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MAJOR ARTICLE

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-naive 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].

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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 nonstructural 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-gamma-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 NS5A 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/mm^3, or platelet count <100,000 platelets/mm^3 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 μg/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/mm^3, neutrophil count decreased to <750 neutrophils/mm^3, or platelet count decreased to <80 × 10^3 platelets/mm^3; PEG-IFN was withdrawn if these factors decreased to <1000 leukocytes/mm^3, 500 neutrophils/mm^3, or 50 × 10^3 platelets/mm^3, 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 $\log_{10}$ 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 ($\times 10^4$/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	8/3/12/2

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-naive 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 naive, 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

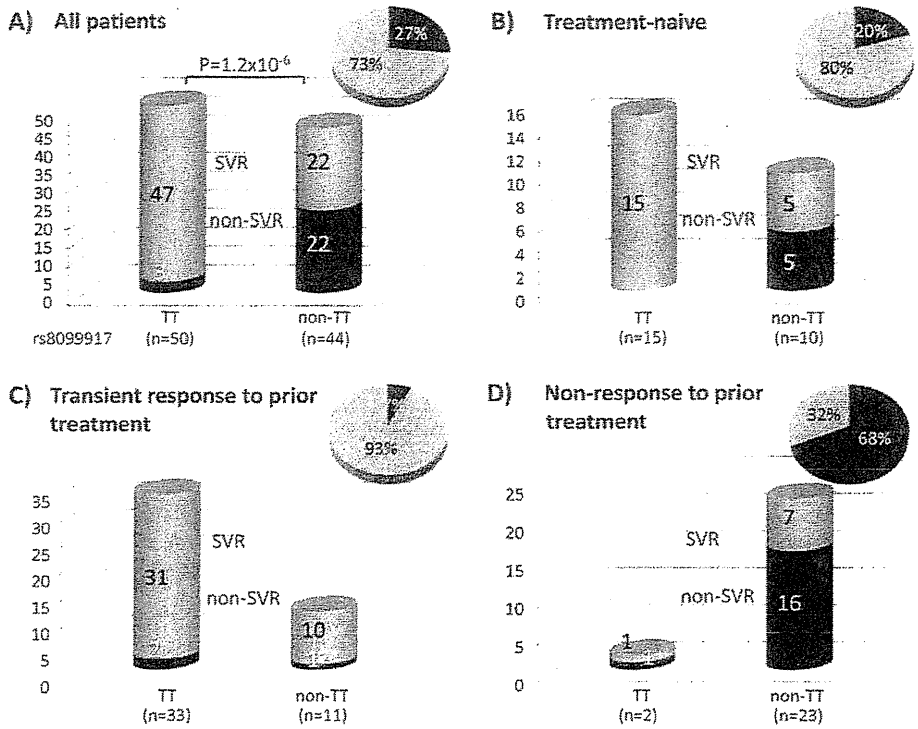

Figure 1. SVR frequency after triple therapy grouped by IL128B SNP rs8099917 genotype and by response to previous interferon (IFN) treatment. *A*, All patients. *B*, Treatment-naive patients. *C*, Previously treated patients who responded transiently to therapy. *D*, Previously treated patients who failed 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 (γGTP level; rs8099917 genotype), viral factors (core70 substitutions), response to prior treatment (relapse or nonresponse), 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

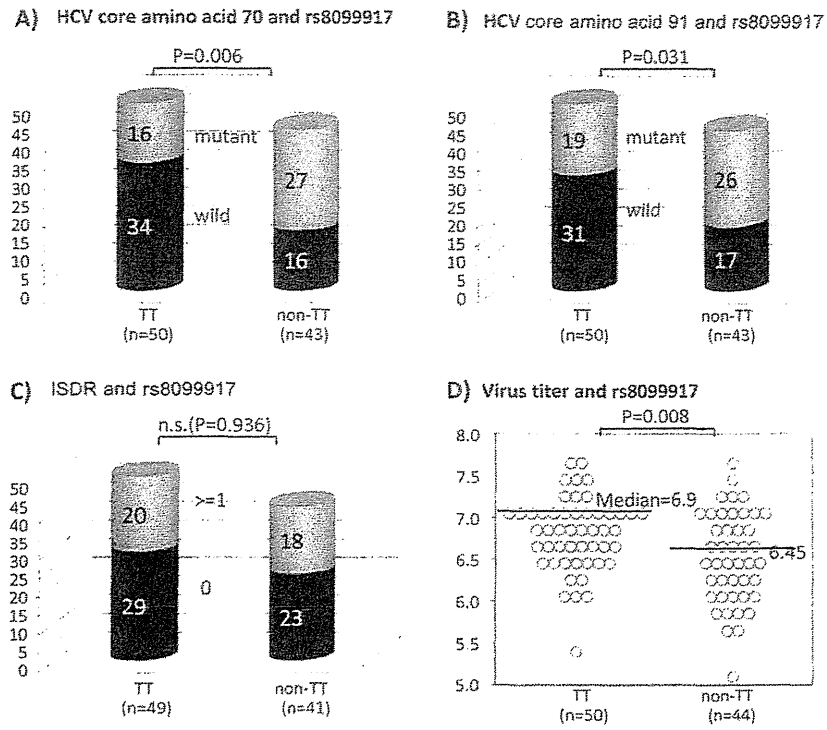

Figure 2. Viral factors and IL128B SNP rs8099917 genotype. *A*, Substitutions at HCV core amino acid 70. *B*, Substitutions at core amino acid 91. *C*, Frequency of patients with ≥2 substitutions in the NS5A interferon sensitivity determining region. *D*, Baseline viral load.

