TABLE I. Characteristics of Patients Infected With HCV Genotype 1b

Characteristic	Group A (n = 288)	Group B (n = 192)	Group C (n = 87)
Gender (male/female)	165/123	108/84	44/43
Age (years)[a]	62 (16–83)	60 (16–77)	59 (16–74)
Body mass index (kg/m^2)[a]	22.5 (15.7–38.9)	22.4 (16.4–34.6)	22.2 (17.3–30.3)
Fibrosis F0/F1/F2/F3/F4/ND	1/65/82/43/37/60	1/49/37/20/7/78	1/29/24/5/4/24
Necroinflammation A0/A1/A2/A3/ND	1/54/136/32/65	0/35/62/17/78	0/23/32/8/24
Duration of therapy (weeks)[a]	47 (1–172)	47 (4–172)	48 (45–52)
Hemoglobin (g/dl)[a]	13.7 (7.8–17.9)	13.8 (9.5–17.9)	13.7 (9.9–17.9)
Platelet count ($\times 10^4/\mu l$)[a]	13.8 (4.0–207.0)	14.7 (5.0–75.9)	15.8 (5.0–75.9)
Alanine aminotransferase (IU/L)[a]	48 (2–504)	49 (2–411)	44 (18–411)
γ-glutamyl transpeptidase (IU/L)[a]	43 (12–1,529)	38 (12–1,529)	32 (12–1,529)
Total cholesterol (mg/dl)[a]	167 (75–274)	171 (104–250)	176 (113–248)
Hyaluronic acid (ng/ml)[a]	111 (5–2,104)	96 (5–1,369)	84 (5–414)
Ferritin (ng/dl)	113 (5–1,611)	120 (5–1,611)	110 (13–833)
α-fetoprotein (ng/ml)[a]	8.5 (3–262)	7 (3–82)	6 (3–29)
HCV core antibody (U)[a]	170 (1–4,300)	180 (2–4,300)	210 (2–4,300)
Viral load (log IU/ml)[a]	6.7 (3.0–8.1)	6.7 (3.0–8.1)	6.7 (5.0–7.4)
NVRs/TVRs/SVRs	68/98/100	39/72/81	12/31/44
HCV core 70 R/Q/H/RQ/ND	154/82/10/4/42	107/51/8/4/24	57/19/3/2/6
HCV core 91 L/M/LM/ND	141/108/3/39	94/74/1/25	47/33/1/6
Mutation of ISDR 0/1/2+/ND	71/87/44/68	49/60/45/40	25/30/20/12
rs8099917 (GG/TG/TT/ND)	6/63/203/16	5/42/138/7	2/19/63/3

NVRs, nonviral response; TVRs, transient viral response; SVRs, sustained viral response; ND, not determined.
For explanation of Groups A, B, and C refer to Figure 1 and Patients and Methods Section.
[a]Median.

Genetic Variation in IL-28B

In this study, genetic variation within the IL-28B locus (SNPs rs8099917 and rs12979860), which has recently been reported as a pretreatment predictor of treatment efficacy and clinical outcome [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2011], was investigated. Genotyping was performed using the Invader assay as described previously [Ohnishi et al., 2001]. Both SNPs were in Hardy–Weinberg equilibrium.

HCV RNA Level

HCV RNA level was analyzed before the start of IFN therapy and at the end of the fourth week using reverse transcription polymerase chain reaction (RT-PCR)-based methods, that is, the original method, the high range method, or TaqMan RT-PCR. The measurement ranges of these assays were 0.5–850 KIU/ml, 5–5,000 KIU/ml, and 1.2–7.8 log IU/ml, respectively. Samples with values exceeding the measurable range were reanalyzed after diluting serum samples stored at −80°C.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

Amino acid substitutions of the core protein and NS5A-ISDR of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by PCR using the primers listed in Table II. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second set of PCR primers were used instead of the first set. The amplified PCR products were purified

TABLE II. Primers Used for Nested PCR

Locus	Primer
Core region	
Outer forward	5'-GCC ATA GTG GTC TGC GGA AC-3'
Outer reverse	5'-GGA GCA GTC CTT CGT GAC ATG-3'
Inner forward	5'-GCT AGC CGA GTA GTG TT-3'
Inner reverse	5'-GGA GCA GTC CTT CGT GAC ATG-3'
ISDR	
Outer forward	5'-TTC CAC TAC GTG ACG GGC AT-3'
Outer reverse	5'-CCC GTC CAT GTG TAG GAC AT-3'
Inner forward	5'-GGG TCA CAG CTC CCA TGT GAG CC-3'
Inner reverse	5'-GAG GGT TGT AAT CCG GGC GTG C-3'

by the QIAquick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan). The sequence of amino acids 1–191 in the core protein of HCV-1b was determined, using HCV-J accession number D90208 as a reference sequence [Kato et al., 1990]. Arg70 and Leu91 are thought to be wild-type for aa 70 and aa 91, respectively [Akuta et al., 2005]. The sequence of amino acids 2,209–2,248 in the HCV-1b NS5A IFN-sensitivity determining region (ISDR) was determined [Enomoto et al., 1995; Shirakawa et al., 2008] and, based on the number of amino acid substitutions in the ISDR, sequences were classified as wild-type (0, 1 mutations) or nonwild-type (≥2) [Nakagawa et al., 2010].

Liver Biopsy

Liver biopsy specimens were evaluated by a pathologist at each institution and were scored for necroinflammatory activity and fibrotic stage according to the classification of Desmet et al. [1994].

Statistical Analysis

Continuous data were analyzed using the Mann–Whitney *U*-test. Categorical variables were analyzed with the Pearson chi-squared test or the Fisher exact test as appropriate. Odds ratios and 95% confidence intervals (95% CI) were also calculated. All *P* values <0.05 by two-tailed tests were considered significant. Regression analysis was used to identify factors associated with viral dynamics during the early phase of treatment. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) in univariate analysis were analyzed using multiple regression analysis to account for correlations among factors. Correlations among variables were evaluated by Spearman rank correlation analysis. Statistical analyses were performed using the PASW software (SPSS, Inc., Chicago, IL).

RESULTS

Factors Associated With Sustained Viral Response and Nonviral Response to PEG-IFN and RBV Combination Therapy

Factors associated with SVR and NVR are summarized in Tables III and IV, respectively. In univariate analysis, age, platelet count, γ-glutamyl transpeptidase, hyaluronic acid, and rs8099917 TT genotype were associated with SVR (Table III). In multivariate analysis, TT genotype was the only significant factor associated with SVR ($P = 0.016$, OR 61.5) (Table III). In contrast, γ-glutamyl transpeptidase, wild-type ISDR, aa 70 mutation and non-TT rs8099917 genotypes were associated with NVR in univariate analysis (Table IV). Under multiple logistic regression, both aa 70 mutation ($P = 0.038$, OR 5.9) and non-TT genotypes ($P = 0.002$, OR 17.2,) were associated with NVR (Table IV). Next, patients were divided into four groups according to host

TABLE III. Factors Associated With Sustained Viral Response to the Therapy (n = 87)

Factor	SR (n = 44)	Non-SR (n = 43)	*P*-value
Univariate analysis			
Gender (male/female)	22/22	22/21	0.914[b]
Age (years)[a]	54 (16–73)	65 (45–74)	<0.001[c]
Body mass index (kg/m^2)[a]	22.2 (17.6–30.3)	22.7 (17.3–29.7)	0.882[c]
Fibrosis: F0, F1/F2–F4	15/14	15/19	0.547[b]
Necroinflammation: A0, A1/A2–A3	13/16	10/24	0.205[b]
Duration of therapy (weeks)[a]	47 (45–52)	48 (45–52)	0.385[c]
Hemoglobin (g/dl)[a]	13.8 (9.9–17.9)	13.7 (10.6–17.5)	0.730[c]
Platelet count (× 10^4/μl)[a]	17.2 (5.0–75.9)	14.9 (6.8–31.1)	0.026[c]
Alanine aminotransferase (IU/L)[a]	49 (20–411)	41 (18–181)	0.465[c]
γ-glutamyl transpeptidase (IU/L)[a]	30 (12–103)	45 (15–1,529)	0.004[c]
Total cholesterol (mg/dl)[a]	178 (113–235)	174 (127–248)	0.536[c]
Hyaluronic acid (ng/ml)[a]	61 (5–414)	97 (32–414)	0.018[c]
Ferritin (ng/dl)	105 (15–694)	110 (13–833)	0.952[c]
α-fetoprotein (ng/ml)[a]	5.2 (3–29)	8.2 (3–29)	0.087[c]
HCV core antibody (U)[a]	195 (2–4,300)	225 (76–330)	0.987[c]
Viral load (log IU/ml)[a]	6.7 (5.0–7.3)	6.8 (5.0–7.4)	0.255[c]
Mutation of ISDR 0–1/2+	24/13	31/7	0.102[b]
HCV core 70 aa wild (R)/mutant (Q or H)	28/9	29/13	0.512[b]
HCV core 91 aa wild (L)/mutant (M)	26/13	21/20	0.161[b]
rs8099917 (GG, TG/TT)	5/38	16/25	0.004[b]

Factors	Odds ratio	95% CI	*P*-value
Multivariate analysis			
rs8099917 (TT)	61.5	2.2–1735.8	0.016

[a]Median.
[b]χ^2 test.
[c]Mann–Whitney *U*-test.

