

Table 2 Predictors for a sustained virological response

Variable	Simple			Multiple			
	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 ($\times 10^4/L$)	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**				
γ GTP (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; γ GTP, 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

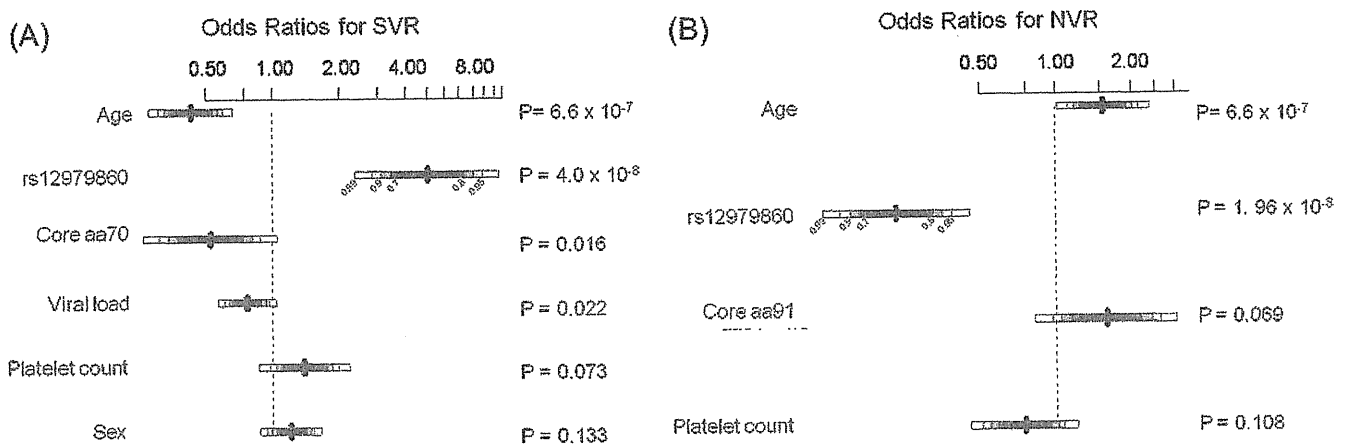


Figure 1 ORs for predictive factors response to treatment. ORs and 95% CIs are shown for predictive factors for (A) sustained virological response (SVR) and (B) non-virological response (NVR) based on multiple logistic regression with stepwise selection.

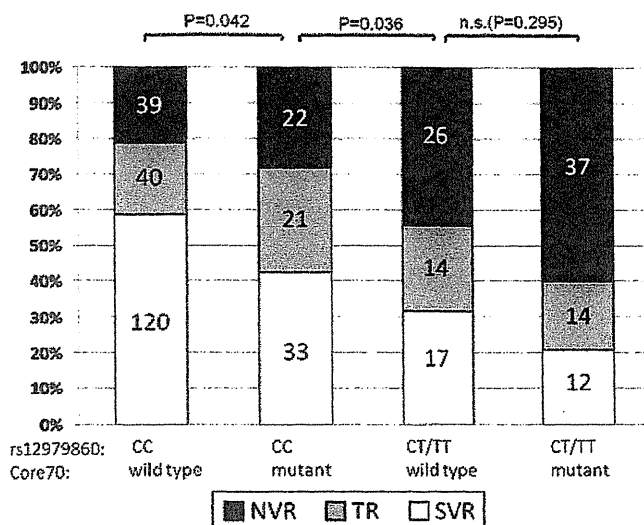


Figure 2 Cumulative effects of rs12979860 genotype and core protein amino acid 70 substitutions. The relative effects of rs12979860 genotype (favourable CC vs non-favourable CT/CC) and core amino acid 70 substitutions (favourable wild type vs unfavourable substitutions) on response to treatment are shown. 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- κ B (NF- κ B)-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

Variable	Simple			Multiple			
	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				
γ GTP (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; γ GTP, γ -glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

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

Variable	Simple			Multiple		
	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 ($\times 10^4/L$)	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*			
γ GTP (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; γ GTP, γ -glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

strain. Nonetheless, the NS5A protein has been shown to be under purifying selection⁴⁴ 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

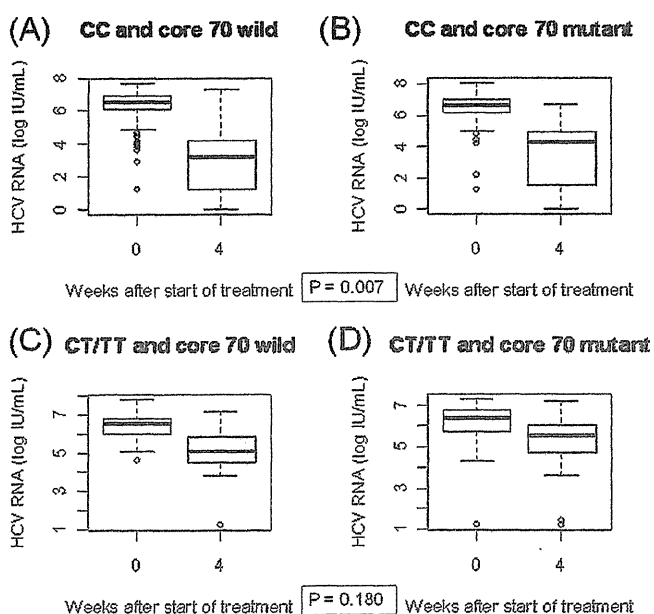


Figure 3 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and hepatitis C virus (HCV) core protein substitutions. The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and wild/mutant amino acid at core70 is shown.

