inhibitor (sitagliptin), is minimally metabolized.[21,22] Hence, sitagliptin raises the possibility for use in patients with T2DM complicated with chronic liver disease.

With this background in mind, the case–control study was initiated to investigate the efficacy and safety of DPP-4 inhibitors for T2DM patients with HCV positive chronic liver disease.

METHODS

Patients

SIXTEEN PATIENTS WITH T2DM complicated with HCV positive chronic liver disease started the treatment with oral DPP-4 inhibitor (sitagliptin; MDS, Tokyo, Japan) of 50 mg/day from December 2009 to January 2010 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. These 16 consecutive patients treated with sitagliptin of 50 mg/day were regarded as the sitagliptin group. Inclusion criteria of DPP-4 inhibitor administration were as follows: (i) evidence of diabetes mellitus (i.e. plasma glucose concentration of ≥126 mg/dL [6.9 mM) in the fasting state, ≥200 mg/dL [11.0 mM] in casual state and/or 2 h after a 75-g oral glucose load; (ii) a diabetic history of less than 2 years; (iii) features of chronic hepatitis or cirrhosis diagnosed by ultrasonography and/or computed tomography; (iv) positive for serum HCV RNA; (v) negativity for hepatitis B surface antigens (HBsAg), antinuclear antibodies or anti-mitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (vi) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; and (vii) no underlying systemic disease, such as systemic lupus erythematosus and rheumatic arthritis. The distinction between chronic hepatitis and liver cirrhosis in patients was done by discriminant function using platelet, hyaluronic acid, and γ-globulin.[23] Patients with either of the following criteria were excluded from the study: (i) they were taking medicines except DPP-4 inhibitors known to alter glucose tolerance; and/or (ii) they had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial. Patients in the sitagliptin group exercised and participated in diet therapy in addition to administration of sitagliptin. In the same period, 303 patients with T2DM and chronic liver disease type C were not treated with antidiabetic drugs. These patients exercised and participated in diet therapy for T2DM. Seventy-three of these 303 patients were applied with seven inclusion criteria and two exclusion criteria as described above. Sixteen subjects in the control group were selected from these 73 patients by matching 1:1 with the sitagliptin group for age and sex. Patients who belonged to the control group or sitagliptin group had been subjected to lifestyle intervention of diet and physical exercise after the diagnosis of T2DM. The diet prescription included daily calorie intake of 125.6 kJ/ideal body-weight (kg), a protein energy fraction of 15% and a fat energy fraction of 25%. Physical activity was recommended as at least 120 min of aerobic exercise a week. The physicians in charge explained the methods and side-effects of sitagliptin therapy to each patient and/or patient's family before sitagliptin therapy. Informed consent was obtained from 16 patients of the sitagliptin group before the initiation of sitagliptin therapy. All of the studies in the control group were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by the Institutional Review Board of Toranomon Hospital.

Outcome measures

Type 2 diabetes mellitus was diagnosed by the 2003 criteria of the American Diabetes Association:[24] (i) casual plasma glucose of 200 mg/dL or more; (ii) fasting plasma glucose (FPG) of 126 mg/dL or more; and/or (iii) 2-h post-glucose (oral glucose tolerance test) of 200 mg/dL or more. Hemoglobin A1c (HbA1c) was measured using a high-performance liquid chromatography method.

Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test v2.0; Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society, JDS) + 0.4%, considering the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard materials and measurement methods and HbA1c (NGSP).[25] Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (kg) / height (m^2).

Follow up

The starting time of follow up in the sitagliptin group was the initiation of sitagliptin therapy. That is, the time

Table 1 Clinical characteristics at the starting time of follow up

	Sitagliptin group	Control group	*P*-value
n	16	16	
Age (years)	65.3 ± 9.1	65.2 ± 9.5	1.0
Sex (male/female)	8/8	8/8	1.0
Chronic hepatitis/liver cirrhosis	13/3	13/3	1.0
BMI	23.0 ± 3.5	23.5 ± 2.9	0.713
BMI (post-intervention)	22.4 ± 2.4	22.6 ± 2.3	1.0
AST (IU/L)	43 ± 34	34 ± 21	0.170
ALT (IU/L)	45 ± 31	40 ± 31	0.423
Albumin (g/dL)	3.8 ± 0.4	3.9 ± 0.4	0.873
Total bilirubin (mg/dL)	0.9 ± 0.5	0.8 ± 0.3	0.167
Platelets ($\times 10^4/mm^3$)	15.1 ± 5.3	17.0 ± 6.7	0.208
Hyaluronic acid (ng/mL)	132 ± 80	112 ± 62	0.637
HbA1c (NGSP value)	7.4 ± 0.8	7.2 ± 0.9	0.552
FPG (mg/dL)	142.1 ± 24.1	140.0 ± 25.7	0.951

Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; NGSP, National Glycohemoglobin Standardization Program.

was from December 2009 to January 2010. The starting time of follow up in the control group was the same as that in the sitagliptin group. Patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check up. An overnight (12 h) fasting blood sample and HbA1C sample were taken for routine analyses. These included transaminase activities.

Statistical analysis

Clinical differences between the sitagliptin group and control group were evaluated by Wilcoxon rank sum test or Fisher's exact test. Changes in serum HbA1C and FPG level between the sitagliptin group and control group during follow up were analyzed by one-way repeated measurement ANOVA. Next, predictive factors for responders were assessed. A $P < 0.05$ was considered to be statistically significant. SPSS ver. 11.5 for Windows was used to perform statistical analysis.

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics before follow up in the 32 patients with T2DM and HCV positive chronic liver disease. There were no significant differences in clinical profiles between the sitagliptin group and control group.

Change of HbA1c and FPG

Change of average HbA1C and FPG level are plotted in Figures 1 and 2 in the sitagliptin group and control group. In the sitagliptin group, average HbA1C level decreased from 7.4% to 6.5% at 48 weeks after the initiation of sitagliptin. Moreover, average FPG level could be deduced at approximately 20 mg/dL during follow up after the initiation of sitagliptin. The HbA1C and FPG level in the sitagliptin group were statistically lower than those in the control group.

