applied to amplicons of *IL-28A* or *28B* separated by gel electrophoresis. Homozygotes of TA repeat showed clear patterns and a high quality value in the bar above, whereas the patterns of heterozygotes were mixed because the length differed between alleles. The mixed patterns are shown in dashed boxes. These mixed products were cloned into the pGEM-Teasy vector to isolate and count the $(TA)_n$ number by sequencing of both alleles.
(PDF)

Table S1
(DOC)

Table S2
(DOC)

Table S3
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Table S4
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Author Contributions

Conceived and designed the experiments: MS YT MN TW MM. Performed the experiments: MS YT. Analyzed the data: MS YT. Contributed reagents/materials/analysis tools: MS YT MN TW MM. Wrote the paper: MS YT MN TW MM.

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Original article

Relationship between polymorphisms of the inosine triphosphatase gene and anaemia or outcome after treatment with pegylated interferon and ribavirin

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Background: A genome-wide association study revealed an association between variants of the inosine triphosphatase (*ITPA*) gene and ribavirin (RBV)-induced anaemia. The aim of this study was to replicate this finding in an independent Japanese cohort and to define a method to allow pretreatment prediction of anaemia in combination with other factors.

Methods: Genotype 1b chronic hepatitis C patients (n=132) treated with pegylated interferon (PEG-IFN)-α and RBV for 48 weeks were genotyped for *ITPA* rs1127354 and examined for anaemia and treatment outcome.

Results: Variants of the *ITPA* gene protected against severe anaemia throughout the 48-week treatment period and were associated with lower incidence of anaemia-related RBV dose reduction. A combination of the *ITPA* genotype with baseline haemoglobin (Hb) and creatinine clearance (CLcr) levels predicted severe anaemia with high accuracy (90% sensitivity and 62% specificity). Among a subset of patients with the *IL28B* genotype of TT at rs8099917, patients with variants of the *ITPA* gene were associated with a higher rate of receiving >80% of the expected RBV dose, a higher rate of sustained virological response (SVR), and a lower rate of relapse.

Conclusions: The variants of the *ITPA* gene, which could protect against haemolytic anaemia and RBV dose reduction, were associated with a high rate of SVR by standard PEG-IFN and RBV therapy in a subset of Japanese patients with the favourable TT genotype at rs8099917 of *IL28B*. A combination of *ITPA* genetic polymorphisms with baseline Hb and CLcr levels further improves the predictive accuracy of severe anaemia.

Introduction

Treatment with pegylated interferon (PEG-IFN) combined with ribavirin (RBV) is the most effective standard treatment for chronic HCV infection. Successful eradication of HCV is associated with a reduced risk of developing hepatocellular carcinoma. However, the rate of sustained virological response (SVR) is approximately 50% in patients with HCV genotype 1 [1,2]. The probability of SVR decreases when the patients become intolerant to therapy and receive <80% of the planned dose of PEG-IFN and/or RBV [3]. One of the major reasons for intolerance to therapy is severe haemolytic anaemia induced by RBV [1]. The degree of haemolytic anaemia caused by RBV varies among individuals, and no reliable baseline predictors exist for this severe anaemia.

Recently, a genome-wide association study revealed that a single nucleotide polymorphism (SNP) at rs6051702 is strongly associated with RBV-induced haemolytic anaemia at week 4 of treatment [4]. This SNP was linked to two functional SNPs (rs1127354 and rs7270101) in the inosine triphosphatase (*ITPA*)

gene on chromosome 20, which had previously been well-characterized in studies of patients with ITPase deficiency [5–8]. Subsequent studies confirmed independently that variants of the *ITPA* gene are protective against haemolytic anaemia during the early weeks of treatment [9,10]. Furthermore, Thompson *et al.* [9] showed that the variants are protective against anaemia over the entire 48-week course of therapy and are associated with reduced requirement for an anaemia-related dose reduction of RBV. Notably, despite these protective effects, variants in the *ITPA* gene were not associated with treatment outcome [4,9] or showed only a marginal association [10].

In the present study, we aimed to replicate the association between *ITPA* genetic polymorphisms and RBV-induced anaemia in the early weeks, as well as throughout the entire course, of therapy in an independent Japanese cohort. In addition, for the general application of these genetic associations in clinical practice, we aimed to define a pretreatment prediction for severe anaemia in combination with other clinical covariates.

Methods

Patients

Data were collected retrospectively from a total of 132 genotype 1b chronic hepatitis C patients who were treated with PEG-IFN-α and RBV at Musashino Red Cross Hospital (Tokyo, Japan) and at Nagoya City University Graduate School of Medical Sciences (Nagoya, Japan). The inclusion criteria were: genotype 1b, HCV RNA titre >100 KIU/ml by quantitative PCR (Cobas Amplicor HCV Monitor version 2.0; Roche Diagnostic Systems, Indianapolis, IN, USA), no coinfection with HBV or HIV, no other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis, and availability of DNA for the analysis of the genetic polymorphism of *ITPA*. Patients received PEG-IFN-α2a (180 μg) and -α2b (1.5 μg/kg) subcutaneously every week and were administered a daily weight-adjusted dose of RBV (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg, and 1,000 mg for patients weighing >80 kg) for 48 weeks. Dose reduction of RBV was considered by physicians based on the clinical conditions of the individual patients or the recommendations on the package inserts: dose reduction from 800 mg and 1,000 mg to 600 mg or from 600 mg to 400 mg for haemoglobin levels <10 g/dl and drug discontinuation when haemoglobin levels drop to <8.5 g/dl. No patient received erythropoietin or other growth factors for the treatment of anaemia. PEG-IFN and RBV was stopped prematurely in 22 patients: in 15 patients due to non-virological response and in 7 patients due to adverse events. Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees.

Laboratory and histological tests

Blood samples were obtained before therapy and at 1, 2, 4, 6, 8, 12, 16, 20, 24, 36 and 48 weeks after the start of therapy, and were analysed for haematological tests, blood chemistry and HCV RNA. Genetic polymorphisms in an SNP located in exon 2 (rs1127354) and in intron 2 (rs7270101) of the *ITPA* gene were determined using ABI TaqMan Probes (Applied Biosystems, Carlsbad, CA, USA) [4]. Since a recent paper studying Japanese patients showed no variants in rs7270101 [10] and our preliminary genotyping data for 100 Japanese patients also showed no variations in rs7270101, rs1127354 was used for further analysis (major allele =C and minor allele =A). Genetic polymorphisms in the *IL28B* gene (rs8099917), an SNP recently identified to be associated with hepatitis C treatment response [11–14], was also determined by a DigiTag2 assay [15]. Viral factors affecting therapeutic efficacy was determined. A stretch of 40 amino acids in the NS5A region of HCV, designated as the interferon sensitivity-determining region (ISDR) [16,17] and amino acid substitutions at positions 70 of the core region (Core70) [18] were determined by direct sequencing after amplification by reverse transcription and PCR as reported previously. Arginine at Core70 was defined as the wild type, and glutamine or histidine was defined as the mutant type. Baseline creatinine clearance (CLcr) levels were calculated using the formula of Cockcroft and Gault [19]: for males, CLcr= ([140 - age in years] × body weight in kg)/(72× serum creatinine in mg/dl) and for females, CLcr=0.85× ([140 - age in years]× body weight in kg)/(72× serum creatinine in mg/dl). Fibrosis was evaluated on a scale of 0–4: F0 indicates no fibrosis, F1 indicates mild fibrosis, F2 indicates moderate fibrosis, F3 indicates severe fibrosis and F4 indicates cirrhosis according to the Metavir scoring system [20]. The end of treatment response was defined as an undetectable HCV RNA level by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor; Roche Diagnostic Systems) at the end of therapy. SVR was defined as an undetectable HCV RNA level 24 weeks after the completion of therapy. A relapse was defined as the reappearance of HCV RNA after the completion of therapy.

Statistical analysis

We analysed the association between an SNP of the *ITPA* gene (rs1127354) and the following: the incidence of haemoglobin (Hb) reduction of >3.0 g/dl at week 4 and the incidence of severe anaemia (Hb<10 g/dl) at week 4 or at any time point during the therapy; the time-dependent decrease in Hb levels throughout

the treatment period; the time-dependent requirement for RBV dose reduction throughout the treatment period; and the rate of virological response or relapse. Associations between pretreatment variables and anaemia were analysed by multivariable regression. The association between the *ITPA* polymorphisms and anaemia or treatment outcome was analysed by Fisher's exact test. The association between the *ITPA* polymorphisms and the time-dependent reduction in Hb levels or the requirement for RBV dose reduction was analysed by Kaplan–Meier survival analysis. SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA) was used for these analyses.