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

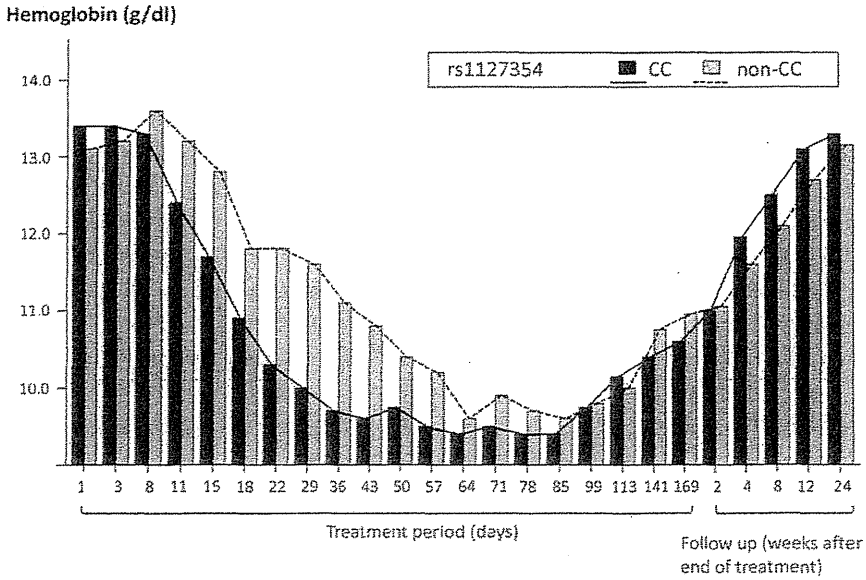

Figure 3. Change in hemoglobin level by ITPA SNP during triple therapy. Hemoglobin levels in patients grouped by ITPA SNP rs1127354 genotype (solid line represents CC; dashed line represents non-CC).

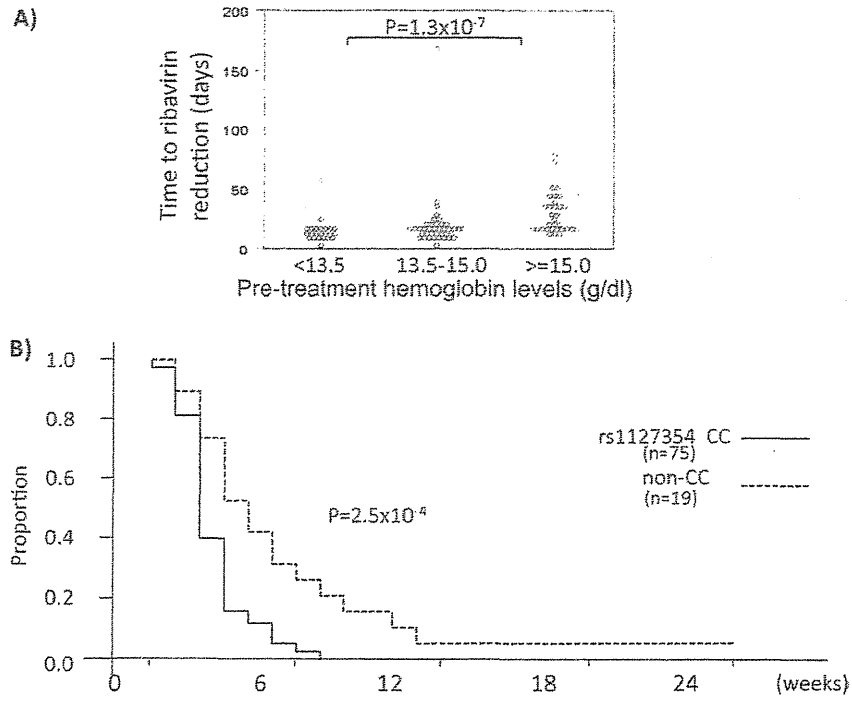

Figure 4. Ribavirin dose reduction during triple therapy. *A*, Number of days of treatment until first ribavirin dose reduction, by pretreatment hemoglobin levels. *B*, Kaplan-Meier curve for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line represents CC; dashed line represents non-CC).

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

	Simple				Multiple			
Variable	*n*	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/m2)	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 (×104/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.

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nature genetics

Variation in the *DEPDC5* locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers

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Chronic viral hepatitis is the most important risk factor for progression to hepatocellular carcinoma (HCC). To identify genetic risk factors for progression to HCC in individuals with chronic hepatitis C virus (HCV), we analyzed 467,538 SNPs in 212 Japanese individuals with chronic HCV with HCC and 765 individuals with chronic HCV without HCC. We identified one intronic SNP in the *DEPDC5* locus on chromosome 22 associated with HCC risk and confirmed the association using an independent case-control population (710 cases and 1,625 controls). The association was highly significant when we analyzed the stages separately as well as together (rs1012068, $P_{combined} = 1.27 \times 10^{-13}$, odds ratio = 1.75). The significance level of the association further increased after adjustment for gender, age and platelet count ($P = 1.35 \times 10^{-14}$, odds ratio = 1.96). Our findings suggest that common variants within the *DEPDC5* locus affect susceptibility to HCC in Japanese individuals with chronic HCV infection.

