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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INTERNAL MEDICINE

ORIGINAL ARTICLE

The Development of Chronic Kidney Disease in Japanese Patients with Non-alcoholic Fatty Liver Disease

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Abstract

Objective Chronic kidney disease (CKD) is present in patients with nonalcoholic fatty liver disease (NAFLD). The aim of this retrospective study was to assess the cumulative development incidence and predictive factors for new onset of CKD in Japanese patients with NAFLD.

Methods A total of 5,561 NAFLD patients without CKD were enrolled. CKD was defined as either an estimated glomerular filtration rate of <60 mL/min/1.73 m^2 or dipstick proteinuria ($\geq +1$). A blood sample and a urine sample were taken for routine analyses during follow-up. The mean observation period was 5.5 years. The primary goal is the new development of CKD. Independent factors associated with new development of CKD were analyzed by using the Kaplan-Meyer method and the Cox proportional hazards model.

Results Of 5.561 NAFLD patients, 263 patients developed CKD. The cumulative development rate of CKD was 3.1% at the 5th year and 12.2% at the 10th year. Multivariate Cox proportional hazards analysis showed that CKD development in patients with NAFLD occurred when patient had low level of GFR of 60-75 mL/min/1.73 m^2 [hazard ratio:2.75; 95% confidence interval (CI) =1.93-3.94; p<0.001], age of $\geq$50 years (hazard ratio: 2.67; 95% CI=2.06-3.46; p<0.001), diabetes (hazard ratio: 1.92; 95% CI=1.45-2.54; p<0.001), hypertension (hazard ratio: 1.69; 95% CI=1.25-2.29; p<0.001), and elevated serum gamma-glutamyltransferase of $\geq$109 IU/L(hazard ratio: 1.35; 95% CI=1.02-1.78; p=0.038).

Conclusion Our retrospective study indicates that the annual incidence of CKD in Japanese patients with NAFLD is about 1.2%. Five factors of low eGFR level, aging, type 2 diabetes, hypertension, and elevated gamma-glutamyltransferase, increases the risk of the development of CKD.

Key words: nonalcoholic fatty liver disease, chronic kidney disease, gamma-glutamyltransferase

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the more common causes of chronic liver disease in Western world (1-4) and in many Asian nations (5, 6). NAFLD is considered to be the liver component of metabolic syndrome (7-9). It is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, and type 2 diabetes mellitus (T2DM) (10-16). Moreover, NAFLD often causes cardiovascular disease and stroke (17, 18). Thus, NAFLD is emerging as a new significant health problem in many countries.

On the other hand, there has been a recent dramatic in-

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crease in the prevalence of end-stage renal disease (ESRD) in USA and Asia (19-22). Chronic kidney disease (CKD) often progresses to ESRD with its attendant complications. CKD, a disease entity including mild to ESRD due to any etiology, was defined as an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and/or the presence of proteinuria (21). Recently, metabolic syndrome and NAFLD have been reported to enhance the new onset of CKD (23, 24). Although there is growing evidence to support the concept that metabolic syndrome is a risk factor for developing CKD, little research has been done to evaluate whether NAFLD is associated with the long-term development of CKD.

The present cohort study was initiated to investigate the cumulative incidence and risk factors of CKD after long-term follow-up in patients with NAFLD. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Methods

Patients

The number of Japanese patients who were diagnosed with fatty liver by ultrasonography (25) between January 1997 and December 2007 in the Department of Hepatology and/or Health Management Center, Toranomon Hospital, Tokyo, Japan was 9,120. Of these, 5,561 Japanese patients satisfied with the following enrolled criteria; 1) no evidence of CKD based on eGFR calculated with serum creatinine level (eGFR≥60 [mL/min/1.73 m^2]); 2) the absence of proteinuria (≥ +1); 3) current and past daily alcohol intake of <20 g/day; 4) negativity for hepatitis B surface antigens (HBsAg), hepatitis C virus antibodies, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or spot hybridization; 5) no underlying neoplasm or systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis; 6) no evidence of nodules of hepatocellular carcinoma as shown by ultrasonography and/or computed tomography. Patients with the above criteria were enrolled regardless of whether the serum level of aminotransferase was normal or abnormal. Patients with any of the following criteria were excluded from the study: 1) illness that could seriously reduce their life expectancy, 2) findings suggestive of other chronic liver disease, and 3) refusal to be followed up after the diagnosis of NAFLD. A total of 3,559 out of 9,120 patients were excluded based on the following findings; 169 had a dipstick-positive proteinuria; 1,685 had an eGFR of <60 mL/min/1.73 m^2; 2,098 had alcohol intake of ≥20 g/day; 133 had positive serologic findings for either hepatitis B or C virus, a reported history of known liver disease, or decompensated liver cirrhosis; 36 had a history of malignancy; 26 had a history of cardiovascular disease; 11 refused the participation of prospective follow-up. Because some individuals were excluded for multiple reasons, the total number of eligible patients for the study was 5,561.