Table 1. Clinical characteristics of the study population

Characteristic	Value
Age, years	57.5 (±9.5)
Sex, male/female	50/82
Baseline platelet count, 10^9/l	150.4 (±55.8)
Baseline Hb, g/dl	14.0 (±1.5)
Baseline creatinine clearance, ml/min	94.8 (±24.1)
Baseline liver fibrosis, F0–2/F3–4	102/30
Initial ribavirin dose	
600 mg/day, *n* (%)	91 (69)
800 mg/day, *n* (%)	38 (29)
1,000 mg/day, *n* (%)	3 (2)
Dose reduction of ribavirin, *n* (%)	58 (43)
Hb reduction at week 4, g/dl	2.2 (±1.4)
Hb reduction >3.0 g/dl at week 4, *n* (%)	37 (28)
Severe anaemia at week 4, *n* (%)[a]	21 (16)
Severe anaemia at any time point, *n* (%)[a]	57 (43)
ITPA rs1127354, AA/CA/CC	4/33/95
ISDR mutation ≤1, *n*/total *n* (%)	96/114 (84)
Core70 mutant type, *n*/total *n* (%)	42/105 (40)

Continuous variables were described as mean (±SD) and categorical variables were described as frequency and percentage. [a]Severe anaemia defined as haemoglobin (Hb)<10 g/dl. Core70, amino acid substitutions at position 70 of the core region; ISDR, interferon sensitivity-determining region; *ITPA*, inosine triphosphatase gene.

Results

ITPA rs1127354 minor genotype alleles AA and CA were protective for anaemia during drug therapy

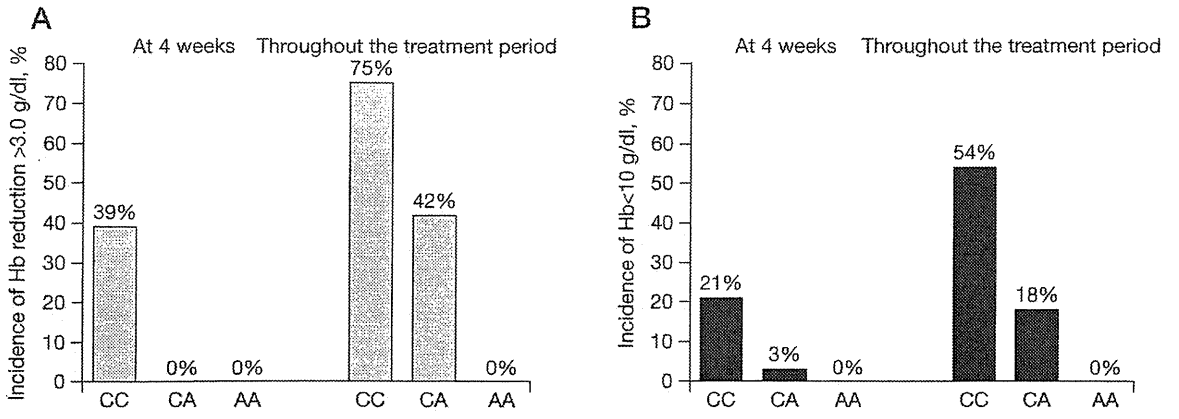
The baseline characteristics are listed in Table 1. Genotyping of rs1127354 revealed that 4 patients were homozygous for the minor allele (AA), 95 were homozygous for the major allele (CC) and 33 were heterozygous (CA). The frequency of the minor allele A was 0.16. The *ITPA* genotype was not associated with any baseline factors including age, gender, Hb levels, CLcr, platelet counts, liver fibrosis, mutations in the ISDR and Core70 (Table 2). The mean value of Hb reduction at week 4 was 2.2 g/dl and a reduction of >3.0 g/dl developed in 37 patients (28%) at week 4. Severe anaemia (Hb<10 g/dl) developed in 21 (16%) patients at week 4 of therapy and in 57 (43%) patients at any time point during the entire 48 weeks of therapy. Figure 1A and 1B shows the percentages of patients with anaemia according to the rs1127354 genotypes. At week 4, Hb reduction of >3.0 g/dl developed in 37 patients (39%) with the CC genotype, which is in contrast to 0 patients with the CA or AA genotypes (Figure 1A). Severe anaemia developed in 20 (21%) patients with the CC genotype, which is in contrast to only 1 (3%) patient with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, P=0.008; Figure 1B). Throughout the course of the 48-week therapy, Hb reduction of >3.0 g/dl developed in 71 (75%) patients with the CC genotype in contrast to 14 (42%) patients with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, P=0.0001). Severe anaemia was observed in 51 (54%) patients with the CC genotype, which is in contrast to 6 (18%) patients with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, P<0.0001). The mean reduction of Hb levels and the time course of therapy are shown in Figure 2. Patients with genotypes AA and CA showed less Hb reduction at weeks 2, 4, 6, 8 and 12 during drug therapy compared to those with the

Table 2. Clinical characteristics of patients according to *ITPA* genotype

Characteristic	rs1127354		*P*-value
	AA/CA	CC	
Age, *n* (%)	56.0 (10.9)	58.1 (8.8)	0.316
Sex, male/female	17/20	33/62	0.239
Baseline platelet count, 10^9/l	153.3 (±48.5)	149.2 (±58.5)	0.711
Baseline Hb, g/dl	14.3 (±1.4)	13.8 (±1.5)	0.132
Baseline creatinine clearance, ml/min	93.4 (±23.3)	95.3 (±24.5)	0.692
Baseline liver fibrosis, F0–2/F3–4	33/4	69/26	0.063
ISDR mutation ≤1, *n*/total *n* (%)	26/30 (87)	70/84 (83)	0.777
Core70 mutant type, *n*/total *n* (%)	11/27 (41)	31/78 (40)	1.000

Continuous variables were described as mean (±SD) and categorical variables were described as frequency and percentage. Core70, amino acid substitutions at position 70 of the core region; Hb, haemoglobin; ISDR, interferon sensitivity-determining region.

Figure 1. *ITPA* rs1127354 genotypes and anaemia during drug therapy



The percentage of patients with (A) haemoglobin (Hb) reduction of >3.0 g/dl or (B) Hb concentrations of <10 g/dl at week 4 and at any time point throughout the treatment period is shown for rs1127354 genotypes. Severe anaemia was less frequent in patients with the rs1127354 genotypes AA and CA (Hb reduction >3.0 g/dl at any time point: CC versus AA/CA, P=0.0001; Hb concentrations <10 g/dl at week 4: CC versus AA/CA, P=0.008; and Hb concentrations <10 g/dl at any time point: CC versus AA/CA, P<0.0001). *ITPA*, inosine triphosphatase gene.

CC genotype (P<0.0001 for weeks 2, 4 and 6; P=0.02 for weeks 8 and 12). These results show that the AA and CA genotypes are significantly associated with less absolute reduction in Hb levels, especially during the early weeks of therapy, and are protective against the development of severe anaemia. The sensitivity and specificity of the *ITPA* genotype for the prediction of severe anaemia (Hb<10 g/dl) throughout the course of treatment was 89% (51/57) and 41% (31/75), respectively.

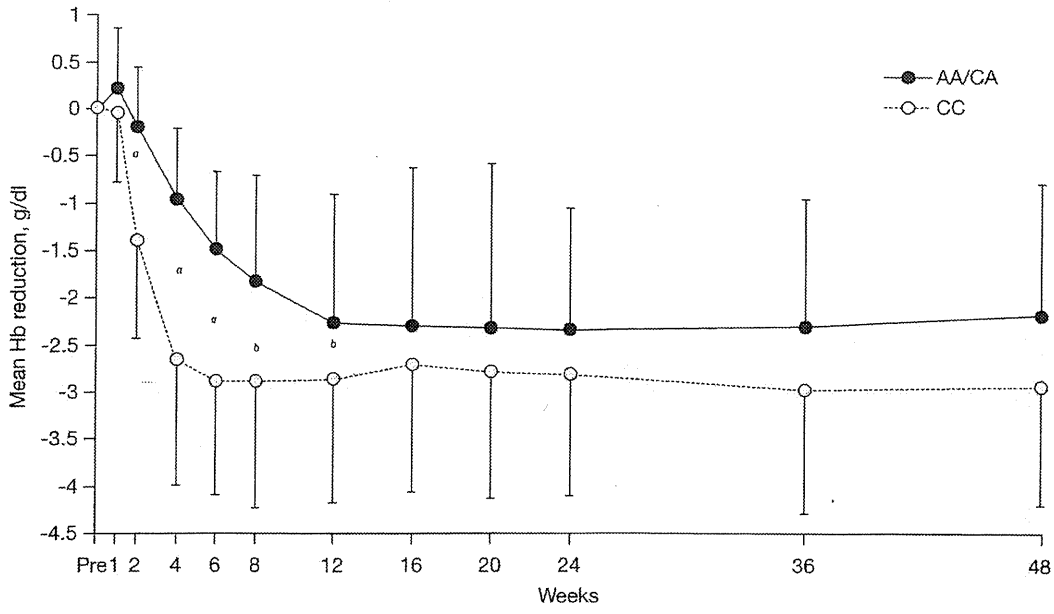
ITPA rs1127354 minor genotypes AA and CA were protective against the requirement for RBV dose reduction

The dose of RBV was reduced in 58 (43%) patients. Severe anaemia was the indication for dose reduction in 45 of the 58 (78%) patients. In the remaining 13 patients, the RBV dose was reduced because of other adverse events such as fatigue, skin eruption or loss of appetite. Figure 3 shows the time to the first RBV dose reduction during the 48 weeks of therapy. A dose reduction of RBV for any reason was less frequent and delayed in patients with the AA and CA genotypes compared to those with the CC genotype (Figure 3A; P=0.048). The difference was more significant for anaemia-related RBV dose reduction (Figure 3B; P=0.004).