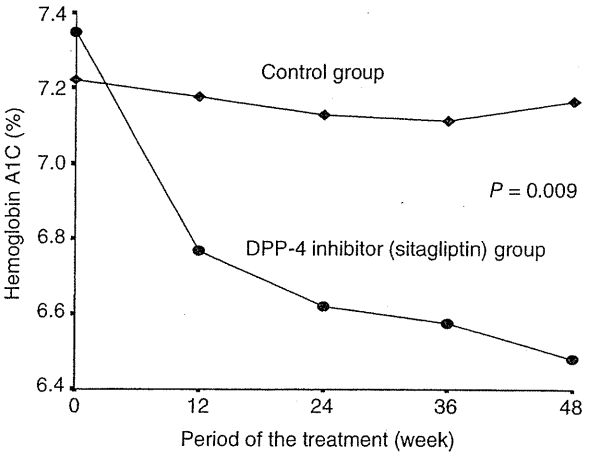

Figure 1 Change of average hemoglobin A1c (HbA1c) level during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group.

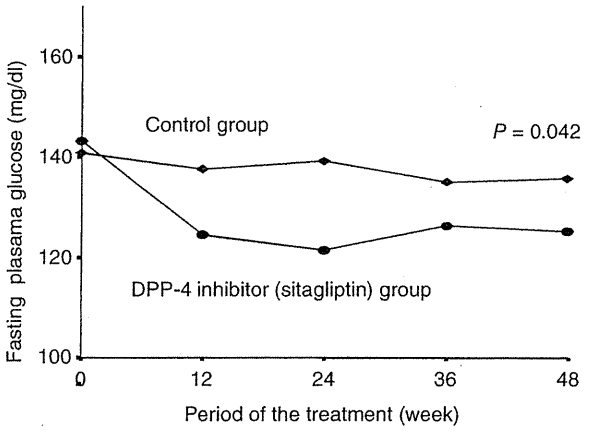

Figure 2 Change of average fasting plasma glucose during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group.

Adverse events of sitagliptin

Regarding side-effects, none of the patients treated with DDP-4 inhibitor had sitagliptin-related episodes severe enough to stop the treatment of sitagliptin. Thus, all the patients were able to take sitagliptin 50 mg/day for 48 weeks without reduction. Next, changes of average AST and ALT level during follow up are plotted in Figure 3. There were no significant changes of average AST and ALT level during follow up in either the sitagliptin or control group.

DISCUSSION

WE HAVE DESCRIBED the efficacy and side-effects of sitagliptin for T2DM patients with HCV positive chronic liver disease in the present study. The present study was limited by being a case–control study. Another limitation of the study was that patients were treated with different types of diet and different excise. This heterogeneity makes it slightly difficult to interpret the results of the study.

On the other hand, the present study shows several findings with regard to the efficacy and side-effects of sitagliptin for T2DM patients with HCV positive chronic liver disease. First, in the sitagliptin group, average HbA1C and FPG levels after the initiation of sitagliptin were statistically lower than those at the starting time of DPP-4 inhibitor. It is suggested that sitagliptin increases active glucagon-like peptide-1, stimulates insulin secretion and inhibits glucagon secretion.[21,22] Thus, it is accepted that sitagliptin could improve both HbA1C and glucose level in patients with T2DM and HCV positive chronic liver disease.

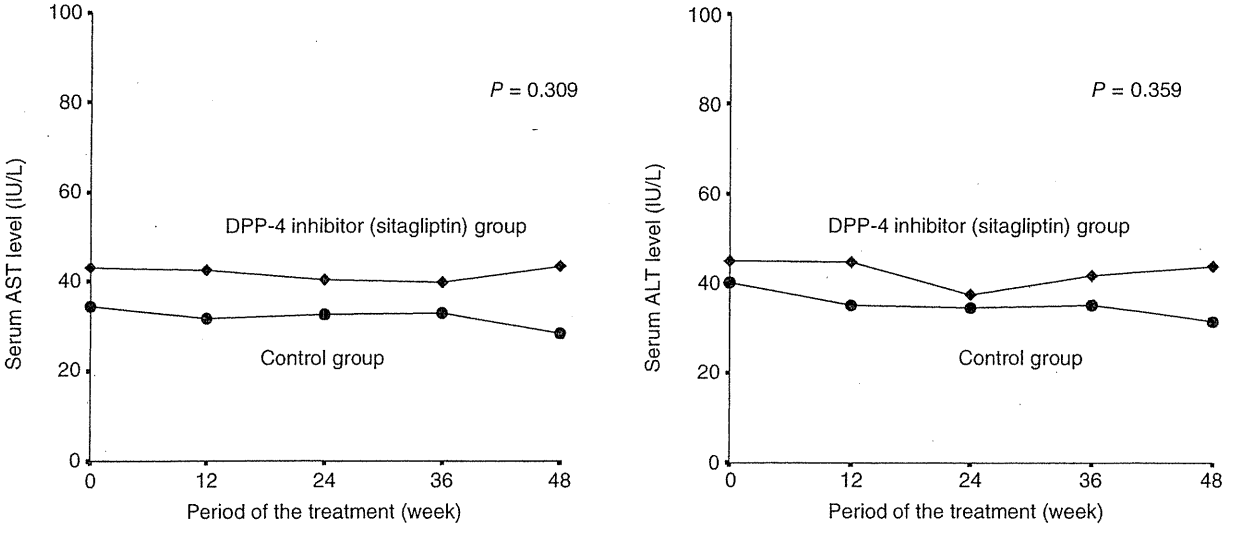

Figure 3 Change of average aminotransferase level during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group. (a) Change of average aspartate aminotransferase (AST) level during follow up was plotted in both the DPP-4 inhibitor group and control group. (b) Change of average alanine aminotransferase (ALT) level during follow up was plotted in both the DPP-4 inhibitor group and control group. Patients who belonged to the control group or sitagliptin group were subjected to lifestyle intervention of diet and physical exercise. The diet prescription included daily calorie intake of 30 kcal/ideal bodyweight, a protein energy fraction of 15% and a fat energy fraction of 25%.