TABLE IV. Factors Associated With Nonviral Response to the Therapy (n = 87)

Factor	NR (n = 12)	Non-NR (n = 75)	*P*-value
Univariate analysis			
Gender (male/female)	8/4	36/39	0.230[b]
Age (years)[a]	62 (45–72)	59 (16–74)	0.347[c]
Body mass index (kg/m)[a]	22.2 (17.3–25.8)	22.2 (17.6–30.3)	0.442[c]
Fibrosis: F0, F1/F2–F4	6/4	24/29	0.305[d]
Necroinflammation: A0, A1/A2–A3	4/6	19/34	0.534[b]
Duration of therapy (weeks)[a]	47 (45–51)	47 (45–52)	0.288[c]
Hemoglobin (g/dl)[a]	14.1 (10.6–17.5)	13.6 (9.9–17.9)	0.600[c]
Platelet count ($\times 10^4/\mu l$)[a]	15.8 (8.9–27.6)	15.8 (5.0–75.9)	0.911[c]
Alanine aminotransferase (IU/L)[a]	43 (20–179)	45 (18–411)	0.580[c]
γ-glutamyl transpeptidase (IU/L)[a]	52 (23–1,529)	32 (17–171)	0.026[c]
Total cholesterol (mg/dl)[a]	169 (129–222)	177 (113–248)	0.539[c]
Hyaluronic acid (ng/ml)[a]	151 (42–247)	73 (5–414)	0.121[c]
Ferritin (ng/dl)	112 (18–489)	108 (13–833)	0.665[c]
α-fetoprotein (ng/ml)[a]	7.2 (3–16)	6.1 (3–29)	0.732[c]
HCV core antibody (U)[a]	240 (76–250)	210 (2–4,300)	0.790[c]
Viral load (log IU/ml)[a]	6.8 (6.0–7.4)	6.8 (5.0–7.3)	0.226[c]
Mutation of ISDR 0–1/2–	10/0	45/20	0.035[d]
HCV core 70 aa wild (R)/mutant (Q or H)	4/7	53/15	0.009[d]
HCV core 91 aa wild (L)/mutant (M)	6/5	41/28	0.504[d]
rs8099917 (GG, TG/TT)	8/4	13/59	0.001[d]

Factor	Odds ratio	95% CI	*P*-value
Multivariate analysis			
rs8099917 (GG and TG)	17.2	1.9–100.0	0.002
Core aa 70 mutant	5.9	1.1–31.5	0.038

[a]Median.
[b]χ^2 test.
[c]Mann–Whitney *U*-test.
[d]Fisher's exact test.

(rs8099917) and viral (aa 70) factors (Fig. 2). Fifty-seven percent of patients with genotype TT achieved SVR, whereas only 19% of patients with genotype non-TT achieved SVR. Moreover, none of the patients with genotype non-TT and aa 70 mutant achieved SVR (Fig. 2).

Viral Load Decline During the Initial 4 weeks of the Therapy

The decline of viral load at 4 weeks after initiation of therapy was evaluated according to IL-28B variants and/or aa substitution of HCV core region. There seemed to be a significant difference between wild and mutant type of aa 70 in the HCV core region, as well as between rs8099917 genotypes (Fig. 3A,B). Stratified analysis according to IL-28B variant revealed that there was no significant difference in viral decline between HCV core aa 70 wild and mutant types (Fig. 3C).

Correlation Between IL-28B and Other Factors

Since only IL-28B genotype and aa 70 substitutions were significant in multivariate analysis among variables that were found to be associated with therapy outcome in the univariate analysis, relationships between these variables were examined. The results of the univariate correlations between the variables indicated that γ-glutamyl transpeptidase ($P = 0.004$), hyaluronic acid ($P = 0.005$), and aa 70 substitutions

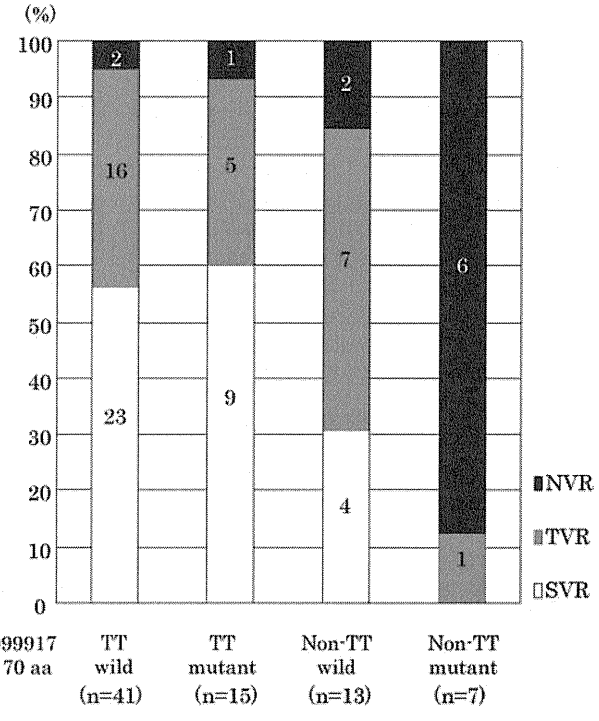

Fig. 2. Response to the combination therapy. Patients were divided into four groups according to rs8099917 genotype and core 70 amino acid type. SVR, sustained viral response; TVR, transient viral response; NVR, nonviral response.

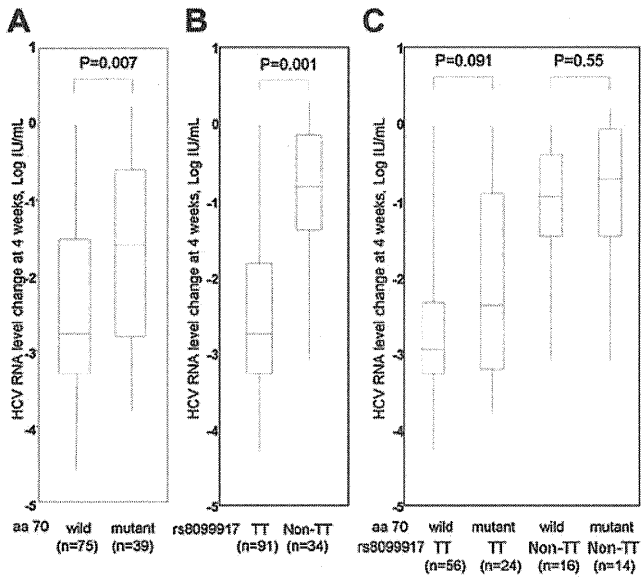

Fig. 3. Viral decline during the initial 4 weeks of the combination therapy. One hundred ninety-two patients (Group B) who were treated with the standard dose of peg-interferon and ribavirin were analyzed. Boxes show 25–75 percentiles with the inner line representing the median. The whiskers indicate range.

($P = 0.001$) were significantly correlated with IL-28B genotype (Table V).

DISCUSSION

IL-28B, also known as IFN-lambda 3, is a member of the Type-III interferon family which was recently described as a novel group of cytokines. They differ structurally and genetically from type-I IFNs but are similar in terms of biological activity and mechanism of action. Recently, several studies have reported that genetic variants near IL-28B are associated with response to treatment of chronic HCV infection with PEG-IFN and RBV combined therapy.

In this study, IL-28B variants and HCV core aa 70 substitutions were found to be independent predictive factors of NVR. In addition, the proportion of aa 70 wild-type was significantly higher in patients with the TT genotype of rs8099917 compared to the other genotypes. Interestingly, there was a significant difference in the proportion of aa 70 substitutions between rs8099917 genotypes. The mechanism by which IL-28B polymorphism affects the proportion of HCV core variants is currently unclear. Thomas et al. [2009] have reported that IL-28B variation is associated with spontaneous eradication of HCV virus among European and African populations. On the other hand, HCV core protein has been reported to inhibit activation of STAT1 [Lin et al., 2006] and induce SOCS3 [Bode et al., 2003], resulting in suppression of IFN signaling. Thus, effects of IL-28B variants on the immune response might be modulated by amino acid substitutions in the HCV core region, although further studies are needed.

Mori et al. [2009] have observed that the viral decline during the initial 4 weeks of combination therapy was significantly greater in patients with aa 70 wild-type than in those with mutant type. Amino acid substitutions in HCV core region have been reported to be a useful predictor of response to PEG-IFN plus ribavirin therapy [Akuta et al., 2009]. We found what appeared to be a significant difference between wild and mutant type of aa 70 in the HCV core region, as well as between rs8099917 genotypes (Fig. 3A,B). However, stratified analysis according to IL-28B variant revealed that there was not significant difference in viral decline at 4 weeks between aa 70 wild and mutant types (Fig. 3C). Therefore, these data emphasize that studies evaluating the effect of viral factors, such as 70 aa in the HCV core region should consider the confounding effect of IL-28B variant.

Based on the above host and viral factors, patients were classified into four categories. As shown in Figure 2, about 60% of the patients in each category with the TT genotype showed SVR. In contrast, none of the patients with aa 70 mutant HCV who lacked the TT genotype achieved SVR. These results may provide useful information for the development of a more effective and personalized treatment regimen. Further investigation would be required to assess whether a longer period of treatment for the patients with non-TT genotypes could improve SVR rate in spite of aa 70 mutation.

TABLE V. Correlations Between Biomarkers and Clinical Parameters in 288 Patients

	HCV core 70 aa wild (R)/mutant (Q or H)	Age	Fibrosis	γ-glutamyl transpeptidase	Hyaluronic acid	Platelet count
rs8099917 (TT/TG/GG)[a]	0.212 (0.001)	−0.135 (0.026)	0.036 (0.588)	0.178 (0.004)	−0.220 (0.005)	0.008 (0.896)
HCV core 70 aa wild (R)/mutant (Q or H)[b]		−0.011 (0.862)	0.149 (0.033)	0.134 (0.039)	0.036 (0.667)	0.003 (0.968)
Age			0.076 (0.253)	−0.116 (0.054)	0.435 (<0.001)	−0.114 (0.060)
Fibrosis[c]				0.174 (0.010)	0.337 (<0.001)	−0.229 (0.001)
γ-glutamyl transpeptidase					0.210 (0.007)	−0.221 (<0.001)
Hyaluronic acid						−0.519 (<0.001)

Values are r (*P*-value), determined with Spearman's rank correlation test.
[a]rs8099917 genotypes were coded as 0, 1, or 2 according to the number of the minor allele G.
[b]HCV core aa substitution were coded as 0 for wild (R) and 1 for mutant (Q or H).
[c]Stages of liver fibrosis were coded as 0, 1, 2, 3, 4 according to F0, F1, F2, F3, F4, respectively.

Another interesting finding was that rs8099917 was correlated not only with aa 70 but also with γ-glutamyl transpeptidase and hyaluronic acid (Table V). HCV core protein has been noted to induce liver steatosis [Moriya et al., 1998], and γ-glutamyl transpeptidase is known to be associated with steatosis. Hyaluronic acid is a marker of liver fibrosis, and patients with advanced liver disease have high hyaluronic acid levels. Nevertheless, rs8099917 was correlated with hyaluronic acid levels but not with fibrosis. The reason for this discrepancy is not clear. These correlations suggest that IL-28B polymorphism may also affect disease progression. Further study is required to understand these issues.