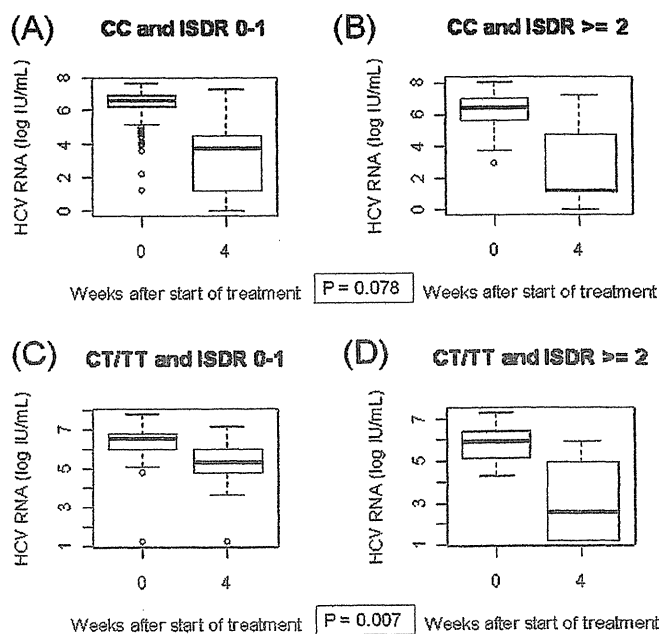


Figure 4 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and substitutions in the interferon sensitivity-determining region (ISDR). The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and the number of substitutions in the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

Acknowledgements We thank Mika Tsuzuno, Sakura Akamatsu, Sanae Furuya and other members of the clerical staff, and Yoshiiku Kawakami and other physicians at Hiroshima University Hospital for their help.

Funding This work was supported in part by Grants-in-Aid for scientific research and development from the Ministry of Health, Labor and Welfare, Government of Japan.

Competing interests None.

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

Provenance and peer review Not commissioned; externally peer reviewed.

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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.

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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

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

Received 12 March 2010; received in revised form 1 July 2010; accepted 2 July 2010; available online 19 September 2010

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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.



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 mono-

therapy 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.

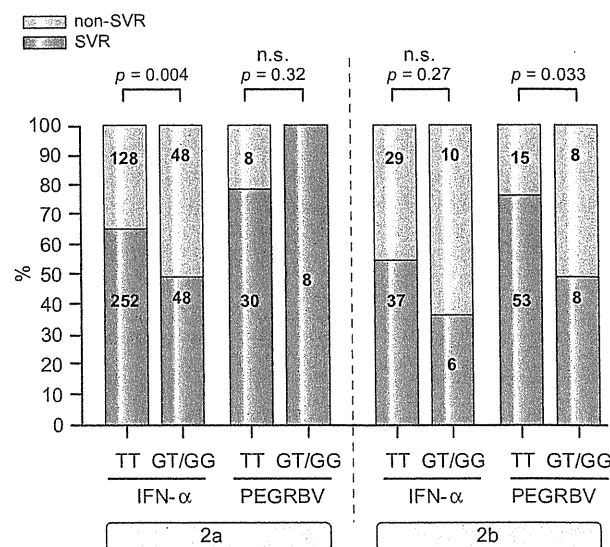


Fig. 1. Effect of interferon therapy on patients with genotype 2a and 2b infection. Sustained viral responders (SVR) and non-responders (non-SVR) were analyzed by *IL28B* SNP rs8099917 genotype, viral genotype, and treatment type. All patients were interferon-naïve.

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

Genotype	Variable	Simple		Multiple			
		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

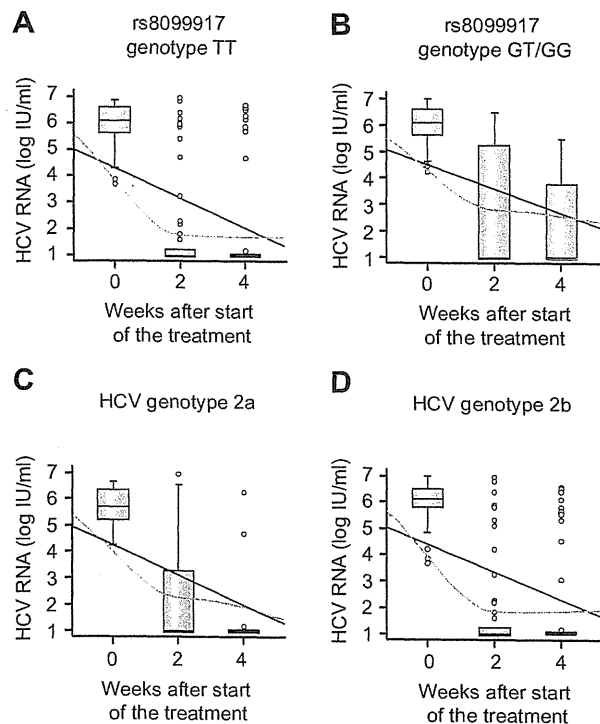


Fig. 2. Effect of rs8099917 genotype and HCV genotype on change in HCV RNA levels. HCV RNA levels at 0, 2, and 4 weeks after the start of peg-interferon plus ribavirin combination therapy in treatment-naïve patients. (A and B) Change in viral load for patients with the protective TT genotype for rs8099917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

Genotype	Variable	Simple		Multiple			
		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 **
2a	rs12980275	558	0.009 **				
	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 *
2b	rs12980275	476	0.01 **				
	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: $p = 4.9 \times 10^{-5}$; 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.

Table 4. Comparison of predictive factors for SVR based on prior treatment with interferon.

	Variable	Simple		Multiple			
		n	p	n	OR	(95% CI)	p
All	Age	719	0.70				
	Sex	719	0.28				
	Genotype	719	0.42				
	Viral load	663	6.00E-02 ***	662	0.63	(0.51-0.79)	4.30E-05 ***
	Fibrosis	585	0.83				
	rs8099917	718	0.002 **	662	0.57	(0.38-0.85)	0.0055 **
	rs12980275	717	0.03 *				
	Treatment	719	0.054				
Naive	Age	689	0.58				
	Sex	689	0.18				
	Genotype	689	0.62				
	Viral load	634	0.0011 **	633	0.53	(0.41-0.69)	2.00E-06 ***
	Fibrosis	560	0.95				
	rs8099917	688	0.00059 ***	633	0.5	(0.33-0.77)	0.0015 **
	rs12980275	687	0.013 *				
	Treatment	689	0.0013 **	633	3.01	(1.82-4.99)	1.80E-05 ***
Experienced	Age	30	0.91				
	Sex	30	0.75				
	Genotype	30	0.14				
	Viral load	29	0.032 *	29	0.21	(0.05-0.87)	0.032 *
	Fibrosis	25	0.53				
	rs8099917	30	0.12				
	rs12980275	30	0.1				
	Treatment	30	N/A				

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

Acknowledgments

This work was supported in part by Grants-in-Aid for scientific research and development from the Ministry of Health, Labor and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology, of the Government of Japan. Part of this work was carried out at the Analysis Center of Life Science, Hiroshima University. The authors thank Yasufumi Hayashida, Rie Akiyama, Yoshie Yoshida, Kazuyo Hattori, Mariko Shiota, Hiromi Ishino, and Takako Yokogi for excellent technical assistance, and Yuko Nagai, Junko Sakamiya and Aya Furukawa for clerical assistance.