Second, administration of sitagliptin is minimal risk and highly tolerable for T2DM patients with HCV positive chronic liver disease. In the present study, none of the patients treated with DDP-4 inhibitor had sitagliptin-related episodes severe enough to stop the sitagliptin therapy. Thus, all the patients could take sitagliptin of 50 mg/day over 48 weeks without reduction or stopping. This new oral hypoglycemic agent, sitagliptin, is minimally metabolized and over 80% of it is excreted in the urine. It seems not to alter pharmacokinetics in hepatic insufficiency.[22] Thus, sitagliptin has few possibilities to cause the aggravation of the chronic liver damage. In fact, in the present study, three patients with liver cirrhosis did not have elevation of aminotransferase during the treatment by sitagliptin. This result indicates that sitagliptin is valuable for treating T2DM with HCV positive liver cirrhosis.

Type 2 diabetes mellitus has been increasing dramatically in many nations including Japan over the past decades.[26] It is widely accepted that approximately 7–8 million people are affected by DM in Japan. Approximately 8–10% of adults in Japan have T2DM. Recently, it has been reported that T2DM has occurred in HCV positive chronic liver disease.[8–13] Moreover, HCV patients with T2DM are at major risk for HCC.[15–17] So, in patients with T2DM and HCV positive chronic liver diseases, the management of DM is very important to improve the prolonged prognosis. However, most oral hypoglycemic agents (thiazolidines, sulfonylurea and biguanides) are metabolized in the liver. Thus, it is suggested that most oral hypoglycemic agents often induce liver damage. The new oral hypoglycemic agent, DPP-4 inhibitor (sitagliptin), is minimally metabolized. Hence, this drug raises the possibility of being used for T2DM patients with HCV positive chronic liver disease.

In conclusion, our retrospective study suggests that sitagliptin is effective and safe for the treatment of T2DM complicated with HCV positive chronic liver disease.

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

Takahiro Azakami,[1,2,3] C. Nelson Hayes,[1,2,3] Hitomi Sezaki,[4] Mariko Kobayashi,[4] Norio Akuta,[4] Fumitaka Suzuki,[4] Hiromitsu Kumada,[4] Hiromi Abe,[1,2,3] Daiki Miki,[1,2,3] Masataka Tsuge,[1,2,3] Michio Imamura,[1,2,3] Yoshiiku Kawakami,[1,2,3] Shoichi Takahashi,[1,2,3] Hidenori Ochi,[1,2,3] Yusuke Nakamura,[5] Naoyuki Kamatani,[6] and Kazuaki Chayama[1,2,3]*

[1]*Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN, Hiroshima, Japan*
[2]*Programs for Biomedical Research, Division of Frontier Medical Science, Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*
[3]*Liver Research Project Center, Hiroshima University, Hiroshima, Japan*
[4]*Department of Hepatology, Toranomon Hospital, Tokyo, Japan*
[5]*Laboratory of Molecular Medicine, Human Genome Center, The Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[6]*Laboratory for Statistics, RIKEN Center for Genomic Medicine, Yokohama, Japan*

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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*Correspondence to: Prof. Kazuaki Chayama, MD, PhD, Division of Frontier Medical Science, Department of Medical and Molecular Science, Programs for Biomedical Research, Graduate school of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
E-mail: chayama@hiroshima-u.ac.jp

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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 (×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

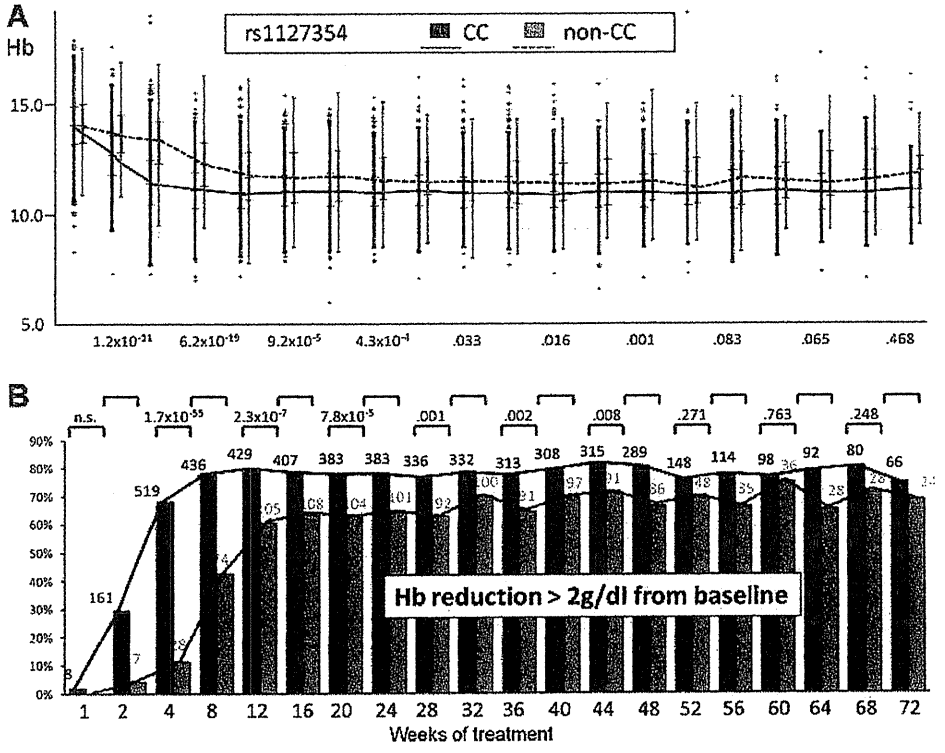

Fig. 1. Reduction of hemoglobin levels by ITPA polymorphism during peg-interferon plus ribavirin combination therapy. **A**: Hemoglobin levels in patients who were treated during the course of therapy. Patients were grouped by ITPA SNP rs1127354 genotype (CC or non-CC). Follow-up hemoglobin levels following 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