HCC is the third leading cancer-related cause of death and the seventh most common form of cancer worldwide[1]. In many western countries and Japan, HCV infection is the most common risk factor for HCC[2,3]. Chronic hepatitis caused by HCV often leads to fibrosis and cirrhosis (stage F4 fibrosis), which markedly increase the risk of developing HCC. The annual incidence of HCC correlates with the severity of liver fibrosis, from 0.5% among individuals with stage F0 or F1 fibrosis to 7.9% among individuals with stage F4 fibrosis[4]. Recently, age at initial diagnosis of HCV-related HCC has been increasing in Japan, and most affected individuals are diagnosed at age 55 or older[5–8]. To date, many studies have examined individuals with HCV and identified several predictive factors for HCC in addition to fibrosis and age, including male gender, alcohol consumption, diabetes mellitus, obesity, ethnicity and co-infection with hepatitis B virus (HBV)[1,5,7,9]. In spite of recent progress in anti-HCV therapy, it remains difficult to achieve complete eradication of the virus[10]. Particularly among individuals with HCV who are unable to clear the virus, screening of any SNPs associated with susceptibility to HCC may help improve prognosis and target surveillance efforts more efficiently to high-risk individuals. Researchers from another study[11] recently identified a SNP within the *KIF1B* locus associated with progression to HCC among chronic HBV carriers; however, the virological effects of HBV and HCV are entirely different[12], and so far, SNPs associated with risk of HCC among individuals with chronic HCV have not been fully investigated. To identify genetic markers associated with risk of HCV-related HCC development in the Japanese population, we conducted a two-phase case-control study consisting of a genome-wide association study (GWAS) and a replication study using a total of 3,312 Japanese individuals over the age of 55 with chronic HCV infection. In the GWAS phase, we performed SNP genotyping using the Illumina HumanHap610-Quad BeadChip. We analyzed 467,538 SNPs that passed quality control filters using an additive model for genotype-phenotype association in 212 chronic HCV carriers with HCC (cases) and 765 chronic HCV carriers without HCC (controls). Principal component analysis revealed no population substructure in our population. In addition, a quantile-quantile plot using the results of the Cochran-Armitage trend test showed that the inflation factor, λ, was 1.00, indicating a low probability of false-positive associations resulting from population stratification (**Supplementary Fig. 1a**). Using the additive model, one intronic SNP, rs1012068, within isoform 1 of the *DEPDC5* locus on chromosome 22, showed strong association with HCC ($P = 8.05 \times 10^{-8}$) with odds ratio (OR) = 2.20 (95% confidence interval (CI) 1.64–2.97) (**Table 1**). We also found that rs1012068 showed a statistically significant association

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Table 1 Summary of GWAS and replication study

SNP	Gene	Study	Allele (1/2)	Case 11	Case 12	Case 22	Control 11	Control 12	Control 22	MAF Case	MAF Control	OR	95% CI[a]	P^b	P^c_{het}
rs1012068	*DEPDC5*	GWAS	T/G	138	68	6	624	136	5	0.189	0.095	2.20	1.64–2.97	8.05×10^{-8}	
		Replication		470	221	19	1262	334	29	0.182	0.121	1.63	1.37–1.93	2.41×10^{-8}	
		Combined studies[d]										1.75	1.51–2.03	1.27×10^{-13}	0.082

MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.
[a]Odds ratios of risk allele from two-by-two allele frequency table. [b]*P* value of Cochran-Armitage trend test. [c]Result of Breslow-Day test. [d]Combined meta-analysis was performed using the Mantel-Haenszel method.

with HCV-related HCC after Bonferroni correction for multiple testing (calculated as $P < 0.05/467{,}538 = 1.07 \times 10^{-7}$)[13,14]. No other SNPs reached genome-wide significance (**Supplementary Table 1** and **Supplementary Fig. 1b**). As shown in **Table 1**, we next performed a replication study using 710 cases and 1,625 controls and again found that rs1012068 was strongly associated with HCC ($P = 2.41 \times 10^{-8}$, OR = 1.63). The association between rs1012068 and HCC remained highly significant when we combined results of the GWAS and replication sets using the Mantel-Haenszel method (combined $P = 1.27 \times 10^{-13}$, OR = 1.75). We observed no heterogeneity across the two studies (heterogeneity test $P = 0.082$).

On the other hand, platelet count is known to correlate significantly with the stage of liver fibrosis in individuals with HCV, and a platelet count of $<10 \times 10^4/\mu l$ has also been used as a marker for cirrhosis[4,15–19]. After adjusting for age, gender and platelet count using multiple logistic regression analysis, the significance level of rs1012068 increased ($P = 1.35 \times 10^{-14}$, OR = 1.96) (**Supplementary Table 2**). Other predictive factors for HCV-related HCC have been reported, including alcohol consumption, diabetes mellitus, obesity, ethnicity and co-infection with HBV[1,5,7,9]. As all subjects enrolled were of Japanese ethnicity, and there were no HBV co-infected subjects, the effect of the SNP was reevaluated using only 994 subjects (480 cases and 514 controls) for whom data was fully available for other factors (**Supplementary Table 3**). After adjusting for each of these six factors using multiple logistic regression analysis, rs1012068 remained highly significant with an OR = 1.87 (95% CI 1.39–2.52) (**Supplementary Table 4**). However, we cannot rule out the possibility that other confounding factors influenced the results. In addition to examining the effect of rs1012068 on HCC independently of other predictive factors, we performed stratified analysis using gender, age and platelet count (**Supplementary Table 5**). Notably, this SNP tended to show a greater effect in males (OR = 1.99 (95% CI 1.63–2.42)) than females (OR = 1.51 (95% CI 1.18–1.93)), as well as in elderly subjets (age ≥65 years: OR = 1.84 (95% CI 1.52–2.24) compared to age <65: OR = 1.73 (95% CI 1.36–2.19)) and in subjects with low platelet counts ($<10 \times 10^4/\mu l$: OR = 2.35 (95% CI 1.67–3.31) compared to $\geq 10 \times 10^4/\mu l$: OR = 1.71 (95% CI 1.42–2.05)). Each of these factors (male gender, older age and lower platelet count) are well known risk factors for HCV-related HCC. rs1012068 seems to more strongly affect individuals with multiple risk factors for HCC, but we detected no heterogeneity among subgroups stratified by gender, age and platelet count (heterogeneity test $P = 0.086$, $P = 0.675$ and $P = 0.103$, respectively, for each factor).

