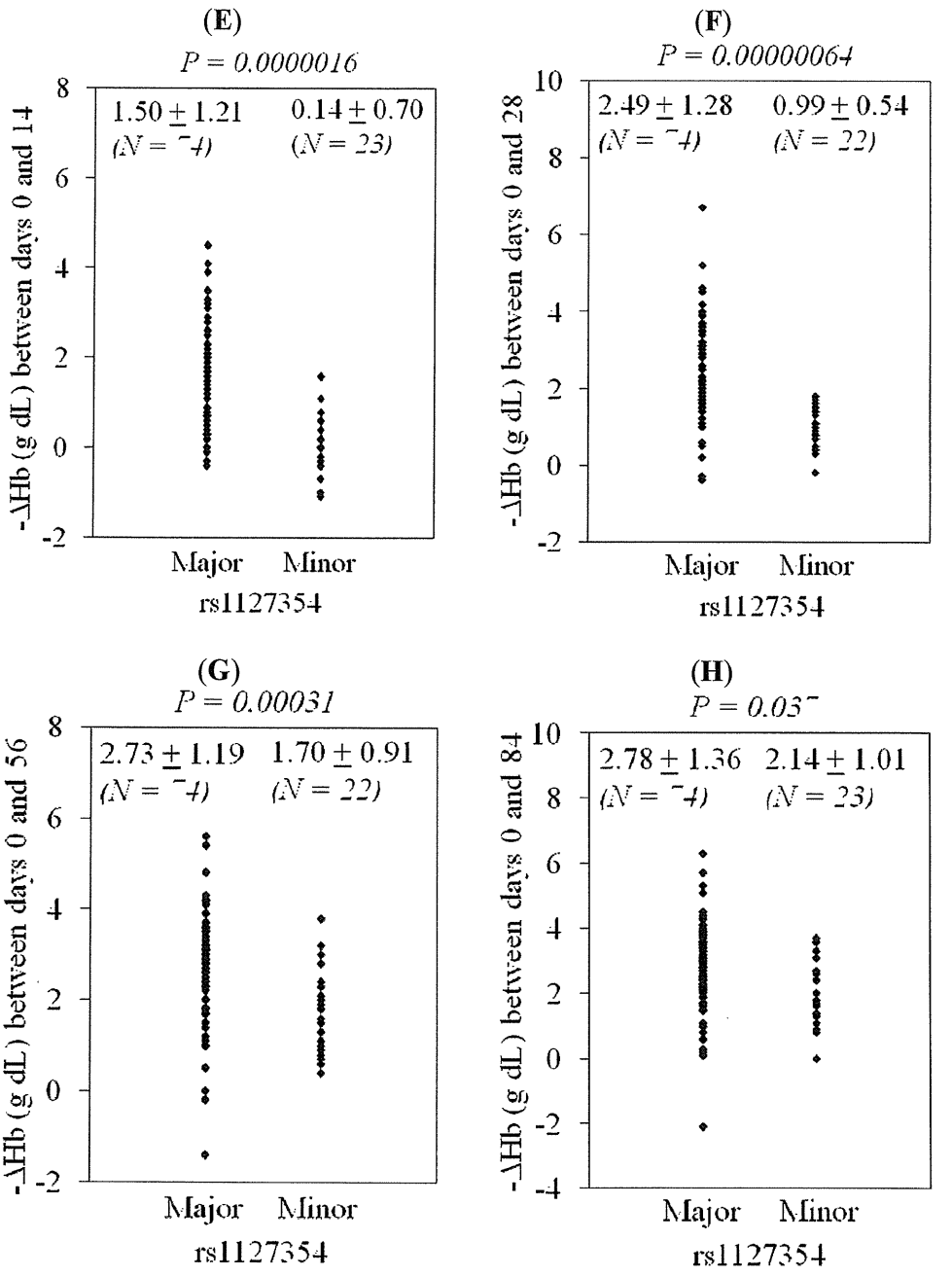
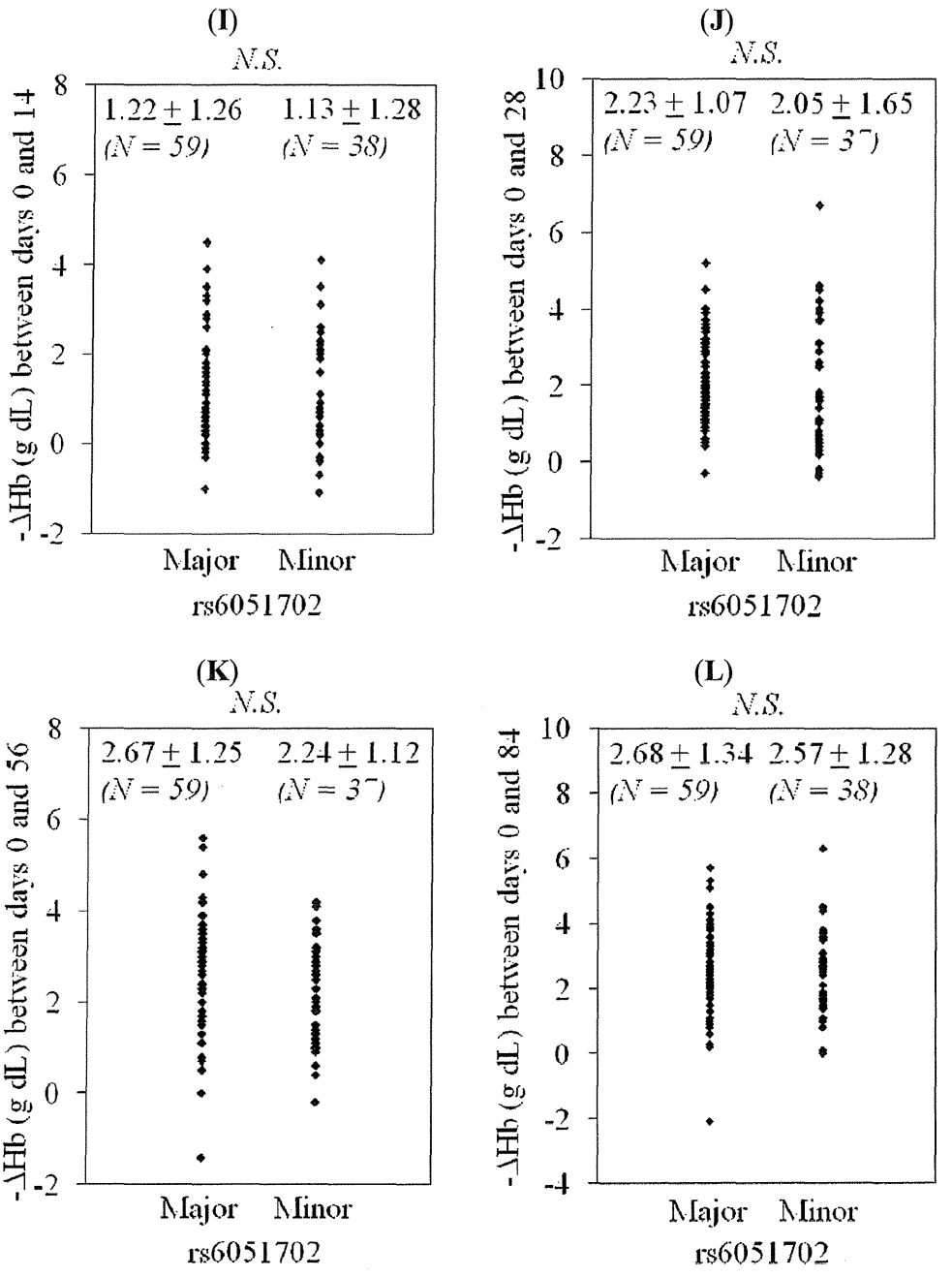