In summary, the present study demonstrated that IL-28B genotype correlates with viral and biochemical markers and is a strong predictor of response to combination therapy. Assessment of these factors in a large-scale prospective study will help facilitate development of personalized therapy for chronic hepatitis C.

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REFERENCES

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: Amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 46:403–410.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2009. A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: Amino acid substitutions in HCV core region as predictor of sustained virological response. J Med Virol 81:452–458.

Alter H. 2006. Viral hepatitis. Hepatology 43:S230–S234.

Bode JG, Ludwig S, Ehrhardt C, Albrecht U, Erhardt A, Schaper F, Heinrich PC, Haussinger D. 2003. IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J 17:488–490.

Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, Howell CD. 2006. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 131:470–477.

Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ. 1994. Classification of chronic hepatitis: Grading and staging. Hepatology 19:1513–1520.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C. 1995. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 96:224–230.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347:975–982.

Gao B, Hong F, Radaeva S. 2004. Host factors and failure of interferon-alpha treatment in hepatitis C virus. Hepatology 39:880–890.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Hijikata M, Ohta Y, Mishiro S. 2000. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt -88) correlated with the response of hepatitis C patients to interferon. Intervirology 43:124–127.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 87:9524–9528.

Knapp S, Yee LJ, Frodsham AJ, Hennig BJ, Hellier S, Zhang L, Wright M, Chiaramonte M, Graves M, Thomas HC, Hill AV, Thursz MR. 2003. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: Roles of MxA, OAS-1 and PKR. Genes Immun 4:411–419.

Lin W, Kim SS, Yeung E, Kamegaya Y, Blackard JT, Kim KA, Holtzman MJ, Chung RT. 2006. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. J Virol 80:9226–9235.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

Matsuyama N, Mishiro S, Sugimoto M, Furuichi Y, Hashimoto M, Hijikata M, Ohta Y. 2003. The dinucleotide microsatellite polymorphism of the IFNAR1 gene promoter correlates with responsiveness of hepatitis C patients to interferon. Hepatol Res 25:221–225.

McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS. 2009. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 361:580–593.

Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, Aikata H, Takahashi S, Chayama K. 2009. Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. J Med Virol 81:640–649.

Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. 1998. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 4:1065–1067.

Naito M, Matsui A, Inao M, Nagoshi S, Nagano M, Ito N, Egashira T, Hashimoto M, Mishiro S, Mochida S, Fujiwara K. 2005. SNPs in the promoter region of the osteopontin gene as a marker predicting the efficacy of interferon-based therapies in patients with chronic hepatitis C. J Gastroenterol 40:381–388.

Nakagawa M, Sakamoto N, Ueyama M, Mogushi K, Nagaie S, Itsui Y, Azuma S, Kakinuma S, Tanaka H, Enomoto N, Watanabe M. 2010. Mutations in the interferon sensitivity determining region and virological response to combination therapy with pegylated-interferon alpha 2b plus ribavirin in patients with chronic hepatitis C-1b infection. J Gastroenterol 45:656–665.

Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. 2001. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477.

Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, Berg T. 2004. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: A meta-analysis focused on geographical differences. Gut 53:1345–1351.

Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Gunthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Mullhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: A genome-wide association study. Gastroenterology 138:1338–1345, 1345 e1331-1337.

Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, Ichijo T, Yoshizawa K, Kiyosawa K, Tanaka E. 2008. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 48:1753–1760.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41: 1100–1104.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461:798–801.

Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromitsu K. 1994. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. Hepatology 19:1088–1094.

Tsukada H, Ochi H, Maekawa T, Abe H, Fujimoto Y, Tsuge M, Takahashi H, Kumada H, Kamatani N, Nakamura Y, Chayama K. 2009. A polymorphism in MAPKAPK3 affects response to interferon therapy for chronic hepatitis C. Gastroenterology 136:1796–1805 e1796.

Walsh MJ, Jonsson JR, Richardson MM, Lipka GM, Purdie DM, Clouston AD, Powell EE. 2006. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. Gut 55:529–535.

Welzel TM, Morgan TR, Bonkovsky HL, Naishadham D, Pfeiffer RM, Wright EC, Hutchinson AA, Crenshaw AT, Bashirova A, Carrington M, Dotrang M, Sterling RK, Lindsay KL, Fontana RJ, Lee WM, Di Bisceglie AM, Ghany MG, Gretch DR, Chanock SJ, Chung RT, O'Brien TR. 2009. Variants in interferon-alpha pathway genes and response to pegylated interferon-Alpha2a plus ribavirin for treatment of chronic hepatitis C virus infection in the hepatitis C antiviral long-term treatment against cirrhosis trial. Hepatology 49: 1847–1858.

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Common Genetic Polymorphism of ITPA Gene Affects Ribavirin-Induced Anemia and Effect of Peg-Interferon Plus Ribavirin Therapy

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An association between a single nucleotide polymorphism (SNP) in the inosine triphosphate pyrophosphatase (ITPA) gene and reduction of hemoglobin during peg-interferon plus ribavirin combination therapy for patients with chronic hepatitis C virus (HCV) infection has been reported. However, the effect of the SNP on outcome of therapy has not been fully elucidated. Factors associated with anemia during combination therapy, including rs1127354 genotype, were analyzed in 1,002 treated patients. The effect of the SNP on outcome of therapy was analyzed in a subset of 830 patients with genotype 1. A rapid initial decrease in hemoglobin levels was observed in patients with rs1127354 genotype CC compared with a slow decrease in non-CC patients. Cumulative reduction of ribavirin was significantly more frequent in genotype CC patients than non-CC patients (odds ratio 1.928, $P = 8.6 \times 10^{-8}$). The frequency of patients who received at least the recommended 80% of scheduled ribavirin was significantly lower among genotype CC patients, especially among those who had pretreatment hemoglobin levels between 13.5 and 15 g/dl ($P < 0.03$), and the sustained viral response rate was significantly lower in this group of patients. Independent predictive factors for sustained virological response included a SNP in the IL28B locus (rs809991), age, fibrosis, ITPA SNP rs1127354 as well as pretreatment hemoglobin levels. Our data suggests that measures to prevent anemia should be considered for patients who have pretreatment hemoglobin levels less than 13.5 g/dl or who have rs1127354 genotype CC and pretreatment hemoglobin levels between 13.5 and 15 g/dl. ***J. Med. Virol. 83:1048–1057, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: inosine triphosphate pyrophosphatase; single nucleotide polymorphism; peg-interferon; anemia; dose reduction

INTRODUCTION

Hepatitis C virus (HCV), a positive-strand RNA flavivirus, chronically infects 170 million people worldwide and is responsible for up to 300,000 deaths due

Abbreviations: HCV, hepatitis C virus; ITPA, inosine triphosphate pyrophosphatase; SNP, single nucleotide polymorphism.

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to progression to liver cirrhosis and hepatocellular carcinoma [Alter, 1995; Chevaliez and Pawlotsky, 2007]. Currently, peg-interferon plus ribavirin combination therapy (PEG-RBV) is the most effective treatment, but it is only effective in 50% of patients with genotype 1b, and the therapy has severe side effects often requiring dose modification or discontinuation [Hadziyannis et al., 2004]. However, there are several factors that may help predict outcome of therapy, including HCV genotype [Zeuzem et al., 1996], virus titer [Zeuzem et al., 1996; Dienstag and McHutchison, 2006], age, fibrosis of the liver, obesity, race, hepatic steatosis [Dienstag and McHutchison, 2006], LDL cholesterol, gamma-GTP [Akuta et al., 2007], insulin resistance [Romero-Gómez et al., 2005], amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity determining region (ISDR) of the NS5A protein [Enomoto et al., 1995a; Akuta et al., 2005]. A series of recent studies have also identified common genetic variants in the IL28B locus on chromosome 19 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009] that are strongly associated with outcome of combination therapy.

Ribavirin-induced anemia is a serious side effect of therapy which results in dose reduction of ribavirin and possibly of interferon as well. The precise mechanism of induction of anemia remains to be determined. Ribavirin-induced hemolytic anemia accompanied by an increase in reticulocyte counts has been reported to be associated with membrane oxidative damage as well as impairment of erythrocyte Na–K pump activity and increase in dithiotreitol-sensitive fraction, malondialdehyde, and methemoglobin levels [De Franceschi et al., 2000]. Treating patients with erythropoietin, which induces erythropoiesis and helps alleviate anemia, has been reported to be effective in preventing ribavirin dose reduction and leads to better therapy outcome [Dieterich et al., 2003].

Recently, single nucleotide polymorphisms (SNPs) in the inosine triphosphate pyrophosphatase (ITPA) locus have been found to be associated with anemia in patients treated with combination therapy [Fellay et al., 2010; Ochi et al., 2010; Thompson et al., 2010]. In Caucasian patients there are two SNPs that are associated with ITPA enzyme activity [Fellay et al., 2010; Thompson et al., 2010], although one of these SNPs appears to be absent in Japanese patients [Ochi et al., 2010]. Although the effect of the ITPA polymorphism on ribavirin-induced anemia has been clearly demonstrated by these studies, the effect of the SNP on outcome of therapy has not been fully explored. Our previous report suggested an association of the polymorphism with sustained virological response (SVR) [Ochi et al., 2010], whereas other reports found no association [Fellay et al., 2010; Thompson et al., 2010].

In the current study, 1,002 patients who were treated with peg-interferon 2b plus ribavirin combination therapy were analyzed to elucidate the precise effect of the ITPA SNP on hemoglobin reduction. A subset of 830 of the patients with genotype 1 were further examined to assess the effect of the SNP on therapy outcome. The results show that reduction of ribavirin was frequent among patients with low pretreatment hemoglobin levels (<13 g/dl) as well as those with the ribavirin-sensitive ITPA genotype (rs1127354 CC) and intermediate pretreatment hemoglobin levels (13.5–15 g/dl). Our results suggest that anemia-preventing measures, such as administration of erythropoietin, should be considered for patients likely to develop anemia.

