③またウイルス肝炎については，重症化の問題，肝移植後の治療の問題，自然経過の問題，インターフェロン抗体の問題など残された懸案が多岐にわたるが，それについても症例を呈示しながら詳しく解説して頂いた．いわば応用篇といえるような症例である．

④肝癌の三次予防としての肝癌根治後のインターフェロン治療についても，evidence は集積されつつあるものの，個々の症例がどのような経過を辿り，どのように治療に工夫がなされているかという点を症例ベースで実際の症例から学ぶことは極めて意義深い．ゆえに，その点についても詳述していただいた．

⑤また，E 型肝炎は輸入感染例に加えて近年，日本土着の E 型肝炎が報告されており，急速に注目を浴びるようになってきている．従来の検査で A 型，B 型，C 型肝炎が否定された急性肝炎においては E 型肝炎も念頭に置くことも重要であろう．したがって，この点についても的確な解説をお願いした．

本書の全体の構成は，上記のようなウイルス肝炎の最近のトピックスを網羅した「第 1 部　総論」とともに，その最近のトピックスを踏まえた「第 2 部　症例呈示」では，各領域のエキスパートに示唆に富む症例を様々な角度から呈示して頂き，その経過，問題点(problem lists)および解決方法(solution)，具体的な治療などについて解説を交えて示したものとなっている．

したがって本書では，ポイントを絞った最新の知識が得られるものと確信する．

そのような点で本書は，消化器病，肝臓病の初学者・一般内科医はもちろん，肝臓専門医にも勧められるアップデートな知識・情報が盛り込まれており，必ずや診療の一助となると信じるものである．

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Influence of *ITPA* Polymorphisms on Decreases of Hemoglobin During Treatment with Pegylated Interferon, Ribavirin, and Telaprevir

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

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

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

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

exons in the *ITPA* gene, as well as that at rs7270101 in the second intron, affects the expression of ITPA.[8-11] Patients infected with HCV-1 carrying the CC genotype at rs1127354 are more prone to develop anemia than those with CA/AA genotypes during the combination therapy, and the decrease in hemoglobin is greater in patients with the AA than AC/CC genotypes at rs7270101.[7] Their observations have been extended to many patients in a large-scale trial with pegIFN-α-2a on Caucasian and African Americans,[12] as well as in the Japanese receiving PEG-IFN-α-2b and RBV who were infected with HCV-1.[13]

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

Patients and Methods

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

Of the 61 patients, 44 (72%) had received IFN-based treatment before. Relapse occurred in 29 (47%) and the remaining 15 (25%) did not respond (null-responders). All patients gave consent for analysis of SNPs in *ITPA* and interleukin 28 (*IL28B*) genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Toranomon Hospital. Written informed consent was obtained from each patient.

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

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

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

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

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

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

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

Data are median values (range) or n.

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

Results

Triple Therapy in Patients with HCV-1 Infection. Baseline characteristics of the 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 in the *ITPA* gene are compared in Table 1. They all were infected with HCV-1. There were no significant differences between them, except that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were higher in patients with CC than CA/AA genotypes ($P = 0.041$ and $P = 0.008$, respectively). Overall, *IL28B* genotypes resistant to PEG-IFN and RBV, TT/TG at rs8099917, and CC/CT at rs12979860 were rather frequent, and possessed by 44% and 47%, respectively, of the patients. This was due to inclusion of 15 nonresponders to previous IFN-based therapies, corresponding to 25% of the 61 patients studied, most of whom (14/15 [93%]) possessed IFN-resistant genotypes (TT/TG and CC/CT). Six of them had low hemoglobin levels (<13 g/dL) at baseline and were started with an RBV dose decreased by 200 mg; they included five with CC and one with CA genotypes of the *ITPA* gene.

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

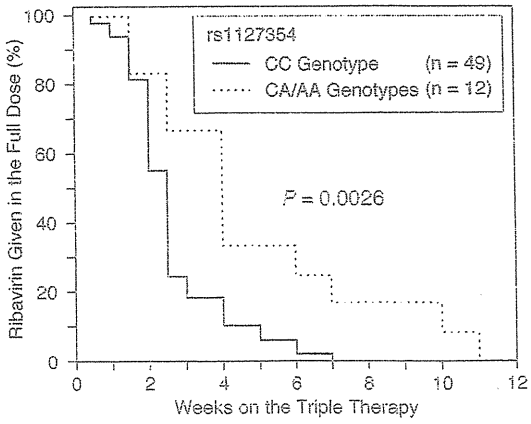

Fig. 1. Patients who received the full ribavirin dose during 12 weeks on triple therapy. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared.

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

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

Decreases in Hemoglobin Levels During Triple Therapy. Figure 2 compares decreases in hemoglobin levels between 49 patients with CC and 12 with CA/AA genotypes of the *ITPA* gene. Data of six patients were omitted because the triple therapy was withdrawn 4-10 weeks after the start, including five with CC and one with CA genotype. Hemoglobin decreased more in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$). During week 8 through 12, hemoglobin reached the nadir of approximately −4 g/dL both in patients with CC and CA/AA genotypes. Thereafter, differences in hemoglobin decrease started to widen between patients with CC and CA/AA genotypes and were significant at week 20 (−3.0 ± 1.2 vs. −2.4 ± 0.88 g/dL, $P = 0.048$) and week 24 (−2.9 ± 1.1 vs. −2.0 ± 0.85 g/dL, $P = 0.013$).

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

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

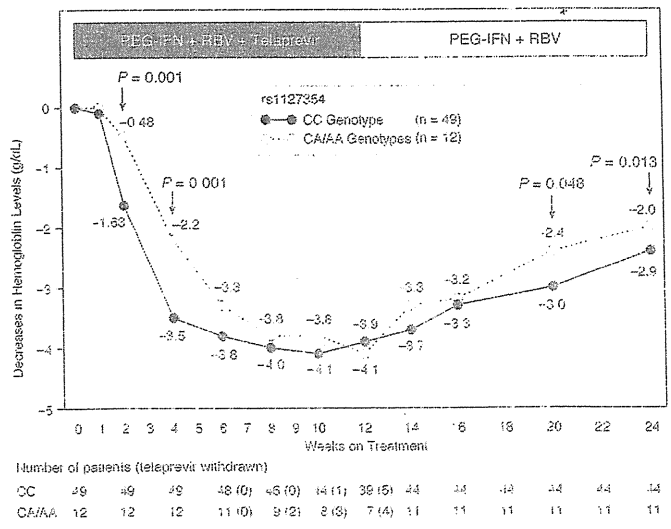

Fig. 2. Decreases in hemoglobin levels during triple therapy with telaprevir, PEG-IFN, and RBV. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared. Patients evaluated at each timepoint are indicated below, with the number of patients in whom telaprevir was withdrawn (PEG-IFN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

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

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

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

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

Discussion

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

In the present study, 61 patients infected with HCV-1 received triple therapy with PEG-IFN, RBV, and telaprevir in the first 12 weeks followed by PEG-IFN and RBV in the second 12 weeks. Then the RBV dose and hemoglobin were compared between patients with CC and CA/AA genotypes in the *ITPA* gene. Two polymorphisms in the *ITPA* gene, in close linkage disequilibrium with an r^2 value of 0.65,[7] have been recognized in Caucasians (rs1127354 and rs7270107); the respective CA/AA and AC/CC genotypes decrease the activity of inosine triphosphatase and protect against anemia induced by RBV.[7,12] Because the Japanese are monoallelic at rs7270107 and possess the AA genotype exclusively,[11,25] only polymorphisms at rs1127354 were examined.