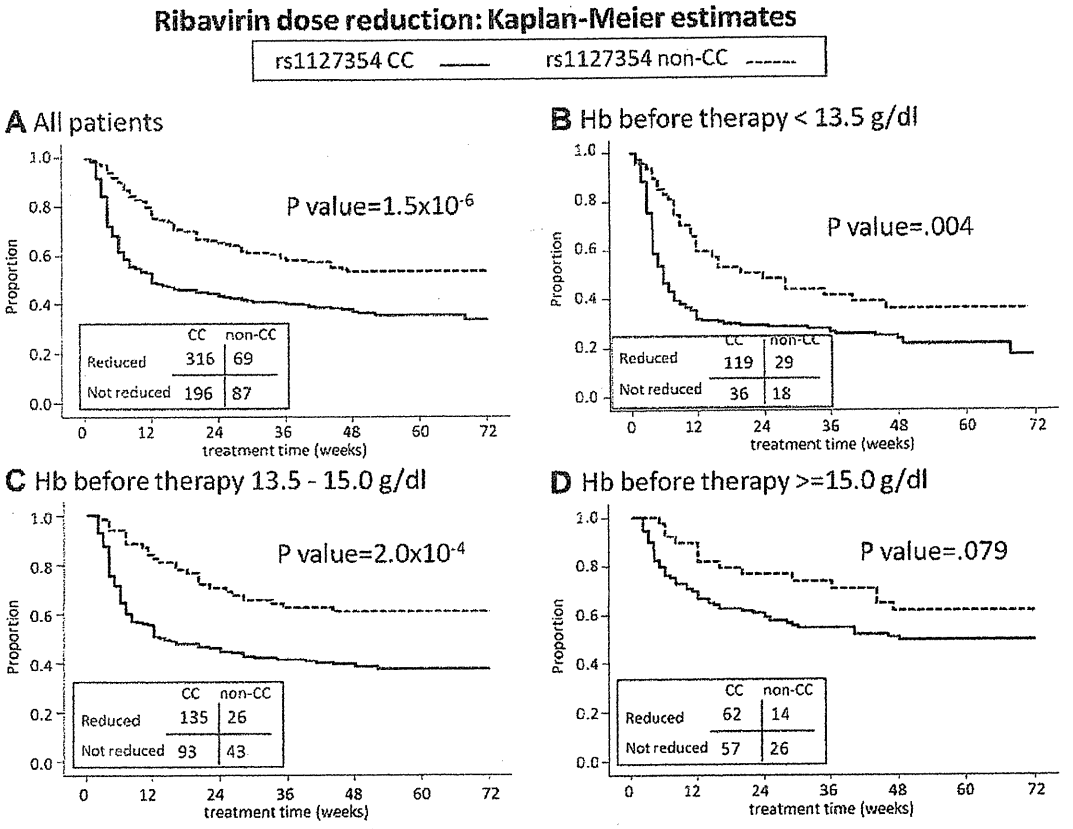

Fig. 2. Dose reduction of ribavirin in patients who were treated with combination therapy. Kaplan–Meier curves for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line: CC, dashed-line: non-CC) among (**A**) all patients, (**B**) patients with low pretreatment hemoglobin levels (<13.5 g/dl),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

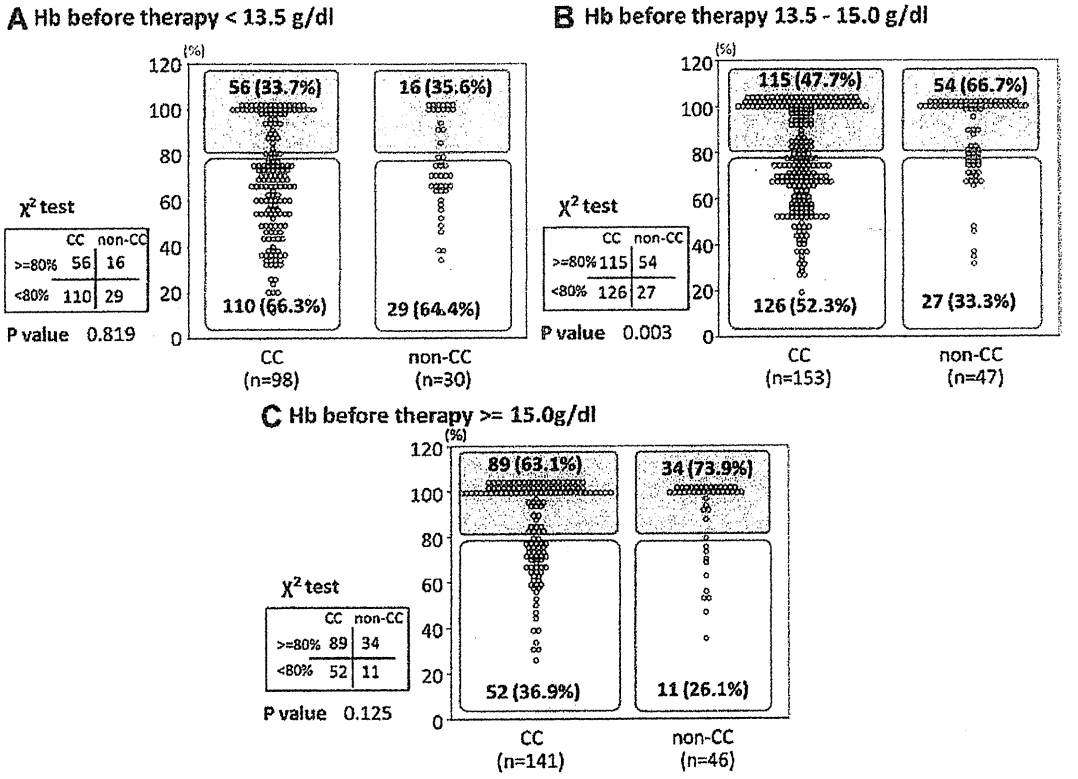

Fig. 3. Dose of ribavirin administered to patients with genotype 1 treated with combination therapy by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (**A**) <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**			