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IL28B But Not ITPA Polymorphism Is Predictive of Response to Pegylated Interferon, Ribavirin, and Telaprevir Triple Therapy in Patients With Genotype 1 Hepatitis C

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Background. Pegylated interferon, ribavirin, and telaprevir triple therapy is a new strategy expected to eradicate the hepatitis C virus (HCV) even in patients infected with difficult-to-treat genotype 1 strains, although adverse effects, such as anemia and rash, are frequent.

Methods. We assessed efficacy and predictive factors for sustained virological response (SVR) for triple therapy in 94 Japanese patients with HCV genotype 1. We included recently identified predictive factors, such as IL28B and ITPA polymorphism, and substitutions in the HCV core and NS5A proteins.

Results. Patients treated with triple therapy achieved comparatively high SVR rates (73%), especially among treatment-naïve patients (80%). Of note, however, patients who experienced relapse during prior pegylated interferon plus ribavirin combination therapy were highly likely to achieve SVR while receiving triple therapy (93%); conversely, prior nonresponders were much less likely to respond to triple therapy (32%). In addition to prior treatment response, IL28B SNP genotype and rapid viral response were significant independent predictors for SVR. Patients with the anemia-susceptible ITPA SNP rs1127354 genotype typically required ribavirin dose reduction earlier than did patients with other genotypes.

Conclusions. Analysis of predictive factors identified IL28B SNP, rapid viral response, and transient response to previous therapy as significant independent predictors of SVR after triple therapy.

Hepatitis C virus (HCV) establishes a chronic infection in 80% of infected individuals, and currently, >100 million persons are chronically infected and at increased risk of cirrhosis, hepatocellular carcinoma, and end-stage

liver disease [1–3]. The current standard of care is combination treatment with pegylated interferon (PEG-IFN) and ribavirin, but this costly and poorly tolerated treatment achieves sustained virological response in only 50% of patients [4]. Options are limited in the event of treatment failure, and alternative therapies are needed.

Of the many drugs under investigation, the most promising are the direct-acting antiviral agents, which directly target essential aspects of viral replication, including internal ribosome entry site inhibitors, protease and polymerase inhibitors, and assembly inhibitors [5]. Several protease inhibitors, including telaprevir and boceprevir, are in phase III clinical trials and will likely become the first direct-acting antiviral agents approved for clinical use [6].

Received 25 November 2010; accepted 11 February 2011.

Potential conflicts of interest: none reported.

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The Journal of Infectious Diseases 2011;204:84–93

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0022-1899 (print)/1537-6613 (online)/2011/2041-0013\$14.00

DOI: 10.1093/infdis/jir210

The HCV genome is initially translated as a large polyprotein and must be processed to produce functional viral proteins. Host proteases cleave the N-terminal structural proteins, but the viral NS3-4A serine protease is essential for cleaving the non-structural proteins. NS3-4A also interferes with the immune response by degrading immune-signaling molecules [7]. Consequently, targeting this protease using the peptidomimetic inhibitor telaprevir both interferes with viral replication and may help rescue immune signaling, leading to a rapid decrease in HCV RNA level [8, 9]. In most patients, however, viral decline after telaprevir monotherapy is short-lived, followed by viral breakthrough because of strong selection for escape mutants within several weeks. Combination therapy with IFN alone yields unsatisfactory results, and ribavirin appears to be required to avoid relapse [10]. Because telaprevir triple therapy is an extension of the current standard of care instead of an IFN-free alternative, it does not address problems associated with the cost or adverse effects of combination therapy and may limit options for retreatment; however, it is particularly promising for patients who showed at least a transient response after prior combination therapy [11]. Nonetheless, telaprevir monotherapy may provide an alternative treatment for patients unable to tolerate IFN and/or ribavirin—at least in patients with low viral loads [12]. Additional research is needed to identify factors predicting outcome of treatment and incidence of adverse effects in different populations.

A number of host factors are known to affect outcome of PEG-IFN plus ribavirin combination therapy, including age, fibrosis, obesity, hepatic steatosis, [13] low-density lipoprotein cholesterol, γ -glutamyl transpeptidase (GTP) [14], and insulin resistance [15]. A number of recent studies have also shown that common genetic variation in the IL28B locus on chromosome 19 is strongly associated with spontaneous clearance and outcome after combination therapy [16–19]. Viral factors have also been shown to predict response to combination therapy, including HCV genotype [20], baseline viral titer [13, 20], amino acid substitutions at positions 70 and 91 of the HCV core protein, and the NSSA IFN Sensitivity Determining Region (ISDR) [21, 22]. Because telaprevir directly targets the virus and often results in selection for escape mutants, it is likely that additional predictive factors affecting response to treatment will be uncovered.

Combination therapy is poorly tolerated among some patients, and ribavirin-induced anemia is a serious adverse effect of the therapy that may result in dose reduction or discontinuation. Recent studies have shown an association between genetic variation in the ITPA locus and change in hemoglobin levels during treatment [23–25]. Although it does not appear to affect outcome of therapy [23, 24] (but see [25]), patients with an anemia-susceptible genotype may require greater reductions in ribavirin dose, which is associated with poorer response to therapy [26]. Telaprevir also moderately affects hemoglobin levels, but rash is the most common side effect of telaprevir therapy [10].

In the current study, we examined 94 patients with genotype 1 who received triple therapy to identify predictors for response to treatment and to assess effects of triple therapy on hemoglobin levels.