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

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

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

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

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

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

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

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

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

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

There are strong points in this study. First, *ITPA* polymorphisms influence RBV-induced anemia in the triple therapy. Second, polymorphisms at rs1127350, without involvement of those at rs7270107, protect against RBV-induced anemia. Third, the triple therapy can be applied with high efficacy by careful monitoring of hemoglobin and prompt modification of RBV dose. There are weak points in this study as well. First, it was a retrospective cohort study conducted in a small size of patients, especially those with CA/AA genotypes at rs1127350, and included null-responders to previous IFN-based therapies; the real impact of *ITPA* polymorphisms on RBV-induced anemia may have been obscured. Second, the study was conducted in Japanese patients, and the results may or may not be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, the results presented herein will promote future studies in which the influence of the *ITPA* polymorphism on RBV-induced anemia will be pursued in larger scale and on patients of various ethnicities around the world.

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HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy

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ABSTRACT

Background and aims A number of recent studies have shown that human polymorphisms near the *IL28B* type III interferon (IFNλ) gene influence the response to peg-interferon plus ribavirin combination therapy for infection with chronic hepatitis C virus (HCV). Viral polymorphisms, including substitutions within the HCV core and NS5A proteins, have also been shown to influence treatment outcome, but it is not known whether these factors act independently of the IL28B polymorphism or if they reflect the same or a different underlying mechanism. Multiple logistic regression was used to determine whether host and viral polymorphisms independently predict sustained virological response (SVR).

Methods Two single nucleotide polymorphisms were genotyped in the IL28B locus (rs12979860 and rs8099917) from 817 patients with chronic HCV infection, and substitutions at amino acids 70 and 91 of the HCV core protein and within the NS5A interferon sensitivity-determining region (ISDR) were analysed.

Results It was found that independent predictors of an SVR included IL28B rs12979860 CC genotype (OR=4.98; p=4.00E-08), core amino acid 70 substitutions (OR=0.53; p=0.016), age and baseline viral load. For non-virological response, the IL28B rs12979860 CT/TT genotype (OR=0.23; p=1.96E-8) and age were independent predictors. IL28B rs12979860 genotype (p=1.4E-8), core amino acid 70 substitutions (p=0.0013), ISDR substitutions (p=0.0019), baseline viral load, γ-glutamyltranspeptidase, alanine aminotransferase and platelet count were independent predictors for change in viral load by week 4 of treatment.

Conclusions IL28B polymorphisms and HCV core amino acid 70 substitutions contribute independently to an SVR to peg-interferon plus ribavirin combination therapy.

Significance of this study

What is already known about this subject?
- Clinical and viral factors influence the outcome of peg-interferon plus ribavirin combination therapy for chronic hepatitis C virus infection.
- Polymorphisms within the human IL28B locus strongly influence treatment outcome.
- Substitutions at amino acids 70 and 91 of the HCV core protein as well as within the interferon sensitivity-determining region (ISDR) also affect response to treatment.

What are the new findings?
- IL28B polymorphisms as well as substitutions at amino acid 70 both independently predict sustained virological response, suggesting that they influence treatment outcome through different mechanisms.
- IL28B polymorphisms, substitutions at core protein amino acid 70 and ISDR substitutions are each independent predictors for change in viral load after 4 weeks of treatment.

How might it impact on clinical practice in the forseeable future?
- The combination of IL28B genotyping and detection of core protein substitutions may yield more accurate pretreatment predictions of treatment efficacy.

INTRODUCTION

Hepatitis C virus (HCV) is a primary cause of chronic hepatitis and often progresses to liver cirrhosis and hepatocellular carcinoma.[1 2] Peg-interferon plus ribavirin combination therapy (PEG-RBV) is the current standard of care, but it is only effective in 50% of patients and has severe side effects often requiring discontinuation or dose modification.[3] Consequently, reliable predictors are needed to identify unsuitable candidates as early as possible.

Genome-wide association studies have reported common single nucleotide polymorphisms (SNPs) predictive of response to interferon treatment. While polymorphisms in MxA,[4 5] interferon α-receptor 1,[6] osteopontin[7] and MAPKAPK3[8] have been reported to be associated with interferon response, several linked SNPs within the IL28B locus on chromosome 19 have recently been shown to be the strongest predictors of early viral kinetics, response to treatment and spontaneous viral clearance.[9–15]

Viral polymorphisms have also been shown to be associated with treatment response. HCV genotypes 1 and 4 in particular are considered more difficult to treat than genotypes 2 and 3,[16 17] and genotype 3 is associated with steatosis.[18] Within genotype 1b, amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity-determining region (ISDR) of the NS5A protein[19 20] have also been shown to be associated with treatment outcome, especially among Japanese patients.

Consequently, a number of human and viral factors are now known to affect response to treatment, but in order to identify the most important independent predictors and to identify which, if any, may be useful in guiding clinical practice, it is necessary to analyse them simultaneously in a multivariate model. In this study we therefore attempted to identify host and viral factors that independently predict treatment outcome.

MATERIALS AND METHODS

Patients

Data from 817 patients who were treated with PEG-RBV combination therapy for chronic hepatitis C genotype 1b infection between 2002 and 2008 were collected from Toranomon Hospital (Tokyo) and hospitals that belong to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Study subjects tested positive for HCV RNA over a span of >6 months, were negative for hepatitis B and HIV, and showed no evidence of other liver diseases. Patients received weekly injections of peg-interferon-α2b at 1.5 g/kg body weight for 48 weeks and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients with low baseline viral load (<5 log IU/ml) were excluded, as were patients who received <0.89 g/kg of peg-interferon or <8.3 mg/kg of ribavirin. Treatment success was evaluated based on a sustained virological response (SVR), defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. Some patients showed a transient response (TR or relapser), in which HCV RNA dropped to undetectable levels during treatment but then later rebounded. In those with a non-viral response (NVR), HCV RNA levels failed to decline by 2 log10 IU/ml by week 12 of treatment and never dropped below detectable levels. Histopathological diagnosis was made according to the criteria of Desmet *et al.*[21] All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA levels

HCV RNA levels were monitored throughout the course of treatment at 1 or 2 month intervals for a total of at least six time points via reverse transcription–PCR (RT–PCR) using the original Amplicor method, the high range method or the TaqMan RT–PCR test. The measurement ranges of these assays were 0.5–850 kIU/ml, 5–5000 kIU/ml and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with phosphate-buffered saline (PBS) and reanalysed. All values were reported as log IU/ml.

ISDR and core amino acid substitutions

Amino acid substitutions in the HCV core and ISDRs were determined by direct sequencing of PCR products following extraction and reverse transcription of serum HCV RNA. Core amino acid substitutions at positions 70 and 91 (core70 and core91) were determined according to Akuta *et al*,[22 23] and the number of ISDR substitutions was established as in Enomoto *et al.*[19 21 24] Of the 817 patients in the study, substitutions for both ISDR and core70 could be determined for 379 patients.

SNP genotyping

We genotyped each patient for two IL28B SNPs previously reported to be associated with treatment outcome, rs12979860 and rs8099917.[9–11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader assay, as described previously.[25 26] The two SNPs are in strong linkage disequilibrium, with a correlation coefficient of 0.99. SNP genotypes for both rs12979860 and rs8099917 were determined for 815 patients (99.7%).

Statistical analysis

All analyses were performed using the R statistical package (http://www.r-project.org). Non-parametric tests (χ^2 and Mann–Whitney U tests) were used to detect significant associations. All statistical analyses were two sided, and $p<0.05$ was considered significant. Simple and multiple logistic regression analyses were used to examine the association between viral substitutions and clinical factors using $p<0.05$ as the criterion for inclusion in the initial multivariate model. Multivariate logistic regression analysis was performed using forward/backward stepwise selection based on Akaike Information Criterion (AIC) score and validated using the rms package in R. ORs and 95% CIs were calculated for each factor.

RESULTS

Patient characteristics

Patient profiles are shown in table 1. Forty-five per cent of patients achieved an SVR, 22% were transient responders and 33% failed to respond to treatment (NVR). Males were significantly more likely to achieve an SVR than females (50% and 38%, respectively; p=0.0011), and younger patients were more likely to achieve an SVR than older patients (59.2% and 40.9% above and below median age 58, respectively; p=1.57E-6). Patients who achieved an SVR also had lower γ-glutamyltranspeptidase (γGTP) levels (36 IU/l vs 45 IU/l; p= 0.008) and higher platelet counts (17.1 vs 15.3×10^{10}/L; p=3.649E-05) than those who did not.