To examine whether rs1012068 genotypes are associated with any specific HCC phenotypes, we analyzed clinical phenotypes of cases with HCC with regard to rs1012068 genotype. We observed no differences between individuals with TT and TG+GG genotypes (**Supplementary Table 6**), but when we evaluated the case to control ratio of each 5-year age group with respect to rs1012068 genotype (TT compared to TG+GG), we found that the ratio was higher among subjects with the TG+GG genotype over all 5-year age ranges, and the slope of the ratio with increasing age was steeper among these individuals (**Supplementary Fig. 2**).

In order to explore the region around the landmark SNP rs1012068 in more detail, we performed fine mapping in the GWAS-stage subjects of a 350-kb genomic region between 22q12.2 and 22q12.3 upstream and downstream of the *DEPDC5* locus, including the neighboring genes *C22orf30* and *YWHAH* (**Fig. 1** and **Supplementary Fig. 3**). We successfully genotyped 43 tagging SNPs and identified another intronic SNP, rs5998152, located about 2.7 kb upstream of the landmark SNP (rs1012068) that is in strong linkage disequilibrium (LD) with rs1012068 ($r^2 = 0.99$). However, no SNPs showed stronger association than rs1012068 (**Supplementary Fig. 3** and **Supplementary Table 7**). To investigate the existence of any functional coding SNPs linked to rs1012068, we resequenced all 42 exons of *DEPDC5* using genomic DNA from 48 individuals

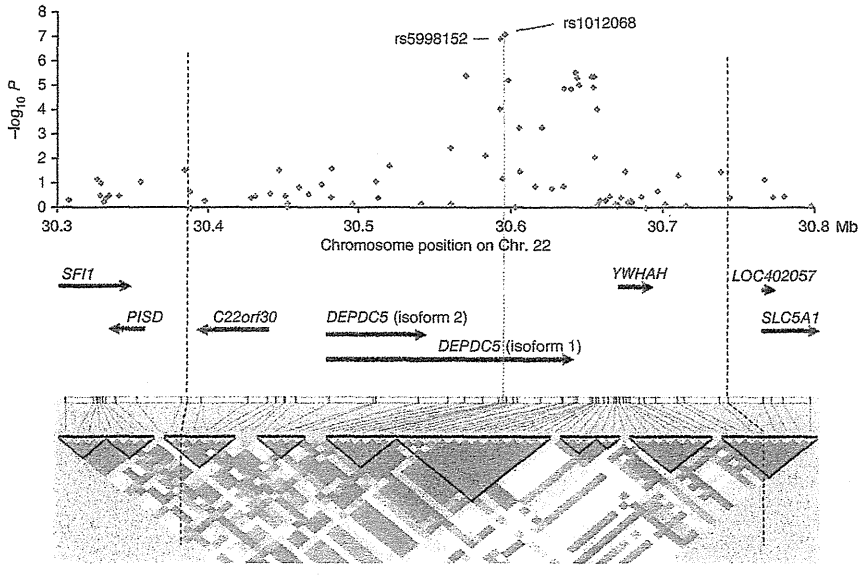

Figure 1 Case-control association plots and linkage disequilibrium (LD) map and genomic structure of the *DEPDC5* region in chromosome 22q12.2–3. The candidate region is indicated by two black dashed lines. We performed fine mapping in the region from 30.39–30.74 Mb. Blue diamonds represent $-\log_{10} P$ obtained from the GWAS and fine mapping using GWAS samples. We drew the LD map based on *D'* values using the genotype data of the cases and controls in the GWAS samples. The landmark SNP (rs1012068) is indicated by the red dotted line.

with HCV. We identified two new SNPs that were not registered in the dbSNP database (**Supplementary Table 8**). However, both SNPs had low minor allele frequencies (MAF) of 0.010 and were not linked to rs1012068 ($r^2 = 0.00$) nor were they significantly associated with HCC. We also performed haplotype analysis to investigate the effect of combinations of SNPs that were strongly associated with HCC susceptibility; however, no haplotype showed stronger association than the single-marker association of rs1012068 (**Supplementary Fig. 4**). Finally, rs1012068 had the strongest independent association with HCV-related HCC in our study.

Then we investigated the association between rs1012068 genotype and *DEPDC5* mRNA expression using paired tumor (HCC) and adjacent non-tumor liver tissues from 43 individuals with HCV. As shown in **Supplementary Figure 5**, real-time quantitative PCR assays revealed a significantly higher level of *DEPDC5* mRNA expression in tumor tissues than non-tumor tissues ($P = 0.025$), but we observed no significant difference with regard to rs1012068 genotype in tumor tissues as well as in non-tumor tissues ($P = 0.610$ and $P = 0.400$, respectively). On the other hand, we also evaluated *DEPDC5* expression using paired tumor and adjacent non-tumor tissues and calculated the tumor to non-tumor ratio as the *DEPDC5* expression level in tumor tissue divided by the expression level in paired non-tumor tissue from the same subject. As shown in **Supplementary Table 9**, we found that the frequency of the risk allele (G) was significantly higher in subjects with a tumor to non-tumor ratio ≥5 as well as a ratio of ≥1 in males ($P = 0.014$ and $P = 0.036$, respectively) but not in females (a ratio of ≥5: $P = 0.500$ and a ratio of ≥1: $P = 0.226$). This finding may suggest a differential effect of the SNP on *DEPDC5* expression due to gender. Although there is insufficient data to show a direct functional effect of rs1012068 on *DEPDC5* expression and HCV-related hepatocarcinogenesis, the data suggest a possible genetic association between a polymorphism within the *DEPDC5* locus and HCV-related HCC that requires further functional analysis.