Figure 1. *Cont.*



2.4. Association Between ITPA rs1127354 Genotype and Dose Reduction of Drugs During Treatment

Next, we investigated the association between ITPA rs1127354 genotype and dose reduction of drugs at day 28 (Table 4). ITPA rs1127354 genotype could not predict the dose reduction of peginterferon (Table 4A), but ITPA rs1127354 major type could predict the dose reduction of ribavirin (Table 4B). We also examined the association between ITPA rs1127354 genotype and dose reduction of drugs at day 84 (data not shown). In patients with reduced ribavirin and/or peginterferon with null response, and in patients relapsed to the treatment, the proportion of patients with ITPA rs1127354

major type was greater among the patients with reduced ribavirin doses than among those with reduced peginterferon doses (20/20, 100% *vs.* 12/15, 80%; $P = 0.036$).

Table 4. Association between ITPA rs1127354 genotype and dose reduction of drugs at day 28. (A) Pegylated interferon (N = 74, no statistically significant difference); (B) Ribavirin (N = 74, $P = 0.0071$)

Study variables	*ITPA rs1127354* major type	*ITPA rs1127354* minor type
A		
Dose reduction (+)	17	4
Dose reduction (−)	57	19
B		
Dose reduction (+)	22	0
Dose reduction (−)	52	23

2.5. Effects of IL28B and ITPA Genotypes on the Reduction of White Blood Cell/Neutrophil Count

Next, we investigated the association between IL28B and ITPA genotypes, and other hematotoxicities between days 0 and 14, 28, 56 and 84 (data not shown). IL28B rs8099917 minor type induced higher reduction of white blood cell count ($P = 0.043$) as well as neutrophil count between days 0 and 14 ($P = 0.034$). We also analyzed the neutropenia, adjusting for background difference, and we confirmed these data. ITPA rs1127354 major type induced higher reduction of white blood cell count ($P = 0.035$) as well as higher reduction of neutrophil count between days 0 and 28 ($P = 0.020$). These genotypes had no effects on the reduction of white blood cell and neutrophil counts at any other time points, and ITPA rs6051702 had no effects on these reductions at any of the time points.