MATERIALS AND METHODS

Patients

Data from 1,002 patients who were treated with peg-interferon alpha 2b and ribavirin combination therapy for chronic hepatitis C infection between December 2004 and January 2010 were collected from Toranomon Hospital (Tokyo) and hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Patient profiles are shown in Table I. All patients tested positive for HCV RNA for more than 6 months and were negative for hepatitis B and HIV and showed no evidence for other liver diseases including alcoholic hepatitis, hemochromatosis, Wilson's disease, and autoimmune hepatitis. Patients received weekly injections of peg-interferon-alpha-2b at 1.5 g/kg body weight for 48 weeks, and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg). Ribavirin dose was reduced when hemoglobin levels fell to 10 g/dl, and both peg-interferon and ribavirin were discontinued when hemoglobin levels dropped to <8.5 g/dl. Patients who remained positive for HCV RNA during the first 12 weeks of treatment but became negative by week 32 received extended administration of both drugs until 72 weeks. The successful endpoint of treatment was considered SVR, defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. A subset of patients showed transient response (TR), in which HCV RNA dropped to undetectable levels but then later rebounded. The remaining patients in which HCV RNA never became undetectable were considered non-responders (NVR). Histopathological diagnosis was made by pathologists at each hospital according to the criteria of Desmet et al. [1994]. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA Levels

HCV RNA levels were measured throughout the course of therapy via RT-PCR using the original

TABLE I. Characteristics of Patients by ITPA rs1127354 SNP Genotype

	All patients	Patients with HCV genotype 1		
	Total (n = 1,002)	Total (n = 830)	CC (n = 628)	CA/AA (n = 202)
Age (years)	58 (51–64)	58 (51–64)	58 (52–64)	58 (51–64)
Sex (M/F)	539/463	448/382	328/300	120/82
Height (cm)	161 (154–168)	161 (154–168)	161 (154–168)	161 (155–168)
Weight (kg)	58.5 (52–67)	58.2 (52–66.2)	58.05 (51.8–66.45)	59 (52–65)
rs8099917 (TT/GT/GG)	720/253/25	585/222/20	437/174/15	148/48/5
rs12979860 (CC/CT/TT)	543/198/52	541/197/52	403/151/44	138/46/8
rs1127354 (CC/CA/AA)	753/227/22	628/183/19	628/0/0	0/183/19
Core70 (W/M/ND)	240/143/619	239/143/448	175/114/339	64/29/109
Core91 (W/M/ND)	217/168/617	216/168/446	168/123/337	48/45/109
ISDR (0–1/≥2/ND)	287/80/635	287/80/463	216/61/351	71/19/112
Fibrosis (1/2/3/4/ND)	252/191/124/29/401	252/190/124/29/230	194/138/90/23/179	58/52/34/6/51
Activity (0/1/2/3/ND)	9/252/280/42/419	9/251/280/42/248	6/187/213/31/191	3/64/67/11/57
WBC (/mm^3)	4,700 (3,900–5,600)	4,700 (3,900–5,600)	4,700 (3,900–5,530)	4,900 (4,000–5,942)
Plt ($\times 10^4$/mm^3)	15.6 (12.2–19.7)	15.4 (12.2–19.35)	15.3 (12.1–19.33)	15.9 (12.45–19.4)
Hb (g/dl)	14 (13.2–14.9)	14 (13.2–14.9)	14.1 (13.2–14.9)	14 (13.4–15)
AST (IU/L)	45 (34–66)	45 (34–66)	45 (34–67)	45.5 (34–64.5)
ALT (IU/L)	53 (36–85)	53 (36–85)	52 (36–84.5)	55 (34.5–85)
γGTP (IU/L)	40 (25–73)	40 (25–73)	39.5 (25–72)	43.5 (25.25–77.25)
Total cholesterol (mg/dl)	172 (151–193)	172 (151–193)	172 (150–194)	171 (154–190)
HDL cholesterol (mg/dl)	51 (40–64)	51 (40–64)	52 (40.25–64)	50 (38–63.75)
Fasting blood sugar (mg/dl)	98 (89–112.8)	98 (89–113)	99 (89–113)	95 (88–108)
Virus titer (log IU/ml)	6.5 (6–7)	6.5 (6–7)	6.5 (6–7)	6.5 (6.1–6.9)
Viral genotype (1b/1a/others)	814/9/179	814/9/7	618/6/4	196/3/3
RBV treatment period (weeks)	48 (37–59)	48 (37–59)	48 (34.75–57)	48 (47–64.75)
RBV reduction (no/yes/ND)	316/450/236	315/448/67	212/366/50	103/82/17
Weeks to first RBV reduction	16 (5–48)	16.5 (5–48)	12 (4–47)	44 (12–51.75)
Outcome of therapy (NR/TR/SVR)	154/157/283	154/156/281	125/120/202	29/36/79

ND, not determined or data unavailable.
Categorical variables are reported as counts, and continuous variables are reported as median and interquartile range.

Amplicor method, the high range method, or the TaqMan RT-PCR test. The measurement ranges of these assays were 0.5–850 KIU/ml, 5–5,000 KIU/ml, and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with PBS and reanalyzed. All values are reported as log IU/ml.

ISDR and Core aa Substitutions

Amino acid substitutions in the HVC core and ISDR regions were determined by direct sequencing of PCR products following extraction and reverse transcription of HCV RNA using 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. [2007, 2006], and the number of ISDR substitutions was established as in Enomoto et al. [1995b, 1996].

SNP Genotyping

Each patient was genotyped for two IL28B SNPs previously reported to be associated with therapy outcome: rs12979860 and rs8099917, and a SNP reported to be associated with ribavirin-induced anemia: rs1127354. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip, the Invader assay, or the TaqMan assay, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003]. The two SNPs in the IL28B locus are in strong linkage disequilibrium, with a correlation coefficient of 0.99.

Statistical Analysis

The χ^2 and Mann–Whitney U-tests were applied to detect significant associations. Simple and multiple regression analyses were used to examine the association between treatment outcome and the values of other markers, using $P < 0.1$ as the criterion for inclusion in the multivariate model. All of the statistical analyses were two sided, and $P < 0.05$ was considered significant. All statistical analysis was performed using the PASW Statistics 18 program (SPSS, Inc., Chicago, IL).

RESULTS

Reduction of Hemoglobin Levels During Therapy by ITPA Genotype

Decrease in hemoglobin levels during therapy was analyzed by rs1127354 genotype (CC vs. non-CC). As shown in Figure 1, a rapid decrease in hemoglobin levels during the initial 4 weeks was observed in genotype CC patients. Hemoglobin levels in genotype

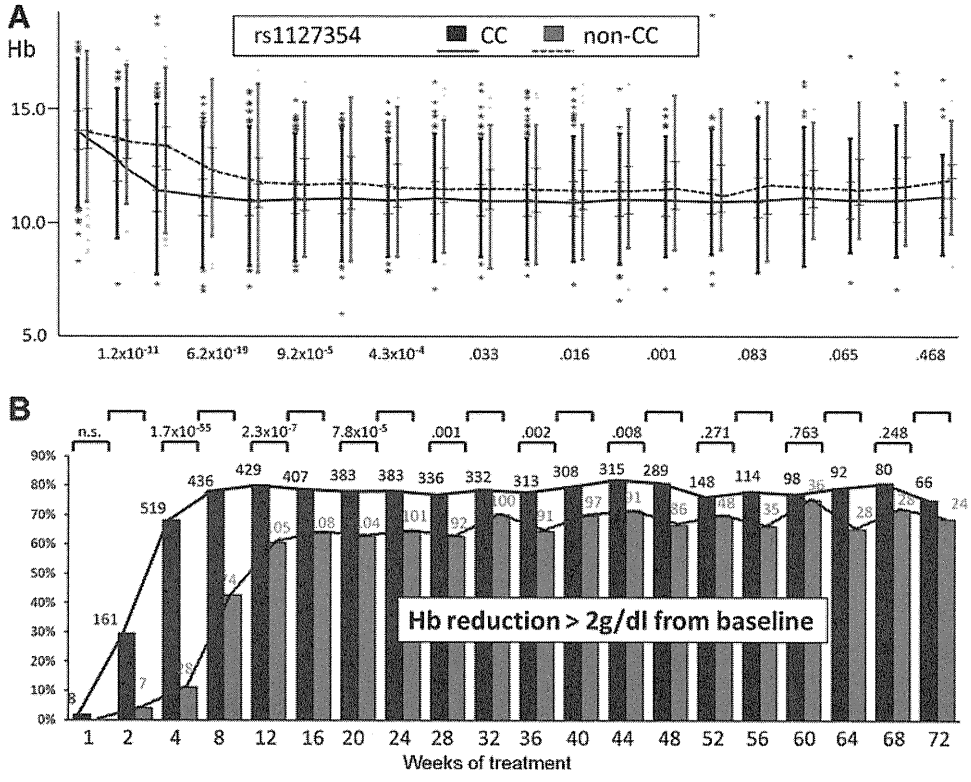

Fig. 1. Reduction of hemoglobin levels by ITPA polymorphism during peg-interferon plus ribavirin combination therapy. **A**: Hemoglobin levels in patients who were treated during the course of therapy. Patients were grouped by ITPA SNP rs1127354 genotype (CC or non-CC). Follow-up hemoglobin levels following cessation of therapy are not shown. **B**: Number of patients who showed >2 g/dl of hemoglobin. Statistical significance was assessed using the χ^2 and Mann–Whitney *U*-tests.

CC patients stabilized by week 8 and did not decrease further. In contrast, a slow but continuous decrease in hemoglobin level was observed in non-CC patients until week 48 (Fig. 1A). Reduction of hemoglobin by more than 2 g/dl was observed significantly more frequently in CC genotype patients than in non-CC patients (Fig. 1B). Differences between the two groups of patients were most pronounced between weeks 2 and 8 (Fig. 1B).

Ribavirin Dose Reduction by ITPA Genotype and Pretreatment Hemoglobin Levels

Decrease in hemoglobin levels resulted in ribavirin dose reduction. The frequency of hemoglobin decrease was higher in genotype CC patients compared with non-CC patients (Fig. 2A). Based on the assumption that initial hemoglobin levels influence incidence of ribavirin dose reduction, reduction frequency was analyzed by initial hemoglobin levels. As shown in Figure 2B–D, reduction of ribavirin was more frequent in genotype CC patients than non-CC patients in all three subsets of patients but was more prominent in patients with intermediate pretreatment hemoglobin levels between 13.5 and 15 g/dl (Fig. 2B–D).