METHODS

Patients

Ninety-four Japanese patients who participated in a phase 3 clinical trial of the triple therapy in 2010 at Hiroshima University Hospital, Sapporo Kosei Hospital, and Toranomon Hospital (16, 17, and 61 patients, respectively) were investigated. Inclusion criteria for the study included remaining positive for genotype 1 HCV RNA for >6 months; having an HCV RNA level ≥ 5.0 log IU/mL, as determined by the COBAS TaqMan HCV test (Roche Diagnostics KK); and being aged 20–65 years, with a body weight >40 kg and <120 kg at the time of entry into the study. Exclusion criteria included cirrhosis; results positive for hepatitis B surface antigen or antibody against HIV; previous or current hepatocellular carcinoma; possible overlapping liver diseases, such as autoimmune hepatitis, hemochromatosis, Wilson disease, alcoholic liver disease, or renal disease; or creatinine clearance ≤ 50 mL/min at baseline, hemoglobin level <12 g/dL, neutrophil count <1500 neutrophils/mm³, or platelet count <100,000 platelets/mm³ at baseline. Patient profiles are shown in Tables 1 and 2.

All patients were treated with PEG-IFN- α -2b, ribavirin, and telaprevir triple therapy. Telaprevir (750 mg; MP-424; Mitsubishi Tanabe Pharma) was administered every 8 h after meals. PEG-IFN- α -2b (Schering Plough) was injected subcutaneously at a median dose of 1.5 μ g/kg per week. Ribavirin (Schering Plough) dose was adjusted by body weight (600 mg for ≤ 60 kg; 800 mg for >60 to ≤ 80 kg; and 1000 mg for >80 kg), based on guidelines by the Ministry of Health, Labor and Welfare of Japan [27], and the drug was administered orally after breakfast and dinner. Triple therapy with telaprevir was given for 12 weeks, followed by an additional 12 weeks of PEG-IFN- α -2b and ribavirin combination therapy. Triple therapy was withdrawn if hemoglobin levels were <8.5 g/dL. Ribavirin dose was reduced by 200 mg/day in patients who were receiving 600 or 800 mg/day (or by 400 mg in those receiving 1000 mg/day) when hemoglobin levels decreased to <12 g/dL and by an additional 200 mg if levels decreased to <10 g/dL. In addition, ribavirin dose was also reduced by 200 mg in patients with a hemoglobin level <13 g/dL at baseline and in those in whom the level decreased by 1 g/dL to <13 g/dL within 1 week. PEG-IFN dose was decreased to one-half when leukocyte count decreased to <1500 leukocytes/mm³, neutrophil count decreased to <750 neutrophils/mm³, or platelet count decreased to <80 $\times 10^3$ platelets/mm³; PEG-IFN was withdrawn if these factors decreased to <1000 leukocytes/mm³, 500 neutrophils/mm³, or 50 $\times 10^3$ platelets/mm³, respectively. Triple therapy was suspended temporarily when

Table 1. Patient Characteristics

	Total (n = 94)	SVR (n = 69)	Non-SVR (n = 25)
Response to previous therapy (naive/relapser/NR)	25/44/25	20/41/8	5/3/17
Age	57 (23–65)	57 (23–65)	56 (40–65)
Sex (M/F)	52/42	42/27	10/15
Height (cm)	163.6 (141.8–189.2)	164.7 (141.8–189.2)	157.7 (148.5–181.5)
Weight (kg)	61 (41–92.5)	61.7 (41–92.5)	58.8 (44.9–80.3)
rs8099917 (TT/TG/GG)	50/41/3	47/21/1	3/20/2
rs1127354 (CC/CA/AA)	75/18/1	55/13/1	20/5/0
Viral genotype (1b/others)	93/1	69/0	24/1
Core 70 (W/M/ND)	50/43/1	43/26/0	7/17/1
Core 91 (W/M/ND)	48/45/1	39/30/0	9/15/1
ISDR (0–1/≥2/ND)	82/8/4	61/5/3	3/21/1
WBC (/mm ³)	4800 (2800–8100)	4900 (2800–8100)	4660 (3000–7900)
Plt (×10 ⁴ /mm ³)	17.7 (9.1–33.8)	18 (9.9–33.8)	16 (9.1–23.9)
Hb (g/dL)	14.3 (12.3–16.6)	14.5 (12.5–16.5)	14.1 (12.3–16.6)
ALT (IU/L)	39 (12–302)	38 (12–302)	46 (17–135)
γGTP (IU/L)	36 (11–233)	33 (11–233)	53 (19–226)
Virus titer (log IU/mL)	6.7 (5.1–7.7)	6.8 (5.1–7.7)	6.7 (5.4–7.6)
Days to first ribavirin reduction	17 (2–168)	18 (2–168)	14 (7–73)
Duration of telaprevir administration (days)	85 (29–85)	85 (29–85)	84 (35–85)
Duration of peg-interferon injection (days)	162 (22–165)	162 (22–165)	162 (30–165)
Duration of ribavirin administration (days)	169 (29–169)	169 (29–169)	168 (36–169)
Effect of therapy (SVR/BT/TR/NR)	69/4/19/2	–	–

NOTE. All patients were infected with genotype 1. Counts are listed for categorical values and the median and range are reported for continuous variables. ND, not determined, data unavailable.

hemoglobin levels decreased to <8.5 g/dL. Treatment was resumed with PEG-IFN and 200 mg ribavirin if hemoglobin levels increased to ≥8.5 g/dL within 2 weeks after withdrawal. Reduction of telaprevir dose was not permitted. It was discontinued if severe adverse effects appeared, and therapy was continued with PEG-IFN and ribavirin alone. Erythropoietin was not used to elevate hemoglobin levels.

Virologic response was analyzed on an intent-to-treat basis. The successful end point of treatment was sustained virological response (SVR) for patients who showed undetectable HCV RNA for 24 weeks after cessation of treatment. In transient responders (or persons who experienced relapse), HCV RNA levels became undetectable by the end of treatment but became positive again during the follow-up period. In patients with viral breakthrough, HCV RNA became undetectable during the treatment period but then became positive again before the end of the treatment period. The remaining patients whose HCV RNA never became undetectable were nonresponders. We also defined rapid virological response (RVR) as undetectable HCV RNA at week 4 of treatment and early virological response as a >2 log₁₀ decrease in HCV RNA levels by week 12 of treatment. All participants 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 measured using the TaqMan reverse-transcription polymerase chain reaction (PCR) test. The measurement range of this assay was 1.2–7.8 log IU/mL. Samples that exceeded the measurement range were diluted with phosphate-buffered saline and reanalyzed.