2.6. Effects of IL28B and ITPA Genotypes on the Reduction of Platelet Count

IL28B rs8099917 minor type induced higher reduction of platelet count between days 0 and 14 ($P = 0.013$) as well as between days 0 and 84 ($P = 0.032$) (data not shown). We also analyzed the thrombocytopenia, adjusting for the background difference, and we confirmed these data. ITPA rs1127354 minor-type induced higher reduction of platelet count between days 0 and 28 ($P = 0.026$) (data not shown). At any other time point these genotypes had no effects on the reduction of platelet count, and ITPA rs6051702 had no effects on this reduction at any time point.

3. Experimental Section

3.1. Patients

Between February 2010 and January 2011, blood samples were obtained from 97 chronic hepatitis C patients at the Department of Gastroenterology, Chiba University Medical School Hospital. Some of these patients had already been included in previous reports [7,8]. Written informed consent was obtained from each patient participating in this study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics review committee of Chiba

University, Graduate School of Medicine. Baseline characteristics are listed in Table 1. Sixty-seven and 30 patients were treatment-naïve and previously treated with interferon therapy, respectively. Previous relapse was defined as undetectable HCV RNA by the end of therapy [2], but then its reappearance after the end of therapy, and the definition of null response was less than 2 $\log_{10}$ decrease in HCV RNA from baseline after 12 weeks of therapy [2]. In 17 relapsers, 7, 3, 2, 2 and 3 received standard interferon monotherapy, standard interferon plus ribavirin, peginterferon monotherapy, peginterferon plus ribavirin and unknown, respectively. In 13 null-responders, 10, 1 and 2 received standard interferon monotherapy, standard interferon plus ribavirin and peginterferon plus ribavirin, respectively. Most patients were infected with HCV genotype 1 (83.5%) with high viral load (>5 log IU/mL) (97.9%). Ultrasound (US) findings showed cirrhosis of the liver in 12 cases (Table 1), 3 of which were also biopsy-proven.

3.2. Treatment

All 97 patients were treated with peginterferon-alfa once weekly and 400–1,000 mg of ribavirin daily [19–21]. Some of them stopped treatment at 12–16 weeks according to the early stopping rule.

3.3. HCV RNA Quantification

HCV RNA was determined by Amplicor HCV monitor assay, version 2.0 (range: 0.5–850 KIU/mL) (Roche Diagnostics, Tokyo, Japan), Amplicor HCV assay (Roche) or COBAS TaqMan HCV test (Roche) (range: 1.2–7.8 log IU/mL). The detection limit of this qualitative assay was 50 IU/mL, corresponding to 1.7 log IU/mL by COBAS TaqMan PCR assay [19]. We defined HCV RNA >5 log IU/mL and <5 log IU/mL as high and low viral titers of HCV RNA, respectively.

3.4. HCV Genotyping

HCV genotype was determined using the antibody-serotyping assay of Tsukiyama-Kohara *et al.* [22]. In this assay, HCV serotypes 1 and 2 correspond to genotypes 1a/1b and 2a/2b, respectively, according to Simmonds' classification [23].

3.5. Classification of Treatment Outcome

Patients were classified as having achieved RVR and early virological response (EVR) if HCV RNA was undetectable (<50 IU/mL) in serum at treatment week 4 and week 12, respectively, and as having SVR if HCV RNA was undetectable in serum 24 weeks after the completion of therapy.

3.6. DNA Extraction and TaqMan SNP Assay

To prepare the DNA sample from blood cells, we used DNA Extract All Lysis Reagents (Applied Biosystems Inc., Foster City, CA, USA). A specific TaqMan genotyping assay was performed for rs1127354, rs6051702 and rs8099917. Primers were manufactured by Applied Biosystems. Thermal cycling was performed with the ABI Step One real-time PCR system according to the manufacturer's protocol. Activation of TaqMan GTXpress Master Mix (Applied Biosystems) and the initial denaturation cycle was at 95 °C for 20 seconds, followed by 40 cycles at 95 °C for 3 seconds and 60 °C

for 20 seconds. We analyzed IL28B rs8099917 TT as major type and TG/GG as minor type, ITPA rs1127354 CC as major type and CA/AA as minor type, and ITPA rs6051702 AA as major type and AC/CC as minor type in the present study.

3.7. Statistical Analysis

Data were expressed as mean ± standard deviation. We used univariate analyses to compare patient characteristics and outcomes, applying Student's t-test or Chi-square test as appropriate. $P < 0.05$ was considered statistically significant.

4. Discussion and Conclusion

In the present study, we also observed that IL28B rs8099917 major genotype was useful for the prediction of treatment response, as in previous studies [4–9], which reported the association between IL28B genotypes and HCV eradication with peginterferon plus ribavirin therapies in chronic hepatitis C patients. SVR was strongly associated with IL28B major genotype (rs8099917 TT). Serum γGTP levels were significantly higher in IL28B rs8099917 minor-type patients, as we reported previously [8].