Other factors associated with severe anaemia during therapy

Since 18% of the patients with the protective *ITPA* genotype of CA developed severe anaemia, we analysed the patients for other predictive factors of severe anaemia. By univariable analysis, the rs1127354 CC genotype, female gender, older age, and lower baseline Hb levels, platelet counts and CLcr levels were associated with severe anaemia. Next, multivariable regression models with backward selection were used to identify the independent predictors of severe anaemia. Covariates included age, sex, fibrosis stage, baseline Hb levels, CLcr levels and platelet counts, and the rs1127354 genotype. The multivariable regression analysis showed that the rs1127354 CC genotype, a baseline Hb of <14 g/dl and a baseline CLcr of ≤95 ml/min were independent predictors of severe anaemia at week 4 and at any time point during the 48 weeks of therapy (Table 3). Figure 4 shows the percentage of patients with Hb concentrations of <10 g/dl at any time point during therapy for the subgroups of patients stratified by rs1127354 genotype, baseline Hb levels and baseline CLcr levels. Among patients with the rs1127354 CC genotype, the risk of developing severe anaemia was more prominent in those with a baseline Hb<14 g/dl and a baseline CLcr≤95 ml/min (88%) compared to those with a baseline Hb≥14 g/dl and a baseline CLcr >95 ml/min (P<0.0001) or those with a baseline Hb<14 g/dl or a baseline CLcr≤95 ml/min (P=0.0036). Notably, the incidence of severe anaemia was only 12% in patients with the rs1127354 CC genotype if the baseline Hb was ≥14 g/dl and the CLcr was >95 ml/min. By contrast, there was a moderate risk of severe anaemia (33%) even in patients with the rs1127354 protective genotypes AA or CA when the baseline Hb was <14 g/dl and the baseline CLcr was ≤95 ml/min. Thus, patients who have >30%

Figure 2. *ITPA* rs1127354 genotypes and the quantitative Hb reduction from baseline



The mean reduction of haemoglobin (Hb) levels along the time points of treatment is shown for the rs1127354 genotypes. Solid and dotted lines indicate patients with the AA/CA and CC genotypes, respectively. The error bars indicate standard deviation. The AA/CA genotype had less of a reduction in the mean Hb levels at weeks 2–12 during therapy compared to the CC genotype. $^{a}P<0.001$; $^{b}P=0.02$. *ITPA*, inosine triphosphatase gene; Pre, pretreatment.

risk of severe anaemia had the following characteristics: rs1127354 CC genotype, baseline Hb<14 g/dl and CLcr≤95 ml/min; rs1127354 CC genotype and baseline Hb<14 g/dl or CLcr≤95 ml/min; and rs1127354 AA or CA genotype, baseline Hb<14 g/dl and CLcr≤95 ml/min. The sensitivity and specificity of the combination of these three factors for the prediction of severe anaemia (Hb<10 g/dl) throughout the course of treatment was 89% (51/57) and 64% (48/75). Compared to the *ITPA* genotype alone, specificity improved from 41% to 64% with the same sensitivity (89%), indicating that the combination of the *ITPA* genotype, baseline Hb levels and baseline CLcr levels could improve the prediction accuracy. The AA/CA genotypes of rs1127354 were protective against the requirement for RBV dose reduction even after standardization by baseline Hb and CLcr (Figure 3C). The predictive model for anaemia and recommendations for monitoring and treatment were made for clinical practice application (Table 4).

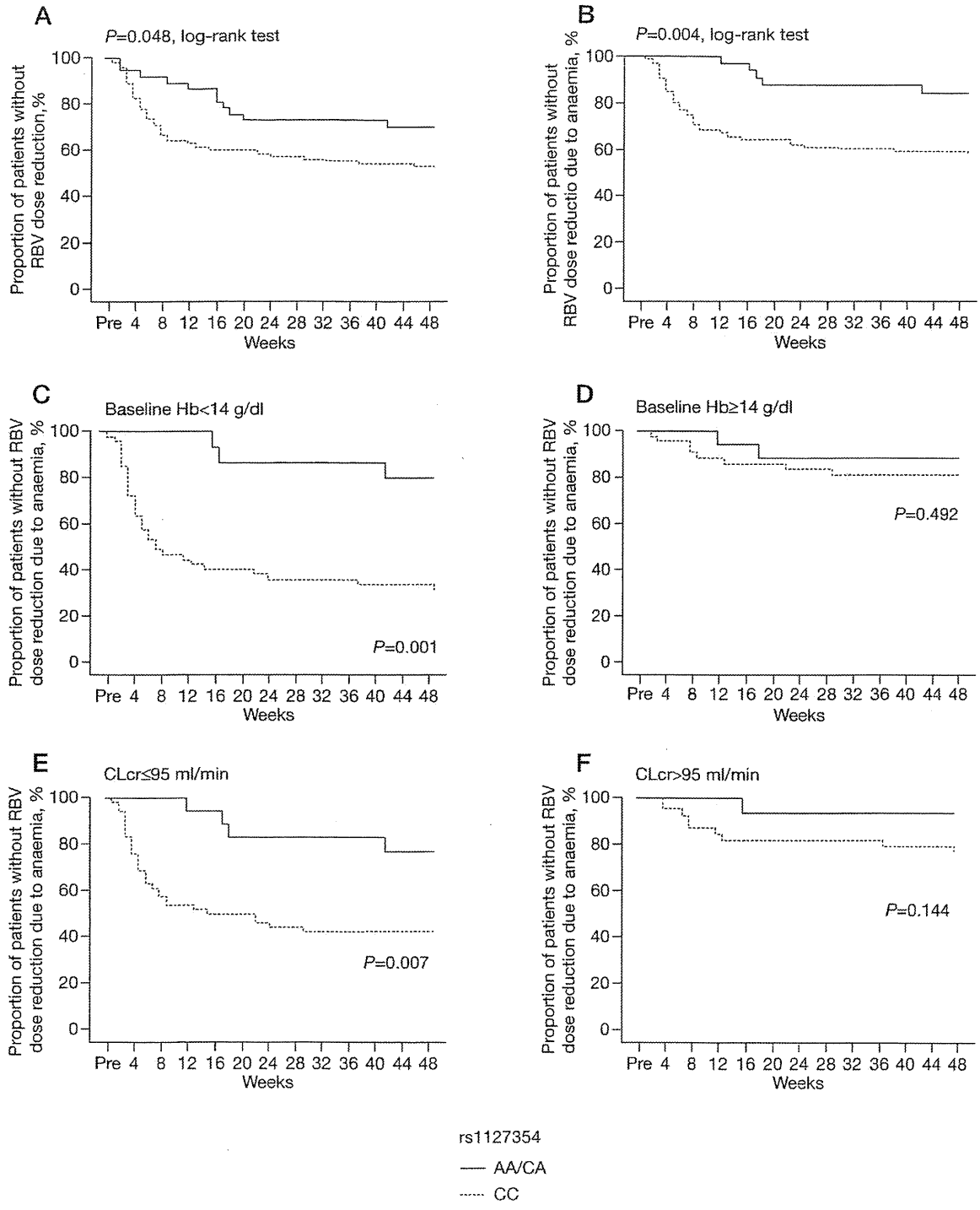
ITPA rs1127354 minor genotypes AA and CA were associated with higher adherence to RBV, higher rate of SVR and lower rate of relapse

The association of the rs1127354 genotype with the adherence to RBV or treatment outcome was analysed. When analysed in the entire population, the percentage of patients receiving >80% of the expected RBV dose, which was reported to be a threshold for an enhanced response to therapy [3], was not significantly different among the rs1127354 genotypes. Treatment outcomes such as the end-of-treatment response, SVR and relapse were also not different among the rs1127354 genotypes (Table 5). By contrast, SVR was closely associated with the *IL28B* genotype [11–14,21]: the rate of SVR was 0% (0/51) for *IL28B* minor type (TG/GG genotype at rs8099917) and 48% (39/81) for *IL28B* major type (TT genotype at rs8099917). This finding confirms that *IL28B* genotype is a significant factor for the prediction of SVR. Thus, we performed a subset analysis on subgroup of patients with the favourable *IL28B* genotype (TT at rs8099917). As a result, patients with the rs8099917 TT genotype and the rs1127354 AA or CA genotypes had a significantly higher rate of receiving >80% of the expected RBV dose ($P=0.016$), a higher rate of SVR ($P=0.031$), as well as a lower rate of relapse ($P=0.046$) compared to patients with the rs8099918 TT and rs1127354 CC genotype (Table 5).