IL28B SNP genotypes

The genotypes of two IL28B SNPs were measured for each patient. Because of linkage disequilibrium, SNP results are nearly interchangeable. However, six patients showed an intermediate haplotype consisting of the favourable genotype for rs8099917 (TT) but an unfavourable genotype for rs12979860 (CT), whereas only one of the six patients achieved an SVR, suggesting that rs12979860 is a better predictor of SVR in this data set.

The frequency of the risk allele (T) for rs12979860 was 0.15 among all patients and 0.08 in SVR patients, 0.14 in TR patients and 0.27 in NVR patients. Patients homozygous for the rs12979860 favourable allele (CC) were significantly more likely to achieve an SVR compared with those with TC or TT genotypes (53% vs 24%, OR=3.55, p=3.95E-13). Conversely, patients with the risk allele (TC or TT) were significantly more likely to show an NVR (55% vs 25%; OR=0.265; p=4.4E-16). Patients with the rs12979860 CC genotype had a marginally lower baseline viral load (6.6 vs 6.4 log IU/ml; p=0.093), but showed significantly greater reduction in viral load by week 4 of treatment (−3.2 vs −0.8 log IU/ml; p<2.2E-16). The rs12979860 CC genotype was also associated with wild type core70 (78% vs 54%; p=1.6E-6) and non-wild type ISDR (67% vs 83%; p=0.007).

The frequency of the rs8099917 risk allele (G) was 0.15 among all patients, 0.08 in SVR patients, 0.13 in TR patients and 0.26 in NVR patients. Patients with the rs8099917 TT genotype were significantly more likely to achieve an SVR than patients with GT or GG genotypes (53% vs 24%, OR=3.43, p=2.18E-12), and GT/GG patients were significantly more likely to show an NVR

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Table 1 Patient profiles by response to treatment

	All (813)	SVR (366)	TR (176)	NVR (271)
Sex (M/F)	459/354	231/135	84/92	144/127
Age	58 (51–65)	56 (47–63)	60.5 (56–65.25)	59 (52.5–66)
Body weight (kg)	59 (52–67)	60 (52–68.25)	58 (51–66)	60 (52–66.4)
BMI (kg/m^2)	22.61 (20.81–24.65)	22.44 (20.46–24.58)	22.85 (20.85–24.89)	22.76 (21.12–24.63)
Hypertension (yes/no)	141/672	61/305	29/147	51/220
Diabetes (yes/no)	97/716	31/335	25/151	41/230
Fibrosis (0–2/3–4)	138/421	52/227	34/81	52/113
Activity (0–1/2–3)	274/272	136/138	53/56	85/78
ISDR (0, 1/≥2)	78/298	43/128	15/71	20/99
Amino acid 70 (wild-type/mutant)	256/139	137/45	54/35	65/59
Amino acid 91 (wild-type/mutant)	221/178	112/72	51/40	58/66
WBC (/L)	4.71×10^9 (3.9×10^9–5.7×10^9)	4.9×10^9 (4.0×10^9–6.0×10^9)	4.6×10^9 (3.8×10^9–5.4×10^9)	4.6×10^9 (3.7×10^9–5.5×10^9)
Haemoglobin (g/dl)	14.1 (13.2–15)	14.2 (13.3–15.22)	13.9 (13.1–14.8)	14.1 (13.05–14.9)
Platelets ($\times10^4$L)	16.1×10^6 (12.5×10^6–19.9×10^6)	17.1×10^6 (13.7×10^6–20.7×10^6)	15.5×10^6 (11.3×10^6–18.8×10^6)	15.1×10^6 (12×10^6–19.2×10^6)
AST (IU/l)	45 (34–65.5)	43 (32.25–64)	43.5 (33.25–66)	48 (37–66.5)
ALT (IU/l)	55 (37–87)	57 (37–92)	50 (33–78)	53 (39–82.5)
γGTP (IU/l)	40 (25–72)	36 (23–65.75)	36 (23–69)	52 (32–86)a
Albumin (g/dl)	3.9 (3.7–4.1)	3.9 (3.7–4.1)	3.8 (3.7–4)	3.8 (3.7–4.1)
Total cholesterol (mg/dl)	171 (150–192)	169 (149.2–192)	175 (158–191)	170 (148.5–192.5)
Viral load (log IU/ml)	6.5 (6.1–6.9)	6.4 (5.9–6.825)	6.6 (6.3–7)	6.6 (6.2–7)
PEG-IFN-α2b (μg)	80 (80–100)	80 (80–100)	80 (75–100)	80 (60–100)
PEG-IFN-α2b/kg (μg/kg)	1.19 (1.19–1.48)	1.36 (1.19–1.48)	1.19 (1.19–1.48)	1.19 (1.02–1.48)
Ribavirin (mg)	600 (600–800)	600 (600–800)	600 (600–800)	600 (400–800)
Ribavirin/kg (mg/kg)	8.9 (8.9–11.87)	10.29 (8.9–11.87)	8.9 (8.9–11.87)	8.9 (7.8–11.86)
rs12979860 (CC/CT/TT)	582/203/27	311/51/4	128/43/4	143/109/19
rs8099917 (TT/TG/GG)	588/199/25	311/51/3	132/40/4	145/108/18

For categorical data, the number of patients in each category is shown. For continuous data, the median and range are displayed.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; M, male; NVR, non-virological response; PEG-IFN, pegylated interferon; SVR, sustained virological response; TR, transient response; WBC, white blood cells.

(56% vs 25%; OR=0.26; p=3.33E-16). Patients with the rs8099917 TT genotype had marginally higher baseline viral load (6.6 vs 6.4 log IU/ml; p=0.077) but showed a significantly greater drop in viral load by week 4 of treatment (−3.1 vs −0.8 log IU/ml; p<2.2E-16). The rs8099917 TT genotype was also associated with wild-type core70 (79% vs 56%; p= 3.1E-6) and non-wild-type ISDR (68% vs 83%; p=0.015).

Viral substitutions

Patients who achieved an SVR had significantly lower initial HCV RNA levels than those who did not (6.4 vs 6.6 log IU/ml; p=2.1E-6). The 140 patients (17%) with a substitution at position 70 of the HCV core protein (core70) were significantly less likely to achieve an SVR than patients with wild type core70 (33% vs 53%; p=0.00019) and were significantly more likely to show an NVR (42% vs 25%; p=0.0013). The 179 (22%) of patients with a substitution at position 91 (core91) were marginally less likely to achieve an SVR (41% vs 50%; p=0.08) but were significantly more likely to show an NVR (37% vs 27%; p=0.039). The 78 (10%) of patients who had two or more substitutions in the ISDR of NS5A were only marginally less likely to achieve an SVR than those with wild-type ISDR (43% vs 55%; p=0.066) and were not more likely to show an NVR (33% vs 26%; p=0.24).

Predictive factors for an SVR

Significant univariate predictors for an SVR included patient clinical factors (age, sex, diabetes, platelet count, white blood cell count, haemoglobin level, γGTP level); SNP genotype (rs12979860 and rs8099917); and viral factors (baseline viral load and core70, core91 and ISDR substitutions) (table 2). Following multivariate analysis, only age, rs12979860 genotype, core70 substitution and baseline viral load were significant independent predictors (figure 1A). The joint effects of rs12979860 and core70 on response to treatments are illustrated in figure 2.

Predictive factors for an NVR

Significant univariate predictors for an NVR included age, rs12979860 and rs8099917 genotypes, core70 and core91 substitutions, diabetes, aspartate aminotransferase (AST), baseline viral load, platelet count, white blood cell count and γGTP levels (table 3). Following multivariate analysis only age and rs12979860 genotype remained as independent predictors (figure 1B).