Effect of ITPA Genotype and Pretreatment Hemoglobin Levels on Patients Receiving at Least 80% of Planned Ribavirin Administration

The reduction of ribavirin dosage during therapy resulted in reduction of the total amount of ribavirin given to each patient. As 80% of planned ribavirin administration appears to be a threshold associated with treatment outcome in patients with genotype 1b [McHutchison et al., 2002], the proportion of patients who received more than 80% of the initially planned dosage of ribavirin in 830 patients with genotype 1 and treated with the combination therapy (Table I) were analyzed. As shown in Figure 3, patients with non-CC genotypes tended to tolerate more than 80% of the predetermined dose of ribavirin compared with patients with CC. The difference was statistically significant only in patients whose pretreatment hemoglobin level was 13.5–15 g/dl, however (Fig. 3).

Factors Associated With Successful Administration of at Least 80% of Planned Ribavirin Dose

As it is possible that several factors including ITPA genotype and pretreatment hemoglobin levels are associated with dose reduction of ribavirin, the

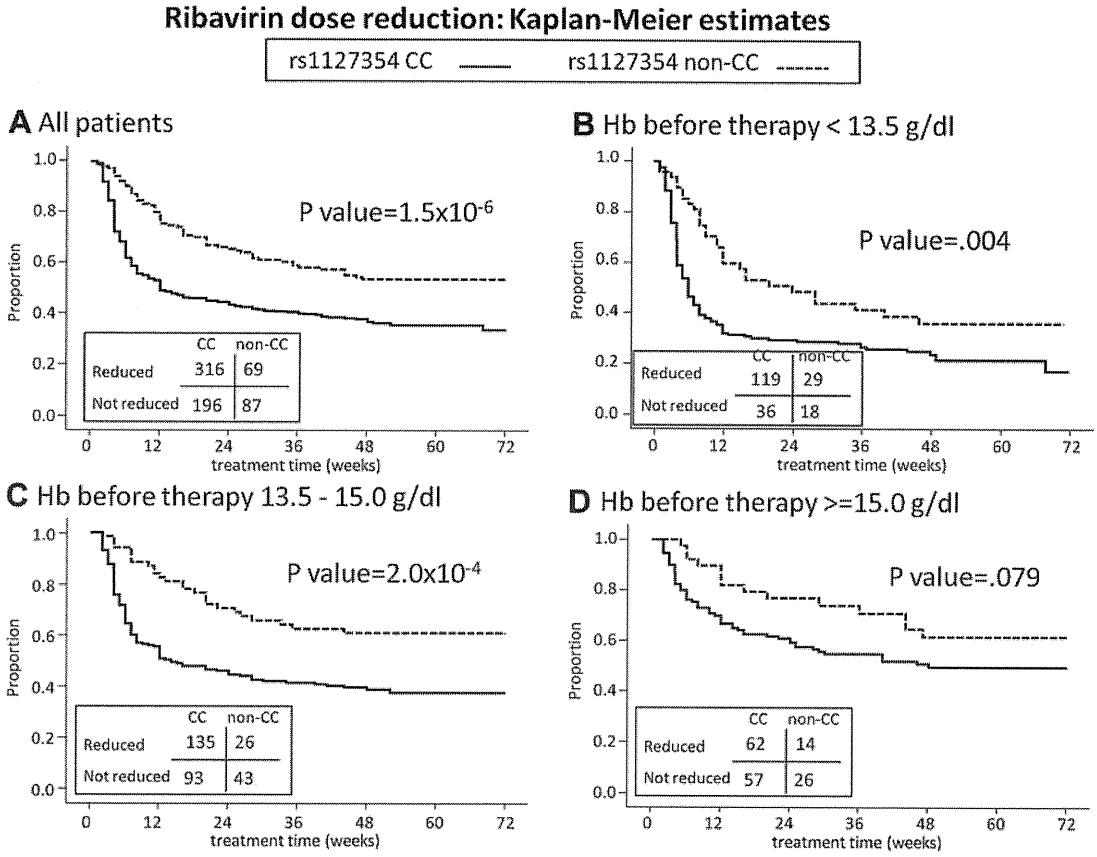

Fig. 2. Dose reduction of ribavirin in patients who were treated with combination therapy. Kaplan–Meier curves for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line: CC, dashed-line: non-CC) among (**A**) all patients, (**B**) patients with low pretreatment hemoglobin levels (<13.5 g/dl), (**C**) patients with intermediate pretreatment hemoglobin levels (13.5–15.0 g/dl), and (**D**) patients with high pretreatment hemoglobin levels (≥15 g/dl).

effect of these factors as well as clinical factors were analyzed for dose reduction of ribavirin. As shown in Table II, univariate analysis identified ITPA SNP rs1127354 genotype, fibrosis stage and inflammatory activity of the liver, white blood cell count, platelet count, hemoglobin, ALT, age, and sex as factors associated with more than 80% ribavirin administration. Multivariate analysis identified age, hemoglobin, and rs1127354 genotype as independent predictive factors.

Effect of ITPA Genotype and Pretreatment Hemoglobin Levels on Outcome of Therapy

As the frequency of patients receiving more than 80% of planned ribavirin administration differed by pretreatment hemoglobin levels and ITPA genotype, treatment outcome might be expected to differ based on these factors. As expected, SVR rate was significantly higher in patients with non-CC genotypes with hemoglobin levels 13.5–15 g/dl, where the frequency of patients receiving 80% ribavirin administration differed most significantly between genotypes CC and non-CC (Fig. 4).

Predictive Factors of the Combination Therapy for SVR and NVR

Predictive factors for SVR and NVR were assessed, including baseline clinical factors, genotype of the recently reported IL28B SNP, and viral factors such as the number of substitutions in the ISDR, and substitutions at core amino acid 70 and 91. By univariate analysis, a number of factors were significantly associated with SVR, including IL28B SNP genotypes (rs8099917 and rs12979860), ITPA SNP rs1127354 genotype, core70 mutation, fibrosis of the liver, white blood cell count, platelet count, hemoglobin, ALT, fasting blood sugar, viral titer, age, sex, body mass index, and duration of the therapy (Table III). Multivariate analysis identified IL28B SNP rs8099917 genotype as the strongest independent predictor for SVR (OR 15.379, $P = 3.48E{-}07$), followed by hemoglobin level, ITPA SNP rs1127354 genotype, fibrosis of the liver, age, and body mass index (Table III). Significant independent predictive factors for NVR included IL28B SNP rs8099917 genotype fibrosis, and age (Table IV).

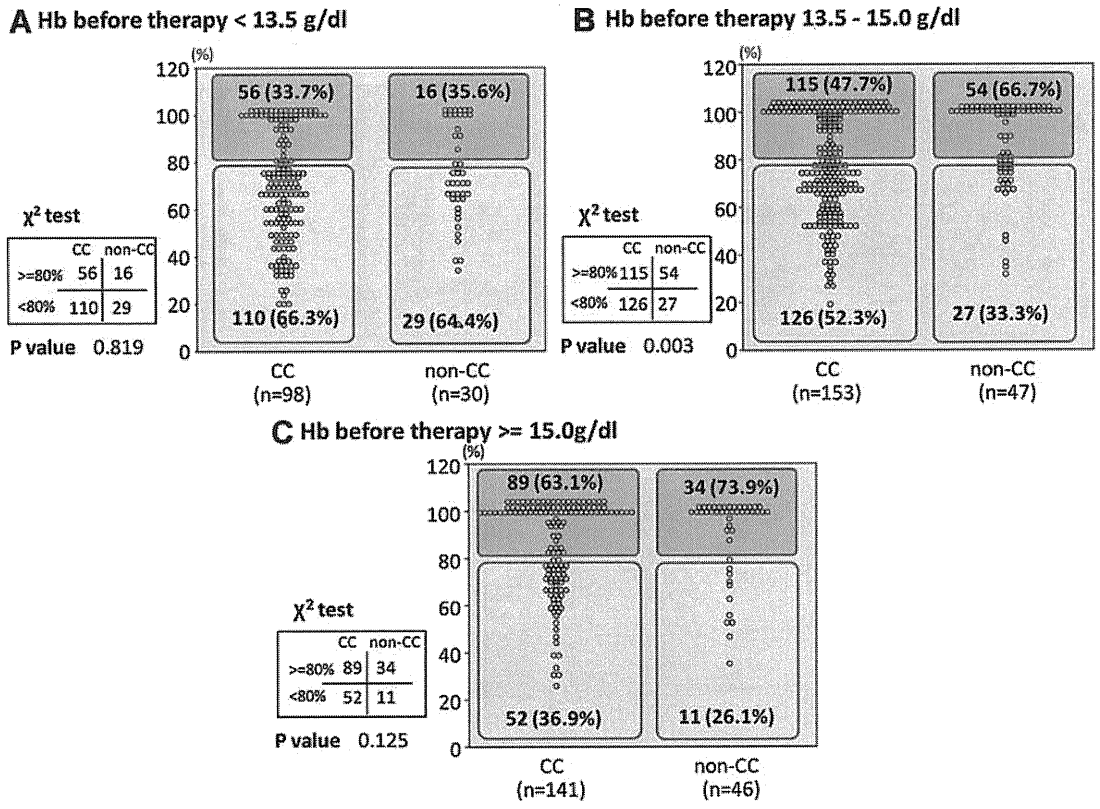

Fig. 3. Dose of ribavirin administered to patients with genotype 1 treated with combination therapy by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (**A**) <13.5 g/dl, (**B**) between 13.5 and 15.0 g/dl, and (**C**) ≥15 g/dl.