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

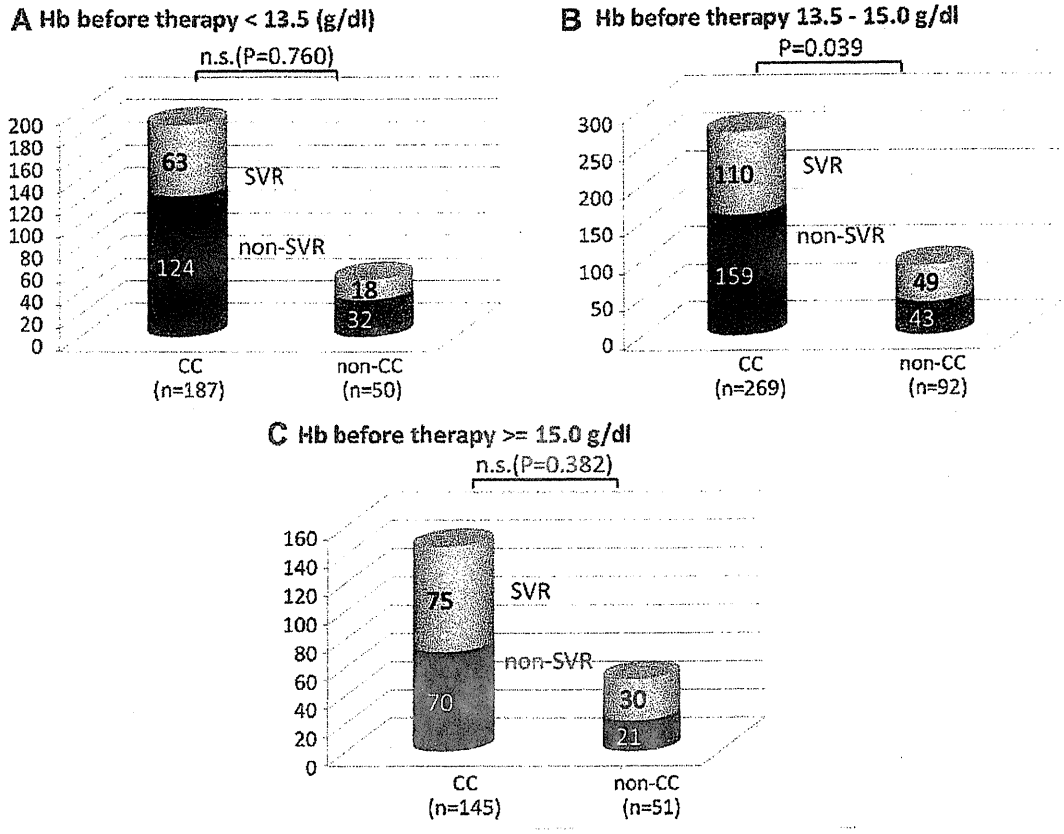

Fig. 4. Effect of combination therapy in patients with genotype 1b by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (A) <13.5 g/dl, (B) between 13.5 and 15.0 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 (×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 (×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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Amino Acid Substitution in HCV Core/NS5A Region and Genetic Variation Near *IL28B* Gene Affect Treatment Efficacy to Interferon plus Ribavirin Combination Therapy

Norio Akuta[a] Fumitaka Suzuki[a] Miharu Hirakawa[a] Yusuke Kawamura[a] Hitomi Sezaki[a] Yoshiyuki Suzuki[a] Tetsuya Hosaka[a] Masahiro Kobayashi[a] Mariko Kobayashi[b] Satoshi Saitoh[a] Yasuji Arase[a] Kenji Ikeda[a] Kazuaki Chayama[c] Yusuke Nakamura[d] Hiromitsu Kumada[a]

[a]Department of Hepatology, and [b]Liver Research Laboratory, Toranomon Hospital, Tokyo, [c]Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, and [d]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Key Words
Hepatitis C virus · Interferon · Ribavirin · Core region · NS5A region · ISDR · IRRDR · *IL28B*

Abstract
Objective: To evaluate predictive factors of treatment efficacy to interferon (IFN)/ribavirin in patients infected with HCV genotype 1b (HCV-1b). ***Methods:*** This study investigated pretreatment predictors, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in 490 Japanese adults infected with HCV-1b. ***Results:*** The proportion of patients who showed end-of-treatment response (ETR), sustained virological response (SVR), and SVR after ETR was 76, 54, and 76%, respectively. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR. Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. Furthermore, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR. By multivariate analysis, rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core aa 70/91 was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. ***Conclusion:*** aa substitution in core/NS5A region and genetic variation near *IL28B* were important predictors of treatment efficacy to IFN/ribavirin.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) combined with ribavirin carries potential serious side effects and is costly, especially when used long enough to achieve a high sustained virological response (SVR) in patients infected with HCV genotype 1b (HCV-1b) and high viral loads. For these rea-

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Norio Akuta, MD
Department of Hepatology, Toranomon Hospital
2-2-2 Toranomon, Minato-ku
Tokyo 105-0001 (Japan)
Tel. +81 ■■■ ■■■■ ■■■■, E-Mail akuta-gi@umin.ac.jp

sons, those patients who do not achieve SVR need to be identified, so as to free them of unnecessary side effects and reduce costs, preferably before the start of the combination therapy.