Previous studies [21,24] showed that HCV-infected patients who can be maintained on >80% of peginterferon and ribavirin dosage for the duration of treatment exhibit enhanced SVR rates. Adherence to therapy decreased over time with both antiviral medications, but more so with ribavirin [25]. Ribavirin could be associated with clinically significant hemolytic anemia, resulting in its necessary dose reduction or discontinuation [26,27]. However, we did not observe any association between ITPA genotypes and SVR.

We also observed that ribavirin-induced anemia is highly dependent on the ITPA rs1127354 genotypes between days 0 and 84, and ITPA rs1127354 major type has been reported to be associated with a reduction in hemoglobin between weeks 0 and 4 [28,29]. In the present study, we observed a difference in age between ITPA rs1127354 major and minor types (Table 2), albeit with a rather limited number of the latter patients. In this respect, further study will be needed, although our previous study showed that the SVR rate of patients aged ≤65 years was similar to that of patients aged >65 years [21]. Genetic variation of ITPA causing an accumulation of inosine triphosphate (ITP) could result in ribavirin-induced anemia. ITP confers protection against ribavirin-induced adenosine triphosphate (ATP) reduction by substituting for erythrocyte GTP, which is depleted by ribavirin, in the biosynthesis of ATP [30]. It is possible that ribavirin-induced anemia is due primarily to the effect of the drug on GTP and consequently ATP levels in erythrocytes [30].

Interestingly, we found that IL28B rs8099917 minor genotype was associated with greater reductions of neutrophils and platelets, although it was reported that IL28B polymorphisms were not associated with interferon-related cytopenia [31]. Our data support the previous reports that patients with ITPA rs1127354 major type had a higher degree of reactive increase in platelet count [32,33]. Further studies will be needed to investigate the potential underlying mechanism and to examine whether there is a synergistic effect of IL28B and ITPA. In the not-too-distant future, HCV therapy will likely move away from interferon-based regimens with increasing numbers of potent antiviral agents being approved, meaning that IL28B and/or ITPA genotyping would not play any additional role and be useful in clinical practice [34–36].

Recent studies revealed that IL28B is associated with hepatic interferon-stimulated gene (ISG) expression [10], hepatic STAT1-nuclear localization [9], hepatic suppressor of cytokine signal 3 (SOCS3) [37] and plasma interferon-gamma inducible protein-10 (IP-10) levels in chronic HCV infection [8]. It is possible that IL28B genotypes affect virus-host interaction through the interaction with interferon signaling pathways. IL28B major type also reported to be associated with a lower prevalence of hepatic steatosis and a less pronounced lipid metabolism, as reflected both by serum lipoprotein levels and hepatic steatosis in HCV infection [38–41]. Insulin resistance is more common in IL28B minor genotype than in major type in treatment-naïve patients with chronic hepatitis C [42,43]. Although there are contrary opinions [44,45], IL28B genotypes influence the stage of liver fibrosis [46,47] and HCV-related hepatocarcinogenesis [48]. Thus, IL28B genotypes play important roles in not only eradication of HCV but also HCV-related pathology.

In HCV infection, patients who developed HCC had lower platelet counts [49]. It is well known that the platelet count decreased with stage advancement of liver diseases in chronic hepatitis C patients [2,49–52]. Chronic hepatitis C is associated with variable degrees of anemia, neutropenia, and/or thrombocytopenia [52]. Multiple factors, including ITPA genotypes, might be involved in this phenomenon.

Our study showed that about 60% of Japanese patients infected with HCV have the preferable allele of IL28B rs8099917, but about 70% of patients also have the undesirable allele of ITPA rs1127354. There seem different distributions between IL28B and ITPA genotypes in the world [6,11]. In conclusion, ITPA rs1127354 is useful for the prediction of ribavirin-induced anemia in the earlier phase of peginterferon plus ribavirin treatment, and IL28B rs8099917 is useful for the prediction of SVR. Use of a combination of these genotypes could lead to a safe and effective treatment for chronic hepatitis C patients.

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Conflict of Interest

The authors declare no conflict of interest.

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Research Paper

Peginterferon Alfa-2a plus Ribavirin in Japanese Patients Infected with Hepatitis C Virus Genotype 2 Who Failed Previous Interferon Therapy

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Abstract

Some patients infected with hepatitis C virus (HCV) genotype 2 could be cured with treatment shorter than 24 weeks using peginterferon plus ribavirin, but there are still treatment-refractory patients. Direct-acting antivirals (DAAs) are not currently available for HCV genotype 2 patients, different from genotype 1 patients, in clinical practice. We investigated 29 HCV genotype 2-infected Japanese patients who had been previously treated and failed to clear HCV. We retreated them with peginterferon alfa-2a plus ribavirin and measured HCV RNA level to assess the efficacy and safety of this treatment in patients who had failed previous therapy. We found that retreatment of HCV genotype 2-infected Japanese patients with peginterferon alfa-2a plus ribavirin for 24-48 weeks led to 60 to 66.6% sustained virological response (SVR) in patients previously treated with (peg-)interferon monotherapy and to 69.9% SVR in relapsers previously treated with peginterferon plus ribavirin. Attention should be paid to certain patients with unique features. Selection of patients according to their previous treatment could lead to optimal therapy in HCV genotype 2 treatment-experienced patients.