In this study, we identified a common SNP associated with HCV-related HCC, and the effect of the SNP remained highly significant even after adjusting for other predictive factors. We observed no significant heterogeneity between the GWAS and replication studies, but the odds ratios for each study differed somewhat, and the 95% CIs for one phase of the study did not include the odds ratio for the other. In addition, the MAFs for the controls differed between the GWAS and replication phases (**Table 1**). We speculate that the differences between the two phases partially explain the different observed effects of the SNP. The female to male ratio was significantly higher in the replication phase than the GWAS phase for both cases and controls (**Supplementary Table 10**). The ages of the cases were significantly lower and the platelet counts of the controls were significantly higher in the GWAS phase than in the replication phase. As shown in **Supplementary Table 5**, rs1012068 showed a weaker risk in females than in males, and the frequency of the risk allele among older cases (≥65 years old) was lower than among younger cases (0.183 compared to 0.186, respectively). The risk allele frequency was also higher among controls with platelet count ≥10 ($\times 10^4/\mu l$) than in controls with platelet count <10 ($\times 10^4/\mu l$) (0.116 compared to 0.088, respectively). These unexpected differences between subjects in the two phases seem to contribute jointly to the observed differences in the effect of the SNP between the two phases. It is important to note that the controls used in this study were not healthy controls (MAF = 0.116 based on HapMap JPT data) but are chronic HCV carriers who still have the potential of developing HCC in the future, especially those who have one or more other strong predictive factors (for example, gender, age or platelet count). When we stratified samples by each predictive factor, the MAFs in the controls were varied (**Supplementary Table 5**). We speculate that after HCV infection becomes chronic, individuals with risk alleles may more easily develop HCC, and conversely, those without risk alleles are relatively less likely to progress to HCC (**Supplementary Fig. 2**), but these other risk factors influence the ultimate course of the disease. Consideration of the genetic background of subjects will likely play a role in personalized medicine, and understanding the mechanism underlying the association may suggest new therapeutic targets.

On the other hand, given the relatively small number of cases in the GWAS phase, we calculated the statistical power to detect an effect caused by rs1012068 to be only 50%, compared to the 80% recommended to detect an association of the expected effect size (**Supplementary Fig. 6**). It remains to be determined whether other SNPs influence susceptibility to HCV-related HCC in the Japanese population. The question also remains whether this susceptibility locus within *DEPDC5* is associated with HCV-related HCC in other ethnic groups, as allele frequencies of rs1012068 vary among ethnic groups, even among those with Asian ethnicities (**Supplementary Table 11**).

DEPDC5 has not been previously reported in association with HCC, but deletion of the region containing *DEPDC* has been reported in malignant brain glioblastomas[20]. Although the function of this gene is still unknown[21], it is noteworthy that DEPDC1, which contains a DEP domain similar to DEPDC5, has been reported to affect bladder carcinogenesis[22,23].

In summary, we conducted a GWAS followed by an independent replication study and fine mapping to detect polymorphisms associated with HCC in Japanese individuals with HCV. We report a common SNP within the *DEPDC5* locus associated with a twofold increased risk of HCC. Further research is required to determine the role of this gene in development of HCV-related HCC.

URLs. PLINK1.03, http://pngu.mgh.harvard.edu/~purcell/plink/; R statistical environment, http://www.cran.r-project.org/; EIGENSOFT, http://genepath.med.harvard.edu/~reich/Software.htm.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

K.C. conceived the study. D.M., H.O. and K.C. designed the study. D.M. and H.O. performed genotyping. D.M., H.O., C.N.H. and K.C. wrote the manuscript. T.M., T.T., M.K. and N.K. performed data analysis at the genome-wide phase. H. Abe and T.Y. performed functional analyses. H. Aikata, K.I., H.K., J.T. and K.C. managed DNA samples. D.M., H.O. and K.C. summarized the whole results. Y.N., N.K. and K.C. obtained funding for the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Samples. We conducted a two-phase case control study consisting of GWAS and replication phases using 3,312 Japanese subjects over the age of 55 with chronic HCV infection diagnosed at Toranomon Hospital Department of Hepatology (n = 727), Sapporo Kosei Hospital (n = 153) and Hiroshima University–affiliated hospitals (n = 2,432) between 2002 and 2010. Individuals with chronic HCV with HCC were enrolled as cases, and those without were enrolled as controls. Cases and controls were then each randomly divided into two sets, totaling 212 cases and 765 controls in the GWAS phase and 710 cases and 1,625 controls in the replication phase. All subjects had abnormal levels of serum alanine transaminase for more than 6 months and were positive for both HCV antibody and serum HCV RNA. All subjects were negative for hepatitis B surface antigen, had no evidence of other liver diseases and had not received immunosuppressive therapy before enrollment in the study. Clinical information such as age, gender and platelet count were available in all subjects. For cases, age and platelet count at initial diagnosis of HCC were used. The subject characteristics are shown in **Supplementary Table 10**. The diagnosis of HCC was based on hypervascularity confirmed by dynamic computed tomography, magnetic resonance imaging, angiography or computed tomography angiography when the serum levels of HCC-related tumor markers, such as alpha fetoprotein or protein induced in the absence of vitamin K or antagonist II (PIVKA-II), were increased or a mass lesion was detected by ultrasonography. When a nodule was not proven to be hypervascular, percutaneous biopsy under ultrasonography was performed for confirmation of the diagnosis of HCC. Staging of HCC adopted in this study was the revised version of Liver Cancer Study Group of Japan[24]. The average quantity of alcohol consumed per day was evaluated regardless of alcohol beverage. Habitual alcohol intake was defined as ≥80 g/day for more than 5 years. All subjects in the present study received a detailed explanation and all signed a written informed consent form. The study was approved by the ethical committee of each participating medical center and by the Ethical Committee at the SNP Research Center, the Institute of Physical and Chemical Research (RIKEN), Yokohama, Japan.