Viral- and host-related factors are useful as predictors of treatment efficacy to 48-week IFN/ribavirin combination therapy. With regard to viral factors, amino acid (aa) substitutions at position 70 and/or 91 in the core region of HCV-1b are pretreatment predictors of virological response to combination therapy [1–4], and also affect clinical outcome, including hepatocarcinogenesis [5, 6]. Furthermore, the NS5A region of HCV-1b, including IFN-sensitivity-determining region (ISDR) [7, 8] and IFN/ribavirin resistance-determining region (IRRDR) [9, 10], are also useful as pretreatment predictors of virological response to combination therapy [11, 12]. With regard to host factors, genetic variations near *IL28B* gene (rs8099917, rs12979860) on chromosome 19, which encodes IFN-λ-3, are pretreatment predictors of virological response to combination therapy in individuals infected with HCV-1 [13–16], and also affect clinical outcome, including spontaneous clearance of HCV [17]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of SVR to triple therapy of telaprevir/pegylated (PEG)-IFN/ribavirin in Japanese patients infected with HCV-1b [18]. However, to our knowledge, there are no previous reports of IFN/ribavirin combination therapy based on multivariate analysis to investigate pretreatment predictors, including all of aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR, and genetic variation near *IL28B* gene.

The aim of the present study was to investigate predictive factors of treatment efficacy, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in Japanese adults infected with HCV-1b.

Patients and Methods

Study Population

A total of 1,249 HCV-1b-infected Japanese adult patients were consecutively recruited into the study protocol of combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) plus ribavirin between December 2001 and January 2009 at Toranomon Hospital, Tokyo, Japan. Among these, 490 patients, who could complete a total of 48 weeks of combination therapy, were enrolled in this retrospective study, and fulfilled the following criteria: (1) negativity for hepatitis B surface antigen (HBsAg) in serum; (2) HCV-1b only confirmed by sequence analysis; (3) HCV-RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan) within the preceding 2 months of enrolment; (4) no hepatocellular carcinoma; (5) body weight >40 kg; (6) lack of coinfection with human immunodeficiency virus; (7) no previous treatment with antiviral or immunosuppressive agents within the preceding 3 months of enrolment; (8) none was an alcoholic; lifetime cumulative alcohol intake was <500 kg; (9) none had other forms of liver diseases, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, or autoimmune liver disease, and (10) none of the females was pregnant or breastfeeding.

The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave their informed consent before participating in this trial.

The treatment efficacy was evaluated in terms of HCV-RNA negativity at the end of treatment (end-of-treatment response (ETR)) and 24 weeks after the completion of therapy (SVR), based on the COBAS TaqMan HCV test (Roche Diagnostics). SVR in patients who achieved ETR was defined as SVR after ETR. ETR, SVR, and SVR after ETR could be evaluated in 487 (99%), 448 (91%), and 321 (66%) of 490 patients, respectively.

422 (86%) patients received PEG-IFNα-2b at a median dose of 1.4 μg/kg (range 0.7–1.9) subcutaneously each week plus oral ribavirin at a median dose of 11.1 mg/kg (range 3.7–15.1) daily for 48 weeks. The remaining 68 (14%) patients received 6 million units of IFNα-2b intramuscularly each day for 48 weeks (daily for the initial 2 weeks, followed by three times per week for 46 weeks), and oral ribavirin at a median dose of 11.3 mg/kg (range 6.8–13.4) daily for 48 weeks.

Table I summarizes the profiles and laboratory data of the 490 patients at the commencement of treatment. They included 310 males and 180 females aged 20–75 years (median 54).

Measurement of HCV RNA

The antiviral effects of treatment on HCV were assessed by measuring plasma HCV-RNA levels. In this study, HCV-RNA levels were evaluated at least once every month before, during, and after therapy. HCV-RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative.

Detection of aa Substitutions in Core, and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [19], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on the previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [1]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [7, 8] was determined, and the number of aa substitutions in ISDR was defined as wild-type (WT) (0, 1) or non-wild-type (non-WT) (≥2) in comparison with HCV-J. Furthermore, the sequence of 2,334–2,379 aa in the NS5A of HCV-1b (IRRDR) reported by El-Shamy et al. [9, 10] was determined and then compared with the consensus sequence constructed on the previous study. In the present study, aa substitutions of the core region and NS5A-ISDR/IRRDR of HCV-1b were analyzed by direct sequencing [10, 18].

Genetic Variation near IL28B Gene

Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of Invader assay, TaqMan assay, or direct sequencing as described previously [20, 21].

In this study, genetic variations near *IL28B* gene (rs8099917), reported as the pretreatment predictors of treatment efficacy in Japanese patients [14, 18], were investigated.

Statistical Analysis

Non-parametric tests (Mann-Whitney U test, χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Correlation analysis was evaluated by the Spearman rank correlation test. Uni- and multivariate logistic regression analyses were used to determine those factors that significantly contributed to ETR, SVR, and SVR after ETR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All p values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for uni- and multivariate analyses. Potential predictive factors associated with ETR, SVR, and SVR after ETR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, γ-glutamyl transpeptidase (GGT), leukocyte count, hemoglobin, platelet count, level of viremia, α-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, ribavirin dose/body weight, genetic variation near *IL28B* gene, and aa substitution in the core region, and NS5A-ISDR/IRRDR. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, Ill., USA).