TABLE II. Factors Associated With Ribavirin Dose Reduction (80%) in Hepatitis C Virus Patients Determined by Logistic Regression Analysis

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs1127354 CC vs. CA/AA	0.580	0.002**	0.578	0.372–0.897	0.014*
Core70	1.007	0.974			
Core91	0.776	0.244			
ISDR 0/1 vs. >1	1.091	0.743			
BMI (kg/m^2)	1.008	0.740			
Fibrosis 1–2 vs. 3–4	1.676	0.009**	1.409	0.902–2.202	0.132
Activity 0–1 vs. 2–3	1.537	0.013*			
WBC (/mm^3)	1.000	1.2E−05**			
Plt (×10^4/mm^3)	1.070	5.2E−06**	1.000	1.000–1.000	0.178
Hb (g/dl)	1.485	1.7E−10**	1.244	1.066–1.453	0.006**
AST (IU/L)	1.001	0.769			
ALT (IU/L)	1.003	0.035*			
γGTP (IU/L)	1.001	0.362			
Albumin (g/dl)	1.549	0.460			
Total cholesterol (mg/dl)	0.997	0.175			
Triglycerides (mg/dl)	1.000	0.935			
HDL cholesterol (mg/dl)	0.989	0.066			
LDL cholesterol (mg/dl)	0.995	0.503			
Fasting blood sugar (mg/dl)	1.001	0.585			
Virus titer (log IU/ml)	1.047	0.567			
Age	0.936	2.1E−15**	0.934	0.914–0.954	3.5E−10**
Sex	0.586	3.9E−04**			

**$P < 0.01$.
*$P < 0.05$.

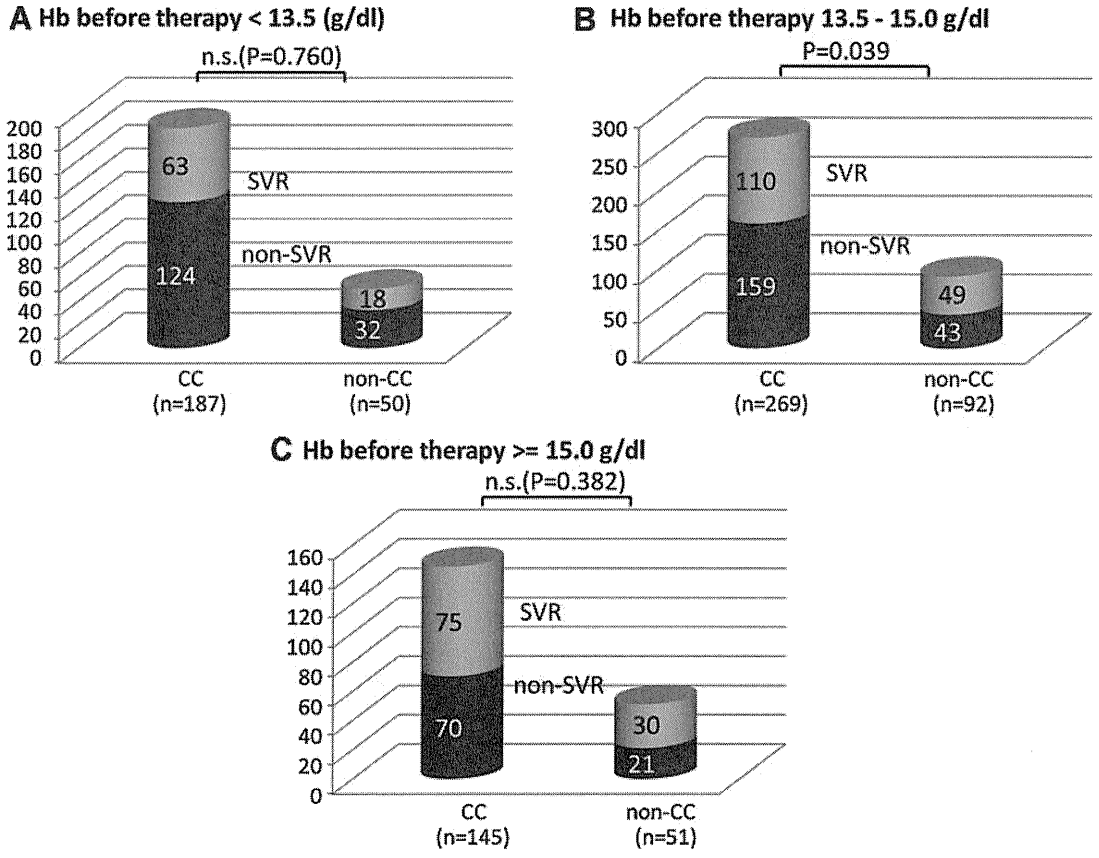

Fig. 4. Effect of combination therapy in patients with genotype 1b by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (**A**) <13.5 g/dl, (**B**) between 13.5 and 15.0 g/dl, and (**C**) ≥15 g/dl.

DISCUSSION

Ribavirin-induced anemia is one of the most serious side effects resulting from combination therapy [De Franceschi et al., 2000], but a polymorphism within the ITPA gene has recently been shown to affect incidence of this form of anemia [Fellay et al., 2010; Ochi et al., 2010; Thompson et al., 2010]. This study showed that hemoglobin decrease is faster and more severe, especially in the first 12 weeks of treatment, in patients with the anemia-susceptible ITPA rs1127354 CC genotype (Fig. 1). The rapid reduction of hemoglobin observed in genotype CC patients persisted to the end of therapy and was associated with early reduction of ribavirin dosage (Fig. 2), resulting in lower total ribavirin administration. The linear and continuous decrease in hemoglobin seen in non-CC patients also contributed to the reduction of ribavirin administration but not as drastically as in patients with the CC genotype (Fig. 2). The other significant ITPA SNP, rs7270101, is associated with splicing variant formation and reduced activity of the ITPA enzyme in patients of European and African ancestry, but this SNP is absent in the Japanese population [Ochi et al., 2010]. Therefore, only the missense SNP rs1127354, which results in a P32T amino acid change and reduced enzyme activity, was analyzed. Thompson et al. [2010] divided patients into four groups (−, +, ++, +++) based on the genotypes of these two SNPs. According to their classification, CC and non-CC genotypes in this study are almost comparable to "−" and "++" in their study because there are no patients with the rs1127354 AA genotype, and there were only two "+++" patients present in their study. Hemoglobin decrease was slightly milder in the this study compared to Thompson et al. [2010], probably due to early reduction in ribavirin dose in Japanese patients resulting from lower pretreatment hemoglobin levels.

Initial hemoglobin levels indeed had a strong influence on reduction of ribavirin dose. As shown in Figure 3, ITPA genotype did not have a significant influence on patients with <80% ribavirin administration when pretreatment hemoglobin levels were <13.5 or >15 g/dl. Accordingly, because reduction of ribavirin to <80% results in decreased rate of SVR [McHutchison et al., 2002], patients with pretreatment hemoglobin levels below 13.5 g/dl or patients with pretreatment hemoglobin levels between 13.5 and 15 g/dl who have the ITPA anemia-susceptible genotype should receive treatment with drugs such as erythropoietin to prevent reduction of ribavirin.

TABLE III. Predictive Factors Associated With Sustained Viral Response in Hepatitis C Virus Patients Determined by Logistic Regression Analysis

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs8099917 TT vs. TG/GG	3.614	1.85E−16**	15.358	5.371–43.919	3.48E−07**
rs12979860 CC vs. CT/TT	4.271	8.87E−16**			
rs1127354 CC vs. CA/AA	0.660	0.006**	0.368	0.161–0.838	0.017*
Core70	1.891	0.005**			
Core91	1.503	.059			
ISDR 0/1 vs. >1	0.660	0.106			
BMI (kg/m^2)	0.944	0.007**	0.865	0.758–0.987	0.032*
Fibrosis 1–2 vs. 3–4	2.290	5.83E−05**	4.540	1.618–12.734	0.004**
Activity 0–1 vs. 2–3	0.869	0.412			
WBC (/mm^3)	1.000	0.008**			
Plt ($\times 10^4$/mm^3)	1.072	4.68E−08**	1.055	0.976–1.141	0.176
Hb (g/dl)	1.172	0.001**	1.505	1.106–2.048	0.009**
AST (IU/L)	1.000	0.824			
ALT (IU/L)	1.003	0.027*			
γGTP (IU/L)	0.998	0.118			
Albumin (g/dl)	2.802	0.089			
Total cholesterol (mg/dl)	1.002	0.345			
Triglyceride (mg/dl)	0.997	0.094			
HDL cholesterol (mg/dl)	1.003	0.670			
LDL cholesterol (mg/dl)	0.999	0.922			
Fasting blood sugar (mg/dl)	0.989	0.001**	0.991	0.977–1.005	0.197
Virus titer (log IU/ml)	0.722	1.83E−04**	0.798	0.567–1.124	0.196
Age	0.960	5.13E−11**	0.957	0.919–0.995	0.028*
Sex	0.713	0.009**			
RBV treatment period (weeks)	1.012	3.86E−04**			

**$P < 0.01$.
*$P < 0.05$.

TABLE IV. Predictive Factors Associated With NVR in Chronic Hepatitis C Virus Patients Treated With Peg-Interferon Plus Ribavirin Combination Therapy

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs8099917 TT vs. TG/GG	6.663	6.00E−32**	7.157	3.592–14.262	2.21E−08**
rs12979860 CC vs. CT/TT	7.589	1.07E−30**			
rs1127354 CC vs. CA/AA	0.673	0.027*			
Core70	2.531	5.25E−05**			
Core91	1.951	0.003**	1.604	0.849–3.033	0.146
ISDR 0/1 vs. >1	0.569	0.053			
BMI (kg/m^2)	0.969	0.189**	0.910	0.822–1.008	0.070
Fibrosis 1–2 vs. 3–4	1.826	0.002**	2.941	1.404–6.162	0.004**
Activity 0–1 vs. 2–3	0.866	0.424			
WBC (/mm^3)	1.000	0.052			
Plt ($\times 10^4$/mm^3)	1.048	0.001**			
Hb (g/dl)	1.112	0.046*			
AST (IU/L)	0.999	0.608			
ALT (IU/L)	1.001	0.651			
γGTP (IU/L)	0.996	0.007**			
Albumin (g/dl)	1.534	0.479			
Total cholesterol (mg/dl)	1.005	0.058			
Triglyceride (mg/dl)	0.998	0.100			
HDL cholesterol (mg/dl)	1.003	0.669			
LDL cholesterol (mg/dl)	0.997	0.664			
Fasting blood sugar (mg/dl)	0.998	0.461			
Virus titer (log IU/ml)	0.753	0.006**	0.744	0.534–1.036	0.080
Age	0.977	0.001**	0.958	0.927–0.99	0.010**
Sex	0.830	0.202			
RBV treatment period (weeks)	1.021	1.86E−07**	1.012	0.996–1.027	0.135

Results of simple and multiple logistic regression are shown. The multivariate model was constructed using stepwise selection of significant univariate terms.
**$P < 0.01$.
*$P < 0.05$.