Predictive factors for change in viral load by week 4 of treatment

Factors influencing virological response were assessed by examining change in viral load between the start of treatment and week 4. Using linear regression, sex, rs12979860, rs8099917, core70, core91, ISDR, baseline viral load, alanine aminotransferase (ALT), platelet count, white blood cell count, haemoglobin level and γGTP were found to be significant univariate predictors of change in viral load by week 4 (table 4). Independent factors included rs12979860, core70, ISDR, ALT, platelet count and γGTP. We also found a significant positive linear relationship between the total number of ISDR substitutions and change in viral load between week 0 and week 4 (slope=0.2; p=0.0047).

In patients with the favourable rs12979860 CC genotype, core70 wild type was a significant predictor of viral decline (p=0.007; figures 3A,B), but in patients with the CT or TT genotypes, viral decline did not vary with respect to core70 substitutions (p=0.18; figures 3C,D). Conversely, ISDR was not

Table 2 Predictors for a sustained virological response

	Simple			Multiple			
Variable	**n**	**OR**	**p Value**	**n**	**OR**	**95% CI**	**p Value**
Age	813	0.58	1.22E-08***	362	0.432	0.31 to 0.60	6.61E-07***
Sex (male vs female)	813	1.28	0.0006***	362	1.2	0.95 to 1.54	0.133
BMI (kg/m^2)	800	0.87	0.1286				
rs12979860 (CC vs TC/TT)	812	3.65	2.67E-14***	362	4.98	2.81 to 8.82	4.00E-08***
rs8099917 (TT vs GT/GG)	812	3.53	1.77E-13***				
Hypertension	813	0.92	0.6452				
Diabetes	813	0.53	0.005907**				
Core amino acid 70 (wild type vs mutant)	395	0.42	5.82E-05***	362	0.527	0.31 to 0.89	0.01575*
Core amino acid 91 (wild type vs mutant)	399	0.66	0.0419*				
ISDR	376	1.12	0.1627				
Viral load (log IU/ml)	695	0.68	2.09E-06***	362	0.77	0.62 to 0.96	0.02249*
Fibrosis (F0–1 vs F2–4)	559	0.74	0.0817				
Activity (A0–1 vs A2–4)	546	0.96	0.7975				
Total cholesterol (mg/dl)	663	0.86	0.2151				
AST (IU/l)	687	1.03	0.1069				
ALT (IU/l)	692	1.26	0.0920				
Platelets (×10^4/L)	694	1.49	3.57E-05***	362	1.39	0.97 to 1.99	0.073
WBC (/L)	693	1.31	0.0014**				
Haemoglobin (g/dl)	693	1.28	0.0043**				
γGTP (IU/l)	646	0.96	0.0052**				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

a significant predictor of viral decline in patients with the rs12979860 CC genotype (p=0.078; figures 4A,B), but patients with the CT or TT genotypes and two or more substitutions in the ISDR showed significantly greater viral decline by week 4 than patients with zero or one ISDR substitution (p=0.007; figures 4C,D).

DISCUSSION

In this study we showed that host factors (younger age, male sex, favourable IL28B SNP genotypes) as well as viral factors (baseline viral load, wild-type core70 and two or more substitutions in the ISDR) contribute to the successful outcome of PEG-RBV combination therapy. Although some of these factors independently predict an SVR or NVR in multivariate analysis, collectively they reflect a complex genotype-by-environment interaction involving common polymorphisms in both the virus and the human host.

Genetic variation within the human IL28 locus has been reported as the strongest pretreatment predictor of an SVR,[15] and the results of this study support this finding. Several tightly linked SNPs in the non-coding region of *IL28A* and *IL28B* have been shown to be associated with spontaneous viral clearance, rapid and early virological response and/or SVR following treatment with interferon and ribavirin for HCV genotype 1b.[9–15] *IL28A*, *IL28B* and *IL29* code for type III (λ) interferons, which are similar to type I interferons but use a different receptor and show high tissue specificity.[27 28] It has not been determined which, if any, of the reported SNPs directly affects function, but the functional SNP probably affects gene expression. IRF3- and IRF7-binding sites near the transcription start

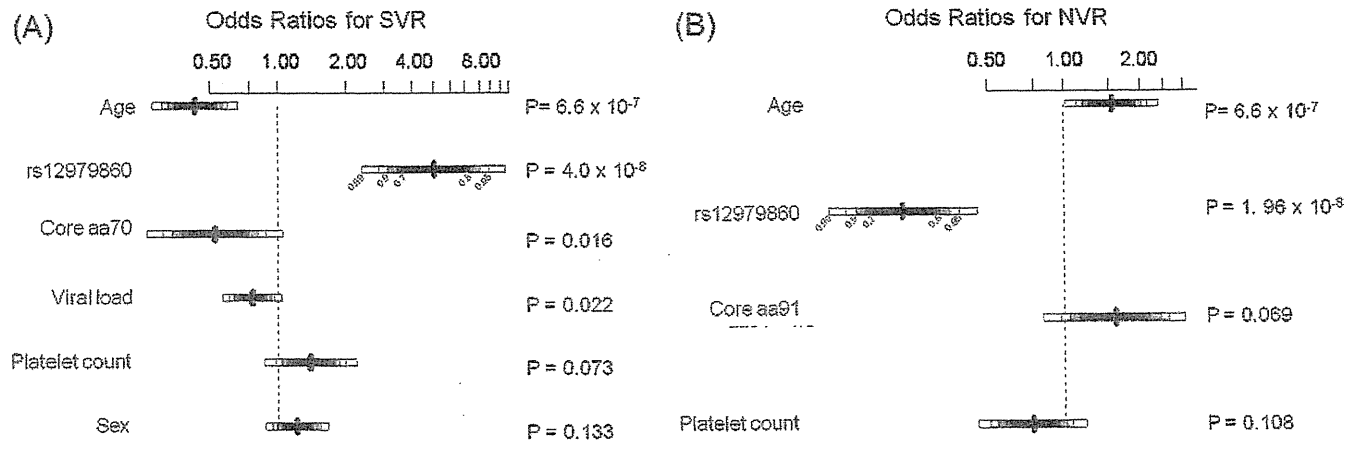

Figure 1 ORs for predictive factors response to treatment. ORs and 95% CIs are shown for predictive factors for (A) sustained virological response (SVR) and (B) non-virological response (NVR) based on multiple logistic regression with stepwise selection.

Hayes CN, Kobayashi M, Akuta N, *et al*. *Gut* (2010). doi:10.1136/gut.2010.223495

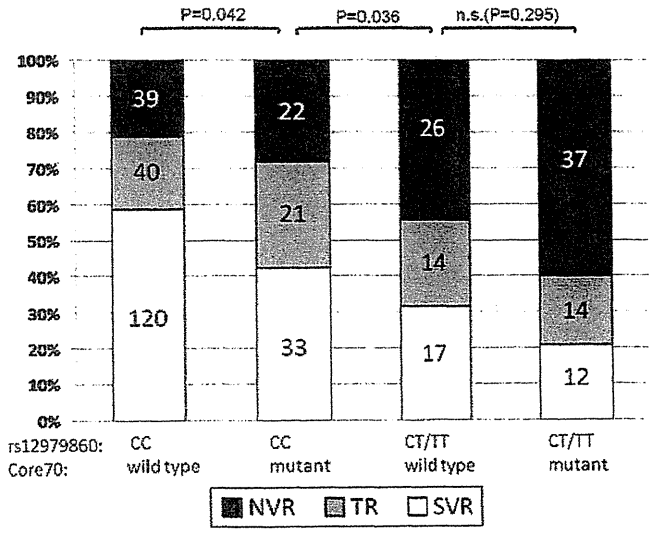

Figure 2 Cumulative effects of rs12979860 genotype and core protein amino acid 70 substitutions. The relative effects of rs12979860 genotype (favourable CC vs non-favourable CT/CC) and core amino acid 70 substitutions (favourable wild type vs unfavourable substitutions) on response to treatment are shown. NVR, non-virological response; TR, transient response/relapser; SVR, sustained virological response.

site of *IL28B* are essential for gene expression, but distal clusters of nuclear factor-κB (NF-κB)-binding sites are necessary for maximal expression,[29 30] suggesting that upstream polymorphisms may potentially disrupt transcription factor-binding sites within a distal promoter or enhancer. Unintuitively, interferon-stimulated genes are downregulated in patients with the favourable rs8099917 TT genotype,[31] implying that responders have a lower baseline expression of immune response genes.[32] This might serve to prevent desensitisation and promote maximal induction of interferon-stimulated genes, but detailed gene regulation studies are needed to resolve the role of IL28B polymorphisms in antiviral defence.