Table 1. Patient profile and laboratory data at commencement of the 48-week combination therapy of IFN + ribavirin in 490 patients infected with HCV-1b

Demographic data	
Number of patients	490
Male/female	310/180
Age, years	54 (20–75)
History of blood transfusion	169 (34%)
Family history of liver disease	96 (20%)
Body mass index, kg/m^2	22.6 (15.7–34.7)
Laboratory data	
Level of viremia, log IU/ml	6.4 (2.2–7.7)
Serum AST, IU/l	50 (16–296)
Serum ALT, IU/l	67 (12–836)
Serum albumin, g/dl	3.9 (3.1–4.7)
GGT, IU/l	44 (10–592)
Leukocyte count, n/mm^3	4,700 (1,200–10,900)
Hemoglobin, g/dl	14.4 (10.6–18.1)
Platelet count, $\times 10^4$/mm^3	16.7 (6.4–37.5)
α-Fetoprotein, μg/l	5 (1–459)
Total cholesterol, mg/dl	170 (96–284)
High-density lipoprotein cholesterol, mg/dl	46 (13–95)
Low-density lipoprotein cholesterol, mg/dl	100 (32–190)
Triglycerides, mg/dl	90 (33–416)
Uric acid, mg/dl	5.5 (2.3–9.4)
Treatment	
PEG-IFNα-2b/IFNα-2b	422/68
Ribavirin dose, mg/kg	11.2 (3.7–15.1)
aa substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	266/151
Core aa 91, leucine/methionine	246/169
ISDR of NS5A, 0/1/≥2	227/95/84
IRRDR of NS5A, ≤3/4–5/≥6	73/67/60
Genetic variation near IL28B gene	
rs8099917 genotype, TT/TG/GG	150/65/4

Data represent number of patients with percentages in parentheses, or median (range) values.

Results

Response to Therapy

ETR was achieved by 372 of 487 (76%) patients, SVR by 244 of 448 (54%), and SVR after ETR by 244 of 321 (76%).

Number of aa Substitutions in NS5A-ISDR and NS5A-IRRDR

As a whole, 0, 1, and ≥2 aa substitutions in ISDR were found in 56% (227 of 406), 23% (95 of 406), and 21% (84 of 406) of patients, respectively. Thus, the percentage of patients with ≤1 aa substitution in ISDR (WT) was 79% (322 of 406). Furthermore, ≤3, 4–5, and ≥6 aa substitutions in IRRDR were found in 36% (73 of 200), 34% (67 of 200), and 30% (60 of 200) of patients, respectively (fig. 1).

The correlation between ISDR and IRRDR was analyzed. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR ($r = 0.308$, $p < 0.001$) (fig. 2).

aa Substitutions in the Core Region and NS5A-ISDR/IRRDR

Concerning the substitution of core aa 70, the number of aa substitutions in ISDR of 256 patients with Arg70 (median 0) was not significantly different from that of 146 patients with Gln70 (His70) (median 0) (fig. 3a). Fur-

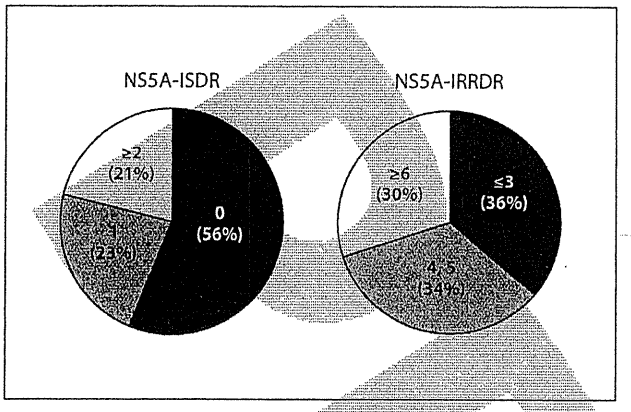

Fig. 1. The number of aa substitutions in NS5A-ISDR and NS5A-IRRDR. The percentage of patients with ≤1 aa substitution in ISDR (WT) was 79%.

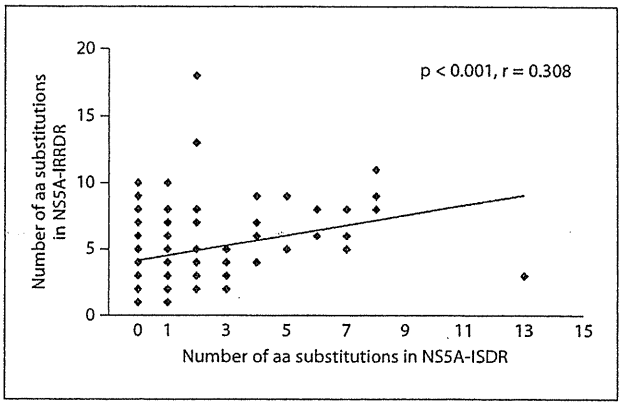

Fig. 2. Correlation between NS5A-ISDR and NS5A-IRRDR. There was a significant positive correlation between the number of aa substitutions in ISDR and that in IRRDR ($r = 0.308$, $p < 0.001$).

thermore, the number of aa substitutions in IRRDR of 123 patients with Arg70 (median 5) was also not significantly different from that of 77 patients with Gln70 (His70) (median 4) (fig. 3b).

Concerning the substitution of core aa 91, the number of aa substitutions in ISDR of 240 patients with Leu91 (median 1) was significantly higher than that of 161 patients with Met91 (median 0) ($p < 0.001$) (fig. 3c). Furthermore, the number of aa substitutions in IRRDR of 111 patients with Leu91 (median 5) was significantly higher than that of 89 patients with Met91 (median 3) ($p < 0.001$) (fig. 3d).

Viremia Level and aa Substitutions in Core Region/ISDR/IRRDR

Concerning the number of substitutions in ISDR, viremia levels of 321 patients with WT (median 6.5) were significantly higher than those of 84 patients with non-WT (median 5.7) ($p < 0.001$) (fig 4a).

Concerning the number of substitutions in IRRDR, viremia levels of 140 patients with ≤5 substitutions (median 6.4) were significantly higher than those of 60 patients with ≥6 (median 6.1) ($p = 0.027$) (fig. 4b).

Concerning the substitution of core aa 70, viremia levels of 265 patients with Arg70 (median 6.4) were not significantly different from those of 151 patients with Gln70 (His70) (median 6.3) (fig. 4c).

Concerning the substitution of core aa 91, viremia levels of 169 patients with Met91 (median 6.5) were significantly higher than those of 245 patients with Leu91 (median 6.2) ($p = 0.028$) (fig. 4d).