ISDR and Core Amino Acid Substitutions

Amino acid substitutions in the HCV core and ISDR regions were determined using direct sequencing of PCR products after extraction and reverse transcription of HCV RNA with use of serum samples kept frozen at –80°C. Core amino acid substitutions at positions 70 and 91 (core70 and core91, respectively) were determined according to Akuta et al [14, 28], and the number of ISDR substitutions was determined using the methods of Enomoto et al [21, 29, 30].

Single-Nucleotide Polymorphism (SNP) Genotyping

We genotyped each patient for 2 SNPs: rs8099917, an IL28B SNP previously reported to be associated with therapy outcome, and rs1127354 [31], an ITPA SNP reported to be associated with ribavirin-induced anemia [23]. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or with the Invader or TaqMan assay, as described elsewhere [32, 33].

Table 2. Patient Characteristics Grouped by Treatment History

	Total (n = 94)	Naive (n = 25)	Relapser (n = 44)	NR (n = 25)
Age	56.5 (23–65)	54 (23–64)	57.5 (44–65)	57 (40–65)
Sex (M/F)	52/42	13/12	27/17	12/13
Height (cm)	163.5 (142–189)	163 (147–189)	167.5 (142–177)	160 (149–174)
Weight (kg)	61 (41–93)	57 (42–80)	63.5 (41–93)	59 (45–77)
rs8099917 (TT/GT/GG)	50/41/3	15/9/1	33/11/0	2/21/2
rs1127354 (CC/CA/AA)	75/18/1	18/6/1	34/10/0	23/2/0
Viral genotype (1b/others)	93/1	25/0	44/0	24/1
Core 70 (W/M/ND)	50/43/1	13/12/0	28/16/0	9/15/1
Core 91 (W/M/ND)	48/45/1	14/11/0	23/21/0	11/13/1
ISDR (0–1/≥2/ND)	82/8/4	25/0/0	38/4/2	19/4/2
WBC (/mm ³)	4800 (2800–8100)	5390 (3000–7500)	4750 (2800–8100)	4700 (3040–8000)
Plt (×10 ⁴ /mm ³)	18 (9–34)	20 (15–30)	16.5 (10–34)	16 (9–24)
Hb (g/dL)	14.3 (12.3–17)	14.1 (12.5–16.1)	14.45 (12.3–17)	14.4 (12.3–16.6)
ALT (IU/L)	38.5 (12–302)	35 (12–113)	39.5 (16–302)	45 (17–135)
γGTP (IU/L)	36 (11–233)	31 (11–141)	34 (14–233)	49 (21–226)
Virus titer (log IU/mL)	6.7 (5.1–7.7)	6.7 (5.1–7.4)	6.7 (5.4–7.6)	6.7 (5.8–7.7)
Days to first ribavirin reduction	18 (3–168)	18 (3–52)	18 (3–168)	15 (8–52)
Duration of telaprevir administration (days)	85 (29–85)	85 (29–85)	85 (32–85)	85 (35–85)
Duration of peg-interferon injection (days)	162 (22–165)	163 (22–165)	162.5 (30–165)	162 (30–165)
Duration of ribavirin administration (days)	169 (29–169)	168 (29–169)	169 (32–169)	169 (36–169)
Effect of therapy (SVR/BT/TR/NR)	69/4/19/2	20/0/5/0	41/1/2/0	8/3/1/2/2

NOTE. Counts are listed for categorical values and the median and range are reported for continuous variables.

Statistical Analysis

Statistical analysis was performed using PASW Statistics, version 18 (SPSS) and R, version 2.11. Categorical data were analyzed using χ^2 and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann-Whitney *U* test. To identify independent predictive factors, variables that were significant at the .05 level in univariate tests were considered as candidate factors for multiple logistic regression analysis. The model was reduced using AIC-based forward and/or backward stepwise selection with bootstrap validation. Odds ratios (ORs) were corrected for over-optimism with use of penalized maximum likelihood.

RESULTS

Effect of the Triple Therapy by Previous Response to PEG-IFN Plus Ribavirin Therapy

Patient profiles are shown in Tables 1 and 2. After triple therapy, 69 (73%) of 94 patients achieved SVR. Of the 25 treatment-naïve patients, 20 (80%) eradicated the virus, and the remaining 5 achieved transient response. Similarly, 49 (71%) of the 69 patients who had received prior treatment achieved SVR with triple therapy. Of note, however, 41 (93%) of 44 patients who had responded transiently to previous treatment were able to eradicate the virus with use of triple therapy. Conversely, only 8 (32%) of 25 patients who had failed to respond to prior treatment were able to achieve SVR with use of triple therapy,

and 2 of these patients also failed to respond to triple therapy. None of the 4 patients in whom viral breakthrough occurred were treatment naïve, and 3 of the 4 were nonresponders to prior treatment.

IL28B SNP Genotypes

The genotype of IL28B SNP rs8099917 was determined for each patient. The frequency of the rs8099917 risk allele (G) was 0.25 among all patients, 0.17 among patients who achieved SVR, 0.38 among patients with viral breakthrough, and 0.5 among both transient responders and nonresponders. Patients with the rs8099917 TT genotype were significantly more likely to achieve SVR (94% vs 50%; $P = 4.6E-6$; Figure 1) and had significantly higher baseline viral loads (6.9 vs 6.45 log IU/mL; $P = .0056$; Figure 2D), compared with patients with GT or GG genotypes.