Discussion

In the present study, we confirmed that variants of the *ITPA* gene protect against severe haemolytic anaemia not

Figure 3. *ITPA* rs1127354 genotypes and the time-dependent incidence of RBV dose reduction



The time to the first reduction of the ribavirin (RBV) dose (A) due to any reason or (B) due to anaemia is shown stratified by the rs1127354 genotypes. Solid and broken lines indicate patients with the AA/CA and CC genotypes, respectively. The AA/CA genotype protected against the requirement for RBV dose reduction. (C–F) Patients were standardized according to the baseline haemoglobin (Hb) and creatinine clearance (CLcr). Even after standardization by baseline Hb and CLcr, the AA/CA genotype protected against the requirement for RBV dose reduction. *ITPA*, inosine triphosphatase gene; Pre, pretreatment.

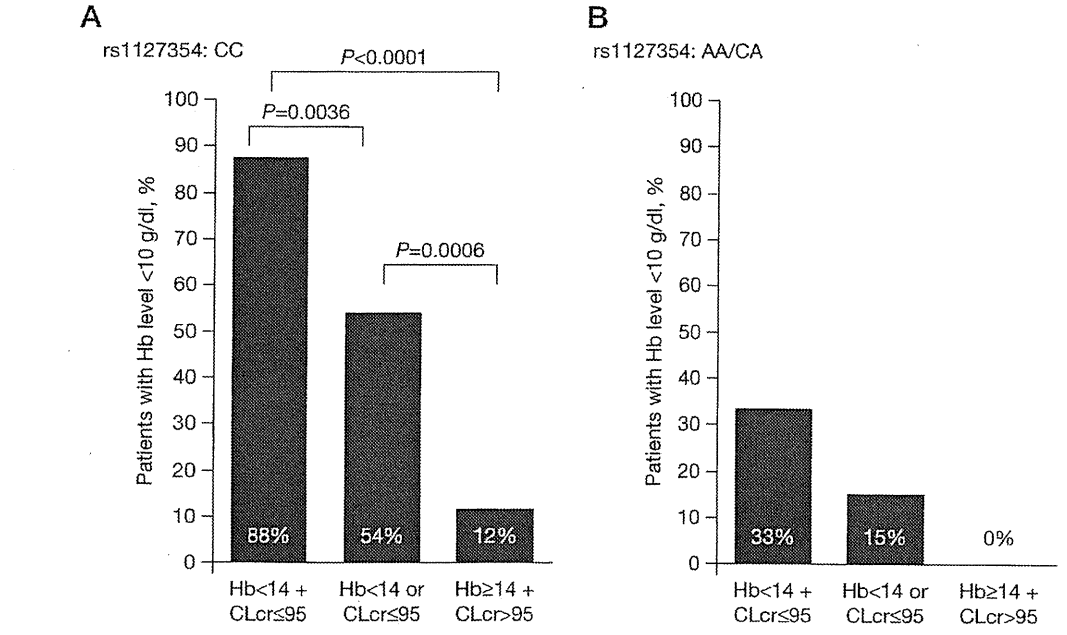
only at the early stage of treatment, but also throughout the 48-week course of treatment in a Japanese cohort of genotype 1b chronic hepatitis C patients treated with PEG-IFN and RBV. We also replicated a previous study [9] that showed that the *ITPA* genotype is significantly associated with a time-dependent reduction of the RBV dose. Furthermore, we found that a combination of the *ITPA* genotype and the baseline Hb and CLcr levels improve the accuracy of predicting RBV-induced severe anaemia. Previous reports on the IDEAL [4] or Vira-Hep-C [9] studies did not find any association between the *ITPA* genotype and treatment outcome; however, we were able to demonstrate the association of the *ITPA* genotype with a higher adherence to RBV, a higher rate

Table 3. Multivariable regression analysis of factors associated with severe anaemia during therapy[a]

Predictor	OR	95% CI	*P*-value
At week 4			
Baseline Hb<14 g/dl	7.18	1.90–27.09	0.004
Baseline creatinine clearance ≤95 ml/min	5.30	1.39–20.26	0.015
ITPA rs1127354: CC	10.17	1.25–82.85	0.030
At any time point			
Baseline Hb<14 g/dl	7.67	3.07–19.12	<0.0001
Baseline creatinine clearance ≤95 ml/min	5.51	2.21–13.73	<0.0001
ITPA rs1127354: CC	9.66	3.11–29.95	<0.0001

[a]Severe anaemia was defined as haemoglobin (Hb)<10 g/dl. *ITPA*, inosine triphosphatase gene.

Figure 4. Combination of the *ITPA* rs1127354 genotype, baseline Hb level and baseline CLcr level is predictive of severe anaemia during the therapy



	A			B		
Total patients, *n*%	32	37	26	9	20	8
Hb<10 g/dl, *n*%	28	20	3	3	3	0

Patients with rs1127354 genotype (A) CC and (B) AA/CA were further stratified by the baseline haemoglobin (Hb) and creatinine clearance (CLcr) levels. The percentage of patients with Hb concentrations of <10 g/dl (severe anaemia) at any time point during therapy is shown for the subgroups of patients. Patients with baseline Hb levels of <14 g/dl and CLcr levels of <95 ml/min had a higher incidence of severe anaemia among patients with the rs1127354 genotype CC (Hb<14 g/dl and CLcr≤95 ml/min versus Hb≥14 g/dl and CLcr>95 ml/min, *P*<0.0001; Hb<14 g/dl and CLcr≤95 ml/min versus Hb<14 g/dl or CLcr≤95 ml/min, *P*=0.0036). *ITPA*, inosine triphosphatase gene.

Table 4. Prediction model for severe anaemia and recommendation for monitoring and treatment

ITPA genotype (rs1127354)	Baseline Hb and CLcr	Risk of anaemia	Recommendation	
			Monitoring	Treatment option
CC	Hb<14 g/dl and CLcr≤95 ml/min	High	Intensive	Consider erythropoietin
	Hb<14 g/dl or CLcr≤95 ml/min	Intermediate	Intensive	Early dose reduction of RBV
	Hb≥14 g/dl and CLcr>95 ml/min	Low	As usual	-
AA/CA	Hb<14 g/dl and CLcr≤95 ml/min	Intermediate	Intensive	Early dose reduction of RBV
	Hb<14 g/dl or CLcr≤95 ml/min	Low	As usual	-
	Hb≥14 g/dl and CLcr>95 ml/min	Absent	As usual	May consider higher RBV dose

CLcr, creatinine clearance; Hb, haemoglobin; *ITPA*, inosine triphosphatase gene; RBV, ribavirin.

Table 5. Treatment response and ribavirin adherence in terms of *ITPA* rs1127354 genotype

Response	rs1127354		*P*-value
	AA/CA, *n*/total *n* (%)	CC, *n*/total *n* (%)	
All patients			
Ribavirin adherence >80%	19/37 (51)	40/95 (42)	0.436
End-of-treatment response	19/37 (51)	58/95 (61)	0.332
Sustained virological response	13/37 (35)	26/95 (27)	0.401
Relapse	6/19 (32)	32/58 (55)	0.112
Subgroup of patients with IL28B rs8099917 TT			
Ribavirin adherence >80%	14/18 (78)	28/63 (49)	0.016
End of treatment response	16/18 (89)	50/63 (79)	0.501
Sustained virological response	13/18 (79)	26/63 (41)	0.031
Relapse	3/16 (19)	24/50 (48)	0.046

ITPA, inosine triphosphatase gene.

of SVR and a lower rate of relapse among a subset of Japanese patients with the favourable *IL28B* genotype (TT at rs8099917).

Haemolytic anaemia induced by RBV is one of the major adverse events of PEG-IFN and RBV therapy leading to dose reduction of RBV or premature termination of therapy [1]. RBV is essential for improving SVR by prevention of relapses and a breakthrough [22], and a reduction of the RBV dose can lower the response rates considerably. It was reported that the maintenance of >80% of the expected RBV dose is associated with an increased SVR [23]. Thus, the prediction and prevention of RBV-induced haemolytic anaemia is clinically important. Previously, no reliable means were available to predict RBV-induced anaemia before therapy, but a recent genome-wide association study identified a strong association between two functional SNPs (rs1127354 and rs7270101) in the *ITPA* gene on chromosome 20 [4] and severe anaemia at week 4 of treatment. This genetic association has been replicated recently by two studies [9,10]. However, the effect of these variants on the long-term development of anaemia or on the requirement for RBV dose reduction has been reported by only one study to date [9]. Therefore, validation of these results by an independent cohort with respect to different geographical areas, age, gender or race is needed. Although the clinical background of our cohort was different from that of the US cohort [9], such as their race, older age (mean age of 57.5 years versus the median age of 48.5 years), and higher predominance of females (62% versus 35%), we were still able to replicate the results that the rs1127354 genotypes AA and CA are protective against anaemia throughout the 48-week course of treatment, especially within the 12 weeks following the initial treatment. We also replicated the association of this genotype with less requirement for RBV dose reduction. These results indicate that the *ITPA* genotype is universally an important determinant of RBV-induced haemolytic anaemia.