Key words: Retreatment, HCV G2, Japanese

INTRODUCTION

Hepatitis C virus (HCV) infection causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) [1]. HCV is also a major causative agent of HCC in Japan [2]. HCV is a positive-sense single stranded RNA virus with ~9.6 kb length, belonging to the genus Hepacivirus, a member of *the family Flaviviridae*. It is known that there exist at least 6 main genotypes of HCV [3]. These approximately equidistant genetic groups each contain a variable number of more closely related, genetically and epidemiologically distinct "subtypes". Genotypes differ from each other by 31 to 33% at the nucleotide level,

compared with 20 to 25% between subtypes [3]. In Japan, HCV genotype 1b, 2a and 2b, respectively, are observed in ~70, 20 and 10% of HCV-infected patients [4].

Treatment with peginterferon plus ribavirin for 24 weeks leads to 70-80% sustained virological response (SVR) in treatment-naïve patients infected with HCV genotype 2 [5-7]. Combination of peginterferon with ribavirin for 24 weeks is the current standard of care (SOC) for treatment-naïve patients infected with HCV genotype 2 or 3. Some selected HCV genotype 2-infected patients achieved SVR with treatment periods shorter than 24 weeks [5-9]. However, in treatment-naïve patients infected with HCV genotype 1, treatment with peginterferon plus ribavirin for 48 weeks leads to only ~50% SVR [7]. Thus, HCV genotype is one of the important factors influencing the outcome of interferon treatments [7,10].

Retreatment of chronic hepatitis C patients failing to achieve SVR with combination peginterferon plus ribavirin could only obtain 10 to 15% SVR in non-responders and 40 to 50% SVR in relapsers [11]. In North America and European countries, retreatment for HCV genotype 2 or 3 patients failing to achieve SVR with combination peginterferon plus ribavirin could lead to 37 to 46% SVR in non-responders and 52 to 63% in relapsers [12, 13], even though retreatment with SOC was performed for 48 weeks. In our previous study [14], we observed that retreatment for HCV genotype 2 Japanese patients who failed to achieve SVR with combination peginterferon alfa-2b plus ribavirin for 16, 24 or 48 weeks resulted in 71.4% SVR, but the proportions of non-responders and relapsers were unclear and HCV RNA was measured with COBAS AMPLICOR HCV Monitor Test v. 2.0 (range: 0.5 - 850 kIU/mL) (Roche Diagnostics, Tokyo, Japan).

In the present study, we investigated 29 HCV genotype 2-infected Japanese patients who had been previously treated and failed to clear HCV. We retreated them with peginterferon alfa-2a plus ribavirin and measured HCV RNA with the more sensitive COBAS TaqMan HCV test (Roche) to assess the efficacy and safety of peginterferon alfa-2a with ribavirin in patients who had failed previous therapy in clinical practice. We focused on 3 females retreated with peginterferon alfa plus ribavirin and resulting in non-SVR, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected. This would indicate that treatment with SOC should be stopped in HCV genotype 2 female patients with these features.

MATERIALS AND METHODS

Patients

Patients were recruited from Chiba University Hospital and 29 hospitals in Chiba, Ibaraki, and Saitama Prefectures between March 2008 and September 2011. Patients were eligible if they met the following inclusion criteria: (i) infected with HCV genotype 2 alone, (ii) age ≥ 20 years, (iii) diagnosed as chronic hepatitis C, (iv) negative for HBs antigen, (V) negative for human immunodeficiency virus, (vi) no autoimmune liver diseases, (vii) no severe renal disease, (viii) no severe heart disease, (ix) no mental disorders, (x) no current intravenous drug abuse, and (xi) no pregnancy. Thirty-four of the patients had previously been included in an investigation of the incidence of HCC during and immediately after peginterferon alfa-2a and ribavirin treatment in patients with chronic hepatitis C in Japan [2].

Study design

We recruited previously treated patients infected with HCV genotype 2. In Japan, combination therapy for treatment-naïve patients infected with HCV genotype 2 was not supported by the Japanese health insurance system at that time [15]. Concerning previously treated patients, they had to have failed previous treatment with either conventional interferon monotherapy, peginterferon monotherapy, conventional interferon/ribavirin combination therapy, or peginterferon/ribavirin combination therapy, different from the previous study by Sherman et al. [12]. Twenty-nine consecutive patients were enrolled in this study. Informed consent was obtained from all patients prior to enrolment. The Ethics Committee of Chiba University School of Medicine approved the study protocol. In this study, 180 μg of peginterferon alfa-2a per week plus 600-800 mg ribavirin per day were usually given in the treatment of patients for as long as 24, 48, or 72 weeks, according to the patient's will, as combination therapy for retreated patients infected with HCV genotype 2 was supported for only 24 weeks by the Japanese health insurance system at that time [15]. Clinical and laboratory assessments were performed at least every 4 weeks during treatment and a 12-week follow-up period. Adverse events were noted by oral inquiry (patient interview), physical examinations and laboratory tests.