Loss of Hemoglobin During and After Triple Therapy

The triple therapy resulted in hemoglobin loss in all patients, but the pattern differed by ITPA SNP rs1127354 genotype (Figure 3). The frequency of the rs1127354 minor allele (A) was 0.11 among all patients, 0.11 among patients who achieved SVR, .13 among transient responders, and 0 in both patients with viral breakthrough and nonresponders. There was no effect of rs1127354 genotype on SVR (73% for both CC and non-CC genotypes), but ribavirin dosage reduction was required significantly earlier in patients with genotype CC than in those with non-CC genotypes (18 days vs 29 days, respectively; $P = 3.2E-5$; Figure 4). Although hemoglobin loss

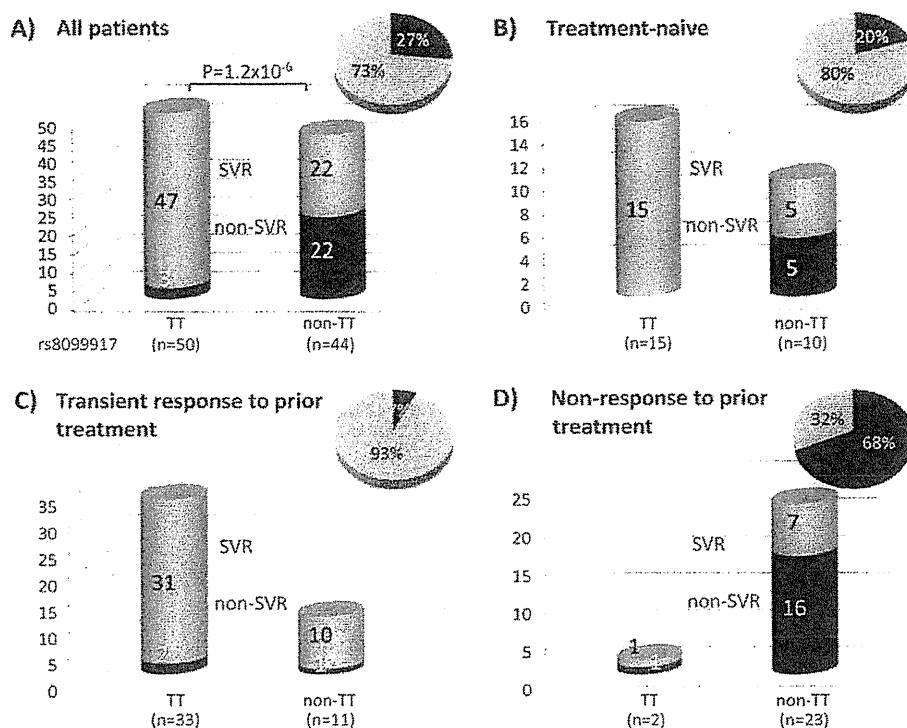


Figure 1. SVR frequency after triple therapy grouped by IL128B SNP rs8099917 genotype and by response to previous interferon (IFN) treatment. *A*, All patients. *B*, Treatment-naive patients. *C*, Previously treated patients who responded transiently to therapy. *D*, Previously treated patients who failed to respond to therapy. Inset pie charts indicate percentage of SVR (light gray) and non-SVR (dark gray) patients.

resulted in dose reduction according to the treatment protocol, no significant effects on SVR rate resulting from dose reduction were observed.

Viral Substitutions

The 43 patients (46%) with a substitution at position 70 of the HCV core protein (core70) were significantly less likely to achieve SVR than were patients with wild-type core70 (60% vs 86%; $P = .01$). There was no difference in SVR rate due to substitution at position 91 (core91; 81% vs 67%; $P = .17$) (Figure 2). There was also no difference in SVR rate due to substitutions in the NS5A ISDR region ($P = .43$). Patients with rs8099917 genotype TT were significantly more likely to be associated with wild-type core70 or core91 ($P = .006$ and $P = .031$, respectively). There was no association between rs809917 genotype and ISDR substitutions ($P = .94$).

Predictive Factors for RVR

RVR, defined as undetectable HCV RNA levels at week 4 of treatment, is a strong on-treatment predictor of SVR [34]. Previous IFN treatment, time to first ribavirin dose reduction, and baseline hemoglobin levels were each significant univariate predictors, but only hemoglobin level was a significant independent predictor of RVR under multiple logistic regression ($P = .028$; OR, 3.11).

Predictive Factors for SVR

Significant univariate predictors for SVR included clinical factors (γ GTP level; rs8099917 genotype), viral factors (core70 substitutions), response to prior treatment (relapse or non-response), and on-treatment factors (RVR) (Table 3). Of these, nonresponse to prior treatment, rs8099917 genotype, RVR, and core70 substitutions were retained in the multivariate model, and nonresponse to prior treatment (OR, .17; $P = .01$), rs8099917 genotype (OR, .12; $P = .014$), and RVR (OR, 14.0; $P = .0064$) were identified as significant independent predictors for SVR. When only pretreatment factors were considered, nonresponse to prior treatment (OR, .14; $P = .0028$) and rs8099917 genotype (OR, .19; $P = .027$) were the only independent predictors.

DISCUSSION

This study showed that patients undergoing PEG-IFN, ribavirin, and telaprevir triple therapy for chronic hepatitis C genotype 1 infection achieve a higher SVR rate than typically expected under combination therapy alone in Japanese patients. Moreover, patients who showed transient response in previous treatment were more likely to achieve SVR after triple therapy, whereas nonresponders to prior treatment remained unlikely to eradicate the virus. Considering that telaprevir has a mode of

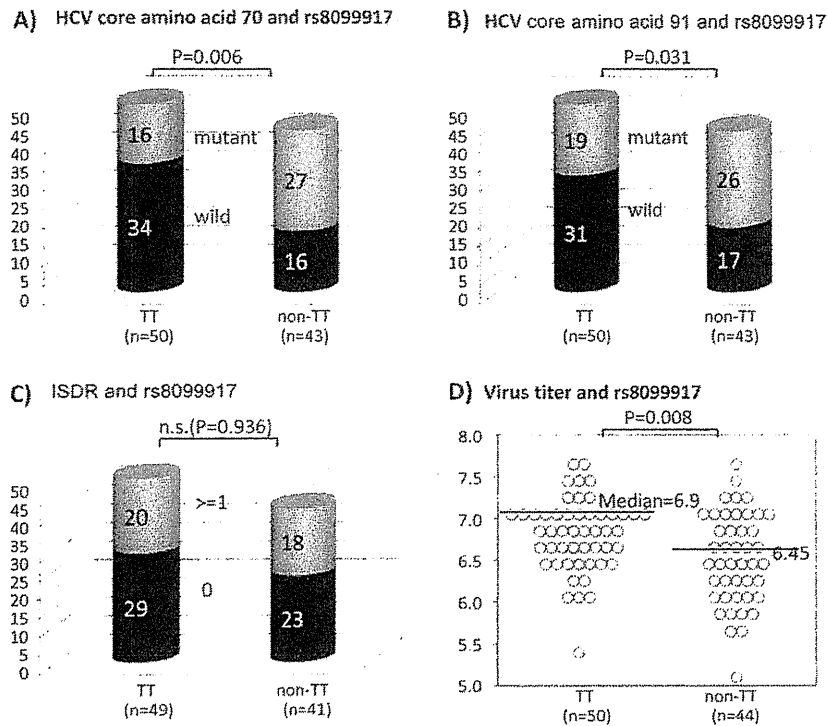


Figure 2. Viral factors and IL28B SNP rs8099917 genotype. *A*, Substitutions at HCV core amino acid 70. *B*, Substitutions at core amino acid 91. *C*, Frequency of patients with ≥ 2 substitutions in the NS5A interferon sensitivity determining region. *D*, Baseline viral load.