For the general application of these genetic associations in clinical practice, we aimed to further improve the accuracy of prediction by combining other clinical covariates. Among the patients with the rs1127354 CC genotype, the risk of developing severe anaemia was as high as 88% in those with baseline Hb levels of <14 g/dl and baseline CLcr levels of ≤95 ml/min, which is in contrast to only 12% in patients with Hb levels of ≥14 g/dl and CLcr levels of >95 ml/min. The rs1127354 AA and CA genotypes were protective against anaemia, but an exception occurred when patients (33%) with a baseline Hb level of <14 g/dl and a CLcr level of ≤95 ml/min developed severe

anaemia. The combination of these three factors may therefore be useful in clinical practice, since it improved the specificity of prediction from 41% to 64% with the same sensitivity (89%) compared to examining just the *ITPA* genotype. These findings may have the potential to support individualized treatment strategies. Patients with the rs1127354 CC genotype, especially those with a baseline Hb level of <14 g/dl and a baseline CLcr level of ≤95 ml/min, require intensive monitoring for anaemia during therapy, and an early dose reduction of RBV or support by erythropoietin may be indicated for safety. By contrast, patients with the AA and CA genotypes, excluding those with a baseline Hb level of <14 g/dl and a baseline CLcr level of ≤95 ml/min, may be candidates for therapy with a higher RBV dose, which may lead to higher rates of SVR. The prediction of RBV-induced anaemia will remain an important issue even in the near future, since direct antiviral agents require RBV and PEG-IFN in combination in order to achieve higher SVR rates for genotype 1 [24,25] and this combination will remain a standard therapy for other genotypes.

In a previous study, there was no clear association between ITPase deficiency and treatment outcome [4,9,10], even after a detailed subset analysis that excluded patients in whom RBV had been reduced for indications other than anaemia or after stratification by the *IL28B* genotype [9]. Thompson *et al.* [9] speculated that the lack of association may derive from several reasons such as an underpowered error due to the small number of patients, a high incidence of RBV dose reduction unrelated to anaemia, and the possibility that the ITPase deficiency may reduce antiviral efficacy. In the present study, we also failed to show associations between the *ITPA* genotype and treatment outcomes among the entire cohort. However, when patients were stratified by the *IL28B* genotype, which is now recognized as the major determinant of treatment outcome [11–14,21], the AA and CA genotypes at rs1127354 were linked to a higher adherence to RBV, a lower rate of relapse and a significantly higher rate of SVR. One of the reasons for this discrepancy may be the lower incidence of anaemia-unrelated RBV dose reduction in our study compared to the participants of the Vira-Hep-C study (22% versus 48%) [9]. The effect of the *ITPA* genotype on RBV adherence and treatment outcome may be less apparent in patients who reduced their RBV dose in the absence of anaemia. Another possibility is that the difference in mean age may have some effect on this association between the *ITPA* genotype and treatment outcome since older age has been reported to compromise drug adherence or treatment outcomes [26,27]. Our results indicated that, although IL28B genotype is the major determinant of SVR, the *ITPA* genotype may be used supplementary to predict the treatment outcome in patients with a favourable *IL28B* genotype (TT at rs8099917), as long as the RBV dose is not reduced in the absence of anaemia. Further studies involving larger populations in different geographical areas or races may be necessary to confirm this speculation.

In conclusion, variants of the *ITPA* gene, which could protect against haemolytic anaemia and RBV dose reduction, were associated with a high rate of SVR by standard PEG-IFN and RBV therapy in a subset of Japanese patients with the favourable *IL28B* genotype. A combination of the *ITPA* genetic polymorphism with baseline Hb and CLcr levels further improved the predictive accuracy of severe anaemia. These findings may have the potential to support selection of the optimum and personalized treatment strategy for individual patients.

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Disclosure statement

The authors declare no competing interests.

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Original Article

Favorable factors for re-treatment with pegylated interferon α2a plus ribavirin in patients with high viral loads of genotype 1 hepatitis C virus

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Aim: Effect of re-treatment for pegylated interferon (PEG-IFN) plus ribavirin was not fully evaluated. We examined the effects of re-treatment with PEG-IFN plus ribavirin in patients with high viral loads of genotype 1 hepatitis C virus who failed to achieve a sustained virological response (SVR) with combination therapy.

Methods: We examined 38 patients who were re-treated with PEG-IFN α2a plus ribavirin for more than 60 weeks, among whom 14 were non-responders and 24 were relapsers after previous treatment with PEG-IFN α2b plus ribavirin. IL28B genotyping was done in 21 patients.

Results: The overall SVR rate was 34%. Analysis of baseline characteristics showed that the relapsers had a significantly higher SVR rate than the non-responders (50.0%, 12/24 vs. 7.1%, 1/14, respectively, $P = 0.012$) The SVR rates of re-treated patients who had turned hepatitis C virus (HCV) RNA-negative at weeks 8, 12, 24, and 48 of the previous therapy were 67% (4/6), 67% (4/6), 29% (2/7), and 25% (1/4), respectively. Re-treatment achieved an SVR in five of 12 patients with IL28B major alleles and three of nine patients with IL28B minor alleles. During the re-treatment, patients with complete viral suppression at week-12 achieved a significantly higher SVR rate ($P = 0.001$).

Conclusions: Re-treatment with PEG-IFN α2a plus ribavirin therapy is effective in patients who relapse after a course of PEG-IFN α2b plus ribavirin therapy. Re-treatment is a particularly useful option for patients who achieve early viral clearance during previous therapy.

Key words: hepatitis C virus, IL28B, peginterferon-alpha-2a, relapse, re-treatment.

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a major cause of liver cancer and other chronic liver diseases. An estimated 170 million individuals are chronically infected with HCV worldwide.[1,2] Interferon (IFN) treatment has been the mainstay of antiviral therapy for chronic HCV infection since the 1990s, producing a variety of clinically significant findings. The antiviral effect of IFN is known to depend on HCV genotype and viral load.[3,4] Recently, IL28B polymorphisms have been recognized to be one of the most reliable host predictors of the response to anti-HCV therapy.[5–7] A combination of pegylated-IFN (PEG-IFN) and ribavirin, currently considered standard therapy, completely eradicates HCV in up to 40% to 50% of treatment-naive patients with high viral loads of HCV genotype 1b.[8,9] However, approximately half of all patients who receive PEG-IFN plus ribavirin remain HCV-positive or have recurrence of hepatitis C. To prevent HCV relapse, 72-week extended treatment has been tried for HCV genotype 1b patients with slow viral response.[10,11] Several clinical studies have been conducted in Europe and the United States to

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investigate the effect of anti-HCV re-treatment with PEG-IFN α2a plus ribavirin in patients who previously did not respond to, or had relapsed after, PEG-IFN plus ribavirin combination therapy.[12–14] In one multicenter, randomized trial, Jensen *et al.* re-treated 942 chronic hepatitis C patients who did not respond to previous PEG-IFN α2b plus ribavirin therapy with PEG-IFN α2a plus ribavirin.[12] The resulting rates of sustained virological response (SVR) in these studies were approximately 10%. Berg *et al.* reported the effects of 48-week re-treatment with PEG-IFN α2a plus ribavirin in 64 patients who had relapsed during follow-up after 24 weeks of treatment with PEG-IFN α2a plus ribavirin.[13] The overall SVR rate was 55%. These studies provide evidence that re-treatment with PEG-IFN α2a plus ribavirin is effective in a considerable proportion of patients with chronic hepatitis C who do not respond to, or relapse after, prior treatment with PEG-IFN (α2a or α2b) plus ribavirin. However, previous studies did not clearly define the baseline characteristics of patients most likely to respond to re-treatment. In the present study, we evaluated the results of a retrospective, exploratory analysis designed to assess the effects of PEG-IFN α2a combined with ribavirin in patients with high viral loads of genotype 1 HCV who did not achieve an SVR in response to prior treatment with PEG-IFN α2b plus ribavirin. In addition, we examined the IL28B genotype of the patients and compared antiviral effectiveness between initial combination therapy and re-treatment.

METHODS

Patients

THIS EXPLORATORY STUDY was performed as part of a 16-center trial of PEG-IFN α2a plus ribavirin combination therapy, which registered 138 patients from 1 April 2007 through 20 July 2010. Thirty-eight patients (18 men and 20 women; mean age, 58.8 years) who did not achieve an SVR after prior treatment with PEG-IFN α2b plus ribavirin were identified retrospectively. In 14 of these patients (non-responders) HCV RNA remained positive during previous combination therapy for more than 24 weeks. In the other 24 patients (relapsers), HCV RNA re-appeared after 48 weeks' combination therapy. All patients were infected with viral loads (HCV RNA) ≥ 5 log copies/mL of genotype 1 HCV. The inclusion criteria for hematologic variables were as follows: white blood cell count ≥ 3000/mm^3; neutrophil count ≥ 1500/mm^3; platelet count ≥ 90 000/mm^3; and hemoglobin concentration ≥ 12 g/dL. The patients' characteristics at enrollment are summarized in Table 1.