In addition to effects of human genetic polymorphisms, a number of studies have reported significant association between HCV core70/core91 substitutions and treatment outcome.[20 33 34] We found significant independent associations between core70 substitutions and an SVR, as well as change in viral load by week 4, but the association was not significant for an NVR under multivariate analysis despite being highly significant in univariate analysis. Although the role of core70 substitutions is unclear, the core protein interacts with a number of viral and host proteins and disrupts the interferon signalling pathway.[35–37] The proportion of core70 substitutions in the host viral population has been reported to increase during treatment with PEG-RBV therapy, which may indicate positive selection at this position in response to treatment.[38] Substitutions at these positions appear to affect the antiviral response during the early stages of treatment, as wild-type core70 and core91 are associated with a rapid decrease in HCV RNA levels during the first 4 weeks of treatment.[39 40] Because a rapid virological response is also a strong predictor of SVR and NVR, core70 and core91 substitutions may affect treatment outcome either directly or indirectly.[40 41]

Unlike HCV core70 substitutions, we found only a marginal association between ISDR substitutions and SVR, and no association with NVR. However, ISDR substitution was a significant independent predictor of change in viral load by week 4. The presence of two or more mutations in this 40 amino acid stretch of the NS5A protein is associated with an SVR.[24 42] Other studies have found no significant association between ISDR and SVR but have found a higher overall mutation rate in the NS5A protein among SVR patients,[43 44] and one study suggests that the association with ISDR varies by strain and is more pronounced in Japan than in Europe.[45] It is not clear whether mutations in ISDR directly affect function or whether they reflect the genetic distance from an interferon-resistant

Table 3 Predictors for a non-virological response

	Simple			Multiple			
Variable	**n**	**OR**	**p Value**	**n**	**OR**	**95% CI**	**p Value**
Age	813	1.30	0.01306*	370	1.55	1.12 to 2.15	0.008367**
Sex (male vs female)	813	0.90	0.178				
BMI (kg/m^2)	800	1.07	0.3899				
rs12979860 (CC vs TC/TT)	812	0.26	2.73E-17***	370	0.231	0.14 to 0.39	1.96E-08***
rs8099917 (TT vs GT/GG)	812	0.26	1.51E-17***				
Hypertension	813	1.16	0.4323				
Diabetes	813	1.55	0.04685*				
Core amino acid 70 (wild type vs mutant)	395	2.17	0.000496***				
Core amino acid 91 (wild type vs mutant)	399	1.66	0.02029*	370	1.58	0.96 to 2.60	0.06943
ISDR	376	0.92	0.06197				
Viral load (log IU/ml)	695	1.32	0.01716*				
Fibrosis (F0–1 vs F2–4)	559	1.24	0.2608				
Activity (A0–1 vs A2–4)	546	1.12	0.5499				
Total cholesterol (mg/dl)	663	0.98	0.5824				
AST (IU/l)	687	1.02	0.03148*				
ALT (IU/l)	692	0.91	0.8772				
Platelets ($\times 10^4$/L)	694	0.76	0.008222**	370	0.739	0.51 to 1.07	0.1077
WBC (/L)	693	0.83	0.04617*				
Haemoglobin (g/dl)	693	0.84	0.1201				
γGTP (IU/l)	646	1.15	1.23E-05***				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

Table 4 Predictors for change in viral load by week 4 of treatment

	Simple			Multiple		
Variable	**n**	**Coefficient**	**p Value**	**n**	**Coefficient**	**p Value**
Age	500	−0.01	0.138			
Sex (male vs female)	500	−0.23	0.005**			
BMI (kg/m^2)	494	0.00	0.958			
rs12979860 (CC vs TC/TT)	500	2.11	5.18E-38***	221	1.37	1.35E-08***
rs8099917 (TT vs GT/GG)	499	2.10	1.40E-36***			
Hypertension	500	−0.25	0.249			
Diabetes	500	−0.31	0.19			
Core amino acid 70 (wild type vs mutant)	259	−1.01	1.38E-05***	221	−0.665	0.001328**
Core amino acid 91 (wild type vs mutant)	262	−0.77	0.000***			
ISDR	247	0.20	0.006**	221	0.186	0.001878**
Viral load (log IU/ml)	500	0.37	0.000***	221	0.414	0.00012***
Fibrosis (F0−1 vs F2−4)	397	−0.22	0.217			
Activity (A0−1 vs A2−4)	389	−0.10	0.578			
Total cholesterol (mg/dl)	472	0.00	0.064			
AST (IU/l)	490	0.00	0.442			
ALT (IU/l)	493	0.00	0.005**	221	0.00606	0.008895**
Platelets (×10^4/L)	495	0.03	0.048*	221	0.0701	7.24E-05***
WBC (/L)	495	0.00	0.027*			
Haemoglobin (g/dl)	495	0.13	0.013*			
γGTP (IU/l)	460	0.00	0.001***	221	−0.00634	0.002095**

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

strain. Nonetheless, the NS5A protein has been shown to be under purifying selection[44] and plays a critical role in both viral replication[46 47] and modulation of the immune response.[48] Therefore, the number of substitutions in one or more variable regions of the NS5A may be a useful predictor of early viral dynamics and an indirect predictor of SVR, although in this study we found a significant effect only for change in viral load by week 4 of treatment.

A number of factors have now been reported to influence outcome of PEG-RBV therapy, and it is important to determine which of these factors represent independent, clinically useful predictors. Because of the expense and occasionally severe side effects of the current standard of care, reliable pretreatment indicators, especially of poor response, will help guide treatment decisions and steer difficult-to-treat patients towards more

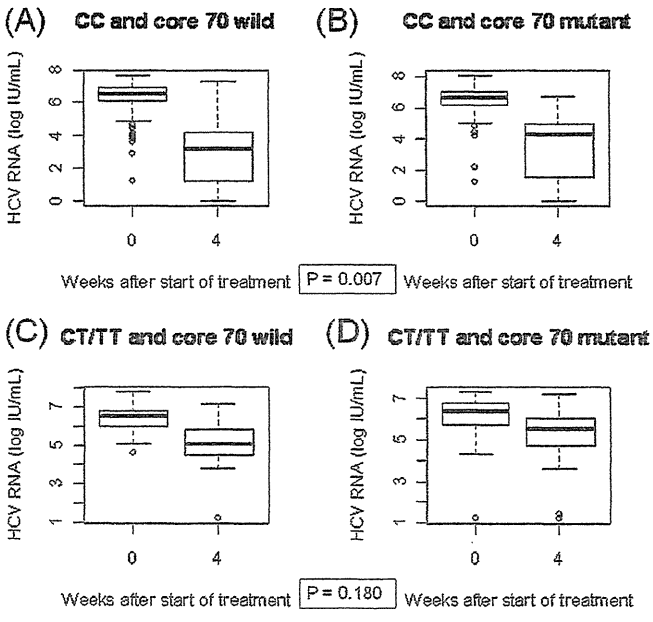

Figure 3 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and hepapitis C virus (HCV) core protein substitutions. The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and wild/mutant amino acid at core70 is shown.