action different from that of IFN and ribavirin, [5] it is surprising that triple therapy does not better improve SVR rates among prior nonresponders, suggesting that additional unknown factors contribute to nonresponse. However, the duration of triple therapy, followed by standard of care, was

limited to 24 weeks in this study; therefore, it is possible that prior nonresponders and patients who experienced relapse may benefit from a longer duration of therapy.

The most interesting result from this study is the high SVR rate among patients who previously experienced relapse, even

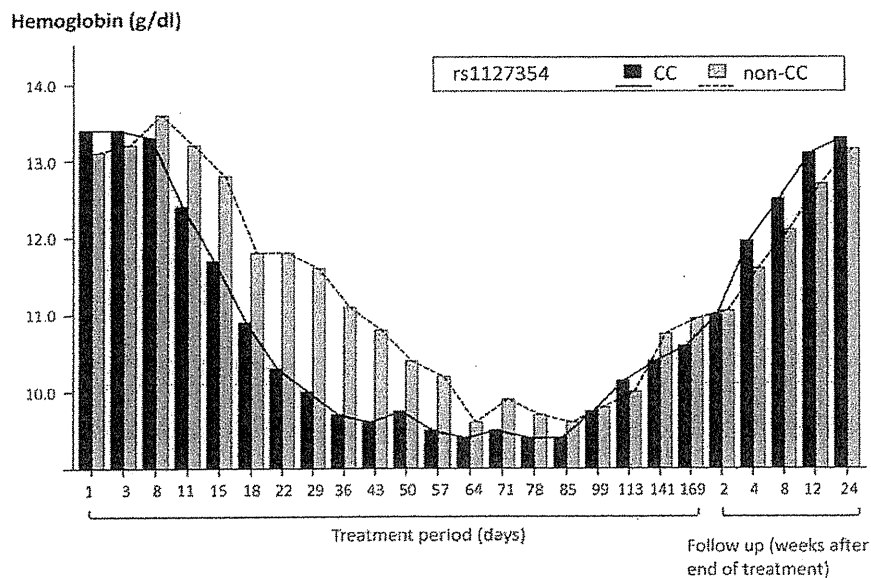


Figure 3. Change in hemoglobin level by ITPA SNP during triple therapy. Hemoglobin levels in patients grouped by ITPA SNP rs1127354 genotype (solid line represents CC; dashed line represents non-CC).

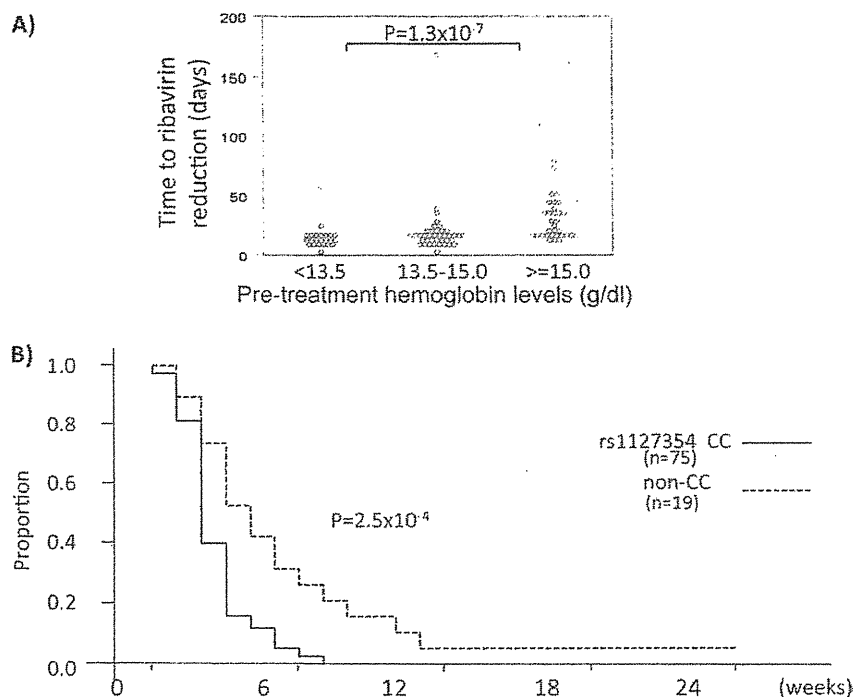


Figure 4. Ribavirin dose reduction during triple therapy. *A*, Number of days of treatment until first ribavirin dose reduction, by pretreatment hemoglobin levels. *B*, Kaplan-Meier curve for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line represents CC; dashed line represents non-CC).

compared with that of naive patients. This is partly because of the higher frequency of the favorable rs8099917 TT genotype among patients who previously experienced relapse (33 [75%] of 44) than among naive patients (15 [60%] of 25), which perhaps reflects the fact that all patients who previously experienced relapse demonstrated at least a transient response to combination therapy and that this group is less likely to include as many patients with non-TT genotypes. All of the treatment-naive patients with the favorable genotype (15 [100%] of 15) achieved SVR, compared with 31 (94%) of 33 patients who previously experienced relapse; conversely, only one-half of the treatment-naive patients with unfavorable rs8099917 genotypes (5 [50%] of 10) achieved SVR, compared with only 1 (9%) of 11 of the patients who previously experienced relapse. This suggests that, although patients who previously experienced relapse have a demonstrated potential to respond to the therapy, there should be more variability among naive patients. Another consideration is that the frequency of the favorable wild-type core70 amino acid was slightly higher among patients who previously experienced relapse (28 [64%] of 44) than among naive patients (13 [52%] of 25). It should be noted, however, that the small number of patients in this study limits the conclusions that can be drawn, and results should be verified in a larger study, perhaps using stratified sampling based on patient background with regard to treatment history to establish more homogeneous patient populations.