Table 1 Baseline characteristics of patients

	$n = 38$
Years of age; median (range)	59 (32–74)
Sex (male/female)	18/20
Effect of PEG2b/RBV (Non-respondents/relapsers)	14/24
BMI (kg/m^2); median (range)	23.5 (18.1–29.0)
Neutrophil count (×10^4/mm^3)	2615 ± 747
Hemoglobin (g/dL)	13.5 ± 1.25
Platelet count (×10^4/mm^3)	15 ± 4.38
HCV RNA (Log IU/mL)	6.2 ± 0.56
ALT (IU/L)	58.1 ± 55.58
γ-GTP (IU/L)	55.6 ± 58.61
Fibrosis (F1/F2/F3/F4)	16/4/6/4 (unknown for 8)
Activity (A0/A1/A2/A3)	2/16/10/0 (unknown for 10)

Values are median ± standard deviation (SD).
ALT, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyl transpeptidase; RBV, ribavirin.

IL28B genotyping

We examined a single nucleotide polymorphism (SNP) of IL28B in 21 patients who consented to genome analysis. Genomic DNA was extracted from whole blood samples of each patient. Genetic polymorphism upstream IL28B gene, rs8099917 was determined by TaqMan polymerase chain reaction (PCR).[15] Heterozygotes (T/G) or homozygotes (G/G) of the minor allele (G) were defined as having the IL28B minor allele, whereas homozygotes for the major allele (T/T) were defined as having the IL28B major allele.

Treatment protocol

Pegylated interferon-IFN α2a was subcutaneously injected at a dose of 180 μg/week. Ribavirin was administered orally in a weight-adjusted dose ranging from 600 to 1000 mg/day, delivered in two fractions. The present Japanese guideline showed that all relapsers of the prior 48-week treatment can be candidates and may benefit from 72-week treatment.[16] At the start of the present trial, there was no consensus in the re-treatment. We designed that the re-treatment period was more than 60 weeks. In particular, the patients were treated for up to 72 weeks if the HCV RNA load decreased to undetectable levels between 13 weeks to 36 weeks from the start of therapy. We decreased the doses of PEG-IFN α2a and ribavirin or withdrew treatment in patients with abnormal clinical laboratory values or adverse events, as appropriate.

Hepatitis C virus-RNA levels were determined by real-time PCR (Taqman, Roche Diagnostics, K.K., Tokyo,

Japan). Patients were judged to have attained SVR status if HCV RNA was not detected for at least 24 weeks after the completion of treatment. Patients were categorized as rapid viral responders (RVR) if HCV RNA was suppressed at week 4 of treatment, early virological responders (EVR) if HCV RNA turned negative at 5 to 12 weeks of treatment, and late viral responders (LVR) if HCV RNA turned negative at 13 to 24 weeks of treatment. Patients continuously positive for HCV RNA during the treatment period were classified as non-virological responders (NVR).

Statistical analysis

Baseline data and treatment factors were statistically compared between relevant groups (e.g. non-respondent versus relapsed patients, SVR versus non-SVR, EVR versus non-EVR). For continuous variables, the Shapiro-Wilk test was used to check whether the data were normally distributed. Variables for which the normality assumption was maintained were tested by Levene's test to evaluate the homogeneity of variance. If variances were assumed to be equal between groups, variables were compared by Student's *t*-test. For continuous variables for which the homogeneity of variance was rejected, Welch's *t*-test was used. Non-normal continuous variables were subjected to Mann–Whitney's *U*-test for comparison between groups. Categorical variables were analyzed by Pearson's χ^2 test if all expected frequencies generated from contingency tables exceeded 5. Otherwise, Fisher's exact test was performed. Differences in the percentage of subjects with viral suppression between the non-responders and relapsers were evaluated for each measurement time point with the use of Fisher's exact test. Multivariate logistic regression analysis was used to determine factors that significantly contributed to SVR.

Ethical considerations

The study protocol complied with the ethical guidelines of the Declaration of Helsinki of 1975 (2004 revision) and was approved by the Ethics Committee of Osaka City University Graduate School of Medicine.

RESULTS

Time course of suppression of HCV

THE OVERALL PROPORTION of the 38 patients who had undetectable levels of HCV RNA increased in a stepwise fashion: 2.6% at week 4; 27.0% at week 8; 38.9% at week 12 (EVR rate); and 63.6% at week 24. The overall rate of patients with undetectable levels of HCV RNA was 68.4% at the end of treatment, and the overall SVR rate was 34.2%.

Characteristics of SVR and non-SVR patients

An analysis of differences in baseline characteristics showed that the relapsers had a significantly higher SVR rate than the non-responders (50.0%, 12/24 vs. 7.1%, 1/14, respectively, $P = 0.012$, Table 2). Among the 36 patients with available data at week 12, HCV RNA status was negative in 14 (38.9%, EVR) and positive in 22 (61.1%, non-EVR). The SVR rate for patients with EVR was 71.4% (10/14), which was significantly higher than the SVR rate for patients with non-EVR (non-EVR, 13.6%, 3/22; $P = 0.001$). A significantly longer duration of the re-treatment was detected in the SVR group (60 weeks) than in the non-SVR group (44 weeks) ($P = 0.024$). In the non-SVR group, five patients prematurely discontinued re-treatment because of insufficient viral response. Multivariate analysis showed that EVR by re-treatment was significantly associated with SVR (odds ratio 11.95, $P = 0.048$, Table 3).

Viral suppression rate in the relapsers and non-responders to previous treatment

The time courses of HCV RNA negative rates were analyzed according to the outcomes of the previous treatment. Among the 24 relapsers, the rate of patients with undetectable levels of HCV RNA increased in a stepwise fashion: 4.2% at week 4, 58.3% at week 12, 81.8% at week 24, and 89.5% at week 48. The final rate of patients with undetectable levels of HCV RNA was 91.7%, and the final SVR rate was 50.0% in this subgroup. Non-responders also showed a stepwise, albeit lower increase in the rate of patients with undetectable levels of HCV RNA: 0% at week 4, 27.3% at week 24, and 50.0% at week 48. The final rate of patients with undetectable levels of HCV RNA was 28.6%, and the final SVR rate was 7.1% for the non-responders (Fig. 1).

Duration of treatment required for a decrease in HCV RNA to undetectable levels in the relapsers and the non-responders to previous treatment

Four (28.6%) of the 14 non-responders became HCV RNA-negative at weeks 18 to 28 of re-treatment: one patient each at weeks 18, 20, 24, and 28. However, most of the non-responders (10/14, 71.4%) did not turn HCV RNA-negative by the end of the treatment. As for the relapsers, the EVR rate (i.e. the proportion of patients who became HCV RNA-negative at or before week 12) was 50.0% (12/24) for previous treatment and 58.3%

Table 2 Characteristics of sustained virological response (SVR) and non-SVR patients

	SVR ($n = 13$)	non-SVR ($n = 25$)	*P*-value
Years of age; median (range)	59 (44–74)	59 (32–71)	0.91
Sex (male/female)	8/5	10/15	0.307
BMI (kg/m^2)	23.8 ± 2.48	23.3 ± 2.52	0.616
Effect of previous PEG2b/RBV			
(Non-responders/relapsers)	1/12	13/12	0.012
(EVR/non-EVR)	8/5	4/21	0.009
Neutrophil count (×10^4/mm^3)	2532 ± 576	2668 ± 856	0.681
Platelet count (×10^4/mm^3)	16.9 ± 5.26	14.1 ± 3.71	0.092
Hemoglobin (g/dL)	13.6 ± 0.94	13.5 ± 1.4	0.922
HCV RNA (Log IU/mL)	6.2 ± 0.5	6.2 ± 0.59	0.658
ALT (IU/L)	53 ± 42	61 ± 62	0.422
γ-GTP (IU/L)	60 ± 81	54 ± 45	0.468
Fibrosis (F1/F2/F3/F4)	6/1/0/2 (unknown for 4)	10/3/6/2 (unknown for 4)	0.286
Activity (A0/A1/A2/A3)	1/6/1/0 (unknown for 5)	1/10/9/0 (unknown for 5)	0.25
IL28B genotyping (major/minor)	5/3 (unknown for 5)	7/6 (unknown for 12)	0.948
EVR† (positive/negative)	10/3	4/19	0.001
Treatment duration (weeks) mean (range)	60 (24–72)	44 (20–72)	0.024
Dose of PEG-IFNα2a (μg/week)	171 ± 10	147 ± 7	0.059
Dose of ribavirin (mg/week)	3534 ± 382	4010 ± 281	0.322

Values are means ± standard deviation (SD). Baseline characteristics were shown above the dot line. Response to the re-treatment, duration, drug adherence were shown below the dot line.
†36 patients with available data on week-12 hepatitis C virus (HCV) RNA status.
ALT, alanine aminotransferase; BMI, body mass index; EVR, early viral response; γ-GTP, γ-glutamyl transpeptidase; RBV, ribavirin.