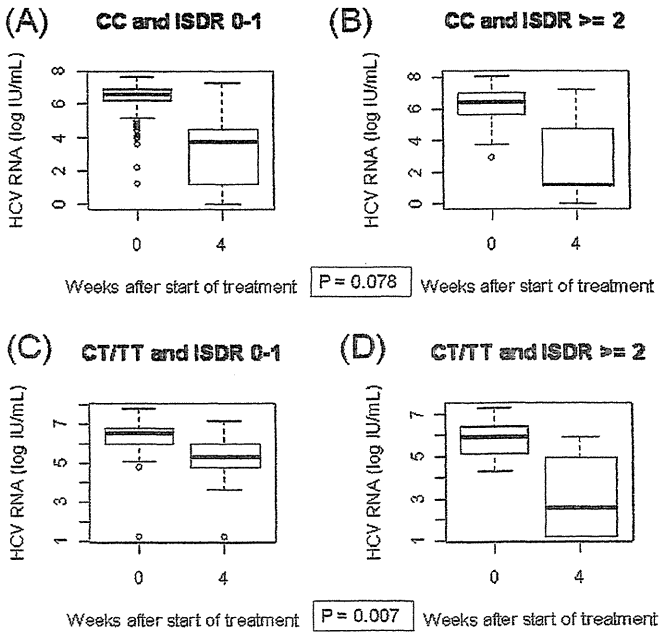

Figure 4 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and substitutions in the interferon sensitivity-determining region (ISDR). The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and the number of substitutions in the ISDR is shown.

effective treatments or enrolment in clinical trials. In order to identify the most important independent predictors, it will be necessary to disentangle the intriguing interactions between human and viral polymorphisms as well as gain better understanding of the role of type III interferon in the immune response against HCV.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Hiroshima University ethics committee.

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Research Article

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Predictive value of the *IL28B* polymorphism on the effect of interferon therapy in chronic hepatitis C patients with genotypes 2a and 2b

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Background & Aims: Common *IL28B* locus polymorphisms (SNPs rs8099917 and rs12979860) have been reported to affect peg-interferon plus ribavirin combination therapy (PEG-RBV) for hepatitis C virus (HCV) genotype 1b, but few reports have examined their effect on other two common genotypes, 2a and 2b.

Methods: We analyzed predictive factors for sustained virological response (SVR) in a retrospective study of 719 patients with either genotype 2a (530) or 2b (189). Of these patients, 160 were treated with PEG-RBV and 559 were treated with interferon monotherapy. We evaluated predictive factors including HCV RNA, histological findings, *IL28B* SNP genotypes (rs8099917, rs12979860, and rs12980275), and the effect of treatment regimen and prior treatment history.

Results: HCV RNA viral load, treatment regimen, and rs8099917 genotypes independently contributed to the effect of the therapy. For patients treated with PEG-RBV, rs8099917 and viral load were independent predictive factors for SVR in genotype 2b but not in genotype 2a. Conversely, in patients treated with interferon monotherapy, viral load and rs8099917 were independent predictive factors for SVR in genotype 2a but not in genotype 2b. The favorable rs8099917 genotype is also associated with a steep decline in viral load by the second week of treatment.

Conclusions: Initial viral load and rs8099917 genotype are significant independent predictors of SVR in genotype 2 patients.

Keywords: Interferon therapy; Single nucleotide polymorphism; Ribavirin; Hepatitis C.

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Abbreviations: HCV, hepatitis C virus; IFN, interferon; PEG-IFN, pegylated interferon; RBV, ribavirin; PEG-RBV, pegylated interferon plus ribavirin combination therapy; SNP, single nucleotide polymorphism; SVR, sustained viral responder; NR, non-responder.

Introduction

Hepatitis C virus (HCV) infection is a major worldwide cause of chronic liver diseases, affecting an estimated 170 million people [1]. Chronic HCV infection may progress to hepatocellular carcinoma (HCC) or liver cirrhosis (LC) [2–6], and in Japan, 60–70% of patients with HCC or LC are HCV carriers [7]. There are two major genotypes (1 and 2) and three sub-genotypes (1b, 2a, and 2b) in Japan as well as in many other countries [8]. Although pathological features of these genotypes are similar [9,10], interferon therapy is more effective against genotype 2 than genotype 1 [11,12]. Compared to the less than 50% of genotype 1 patients who respond to therapy [13–19], more than 80% of genotype 2 patients who received 24-week peg-interferon and ribavirin (PEG-RBV) combination therapy achieved sustained virological response (SVR), defined as absence of HCV RNA six months after the cessation of therapy. Because of this otherwise high success rate, the small subset of genotype 2 patients who fail to respond to therapy should be examined more closely. Although treatment-resistant genotype 2 sub-populations have been reported [20–22], the mechanism underlying variable response to treatment is unclear. Multiple viral (e.g., HCV genotype, amino acid substitutions in the NS5A and core region [22–26]) and host factors (e.g., age [14], body mass index [27], and insulin resistance

Table 1. Baseline characteristics of patients with HCV genotypes 2a and 2b.

	All (n = 719)	2a (n = 530)	2b (n = 189)
Sex (M/F)	403/316	301/229	102/87
Age	57 (49-64)	56 (48-64)	59 (50-66)
Body weight (kg)	59.8 (51-71.4)	60.15 (53.75-71.65)	57.4 (48.5-70)
BMI (kg/m^2)	23.2 (20.3-25.7)	24.48 (21.43-26.4)	21.78 (19.89-24.79)
Fibrosis (F0-2/F3-4)	484/101	359/68	125/33
Treatment (IFN/PEG-RBV)	559/160	477/53	82/107
Treatment naïve (Y/N)	689/30	523/7	166/23
HCV RNA (log IU/ml)	5.3 (4.7-5.9)	5 (4.6-5.7)	5.9 (5.5-6.5)
rs8099917 (TT/GT/GG)	572/135/11	425/97/7	147/38/4
rs12979860 (CC/TC/TT)	565/137/11	422/98/7	143/39/4
rs12980275 (AA/GA/GG)	543/158/16	402/116/10	141/42/6
SVR/non-SVR	455/264	340/190	115/74

IFN, interferon monotherapy; PEG-RBV, peg-interferon plus ribavirin combination therapy; SVR, sustained viral responder.

[28]) have been reported to affect the outcome of interferon therapy in genotype 1-infected patients but such factors have not been closely examined in genotype 2 patients.

Single nucleotide polymorphisms (SNPs) and other genetic factors have been reported to be useful in predicting the outcome of interferon therapy. Polymorphisms in MxA [29,30], interferon alpha-receptor 1 [31], and osteopontin [32] have also been reported to be associated with interferon response. We also identified a MAPKAPK3 SNP [33] that is a predictive factor for interferon mono-therapy. Recently, several groups have reported an association between several SNPs in the *IL28* locus and the effect of PEG-RBV combination therapy for genotype 1b [34–38] but only a few studies have examined the role of these SNPs in the treatment of other genotypes. In this study, we analyzed predictive factors for SVR in genotype 2a and 2b patients treated with PEG-RBV. Because PEG-RBV was only approved for use in Japan in 2005, we also examined predictive factors in patients who were treated with interferon monotherapy, which is still used in the event of an adverse reaction to ribavirin.

Patients and methods

Patients and study design

We studied 719 Japanese patients with chronic hepatitis C (positive for HCV RNA for more than 6 months) who received interferon therapy with or without ribavirin between 2002 and 2008. Patients were treated at Toranomon Hospital in Tokyo, Hiroshima University Hospital, and hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html). All patients were negative for hepatitis B surface antigen, had no evidence of other liver diseases, such as auto-immune hepatitis or alcoholic liver disease, and had not received immunosuppressive therapy before enrollment in the study. All patients gave written informed consent to participate in the study in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and according to the process approved by the ethical committees of Hiroshima University and the SNP Research Center at the Institute of Physical and Chemical Research (RIKEN) in Yokohama.

PEG-RBV patients received weekly injections of peg-interferon-alpha-2b at 1.5 g/kg body weight for 24 weeks. Ribavirin was administered orally, and the dosage was determined based on the patient's body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients receiving interferon monotherapy were treated daily with 6 million units of IFN intramuscularly for 8 weeks, followed by the same dose three times a week for 16 weeks, for a total of 528 million units. Successful treatment was ascertained based on sustained virological response (SVR), defined as HCV RNA-negative six months after cessation of therapy. Fibrosis stage and activity were diagnosed by pathologists at each hospital according to the criteria of Desmet et al. [39]. Patients were classified as interferon treatment naïve or experienced based on prior interferon treatment but only parameters related to the most recent therapy were used in the analysis.