In this and a number of other studies, variation in the IL28B locus remains the strongest predictor of SVR reported to date

[16–18, 35]. It is unclear which, if any, of the reported SNPs is the primary or functional SNP, but most studies report results for rs8099917 and/or rs12979860, which are under strong linkage disequilibrium in Japanese patients and fall within the intergenic region upstream of IL28B. Although the mechanism is unknown, IL28B and the other 2 members of the IFN- λ family, IL28A and IL29, code for type III IFNs, which are similar to type I IFNs but use a highly tissue-specific receptor [36, 37]. IFN-stimulated genes appear to be initially down-regulated in patients with the favorable rs8099917 TT genotype [38], which may help to prevent desensitization and promote maximal induction of IFN-stimulated genes, although mechanistic studies are needed to understand the connection between IL28B and SVR.

In addition to IL28B polymorphisms, a number of studies have reported that amino acid substitutions in the HCV core protein and the ISDR region of NS5A independently predict treatment outcome after combination therapy [14, 22, 28, 30], and these findings have recently been extended to triple therapy [39, 40]. In this study, substitution at core70 was significant in univariate tests and was selected for inclusion in the multivariate model, but it was not significant in multiple logistic regression. One reason for this may be that core substitutions were initially reported to be associated with nonresponse [22], whereas this study focused on SVR because of the very small number of nonresponders. Terms that are significant in univariate but not multivariate tests may be correlated with each

Table 3. Predictive Factors Associated With SVR in Chronic Hepatitis C Virus Genotype 1 Patients Who Received Pegylated Interferon/Ribavirin/Telaprevir Triple Therapy

Variable	n	Simple			Multiple			
		OR	P		OR	(95% CI)	P	
Treatment-naive	94	1.6	.389					
Previous non-responder	94	0.1	5.5E-08	***	0.17	(.04-.66)	.010	*
Previous relapser	94	10.7	5.2E-05	***				
Age	94	0.8	.939					
Sex (male vs female)	94	1.5	.100					
BMI (kg/m ²)	94	0.9	.558					
rs8099917 (TT vs GT/GG)	94	0.1	1.7E-06	***	0.12	(.02-.65)	.014	*
rs1127354 (CC vs AC/AA)	94	1.0	.980					
Core aa70 (wt vs mutant)	93	0.2	.0053	**	0.35	(.09-1.31)	.119	
Core aa91 (wt vs mutant)	93	0.5	.111					
ISDR (0-1 vs ≥2)	90	1.7	.308					
Viral load	94	1.1	.560					
ALT (IU/L)	94	0.9	.142					
gammaGTP	94	0.7	.0009	***				
Hemoglobin (g/dL)	94	1.4	.292					
WBC (/mm ³)	94	1.3	.271					
Platelets (×10 ⁴ /mm ³)	94	1.7	.165					
Total cholesterol (mg/dL)	94	1.7	.160					
LDL cholesterol (mg/dL)	94	2.6	.018	*				
Days to first ribavirin dose reduction	94	1.2	.129					
RVR	94	10.8	4.4E-05	***	14.00	(2.10-93.2)	.006	**
EVR	94	7992.0	.004	**				

NOTE. Results of simple and multiple logistic regression are shown. The multivariate model was constructed using stepwise selection of univariate terms significant at the .05 level. Symbols: * ($P < .05$), ** ($P < .01$), *** ($P < .001$).

other, and only the factor with the strongest effect remains significant. In this case, core70 is significantly correlated with the stronger rs8099917 genotype ($r = .31$; $P = .0027$), although other studies have shown that these terms contribute independently, especially when a larger number of patients are included [39]. Without knowing the mechanism underlying either factor, it is not possible to determine whether the underlying factors that they represent are in fact independent or whether they represent different aspects of a common unknown factor.

Although novel therapies that are not based on IFN and ribavirin are urgently needed, the pending introduction of protease inhibitors represents a pivotal addition to the treatment arsenal, especially for patients who show at least partial response to combination therapy. Because telaprevir is effective as monotherapy, even if only briefly until resistant mutations emerge, alternate combination therapies based on telaprevir and another component designed to raise the barrier to resistance may provide an adequate alternative for older patients and patients unable to tolerate IFN or ribavirin. Furthermore, identification of additional SNPs associated with anemia and other adverse effects will help reduce complications and the need for dose reductions and may lead to treatment guidelines for at-risk

patients, such as administration of erythropoietin to stimulate erythropoiesis [41]. Ribavirin dose reductions were required significantly earlier in patients with ITPA SNP genotype CC, compared with patients with non-CC genotypes, which may contribute to poorer response if cumulative ribavirin administration decreases to <80% of the planned dose [26], although ribavirin dose reduction did not affect SVR rate in this study.

In conclusion, triple therapy with PEG-IFN, ribavirin, and telaprevir resulted in higher rates of SVR, compared with PEG-IFN plus ribavirin combination therapy, especially among treatment-naive patients and patients who showed transient response to prior treatment. ITPA polymorphisms predict ribavirin-induced anemia but are not associated with SVR, whereas IL28B polymorphisms and early viral kinetics remain the strongest predictors of SVR with use of triple therapy. Considering both host and viral factors, we identified 2 subgroups of patients who responded well to triple therapy: patients with the favorable rs8099917 TT genotype (47 [94%] of 50) and patients with non-TT genotypes who had wild-type core70 and core91 amino acids (7 [78%] of 9). Patients matching these conditions would benefit most from this 24-week triple therapy, whereas a longer duration of therapy should perhaps be considered for the remaining difficult-to-treat patients.

Funding

This work was supported by Grants-in-Aid for scientific research and development from the Ministry of Health, Labor and Welfare, Government of Japan.

Acknowledgments

We thank Mika Tsuzuno, Sakura Akamatsu, Sanae Furuya, and other members of the clerical and medical staff at Hiroshima University Hospital for their help.

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