(14/24) for re-treatment. In one relapser, HCV RNA remained detectable during re-treatment. The results are graphically represented in Figure 2.

Comparison of SVR rate according to duration of previous treatment required for decrease in HCV RNA to undetectable levels

Sustained virological response rates in response to re-treatment were investigated according to the duration of previous therapy required to decrease HCV RNA to undetectable levels. SVR rates for patients whose HCV RNA had decreased to undetectable levels at weeks 8, 12, 24, and 48 of previous therapy were 66.6% (4/6), 66.6% (4/6), 28.5% (2/7), and 25.0% (1/4), respectively. Among the 14 non-responders, one patient (7.1%) achieved an SVR. These results indicated a statistically significant trend toward a higher SVR rate with a shorter duration of previous therapy required to

Table 3 Associated factors with sustained virological response (SVR) to re-treatment identified by multivariate analysis

Factor	Category	Odds ratio	95% CI	*P*-value
Effect of previous	1. Non-responder	1	0.012–5.584	0.490
PEG-IFNα2b + RBV therapy	2. Relapser	2.51		
Effect of previous	1. Non-EVR	1	0.1–27.701	0.923
PEG-IFNα2b + RBV therapy	2. EVR	1.04		
Effect of re-treatment with	1. Non-EVR	1	0.003–0.98	0.048
PEG-IFNα2a + RBV therapy	2. EVR	11.95		
Treatment duration (weeks)	per 1 week	1	0.001–1.521	0.086
		1.05		

EVR, early viral response; PEG-IFN, pegylated interferon; RBV, ribavirin.

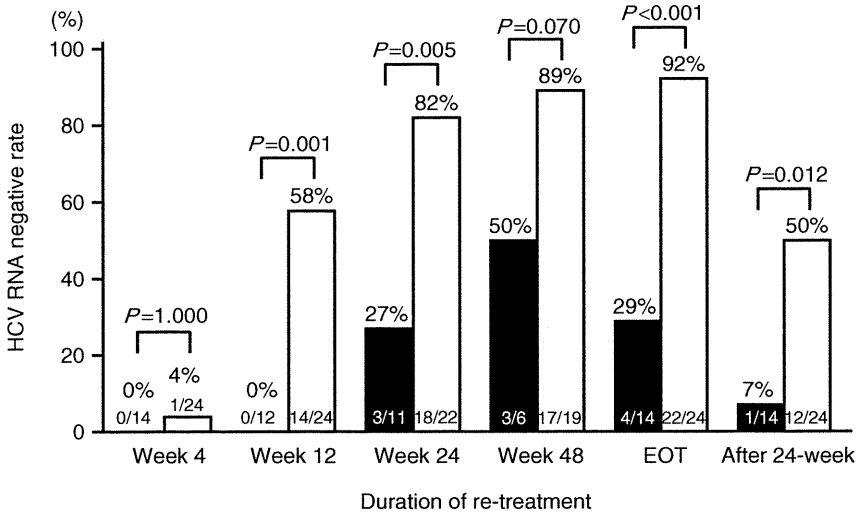

Figure 1 Clinical course of anti-hepatitis C virus (HCV) effect in response to re-treatment with pegylated interferon (PEG-IFN) α2a and ribavirin. ■: Non-responders to previous PEG-IFN α2b and ribavirin therapy ($n = 14$). □: Patients who relapsed after previous PEG-IFN α2b and ribavirin therapy ($n = 24$). HCV RNA negative means an undetectable level of HCV RNA on real-time polymerase chain reaction. EOT, end of treatment.

decrease HCV RNA to undetectable levels ($P = 0.002$, Fig. 3).

IL28B gene polymorphism

Twelve patients had a major allele of IL28B. The other nine patients were heterozygotes (T/G) and were thus defined as having the IL28B minor allele. Anti-viral effect of previous treatment was relapse in 10 of the 12 patients with the IL28B major allele and two of the nine patients with the minor allele ($P = 0.019$, Fig. 4). Re-treatment achieved an SVR in five of the 12 patients with the IL28B major allele and three of the nine patients with the minor allele ($P = 0.948$, Fig. 4). By the re-treatment, two patients with the IL28B major allele and five patients with the minor allele were NVR ($P = 0.161$).

Adverse events

Twenty-two adverse effects that required premature termination or temporary suspension of re-treatment were reported in 14 (36.8%) of the 38 patients. Major adverse effects were anemia (five events, 13.2%), malaise (4, 10.5%), decreased platelet count (2, 5.3%), and hypothyroidism (2, 5.3%). Three patients prematurely discontinued re-treatment because of oral herpes, malaise, and liver function abnormalities in one patient each.

DISCUSSION

RE-TREATMENT WITH PEG-IFN α2a plus ribavirin produced an EVR rate of 38.9% and an SVR rate of 34.2% in patients who had not achieved an SVR by previous treatment with PEG-IFN α2b plus ribavirin. By re-treatment, the previous non-responders had an EVR rate of 0% and an SVR rate of 7.1%, whereas the previous relapsers had an EVR rate of 58.3% and an SVR rate of 50.0%. Relapsers showed consistently higher rates of undetectable levels of HCV RNA than non-responders throughout the study. The results of the present study are consistent with those of earlier investigations.[12–14] Our results also provide compelling evidence that re-treatment with PEG-IFN α2a plus ribavirin has excellent antiviral activity in patients who do not achieve an SVR in response to previous combination therapy, especially in those with relapse after PEG-IFN plus ribavirin.

Italian groups conducted a prospective, investigator-led, randomized clinical trial to compare the effects of PEG-IFN α2a and α2b combined with ribavirin in treatment-naive patients with chronic hepatitis C. That study reported a significantly higher SVR rate for PEG-IFN α2a.[17,18] However, another study failed to demonstrate a significant difference in viral clearance between the two drugs.[19] Past clinical trials seemed to fall short of drawing definite conclusions as to which of these two types of IFN formulations is more beneficial. Recently, a systematic meta-analysis of 12 randomized clinical trials comparing PEG-IFN α2a plus ribavirin with PEG-IFN α2b plus ribavirin was published.[20] The review concluded that PEG-IFN α2a is associated with a higher SVR rate than PEG-IFN α2b, with no significant differences between the two PEG-IFN formulations in the frequency of adverse-event-related treatment discontinuation. In the present study of patients treated with the two PEG-