SNP Genotyping and quality control

We genotyped each patient for three *IL28B* SNPs previously reported to be associated with therapy outcome: rs8099917, rs12979860, and rs12980275. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader assay, as described previously [40,41]. We were unable to determine genotypes for one of the 796 patients for rs8099917, six of the patients for rs12979860, and two for rs12980275.

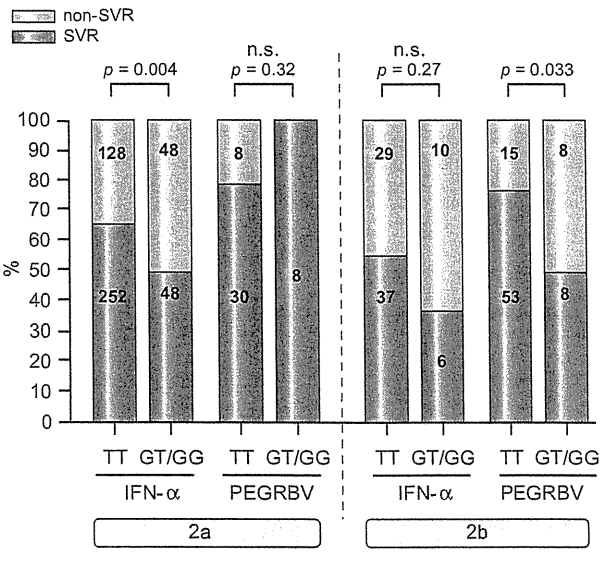

Fig. 1. Effect of interferon therapy on patients with genotype 2a and 2b infection. Sustained viral responders (SVR) and non-responders (non-SVR) were analyzed by IL28B SNP rs8099917 genotype, viral genotype, and treatment type. All patients were interferon-naïve.

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Table 2. Predictors for SVR in treatment-naïve patients treated with peg-interferon plus ribavirin combination therapy.

		Simple		Multiple			
Genotype	Variable	n	*p*	n	OR	(95% CI)	*p*
2a + 2b	Age	130	0.42				
	Sex	130	0.62				
	Genotype	130	0.21				
	Viral load	127	0.002 **	127	0.19	(0.06-0.55)	0.002 **
	Fibrosis	110	0.25				
	rs8099917	130	0.23				
	rs12980275	129	0.79				
2a	Age	46	0.77				
	Sex	46	0.62				
	Viral load	44	0.16				
	Fibrosis	39	0.75				
	rs8099917	46	0.8				
	rs12980275	45	0.77				
2b	Age	84	0.14				
	Sex	84	0.58				
	Viral load	83	0.01 *	83	0.13	(0.03-0.62)	0.01 *
	Fibrosis	71	0.08				
	rs8099917	84	0.03 *	83	0.23	(0.06-0.80)	0.02 *
	rs12980275	84	0.21				

*p <0.05; **p <0.01; ***p <0.001.

HCV RNA levels

HCV RNA levels, corresponding to initial viral load, were measured using one of several RT-PCR-based methods (the original Amplicor method, the high range method, or the TaqMan RT-PCR test). The measurement ranges of these assays were 0.5–850 KIU/ml, 5–5000 KIU/ml, and 1.2–7.8 log IU, respectively. Saturated samples were diluted with PBS and reanalyzed. All values were reported as log IU/ml.

Statistical analysis

Genotype-based associations were tested using the Cochran–Armitage trend test. Combined analysis was performed using the Mantel–Haenszel method. Simple and multiple regression analyses were used to examine the association between viral and clinical factors using p <0.05 as the criterion for inclusion in the multivariate model. HCV RNA was converted into a binary variable based on the median. Multivariate logistic regression analysis was performed using the Design package in *R* (http://www.r-project.org) with fast backward elimination and validation based on AIC score for model construction.

Results

Clinical characteristics are summarized by genotype in Table 1. The SVR rate was slightly but not significantly higher among patients with genotype 2a (340 out of 530; 64%) compared to genotype 2b patients (115 out of 189; 61%) (p = 0.43). Patients who were treated with PEG-RBV had a slightly but not significantly higher rate of SVR (111 out of 160; 69%) than patients treated with interferon monotherapy (344 out of 559, 61%) (p = 0.08). Because the number of patients treated with interferon monotherapy (559) greatly exceeds the number of patients treated with PEG-RBV (160), patients were analyzed separately by treatment type. Because 30 out of the 719 patients (4%) had received prior interferon treatment, only treatment-naïve patients were included in the analyses mentioned below, followed by a separate analysis of the effect of prior interferon treatment on SVR rate.

IL28B polymorphisms

Minor allele frequencies for rs8099917, rs12979860, and rs12980275 were 0.109, 0.112, and 0.132, respectively. The frequency of the rs8099917 risk allele was lower in SVR patients than non-SVR patients (0.089 vs. 0.14; p = 1.03e-05). The risk allele frequency among all patients was slightly higher than in the HapMap-JPT population (0.109 vs. 0.093; p = 0.01) but lower than in the HapMap-CEU population (0.109 vs. 0.183; p = 1.6e-05). We compared rs8099917 allele and genotype frequencies with 900 healthy Japanese subjects but found no significant differences. 67% of patients (372 out of 552) with the favorable rs8099917 TT genotype achieved SVR, compared to 51% (70 out of 136) of patients with GT or GG genotypes. Fig. 1 shows the joint effects of treatment type, viral genotype, and rs8099917 genotype. In every case results for rs8099917 and rs12979860 are the same, but both factors cannot be included in a multivariate model simultaneously due to multicollinearity, so results for rs8099917 are presented due to the higher genotyping success rate.

Predictive factors for SVR in patients treated with PEG-RBV

Among treatment-naïve patients treated with PEG-RBV, 78% (83 out of 106) of patients with rs8099917 TT achieved SVR compared

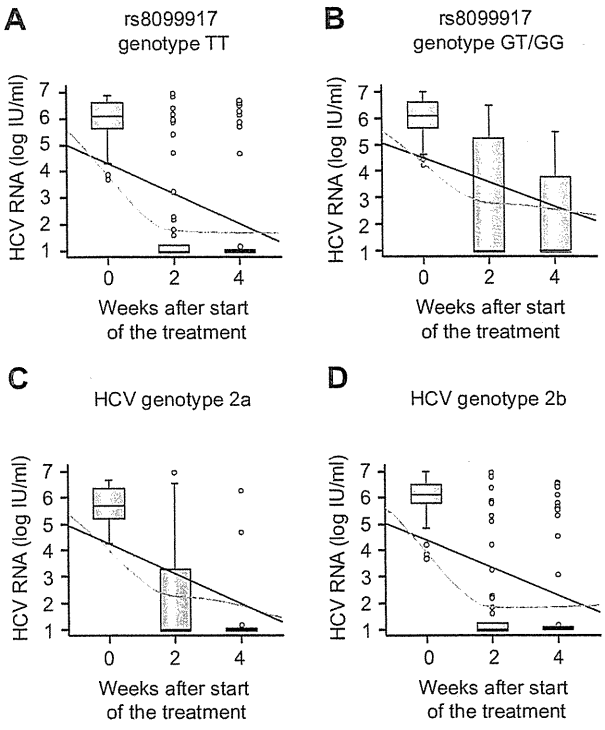

Fig. 2. Effect of rs8099917 genotype and HCV genotype on change in HCV RNA levels. HCV RNA levels at 0, 2, and 4 weeks after the start of peg-interferon plus ribavirin combination therapy in treatment-naïve patients. (A and B) Change in viral load for patients with the protective TT genotype for rs8099917 (A) compared to patients with the GT or GG genotypes (B). (C and D) Change in viral load for patients with HCV genotype 2a (A) versus genotype 2b (B).

to 67% (16 out of 24) of patients with non-TT genotypes ($p = 0.29$). In univariate and multivariate analyses, only viral load was an independent predictive factor for SVR ($p = 0.002$; Table 2), but when we examined genotypes 2a and 2b separately, rs8099917 genotype ($p = 0.02$) and viral load ($p = 0.01$) were both significant independent predictors of SVR for patients with genotype 2b, whereas no significant univariate or multivariate predictors were found for patients with genotype 2a. Notably, however, all 8 patients with genotype 2a with rs8099917 GT/GG achieved SVR (Fig. 1). The same pattern held for patients with rs12979860 TC/TT (9 SVR, 0 non-SVR) and rs12980275 GA/GG (11 SVR, 0 non-SVR) genotypes. Moreover, none of these patients was homozygous for the risk allele at each SNP.