SNP genotyping. Genomic DNA was extracted from peripheral blood leukocytes using a standard method. For the GWAS stage, we genotyped 981 Japanese subjects with chronic HCV infection using the Illumina HumanHap610-Quad BeadChip. We excluded two samples with call rate <0.98, and two other samples suggesting kinship or sample duplication were excluded from the analysis based on PI_HAT value (>0.4). We assessed population stratification using the smartpca program in the EIGENSOFT package using SNPs informative for the Japanese population according to a previously described method[25]. Analysis was performed based on the GWAS data and the Japanese (JPT), Han-Chinese (CHB), European (CEU) and African (YRI) individuals from the HapMap project. Principal component analysis identified no outliers from the JPT/CHB clusters. In total, 467,538 autosomal SNPs passed the quality control filters (call rate ≥ 0.99 in both cases and controls, MAF ≥ 0.01 and a Hardy-Weinberg equilibrium $P \geq 1.0 \times 10^{-6}$ in controls). We used multiplex-PCR–based Invader assays (Third Wave Technologies) for the replication study (710 cases and 1,625 controls) and fine mapping[26]. Samples for both cases and controls were distributed randomly on genotyping plates in both phases of the study, and all persons performing genotyping and interpretation of results were blind to case or control status.

Fine mapping and resequencing. We performed fine mapping using all GWAS-stage case and control samples. Haploview was used to select tag SNPs with a pairwise $r^2 > 0.80$ and MAF ≥ 0.05 on the basis of Phase II HapMap JPT data. Resequencing of candidate regions was performed by direct sequencing of DNA from 48 unrelated Japanese individuals with HCV from among the enrolled subjects.

Quantitative analysis of mRNA of *DEPDC5*. A total of 43 paired primary hepatocellular carcinomas and adjacent non-tumor tissues, derived from 43 unrelated HCC cases enrolled, were examined. Total RNA was extracted from liver tissues using the RNeasy Mini Kit (QIAGEN). One microgram of each RNA sample was reverse transcribed with ReverseTra Ace (TOYOBO Co. Ltd.) and Random Primer (Takara Bio). We quantified the mRNA for *DEPDC5* with the SsoFast EvaGreen Supermix (Bio-Rad Laboratories). Primers were designed for the conserved region between isoforms 1 and 2 (**Supplementary Table 12**). Amplification and detection were performed using a CFX Real-Time PCR Detection System (Bio-Rad Laboratories). Results were normalized to the transcript levels of beta-actin (*ACTB*).

Statistical analysis. Genotype-based associations were tested using a Cochran-Armitage trend test[27,28]. The OR and 95% CI were calculated from a two-by-two allele frequency table. In the GWAS stage, significance levels after Bonferroni correction for multiple testing were $P = 1.07 \times 10^{-7}$ (calculated as 0.05/467,538)[13,14]. Combined analysis was performed following the Mantel-Haenszel method. Heterogeneity among studies was examined using the Breslow-Day test. We used Haploview software to analyze the association of haplotypes and LD values between *DEPDC5* and SNPs. Power analysis was performed using Power for Genetic Association Analyses software[29]. The Mann-Whitney U test was used to analyze continuous variables, and χ^2 or Fisher exact tests were used to analyze categorical data, as appropriate.

Software. For general statistical analysis, we used the R statistical environment version 2.12.0 or PLINK 1.03 (ref. 30). To draw the LD map and analyze the association of haplotypes, we used Haploview software[31].

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Efficacy and Safety of Combination Therapy of Natural Human Interferon Beta and Ribavirin in Chronic Hepatitis C patients

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Abstract

Objective The aim of this study was to evaluate the efficacy and safety of combination therapy of natural human interferon-beta and ribavirin for patients for whom prior interferon therapy was discontinued due to depression induced by interferon-alpha.

Methods Inclusion criteria were as follows; 1) HCV-genotype 1b, 2) serum HCV RNA level of ≥100 KIU/mL, 3) stopping the prior interferon-alpha monotherapy or combination therapy of interferon-alpha and ribavirin due to the appearance of depression. A total of 14 were enrolled in this prospective cohort study. The treatment period of combination therapy was 48 weeks. Depression states, reflected by Beck depression inventories and Hamilton depression rating scale, were assessed during combination therapy. Nonparametric procedures were employed for the analysis of background features of the patients with sustained virological response (SVR) and without SVR. A p value of <0.05 was considered to indicate a significant difference.

Results Five of 14 patients (37.5%) had SVR by the intention to treat analysis. The SVR rate in patients who showed negative HCV RNA at 12 and 24 weeks after the initiation of combination therapy was 100% (4/4) and 83.3% (5/6), respectively. All of the patients continued the combination therapy owing to disappearance of severely adverse events contained the exacerbation of depression. Combination therapy did not yield a statistical difference in Beck depression inventories and Hamilton depression rating scale.

Conclusion The combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

Key words: chronic hepatitis C, depression, natural interferon-beta, ribavirin, HCV genotype 1b

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Introduction

The combination therapy of peginterferon-alpha and ribavirin has been widely recommended as a first choice for chronic hepatitis C patients with high virus-load (1-5). However, one big problem of the combination therapy is the treatment-related side effect (6, 7). In particular, physicians in charge tend to avoid the combination therapy of peginterferon-alpha and ribavirin for chronic hepatitis C patients with depression or interferon (IFN)-reduced depression.