Although the ITPA polymorphism was significantly associated with ribavirin-induced anemia [Fellay et al., 2010; Thompson et al., 2010], no effect on outcome of therapy was found in the two previous studies on ITPA polymorphism from the United States. In contrast, Ochi et al. [2010] reported a possible association between ITPA genotype and outcome of therapy in Japan. Similarly, results of this study suggest an association between ITPA genotype and outcome of combination therapy for HCV genotype 1 in Japanese patients (Table II). There are several potential reasons for the different effects of ITPA genotype among these studies. First, the incidence of anemia-protective (rs1127354 non-CC) genotypes is higher in Japanese patients (20%) compared with patients with European (16.7%) and Sub-Saharan African (6.7%) ancestry [Olivier, 2003], suggesting a lack of power to detect the association in studies based on these populations. Secondly, the age of treated patients is higher in Japan than in the US (50–55 vs. 45) [Kainuma et al., 2010], which may lead to a higher incidence of ribavirin dose reduction during therapy [Hung et al., 2006]. Similarly, lower pretreatment levels of hemoglobin in Japanese patients compared with US patients (13.0 g/dl vs. 14.9 g/dl) [Fellay et al., 2010; Ochi et al., 2010] might result in a greater incidence of ribavirin reduction in Japanese patients and enhance the effects of the ITPA SNP on treatment outcome.

This study showed that a significantly larger number of patients ultimately received <80% of planned ribavirin administration when their hemoglobin levels were either <13.5 g/dl or between 13.5 and 15 g/dl in ribavirin-sensitive patients (ITPA rs1127354 genotype CC) (Fig. 4). As reported previously, administration of <80% of planned ribavirin is associated with poor outcome of therapy, and this study confirmed that reduction of ribavirin is significantly associated with SVR ($P < 0.009$, data not shown). Treatment of these patients with erythropoietin may therefore help prevent ribavirin dose reduction and improve SVR rate. However, in Japan erythropoietin is not available to treat this condition. As erythropoietin has been shown to improve anemia and treatment outcome of combination therapy, administration should be considered, at least for patients matching the criteria in this study, to improve the outcome of therapy.

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REFERENCES

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Arase Y, Ikeda K, Kumada H. 2006. Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol 78:83–90.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: Amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 46:403–410.

Alter MJ. 1995. Epidemiology of hepatitis C in the West. Semin Liver Dis 15:5–14.

Chevaliez S, Pawlotsky JM. 2007. Hepatitis C virus: Virology, diagnosis and management of antiviral therapy. World J Gastroenterol 13:2461–2466.

De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, Stanzial A, Solero P, Corrocher R. 2000. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: Role of membrane oxidative damage. Hepatology 31:997–1004.

Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. 1994. Classification of chronic hepatitis - diagnosis, grading and staging. Hepatology 19:1513–1520.

Dienstag JL, McHutchison JG. 2006. American Gastroenterological Association technical review on the management of hepatitis C. Gastroenterology 130:231–264; quiz 214–237.

Dieterich D, Wasserman R, Bräu N, Hassanein T, Bini E, Bowers P, Sulkowski M. 2003. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. Am J Gastroenterol 98:2491–2499.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C. 1995a. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis-C virus 1b—Sensitivity to interferon is conferred by amino-acid substitutions in the NS5A region. J Clin Invest 96:224–230.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C. 1995b. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 96:224–230.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Fellay J, Thompson A, Ge D, Gumbs C, Urban T, Shianna K, Little L, Qiu P, Bertelsen A, Watson M, Warner A, Muir A, Brass C, Albrecht J, Sulkowski M, McHutchison J, Goldstein D. 2010. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature 464:405–408.

Ge DL, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Jr., Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. 2004. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: A randomized study of treatment duration and ribavirin dose. Ann Intern Med 140:346–355.

Hung CH, Lee CM, Lu SN, Wang JH, Chen CH, Hu TH, Kee KM, Chang KC, Tseng PL, Yen YH, Changchien CS. 2006. Anemia associated with antiviral therapy in chronic hepatitis C: Incidence, risk factors, and impact on treatment response. Liver Int 26:1079–1086.

Kainuma M, Furusyo N, Kajiwara E, Takahashi K, Nomura H, Tanabe Y, Satoh T, Maruyama T, Nakamuta M, Kotoh K, Azuma K, Shimono J, Shimoda S, Hayashi J. 2010. Pegylated interferon alpha-2b plus ribavirin for older patients with chronic hepatitis C. World J Gastroenterol 16:4400–4409.

McHutchison J, Manns M, Patel K, Poynard T, Lindsay K, Trepo C, Dienstag J, Lee W, Mak C, Garaud J, Albrecht J, IHIT Group. 2002. Adherence to combination therapy enhances sustained

response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 123:1061–1069.

Ochi H, Maekawa T, Abe H, Hayashida Y, Nakano R, Kubo M, Tsunoda T, Hayes C, Kumada H, Nakamura Y, Chayama K. 2010. ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy-A genome-wide study of Japanese HCV virus patients. Gastroenterology 139:1190–1197.

Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. 2001. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477.

Olivier M. 2003. A haplotype map of the human genome. Physiol Genomics 13:3–9.

Romero-Gómez M, Del Mar Viloria M, Andrade R, Salmerón J, Diago M, Fernández-Rodríguez C, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez M, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. 2005. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology 128:636–641.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J, Hepatitis CS. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1174.

Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, Ohtsuki M, Furukawa H, Yoshino S, Yukioka M, Tohma S, Matsubara T, Wakitani S, Teshima R, Nishioka Y, Sekine A, Iida A, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K. 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 34:395–402.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Thompson A, Fellay J, Patel K, Tillmann H, Naggie S, Ge D, Urban T, Shianna K, Muir A, Fried M, Afdhal N, Goldstein D, McHutchison J. 2010. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. Gastroenterology 139:1181–1189.

Zeuzem S, Franke A, Lee JH, Herrmann G, Ruster B, Roth WK. 1996. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. Hepatology 24:1003–1009.

5 石川県の肝癌撲滅計画

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肝癌撲滅には背景にある肝炎ウイルスに対する治療導入が重要である。石川県では肝炎ウイルス検診初年度より協議会を設立し，陽性者をフォローアップしてきた。インターフェロン療法導入率向上を目指してさまざまな施策を講じ，導入率は30%を越えるようになった。2010年度よりかかりつけ医と専門医の連携を強化した「石川県肝炎診療連携」を新たに開始して専門医受診勧奨，抗ウイルス療法導入を図ることにより肝癌撲滅を目指している。

はじめに

2009年度人口動態統計では肝癌による死亡者数は男性で第4位，女性では第6位であり，年間3万人を越えている。肝癌の多くはウイルス性慢性肝疾患を背景に発生しており，肝癌撲滅には肝炎ウイルス感染者を早期に発見し，早期に治療することが重要である。国は2002年度より5年間で肝炎ウイルス検診を行い，肝炎ウイルス感染者の発見に努めたが，検診受診率は決して高くなく，また医療機関を受診しても適切な観察，治療導入すなわち抗ウイルス療法が行われてきたとは言い難い。本稿では肝炎ウイルス検診開始当初より石川県で取り組んできた肝炎ウイルス症例への対策について述べる。

肝炎ウイルス検診の方針 1

2002年肝炎ウイルス検診会誌当初より，石川県では肝炎協議会を設置し，県健康福祉部・医師会・保健所・検査センター・学術経験者が一体となって協力した検診体制を確立した。地域により専門医療機関の過不足があるため，精密検査は特に指定医療機関とはせず，かかりつけ医でも可とした。このため検診精度の向上と経過観察の重要性を考え，以下7つの項目を検診事業の柱とした。

1. 検診陽性者への行政の関与することの通知と同意
2. 精密検査の全県での統一
3. 住民，担当医用の診断手引きの作成
4. 精密検査での画像検査の義務付け
5. 全症例を対象とした事例検討会
6. 前年度陽性者に対する事後調査
7. 保健師などを対象とした研修会の開催

このなかで石川県として独自性の高いと考えているものは検診陽性者を行政が継続フォローするために必要な1，6および担当医の肝炎への理解を深めた5である。毎年検診陽性者の医療機関受診・治療状況を把握することと，担当のかかりつけ医が正しく診断，治療導入することへの意識が高まるようこれら事業を継続した。

肝炎ウイルス検診の状況 2

石川県では5年間の肝炎ウイルス検診受診率は36.6～41.5%と全国平均[1]と比べると10%ほど受診率がよかったが半数には満たない。検診陽性者の精密検査受診状況は男性67.6%，女性75.0%，年齢では若年（65歳未満）66.1%，高齢（65歳以上）74.7%であった。性年齢でわけると若年男性53.4%，若年女性71.9%，高齢男性74.0%，高齢女性74.0%と若年男性で精密検査の受診率が低いことが明らかであり，仕事等で忙しく受診機会をつくりにくい状況がうかがえる。図1に性・年齢・医療圏別での精検受診状況を示す。検診自体の受診率は能登地方および南加賀で低い傾向にあった。しかし能登地方はウイルスキャリアと判明すると医療機関をきちんと受診する傾向にある。一方，南加賀ではウイルスキャリアと判明しても医療機関への受診率が悪い。能登地方ではキャリアの発掘が重要であり，南加賀ではキャリアの発掘と受診勧奨の両面が必要なことがう

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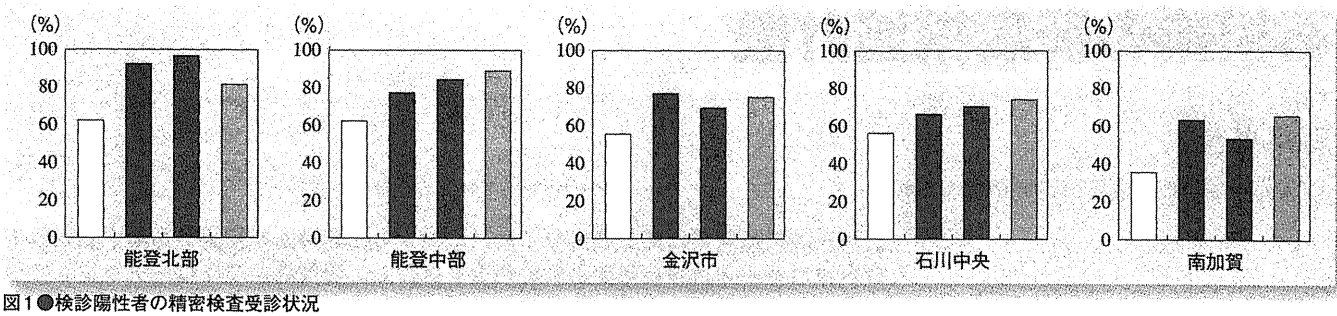