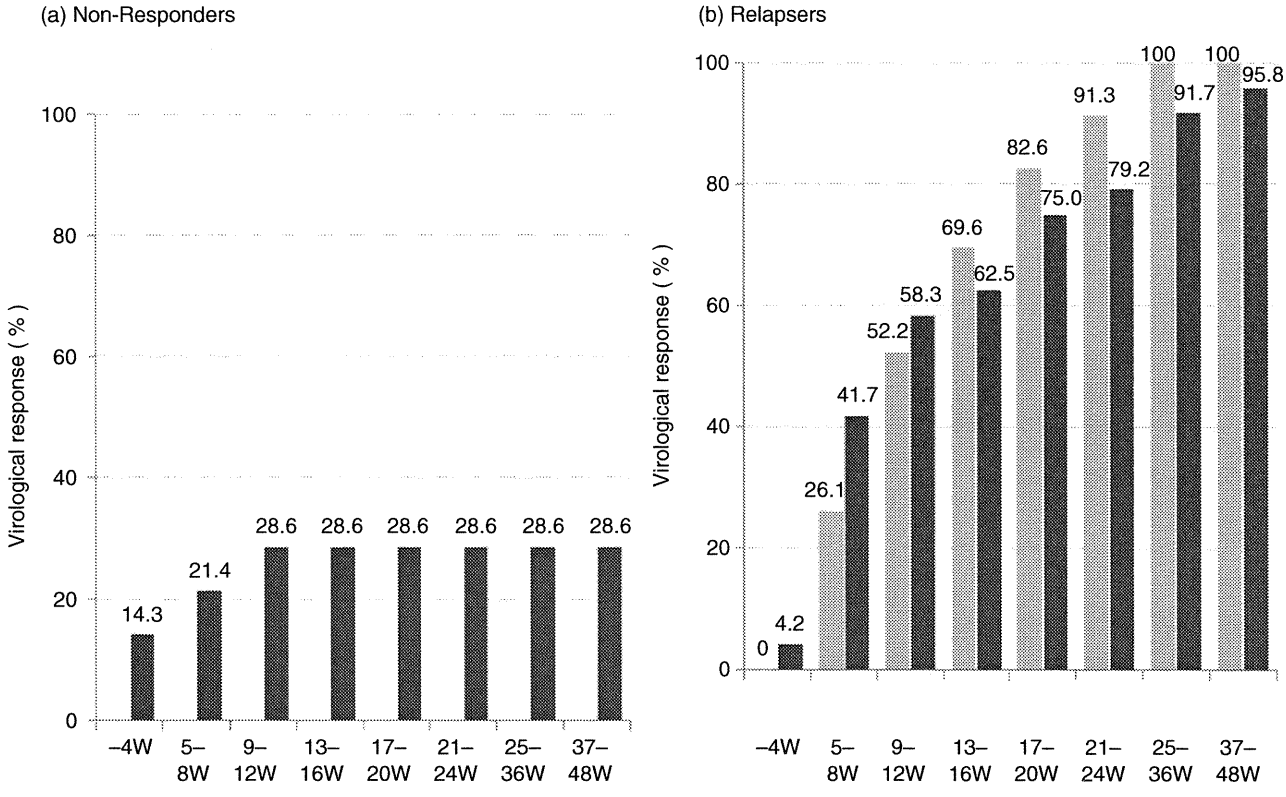

Figure 2 Cumulative rate of decrease in hepatitis C virus (HCV) RNA to undetectable level during the first and the second combination therapy. The duration of treatment required for a decrease in HCV RNA to undetectable levels is shown for non-responders (a) and relapsers (b). (a) Among the 14 non-responders, HCV RNA decreased to undetectable levels in four (28.6%) patients at weeks 18 to 28 of re-treatment: one patient each at weeks 18, 20, 24, and 28. However, in the other 10 non-responders (71.4%), HCV RNA remained detectable during re-treatment. (b) In the 24 relapsers, the EVR rate was 50.0% (12/24) for the previous treatment and 58.3% (14/24) for re-treatment. ▒, Previous treatment (PEG-IFNα-2b); ■, Retreatment (PEG-IFNα-2a).

IFN drugs on different occasions, PEG-IFN α2a achieved earlier HCV RNA suppression than did PEG-IFN α2b. To our knowledge, however, no clinical study has reported the effects of PEG-IFN α2b in patients who did not respond to, or had relapse with, PEG-IFN α2a therapy. This fact precludes us from concluding that the antiviral effects demonstrated in our study are solely attributed to the differences between the two formulations.

It is noteworthy that a certain proportion of relapsers failed to turn HCV RNA-negative in response to re-treatment. Mutation-induced viral resistance to IFN and the emergence of neutralizing anti-IFN antibodies in the host have been proposed as possible causes for the failure of re-treatment with IFN.[21–23] These possibilities should be explored in detail to promote a better understanding of the two PEG-IFNs.

In 10 of the 14 non-responders, HCV RNA did not decrease to undetectable levels during re-treatment. Only one patient (7.1%) achieved an SVR by 72-week extended treatment. Recent studies have reported that adding telaprevir, a protease inhibitor, to standard combination therapy yielded an SVR rate of approximately 40% in previous non-responders.[24,25] It has been suggested that re-treatment with PEG-IFN plus ribavirin is not the optimal treatment for patients with chronic hepatitis C unresponsive to previous combination therapy. However, triple therapy including telaprevir is more frequently associated with anemia, skin disorders, and discontinuation of therapy than standard therapy.[24,25] In Japan, the median age of HCV patients treated with interferon is over 60 years, i.e. older than that in Europe or the United States. Elderly patients may not be able to complete anti-HCV therapy with telaprevir.

The present study showed that relapsers who had EVR with previous therapy had a significantly higher rate of

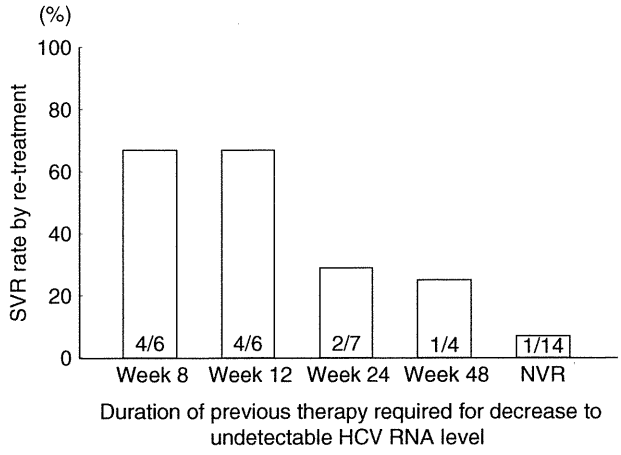

Figure 3 Sustained virological response (SVR) rate according to the anti-hepatitis C virus (HCV) effects of previous treatment. SVR rates were examined according to the duration of previous therapy required to decrease HCV RNA to undetectable levels. SVR rates for patients whose HCV RNA had decreased to undetectable levels at weeks 8, 12, 24, and 48 of the previous therapy were 66.6% (4/6), 66.6% (4/6), 28.5% (2/7), and 25.0% (1/4), respectively (Cochran-Armitage test for trend $P = 0.002$). One patient was excluded from this analysis because of missing data on the time of turning HCV RNA negative during the previous therapy.

SVR on re-treatment with combination therapy. In other words, the antiviral effect of the previous therapy was a useful predictor of the response to re-treatment. Oze *et al.* also reported that patients with a EVR in the previous treatment were good candidates for re-treatment with PEG-IFN α and ribavirin.[26] They concluded that HCV viral loads <5 log copies/mL at the start of re-treatment was also a favorable factor for SVR.

IL28B polymorphism has been reported to contribute to the therapeutic effect of PEG-IFN plus ribavirin in patients with genotype 1 HCV.[27] In a previous study of Japanese patients with chronic hepatitis C, more than 70% of non-responders had a minor rs809917 G allele polymorphism of the IL28B gene, whereas relapsers showed no noteworthy changes in the frequency of polymorphic variants.[7] However, no previous study has performed IL28B genotyping in re-treated patients. In the present study, IL28B gene polymorphism was analyzed in only 21 patients. Recently, it was reported that IL28B genotyping seemed to have limited clinical utility in the arrangement of response-guided therapy for patients with genotype 1.[28] We speculate that the antiviral effect of previous therapy is a more reliable predictor of the response to re-treatment than IL28B genotype. This speculation should be validated by exploring

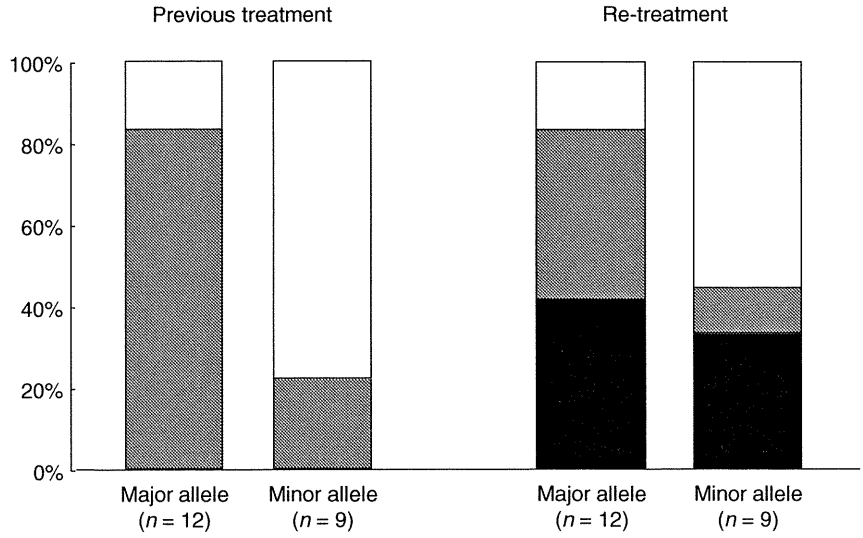

Figure 4 Outcomes of previous treatment and re-treatment according to genotype of IL28B. Twenty-one of 38 patients consented to genome analysis of IL28B. Among 12 patients with major alleles, the antiviral effect of previous treatment was relapse in 10 patients (83%), and non-virological responders (NVR) in two (17%). Among nine patients with minor alleles, two (22%) patients had relapse, and seven (78%) had NVR. Among 12 patients with major alleles, the antiviral effect of re-treatment was sustained virological response (SVR) in five patients (42%), relapse in five (42%), and NVR in two (16%). Among nine patients with minor alleles, three (33%) patients had SVR, one (11%) had relapse, and five (56%) had NVR. (□) NVR; (▩) Relapse; (■) SVR.

factors related to the host, causative virus, and adherence to the protocol that contribute to the outcomes of IFN re-treatment.

In conclusion, re-treatment with PEG-IFN α2a plus ribavirin proved to be effective in relapsers after a previous course of PEG-IFN α2b plus ribavirin. In particular, re-treatment is an effective option for relapsers who achieved viral clearance in an early stage of previous therapy because such patients are more likely to attain SVR with re-treatment than are non-responders. However, non-responders and patients who become HCV-negative after 24 weeks of previous treatment have a low likelihood of achieving SVR with re-treatment. The use of protease inhibitors and other directly acting antiviral agents seems more promising for these patients.

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CONFLICTS OF INTEREST

AKIHIRO TAMORI: SPEAKING and Teaching: MSD K.K., Chugai Pharmaceutical Co., Ltd.

Kiyohide Kioka: none

Osamu Kurai: none

Hiroki Sakaguchi: none

Masaru Enomoto: Speaking and Teaching: MSD K.K., Chugai Pharmaceutical Co., Ltd.

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