Thus, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

Treatment Response according to the Number of aa Substitutions in IRRDR

Concerning the number of aa substitutions in IRRDR, a significantly higher proportion of patients with ≥4 aa substitutions (58%) showed SVR compared to patients with ≤3 (42%) ($p = 0.039$). In contrast, the SVR rate was not significantly different between patients with ≤4 (49%) and those with ≥5 (57%) aa substitutions. Likewise, the SVR rate was not significantly different between patients with ≤5 (51%) and those with ≥6 (55%) aa substitutions (fig. 5a).

The ETR rate was not significantly different between patients with ≤3 (74%) and those with ≥4 (82%) aa substitutions, nor between patients with ≤4 (76%) and those with ≥5 (83%). Likewise, the ETR rate was not significantly different between those with ≤5 (79%) and those with ≥6 (80%) aa substitutions (fig. 5b).

The SVR rate after ETR was not significantly different between patients with ≤3 (61%) and those with ≥4 (74%) aa substitutions, nor between patients with ≤4 (67%) and those with ≥5 (72%). Likewise, they were not significantly different between patients with ≤5 (67%) and those with ≥6 (75%) aa substitutions (fig. 5c).

Thus, it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. However, the ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

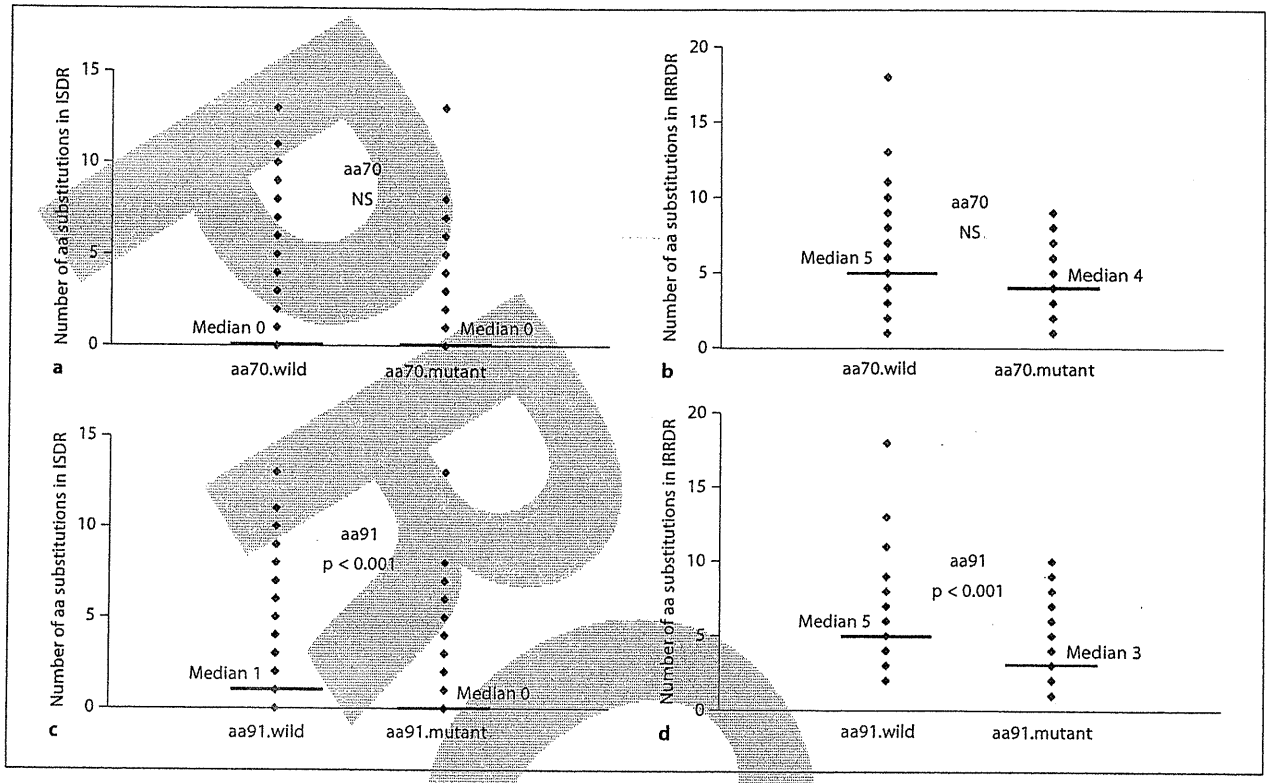

Fig. 3. aa substitutions in the core region and NS5A-ISDR/IRRDR. **a**, **b** Concerning the substitution of core aa 70, the number of aa substitutions in ISDR/IRRDR of patients with Arg70 was not significantly different from that of patients with Gln70 (His70). **c**, **d** Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91 ($p < 0.001$).

Predictors of SVR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 15 parameters that correlate with SVR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.006$), AST (<58 IU/l; $p = 0.039$), leukocyte count (≥4,500/mm^3; $p = 0.043$), hemoglobin (≥14.0 g/dl; $p = 0.001$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), GGT (<50 IU/l; $p = 0.028$), uric acid (≥5.5 mg/dl; $p = 0.005$), level of viremia (<6.0 log IU/ml; $p < 0.001$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), the number of aa substitutions in ISDR (non-WT; $p < 0.001$) and IRRDR (≥4; $p = 0.039$). Figure 6 shows the SVR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 3 parameters that independently influenced SVR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), gender (male sex; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.027$) (table 2).

Predictors of ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 14 parameters that correlated with ETR: gender (male sex; $p = 0.001$), age (<55 years; $p = 0.004$), AST (<39 IU/l; $p = 0.027$), hemoglobin (≥14.0 g/dl; $p = 0.035$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), albumin (≥3.9 g/dl; $p = 0.014$), GGT (<50 IU/l; $p < 0.001$), uric acid (≥5.5 mg/dl; $p = 0.003$), level of viremia (<6.0 log IU/ml; $p = 0.001$), low-density lipoprotein cholesterol (≥85 mg/dl; $p = 0.004$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.021$). Figure 7 shows the ETR rate according to aa