Determination of HCV RNA titers and HCV genotype

Serum HCV RNA titer was measured using COBAS TaqMan HCV test (Roche), with levels ranging from 1.2 to 7.8 log IU/mL [16]. HCV genotype was

determined by the antibody serotyping method of Tukiyama-Kohara et al. [17,18]. According to this assay, HCV serotypes 1 and 2 correspond to HCV genotypes 1a/1b and 2a/2b [3]. HCV RNA titer and HCV genotype were determined before treatment, and HCV RNA was measured every 4 weeks before, during, and for at least 24 weeks after the end of treatment.

Serum liver function tests and hematology tests

Serum aminotransferase concentrations, other liver function tests and hematology tests were performed according to standard methods every 1 to 3 months before, during, and for at least 24 weeks after the end of treatment.

Assessment of efficacy

SVR was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. Relapse was defined as undetectable HCV RNA at the end of therapy, followed by the reappearance of HCV RNA [11]. Non-response was defined as detectable HCV RNA at the end of therapy. Patients with undetectable HCV RNA within the initial 4 weeks of treatment were considered to have had rapid virological response (RVR). Patients who had undetectable HCV RNA within the initial 12 weeks of treatment were considered to have had complete early virological response (cEVR) (described as EVR in this article) [16].

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences were evaluated by Student's *t*-test, chi-square test, or Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The characteristics of the 29 patients in the present study are shown in Table 1. They had a history of peginterferon/conventional interferon with or without ribavirin, and 4 were unknown regarding previous treatment response (Table 1). In these 29 patients, 3 received conventional interferon monotherapy, 10 peginterferon alfa-2a monotherapy, 12 peginterferon alpha-2b plus ribavirin, 3 peginterferon alfa-2a plus ribavirin, and 1 had details unknown. HCV RNA levels (≥5log IU/mL, <5log IU/mL, and unknown) were 24, 4 and 1, respectively. Concerning virological response, 18 (62.0%) had SVR, 9 (31.0%) relapsed, and 2 (6.8%) discontinued treatment due to side effects.

Table 1. Baseline and demographic characteristics of patients in the present study

Number of patients	29
Age (years)	60.1 ± 8.6
Gender (male/female)	15/14
Body mass index (kg/m^2)	26.2 ± 3.6
HCV RNA (log IU/mL)	5.5 ± 2.0
ALT (IU/L)	57.8 ± 50.7
γ-GTP (IU/L)	46.0 ± 40.7
AFP (ng/mL)	5.7 ± 3.4
Leukocyte count (/mm^3)	4940 ± 1670
Hemoglobin (g/dL)	14.0 ± 1.6
Platelet count (x10^4/mm^3)	16.2 ± 5.2
Treatment response	
Duration of treatment (~24/48/72 wks)	9/18/2
RVR rates, %	34.4 (10/29)
HCV RNA negativity at 8 wks	81.4 (22/27)
EVR rates, %	88.8 (24/27)
SVR rates, %	62.0 (18/29)

Data are expressed as mean ± SD. ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transferase; AFP, alpha-fetoprotein; RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response.

Comparison of SVR patients with non-SVR patients among previously treated patients

Next, we compared 18 SVR patients with 11 non-SVR patients among the previously treated patients (Table 2). The platelet count of SVR patients tended to be higher than that of non-SVR patients ($P = 0.061$). We did not see any differences in the baselines of other factors and treatment responses (Table 2). In the 18 SVR patients previously treated, 3 received conventional interferon monotherapy, 5 peginterferon alfa-2a monotherapy, 7 peginterferon alpha-2b plus ribavirin, 2 peginterferon alfa-2a plus ribavirin and 1 with details unknown. In the 11 non-SVR patients previously treated, 5 received peginterferon alfa-2a monotherapy, 5 peginterferon alpha-2b plus ribavirin, and 1 peginterferon alfa-2a plus ribavirin. Concerning previous treatment response of the 29 previously treated patients, 18 were relapsers, 7 non-responders, and 4 had details unknown. In the 18 SVR patients, 12 were relapsers, 4 non-responders, and 2 had details unknown. In 11 non-SVR patients, 6 were relapsers, 3 non-responders, and 2 had details unknown.

Table 2. Baseline and demographic characteristics of SVR- and non-SVR-retreated patients

	SVR	Non-SVR	*P*-value*
Number of patients	18	11	N.S.
Age (years)	60.0 ± 10.0	60.3 ± 6.3	N.S.
Gender (male/female)	8/10	7/3	N.S.
Body mass index (kg/m^2)	26.0 ± 3.4	26.5 ± 4.0	N.S.
HCV RNA (log IU/mL)	5.5 ± 1.9	5.5 ± 2.1	N.S.
ALT (IU/L)	57.8 ± 50.7	55.6 ± 52.8	N.S.
γ-GTP (IU/L)	46.0 ± 40.7	30.4 ± 17.6	N.S.
AFP (ng/mL)	5.7 ± 3.4	6.2 ± 5.7	N.S.
Leukocyte count ($/mm^3$)	4940 ± 1670	4670 ± 940	N.S.
Hemoglobin (g/dL)	14.0 ± 1.6	13.6 ± 2.0	N.S.
Platelet count ($x10^4/mm^3$)	16.2 ± 5.2	12.6 ± 4.1	0.061
Treatment response			
Duration of treatment (~24/48/72 wks)	6/11/1	3/7/1	N.S.
RVR rates, %	44.4 (8/18)	18.1 (2/11)	N.S.
HCV RNA negativity at 8 wks	88.8 (16/18)	66.6 (6/9)	N.S.
EVR rates, %	88.8 (16/18)	88.8 (8/9)	N.S.
Adherence (≥80/≥80/≥80), yes	44.4 (8/18)	54.5 (6/11)	N.S.