IFN-beta-related side effects are mild and few compared to therapy of IFN-alpha (6-8). In particular, IFN-beta-induced mental disorders are mild compared to those induced by IFN-alpha (9). Moreover, IFN-beta could be given to elderly patients aged ≥70 years because of the mild side effects (10). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with genotype 1b and a high virus load (11, 12). The combination therapy of IFN-

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beta and ribavirin has the possibility to show the strong effect for hepatitis C virus (HCV) and mild side effects originating from the treatment (13-15). We have reported that the combination of IFN-beta plus ribavirin therapy is effective and safety for HCV patients with high virus load and depressive state (14). However, the previous study was retrospective and a prospective study is necessary to evaluate the efficacy and safety of combination therapy of IFN-beta and ribavirin for HCV patients with high virus load and depressive state.

Thus, in the present study, we performed a prospective study to examine the efficacy and safety of combination therapy of IFN-beta and ribavirin in HCV genotype 1b patients who had stopped the IFN therapy due to depression induced by IFN-alpha. At the same time, depression states, reflected by Beck depression inventories (BDI) and Hamilton depression rating scale (Ham-D), were assessed during combination therapy (16, 17).

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of ≥100 KIU/mL before treatment; 3) stopping of IFN-alpha therapy due to depression appearance during the prior IFN-alpha treatment; 4) Ham-D of <18; 5) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 6) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 7) white blood cell (WBC) >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/mL; follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy of IFN-beta and ribavirin as well as the potential adverse reactions to each patient and informed consent was obtained from each patient. This study was approved by the Human Ethics Review Committee of Toranomon Hospital.

From December 2007 to May 2008, 14 HCV patients were enrolled in this prospective cohort study at the study hospital. A sustained virological response (SVR) was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (18).

Laboratory investigation

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analysis. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (19). Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (18). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (20). The core protein of HCV-1b was determined by the previous report (21). Next, the genetic variations near the IL28B gene (rs8099917), reported as the pretreatment predictors of treatment efficacy and clinical outcome, were investigated (22-26). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Clinical evaluation and biochemical and hematological tests were performed at 1, 2, and 4 weeks in the first month after the initiation of combination therapy. After that, these evaluations were done at monthly intervals. The patients were followed by both physicians of hepatology and psychiatry.

Combination therapy of IFN-beta and ribavirin

Treatment was provided for 48 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) by six times a week for 4 weeks, followed by three times a week for 44 weeks. The total dose was 936MU. Ribavirin (Rebetol, MSD KK., Tokyo, Japan) was given at the dose prescribed based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1,000 mg for >80 kg).

Evaluation of the psychic state

The psychiatrist in charge evaluated the scores of BDI and Ham-D prospectively. BDI shows the subjective symptom of the depressive patients and Ham-D shows the objective evaluation by the psychiatrist. Scores on the BDI were divided the following; severe, 29-63; moderate, 20-28; mild, 14-19; and minimal, 0-13. Scores on the Ham-D were divided the following; very severe, >23; severe, 19-22; moderate, 14-18; mild, 8-13; and normal ≤7 (27).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher's exact test. The following variables were evaluated as prognostic factors: sex, age, BDI score, Ham-D score, a HCV RNA level, IL28B (genetic variation in rs8099917), variation of HCV-core, biochemical factors (AST, ALT, gamma glutamyltransferase, total cholesterol), white blood cell (WBC), hemoglobin, platelet count, HCV RNA 4, 12, 24 week after the initiation of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Table 1. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR *

	Total	SVR (n=5)	Non-SVR (n=9)	p value†
Age (years old)	62.1 ± 4.3	62.4 ± 4.2	61.9 ± 4.6	0.797
Sex (male/female)	6/8	2/3	4/5	0.898
Previous IFN therapy (combination/monotherapy)	8/6	3/2	5/4	0.898
Duration of previous IFN therapy (week)	11.9 ± 7.8	11.6 ± 10.2	12.0 ± 7.1	0.699
HCV-RNA (KIU/mL)	2588± 1455	2228± 1807	2788± 1296	0.759
Core aa70 (Wild/Mutant)	6/8	3/2	3/6	0.438
BDI score	11.9 ± 10.3	12.2 ± 14.2	11.7 ± 8.4	0.518
Ham-D score	3.5 ± 4.1	3.6 ± 5.5	3.4 ± 3.5	0.606
IL28B (genetic variation in rs8099917, genotype TT/TGorGG)	7/7	5/0	2/7	0.042
AST (IU/L)	50 ± 24	46 ± 37	52 ± 17	0.112
ALT (IU/L)	68 ± 33	60 ± 35	72 ± 32	0.518
GGT (IU/L)	55 ± 59	25 ± 5	72 ± 69	0.813
Total cholesterol (mg/dL)	175 ± 30	166 ± 35	179 ± 28	0.298
White blood cell(10^3/mm^3)	4.39 ± 1.24	4.16 ± 1.02	4.52 ± 1.39	0.898
Hemoglobin (g/dL)	14.1 ± 1.1	14.2 ± 1.5	14.0 ± 0.9	0.898
Platelet(10^4/mm^3)	15.8 ± 4.8	19.9 ± 2.4	13.5 ± 4.1	0.019
HCV RNA (+/-) 4W	11/3	2/3	9/0	0.083
HCV RNA (+/-) 12W	10/4	1/4	9/0	0.012
HCV RNA (+/-) 24W	8/6	0/5	8/1	0.004

Data are number of patients (percentage) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDI, Beck depression inventories; GGT, gamma-glutamyltransferase; Ham-D, Hamilton depression rating; HCV, hepatitis C virus;
*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks.
†Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher' exact test.

Result

Clinical characteristics of the patients

A total of 14 patients treated with IFN-beta +ribavirin were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =62.1 years, male/female =6/8, and HCV-RNA =2,588±1,455 KIU/mL. Patients were classified into two groups according to the difference of response: SVR (n=5), Non-SVR (n=9).