図1●検診陽性者の精密検査受診状況
□：男性・65歳未満 ■：男性・65歳以上 ■：女性・65歳未満 ■：女性・65歳以上

表1●石川県精検未受診者のその後の状況

	検診初年度精検未受診	翌年以降医療機関受診	IFN療法/受診者
能登北部	18（14.8%）	12（66.7%）	3（25.0%）
能登中部	32（17.5%）	17（53.1%）	2（11.8%）
石川中央	71（31.8%）	45（63.4%）	7（15.6%）
南加賀	88（40.6%）	52（59.1%）	10（19.2%）
金沢市	147（28.1%）	39（26.5%）	2（ 5.1%）
合計	356（28.1%）	165（46.3%）	24（14.5%）

かがえる。また医療機関受診の時間がとりにくい若年男性の受診率が悪いのは地域で共通しており，受診動機を促す啓蒙活動が必要である。

フォローアップ事業の有用性 3

前述したように石川県では保健師が面談，電話，手紙などの方法で検診陽性者の状況把握に努めている。継続して医療機関で経過観察されているのはC型肝炎では48.7～63.7%であった。一方，各市町で少なくともフォロー期間（2～7年）中に1度は医療機関を受診した症例はB型肝炎ウイルス陽性者で49～100%，C型肝炎ウイルス陽性者で80～100%であった。表1に初年度精密検査未受診者のその後の状況を示す。受診勧奨を行った結果未受診者のうち能登北部66.7%，能登中部53.1%，金沢市26.5%，石川中央63.4%，南加賀59.1%がその後に医療機関を受診し，さらに受診者のうち能登北部25.0%，能登中部11.8%，金沢市5.1%，石川中央15.6%，南加賀19.2%がインターフェロン（IFN）療法を行っていた。継続した状況把握，受診勧奨が適切な医療へと結びつくことが明らかとなった。

IFN治療状況 4

肝癌撲滅という目標に対してC型肝炎であればIFN療法によりウイルスが排除されることが一番である。年齢，合併症などにより全ての症例でIFN療法を行うのは困難であるが，検診症例のIFN療法の施行率が低いことが問題となっている。厚生労働省研究班の報告では当初3年間では13.8～18.2%であった[1]。石川県でも2002年131例中5例（3.8%），2003年164例中14例（8.5%）とIFN療法施行率は低かった。特に65歳以上の高齢者ではIFN施行率は2.6%と，65歳未満の9.6%に対して有意に低かった[2]。IFN導入率が高齢者を含めて低い理由を検討するために，石川県全下で内科標榜医療機関にアンケート調査を行った。設問「一度はIFN療法を患者に説明するか（複数回答可）」に肝臓専門医の約8割は条件を問わずIFN療法について説明するが，非専門医師は約5割しか条件を問わずにIFN療法を説明していなかった。また「IFN療法を行わない理由」としては高齢であることをあげる医師が多数を占めたが，「何歳までがIFN療法の適応と考えるか」という設問では専門医は70～75歳までを適応と考えているが，非専門医はおおむね70歳以下と考えており，IFN適応年齢を非専門医は低く考えがちであることも明らかとなった[2]。このような実態を踏まえ，一例ごとの事例検討会，IFN療法をテーマにした講習会などを繰り返し行い，2004年102例中24例

表2●全国および石川県の検診C型肝炎陽性者のIFN施行率

		初年度IFN療法施行率	
		精検受診者中	慢性肝炎中
全国[1]	2002年	13.8%	
	2003年	13.3%	
	2004年	18.2%	
	2005年		
	2006年		
石川県	2002年	3.0%	3.8%
	2003年	5.7%	8.5%
	2004年	14.7%	23.5%
	2005年	24.5%	35.3%
	2006年	23.7%	31.0%

表3●「肝炎診療連携」で把握された75歳以下検診C型肝炎陽性症例のIFN治療状況

	キャリア（n：13）＋慢性肝炎（n：75） n＝88
IFN過去にあり	28（著効　6例）
現在投与中	7
投与開始	7
IFN施行数（率）	42/88（48%）
合併症不可（IP，うつなど）	4
IFN可能症例施行数（率）	42/84（50%）
IFN検討中	8

（23.5%），2005年68例中24例（35.3%），2006年71例中22例（31.0%）と後半2年間はIFN療法施行率が30%を超えていた（表2）。

石川県肝炎診療連携 5

年々IFN施行率は上昇してきたが，さらに向上させるには専門医が関わることが重要である。石川県では精密検査を専門医が行った症例では144例中53名（36.8%）がすぐにIFN導入され，翌年以降にさらに26例でIFN療法が施行，計79例（54.9%）でIFN療法が導入されていた。一方，かかりつけ医で診られた41症例では計8例（19.5%）のIFN導入にとどまり，IFN療法施行率をあげるには専門医がその診断，治療方針決定に関わることが重要であった。2007年にでた厚生労働省の肝炎検査後診療体制のガイドラインでも「状態に変化がなくとも年一回の専門医療機関受診が望ましい」とされており，かかりつけ医から患者を年1回の専門医に受診勧奨する「石川県肝炎診療連携」を立案した。個人情報保護の問題をクリアし，行政の保持する検診データを拠点病院と専門医療機関で構成する肝炎診療連携協議会に移行するために，行政・各市町と協議の上，患者より「石川県肝炎診療連携」への参加，データ移行に関して再同意をとり，専門医療機関を受診，順次データ移管することとなった。非同意，または返答のなかった症例は引き続き行政でフォローアップをすることとした。

2,570人の肝炎ウイルス検診陽性者に同意書・調査票が送付され494人が同意，非同意が90人，専門医療機関受診し調査票が回収されたのは328人であった。HBs抗原陽性148人，HCV抗体陽性174人であった。HBs抗原陽性では無症候性キャリアと診断されたのが79例で，そのうち5例でALT31IU/L以上の異常値であったが，4例ではHBV-DNA低値の情報が付加されており，診断が妥当であることが確認された。また核酸アナログ使用率も14%とHBs抗原陽性で治療を必要とする従来の割合と合致しているデータと考えられた。HCV抗体陽性者のうち慢性肝炎またはキャリアと診断された症例の治療方針をみると専門医がIFN療法が望ましいとしたのは全体の33%であった。一方経過観察が選択された症例では，ALT値が低いか，超高齢者が多く含まれていた。今回の専門医受診を契機にIFN療法導入が7例あり，過去のIFN歴も踏まえて現在までにIFN療法が行われたのは75歳以下の検診症例で48%であった（表3）。

おわりに

肝癌撲滅には背景となるウイルス性肝疾患への適切な経過観察，治療の導入が重要である。県下の肝炎ウイルス検診症例を専門医受診勧奨とデータ管理により早期に適切な治療導入に図りたい。

REFERENCES

1) 日野啓輔：肝炎ウイルス検診の実態と要精検者指導に対する今後の問題点．肝炎ウイルス検診の現状把握と評価及び今後のあり方に関する研究（主任研究者　吉澤浩司），厚生労働科学特別研究事業　平成18年度総括・分担報告書．p13-22，2007
2) 酒井明人，他：肝炎ウイルス検診でみる高齢者C型慢性肝炎治療の現状と高齢者IFN療法の成績．消化器科46: 408，2008

Impact of Viral Amino Acid Substitutions and Host Interleukin-28B Polymorphism on Replication and Susceptibility to Interferon of Hepatitis C Virus

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Amino acid (aa) substitutions of core 70 and 91 and in the NS5A (nonstructural protein 5A) interferon sensitivity determining region (ISDR) as well as genetic polymorphisms in the host interleukin-28B (IL28B) locus affect the outcome of interferon (IFN)-based therapies for patients with chronic hepatitis C. The combination of these factors and the quasispecies nature of the virus complicate understanding of the underlying mechanism. Using infectious hepatitis C virus (HCV) genotype 1b clone HCV-KT9, we introduced substitutions at both core aa70 (Arg to Gln) and aa91 (Leu to Met). We also introduced four and nine ISDR aa substitutions into core mutant HCV-KT9. Using human hepatocyte chimeric mice with different IL28B genotypes, we examined the infectivity, replication ability, and susceptibility to IFN of these clones. Although aa substitutions in the ISDR significantly impaired infectivity and replication ability of the virus, core aa70 and 91 substitutions did not. The effect of IFN treatment was similar in core wild-type and mutant viruses. Interestingly, virus titer was significantly higher in mice with the favorable IL28B allele (rs8099917 TT and rs12979860 CC) in the transplanted hepatocytes than in mice with hepatocytes from rs8099917 TG and rs12979860 TT donors ($P < 0.001$). However, the effect of IFN was significantly greater, and intrahepatic expression levels of IFN-stimulated genes were significantly higher in mice with the favorable IL28B allele. *Conclusion*: Our data suggest that HCV replication levels and response to IFN are affected by human hepatocyte IL28B single-nucleotide polymorphism genotype and mutations in the ISDR. The mechanism underlying the clinically observed association of wild-type core protein in eradication-favorable host cells should be investigated further. (HEPATOLOGY 2011;54:764-771)

Chronic hepatitis C virus (HCV) infection is the leading cause of cirrhosis, liver failure, and hepatocellular carcinoma.[1,2] Interferon (IFN) is an essential component of therapy for patients with chronic HCV infection, and the most effective currently available therapy is combination therapy with pegylated (PEG)-IFN and ribavirin (RBV).[3-5] Among HCV genotypes, genotype 1 is the most resistant to

Abbreviations: aa, amino acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCV, hepatitis C virus; HSA, human serum albumin; IFN, interferon; IL28B, interleukin-28B; ISDR, interferon-sensitivity–determining region; ISG, interferon-stimulated gene; MxA, myxovirus resistance protein A; NVR, nonvirological response; OAS, oligoadenylate synthetase; PBS, phosphate-buffered saline; PEG, pegylated; PKR, RNA-dependent protein kinase; RBV, ribavirin; RT-PCR, reverse-transcription polymerase chain reaction; SCID, severe combined immunodeficiency; SNP, single-nucleotide polymorphism; SVR, sustained virological response; uPA, urokinase-type plasminogen activator.

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