Data are expressed as mean ± SD. *P-value, between groups with and without SVR by Student's t-test or chi-square test; N.S., not statistically significant; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transferase; AFP, alpha-fetoprotein; RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response; adherence was classified according to the previous report [19].

Previous treatment response and SVR rates in HCV genotype 2 retreated patients

The relationship between previous treatment response and SVR rates of HCV genotype 2 retreated patients is shown in Table 3. In 1 patient previously treated with peginterferon plus ribavirin and non-response, treatment was discontinued due to side effects by ~8 weeks and SVR was not obtained. Of 13 patients previously treated with peginterferon plus ribavirin who had relapsed, 2 discontinued treatment due to side effects by ~8 weeks.

Female cases retreated, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected

Furthermore, we tried to determine the clinical features of non-SVR HCV genotype 2 patients retreated with peginterferon alfa-2a plus ribavirin. We noticed 3 females retreated with peginterferon alfa-2a plus ribavirin and resulting in non-SVR, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected (Figure 1). HCV RNA finally relapsed in all 3 cases. Treatment with SOC might need to be stopped in HCV genotype 2 female patients with these features.

Table 3. Previous treatment response and SVR rates in 25 retreated patients

Number of patients	Previous treatment (Treatment response)	Formula of re-treatment	SVR rates (%)
6	Peginterferon alfa-2a (NR)	Peginterferon alfa-2a plus ribavirin (~24wks)	66.6
1	Peginterferon plus ribavirin (NR)	Peginterferon alfa-2a plus ribavirin (~24wks)	0
5	(Peg-)interferon (relapse)	Peginterferon alfa-2a plus ribavirin (~48wks)	60
13	Peginterferon plus ribavirin (relapse)	Peginterferon alfa-2a plus ribavirin (24~48wks)	69.9

NR, non-response

DISCUSSION

In the present study, we focused on the virological response in HCV genotype 2-infected Japanese patients retreated with peginterferon alfa-2a plus ribavirin. We did not observe any differences in baseline background between SVR patients retreated and non-SVR patients retreated, although we must admit that the number of patients was small. However, during this study, we did find 3 females who did not obtain SVR by the retreatment and had unique features. That is, in their sera, a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected (Figure 1). These 3 cases did not discontinue peginterferon alfa-2a or ribavirin. In Figure 1, cases 1 and 2 had reduced peginterferon alfa-2a but not reduced ribavirin. On the other hand, case 3 had reduced ribavirin due to anemia, but did not have a reduction of peginterferon alfa-2a. In cases 2 and 3, adherence (≥80/≥80/≥80) [19] based on the calculation at 48 weeks was not lower. These 3 cases were relapsers and seemed different from non-responders having anti-interferon-alfa neutralizing antibody [20]. We do not know the exact reasons at this time.