Change in HCV RNA levels for patients treated with PEG-RBV

HCV RNA levels at the start of PEG-RBV therapy and after 2 and 4 weeks of treatment are plotted by rs8099917 genotype and viral genotype in Fig. 2. Under multivariate analysis, rs8099917 genotype was an independent predictive factor for change in HCV RNA level by week 2 ($p = 0.036$) but viral genotype was not significant ($p = 0.15$). For changes in HCV RNA levels by week 4, neither the rs8099917 genotype nor the viral genotype was significant ($p = 0.17$ and $p = 0.22$, respectively).

Predictive factors for SVR in patients treated with interferon monotherapy

Among patients treated with interferon monotherapy, 65% of patients with rs8099917 TT achieved SVR, compared to only 48% of patients with GT or GG genotypes ($p = 0.002$). Viral load and the rs8099917 and rs12980275 genotypes were significant univariate predictors of SVR, and under multivariate analysis viral load and rs8099917 remained as independent predictors (Table 3). When genotypes 2a and 2b were analyzed separately, viral load ($p = 0.001$) and rs8099917 genotype ($p = 0.014$) were independent predictive factors for SVR in patients with genotype 2a but no significant univariate of multivariate terms were found for genotype 2b.

Effect of prior interferon treatment

Thirty out of the 719 patients (4%) had previously received treatment with interferon. Among these patients, only 40% achieved SVR, compared to the 64% SVR rate among treatment-naïve patients. Initial viral load was the only independent predictor of SVR in these patients, whereas in treatment-naïve patients, viral load, rs8099917 genotype, and treatment type (PEG-RBV vs interferon monotherapy) were independent predictors of SVR (Table 4).

Development of resistance to interferon therapy

Over the course of therapy five patients developed resistance to PEG-RBV treatment. In each case the patient showed an initial drop in viremia followed by viral breakthrough. Three out of the five patients were heterozygous (T/G) for the rs8099917 genotype and two out of the five were homozygous for the favorable allele (T/T).

Discussion

As the effect of *IL28B* polymorphism has not been reported separately for genotype 2 and its subtypes so far, we investigated whether the polymorphism influences treatment outcome in patients with HCV genotype 2a and 2b infections. In addition to previously reported effects for genotypes 1 and 4, our results demonstrate that polymorphisms in the *IL28B* locus are also predictive for SVR in genotype 2 (Table 2). We also showed that the favorable *IL28B* SNP genotype is associated with a rapid decrease in HCV RNA levels, which is itself a predictive factor for SVR [42]. Several studies have reported that polymorphisms at the *IL28B* locus affect the outcome of peg-interferon and ribavirin combination therapy in patients with HCV genotype 1b [34–36,38]. In particular, associations with therapy outcome have been reported for two SNPs in strong linkage disequilibrium, rs8099917 (T/G), and rs12979860 (C/T). Only a few studies have examined the effect of the SNP on the treatment outcome for other genotypes. Rallón et al. reported that the rs12979860 genotype is associated with treatment outcome for genotypes 1 and 4 but not genotype 3 in patients with HIV/HCV co-infection [43]. Similarly Rauch et al. reported an association between rs8099917 polymorphism and NVR for genotypes 1 and 4 (difficult-to-treat) but not for genotypes 2 and 3 (easier-to-treat) but the effect due to genotype 2 alone is unclear [38]. In a recent study, Mangia et al. also exam-

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Table 3. Predictors for SVR in treatment-naïve patients treated with IFN monotherapy.

		Simple			Multiple			
Genotype	Variable	n	p		n	OR	(95% CI)	p	
2a + 2b	Age	559	0.35						
	Sex	559	0.17						
	Genotype	559	0.068						
	Viral load	507	0.0002	***	506	0.59	(0.45-0.77)	0.0001	***
	Fibrosis	450	0.61						
	rs8099917	558	0.001	**	506	0.52	(0.33-0.82)	0.005	**
	rs12980275	558	0.009	**					
2a	Age	477	0.19						
	Sex	477	0.2						
	Viral load	425	0.001	**	424	0.6	(0.44-0.81)	0.001	***
	Fibrosis	382	0.37						
	rs8099917	476	0.003	**	424	0.53	(0.32-0.88)	0.014	*
	rs12980275	476	0.01	**					
2b	Age	82	0.67						
	Sex	82	0.56						
	Viral load	82	0.47						
	Fibrosis	68	0.53						
	rs8099917	82	0.19						
	rs12980275	82	0.44						

*p <0.05; **p <0.01; ***p <0.001.

ined genotypes 2 and 3 and found a significant association between rs12979860 genotype and rapid virological response (RVR) at week 4 for genotype 2 [44]. While rs12979860 was not directly associated with SVR in their study, rs12979860 genotype was significantly associated with SVR among those patients who failed to achieve RVR. In this study, we found a significant association between rs8099917 genotype and RVR in multivariate analysis for genotype 2b ($p = 0.028$, data not shown) but not for genotype 2a. When RVR was included as a factor in multivariate logistic regression analysis for genotype 2b, RVR and rs8099917 genotype were both retained in the final model but only RVR was significant (RVR: $p = 4.9e\text{-}05$; rs8099917: $p = 0.0850$; data not shown). When only non-RVR patients were included, no factors were significant; however, there were only six patients who achieved SVR without RVR and only one patient who achieved RVR but then failed to achieve SVR.

Although SVR rate was generally higher for genotype 2a, as reported previously [20,21], we found few differences between genotypes 2a and 2b. However, when analyzed separately, the results suggest an interesting interaction between the *IL28B* genotype, the viral genotype, and treatment type. In particular, we found that rs8099917 was a predictive factor for genotype 2a treated with IFN but not PEG-RBV, and conversely for genotype 2b treated with PEG-RBV but not IFN. This result is likely due to the relatively small sample sizes, but nonetheless all 8 (100%) of the genotype 2a PEG-RBV patients lacking the favorable rs8099917 genotype achieved SVR, compared to less than 50% for IFN therapy or either type of treatment with genotype 2b. In fact, each patient was heterozygous for each of the three *IL28B* SNPs examined. A further complication is that each of the five patients who developed resistance to interferon therapy was infected with genotype 2a, and two of these patients had the favorable rs8099917 TT genotype while the others were heterozygous (GT). More detailed analysis will be required to interpret these results.

Because PEG-RBV therapy was not covered by insurance in Japan until 2005, we also present data comparing the effects of *IL28B* polymorphisms on treatment with the older IFN monotherapy versus the more recent PEG-RBV combination therapy. Although the small sample sizes within each patient group likely underestimate the effect of SNP genotype, we found that rs8099917 influences response to IFN monotherapy in patients with genotype 2a and also influences the response to PEG-RBV therapy in patients with genotype 2b. Although PEG-RBV is currently the standard treatment for chronic hepatitis C infection, interferon monotherapy may still be used in the case of intolerance to ribavirin; therefore, it is important to understand the direct effects of interferon with and without ribavirin. Moreover, even with the advent of protease inhibitors and other antiviral drugs undergoing clinical trials, they are likely to be co-administered with interferon to prevent the otherwise rapid emergence of resistant quasispecies [45].

In summary, we showed that the *IL28B* SNP genotype is an important predictive factor for SVR and early viral dynamics in patients with HCV genotypes 2a and 2b.

Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.