Efficacy of treatment

Five of 14 patients (37.5%) had SVR by the intention to treat analysis. Table 1 shows the differences in the clinical background between patients with SVR and those without SVR. The negativity rate of HCV RNA 12 weeks after the initiation of combination therapy was 80% (4/5) in SVR group and 0%(0/9) in Non-SVR group (p=0.012). The negativity rate of HCV RNA 24 weeks after the initiation of combination therapy was 100% (5/5) in SVR group and 11.1% (1/9) in Non-SVR group (p=0.004). Next, the platelet count in SVR group was significantly higher than that in Non-SVR group.

On the IL28B (genetic variation in rs8099917), all seven patients with TG or GG at IL28B showed non-SVR. On the other hand, five of the seven patients with TT at IL28B showed SVR. The TT at IL28B that is associated with SVR was statistically significant in the present study (p=0.042).

Safety and tolerance of combination therapy

Of the 14 patients treated with IFN-beta + ribavirin included in this study, four patients necessitated a reduced dose of ribavirin due to the appearance of hemoglobin level <10 g/dL and two patients needed a reduced dose of IFN-beta due to WBC count of <2,000/mm^3. Three patients had dipstick proteinuria of +1 at 4 week after the initiation of combination therapy. This proteinuria continued during combination therapy. However, no patient discontinued combination therapy because of treatment related adverse events related to exacerbation of depression. Fig. 1 shows the changes of BDI scores in 14 patients treated with IFN-beta + ribavirin. BDI scores during combination therapy were lower than that at the initiation time of treatment. Fig. 2 shows the changes of Ham-D scores in 14 patients. There was no statistically significant difference in changes of Ham-D scores during combination therapy compared to that at the initiation time of treatment.

Regarding the prescription of antidepressant and anti-anxiety drugs, antidepressants, such as sulpiride, and amitriptyline hydrochloride, were given to three patients at the

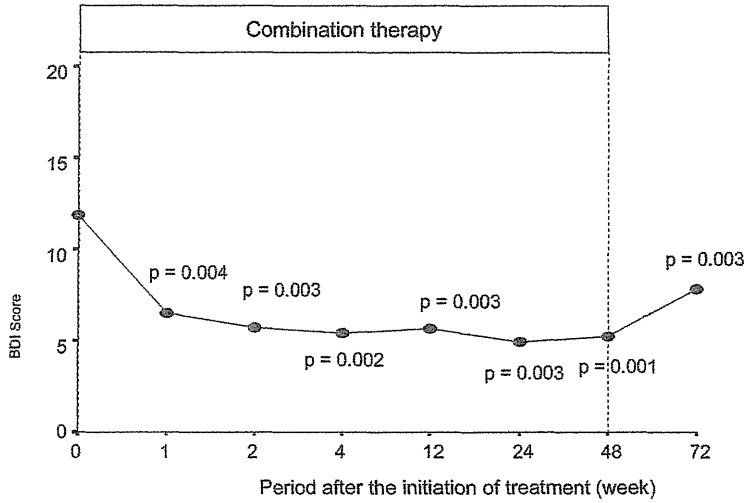

Figure 1. **The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

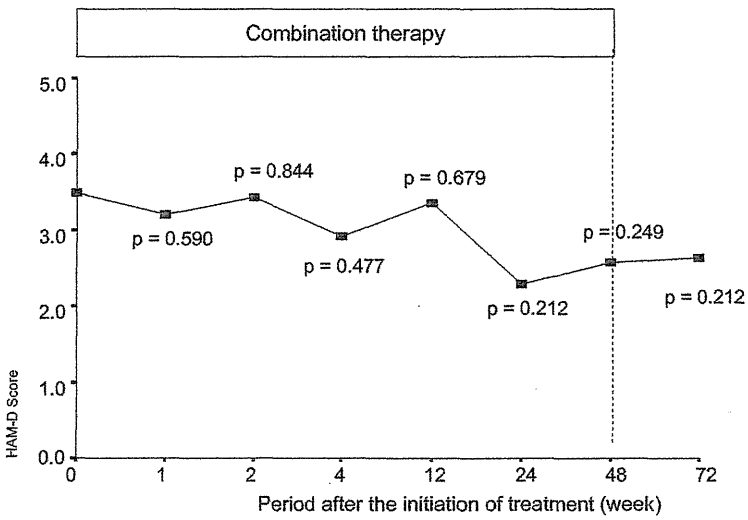

Figure 2. **The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

start of IFN therapy and to four patients during IFN therapy. Anti-anxiety drugs, such as etizolam, alprazolam, were given to four patients at the start of IFN therapy and to five patients during IFN therapy.

The changes of WBC, hemoglobin, and platelet count after the initiation of combination therapy are shown in Fig. 3. WBC and hemoglobin levels were decreased during combination therapy. On the other hand, the platelet count decrease was statistically significant at 1, 2, and 4 weeks after the initiation of combination therapy compared to that at the initiation time of treatment. After that, the platelet count recovered to the base line at 12, 24, and 48 weeks after the initiation of combination therapy.

Discussion

In the present study, we have described the efficacy and safety of combination therapy of IFN-beta and ribavirin for patients for whom IFN therapy was discontinued due to depression induced by IFN-alpha. The patients with HCV genotype 1b and HCV-load of $\geq$100 KIU/mL were enrolled. We could evaluate the relationship between IL-28 or HCV core mutation and SVR in the combination therapy of IFN-beta and ribavirin for genotype 1b and high virus load. The present study was limited to exclude the subjects with Ham-D score of more than 18. Patients with Ham-D score of more than 18 were defined as severe depression state. It is possible that high score of Ham-D enhance the dropout