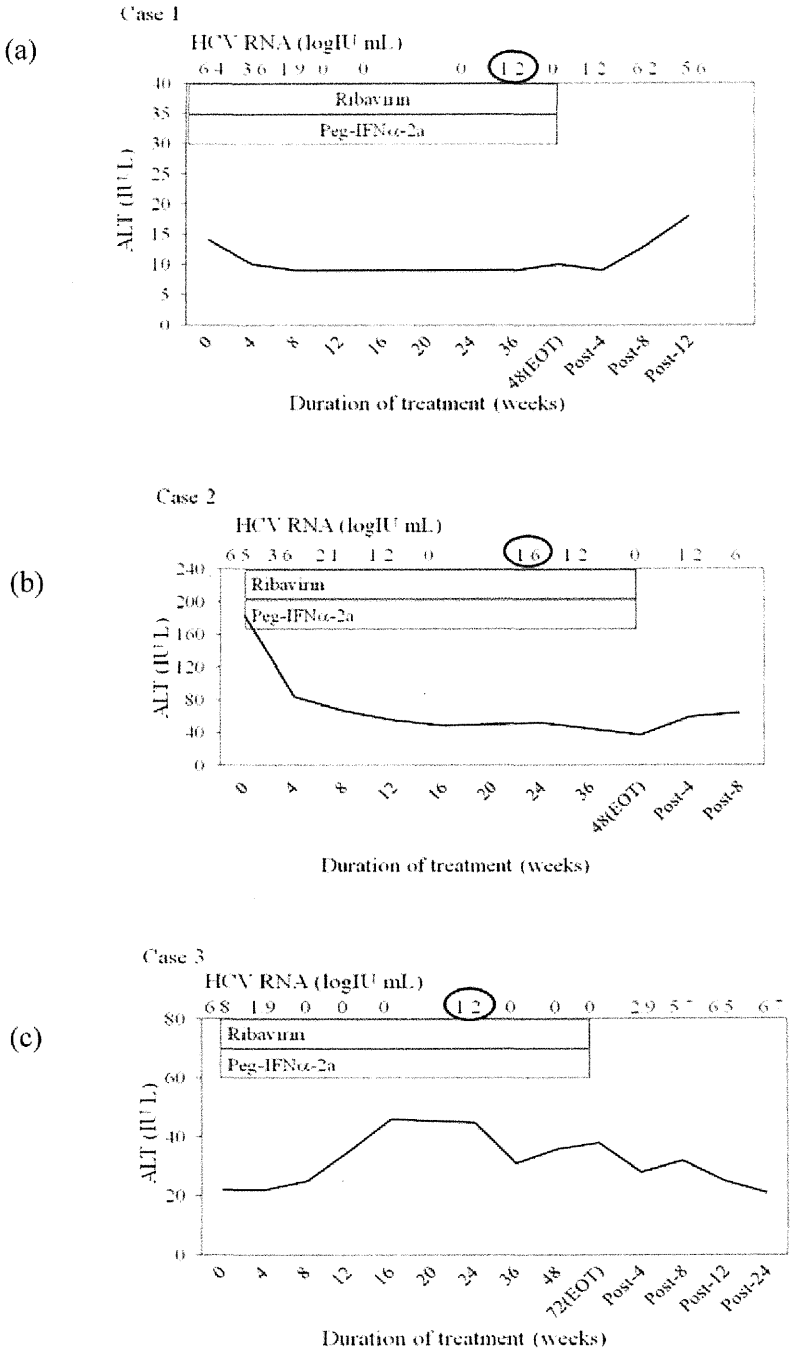

Figure 1. Three females retreated with peginterferon alfa plus ribavirin and resulting in non-sustained virological response, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected. (a) Case 1, 68 years, female, IL28Brs8099917 TT. She was previously treated with peginterferon alfa-2a for 48 weeks, with details unknown. (b) Case 2, 58 years, female, IL28Brs8099977, not determined. She was previously treated with peginterferon alfa-2a for 48 weeks, with relapse. (c) Case 3, 58 years, female, IL28Brs8099917 TG. She was previously treated with peginterferon alfa-2b plus ribavirin, with details unknown. HCV RNA was determined by COBAS TaqMan HCV test (Roche), with levels ranging from 1.2 to 7.8 log IU/mL [16].

In the present study, 44% of patients had rapid virological response (RVR) and 89% of the patients had EVR (cEVR) in the retreated genotype 2 chronic hepatitis C patients with an SVR (Table 2). These results were concordant with previous studies. However, 89% of the non-SVR patients also had EVR (Table 2). Among the 8 non-SVR patients, 3 had lower adherence (≥80/≥80/≥80) (data not shown). In the present study, the adherence rates were quite low (44% in patients with SVR, and 54% in patients without SVR). In certain cases, lower adherence may be one of the reasons for non-SVR.

For HCV genotype 1 patients, direct acting antivirals (DAAs) such as telaprevir and boceprevir have been available in clinical practice [7, 21-23]. The addition of telaprevir or boceprevir to peginterferon plus ribavirin resulted in significantly higher rates of SVR in previously treated patients with chronic HCV genotype 1 infection [7, 21-23]. It will require more time until the more frequent use of DAAs for the treatment of HCV genotype 2 patients will become possible [24, 25]. Until then, we have to retreat HCV genotype 2-infected patients with peginterferon alfa-2a plus ribavirin for 24-48 weeks.

Recently, it was reported by several groups that genetic variations in IL28B-SNP predict HCV genotype 1 treatment-induced viral clearance [7, 26-29]. Yu et al. [30] reported that rs8099917 TT genotype is significantly independently predictive of RVR, which is the single best predictor of SVR, in Asian HCV genotype 2 patients. Further study will be needed.

In conclusion, we showed that retreatment of HCV genotype 2-infected Japanese patients with peginterferon alfa-2a plus ribavirin for 24-48 weeks resulted in 60 to 66.6% SVR in patients previously treated with (peg-)interferon monotherapy and in 69.9% SVR in relapsers previously treated with peginterferon plus ribavirin, which supports the previous reports [12, 13]. Attention should be paid to certain patients with unique features. Selection of patients according to previous treatment could lead to optimal therapy in HCV genotype 2 treatment-experienced patients.

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CONFLICT OF INTEREST

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ABBREVIATIONS

cEVR: Complete early virological response; DAA: Direct-acting antiviral; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; IL28B: Interleukin-28B; RVR: Rapid virological response; SNP: Single nucleotide polymorphism; SD: Standard deviation; SOC: Standard of care; SVR: Sustained virological response.

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