Patients were classified into three groups according to fasting plasma glucose (FPG): 1) those with FPG level of < 110 mg/dL (normal glucose group), 2) those with FPG level of 110-125 mg/dL (pre-diabetes group), and 3) those with FPG level of ≥126 mg/dL (diabetes group) (25). Patients were regarded as hypertension by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic.

The primary goal was the new onset of CKD in patients with NAFLD. The end-point was defined as the first eGFR <60 mL/min/1.73 m^2 or dipstick proteinuria (≥ +1) for more than three months. Serum creatinine level was also measured using an enzymatic method, and the GFR was estimated from the Japanese Society of Nephrology CKD Practice Guide; eGFR (mL/min/1.73 m^2) =194× (serum creatinine level [mg/dL]) -1.094× (age [y]) -0.287. The product of this equation was multiplied by a correction factor of 0.739 for women. CKD and its stages were defined from estimated eGFR of <60 mL/min/1.73 m^2 or dipstick proteinuria (≥ +1) as follows: stage I, eGFR≥90 and proteinuria (≥ +1); stage II, 90>eGFR≥60 and proteinuria (≥ +1); stage III, 60>eGFR≥30; stage IV, 30>eGFR≥15; and stage V, 15>eGFR. Patients with stage III-V were regarded as having CKD regardless of the absence of other markers of kidney damage (21, 22).

All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by Institutional Review Board of our hospital.

Medical evaluation

Fatty liver was diagnosed by the presence of an ultrasonographic pattern consistent with bright liver with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma (26). Ultrasonography test was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka Co., Ltd, Tokyo Japan. Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg) / height (in m^2). All of the patients were interviewed in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and heath-related habits including questions on alcohol intake at the time of diagnosis of fatty liver.

Laboratory investigation

At the first consultation anti-HCV and HBsAg were examined. Anti-HCV was detected using a third-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). Anti-HBs was not evaluated in the present study. Serum creatinine concentration was measured by a modified Jaffe method (creatinine

Table 1. **Characteristics of Subjects Enrolled**

	Total
Number of cases	5561
Age (years)	48.0±8.4
Sex(male/female)	4916/645
Systolic blood pressure(mmHg)	134±18
Diastolic blood pressure(mmHg)	76±10
Hypertension(+)	725(13.0%)
Height(cm)	167.8±7.3
Body Weight (kg)	70.7±9.9
BMI (kg/m^2)	25.1±2.8
Smoking (+)	1028 (18.5%)
FPG(mg/dL)	104.7±24.8
Glucose status (Normal/ preDM/DM)	4436(79.8%)/667(12.0%)/458(8.2%)
eGFR (mL/min/1.73m^2)	74.6±11.9
WBC(×10^3/mm^3)	5.8±1.5
Hemoglobin (g/dL)	15.1±1.2
Platelet (×10^4/mm^3)	23.1±5.0
Triglyceride (mg/dL)	164±117
Total cholesterol (mg/dL)	210±34
HDL cholesterol (mg/dL)	48.1±11.9
Total Protein(g/dL)	7.5±0.4
Albumin (g/dL)	4.2±0.3
Uric Acid (mg/dL)	6.2±1.3
AST (IU/L)	29.2±16.4
ALT (IU/L)	37.5±27.0
GGT(IU/L)	78.2±81.0
Follow-up period (years)	5.5±4.8

Data are number of patients (percent) or mean ± standard deviation
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; WBC, white blood cell;

Figure 1. **Cumulative development rate of CKD in 5,561 patients with NAFLD.**

HR, Wako Pure Chemicals Industries, Ltd, Osaka, Japan) using an autoanalyzer (Hitachi 7350, Hitachi Ltd, Tokyo, Japan or RX-20, JEOL Ltd., Tokyo, Japan). Proteinuria was tested using dipsticks (Ames Hemacombistics; Bayer-Sankyo Ltd, Tokyo, Japan). A test result of $\geq +1$ was defined as positive.

Follow-up

Starting time of follow-up was the day that the fatty liver was confirmed by ultrasonography. After that, patients were followed up monthly to six-monthly in the Toranomon hospital. A blood sample and a urine sample were taken for routine analyses. Four hundred and ninety-two patients were lost to follow-up. Because the appearance of CKD was not identified in these 492 patients, they were considered as censored data in statistical analysis (27).

Statistical Analysis

The cumulative appearance rate of CKD was calculated from the starting time of follow-up to the development of CKD by using the Kaplan-Meier method. Differences in the development of CKD were tested using the log rank test. The Cox proportional hazard model analyzed independent factors associated with the development rate of CKD. The following variables were analyzed for potential covariates for incidence of CKD: age, BMI, T2DM, hypertension, and levels of eGFR, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total protein, triglyceride (TG), total cholesterol level, high density lipoprotein (HDL) cholesterol uric acid, hemoglobin, white blood cell, platelet at the time of diagnosis of NAFLD. A p value of less than 0.05 was considered significant. Data analysis was performed using the computer program SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL).

Results

Patients' characteristics

Table 1 shows the characteristics in the 5,561 patients diagnosed as NAFLD in the present study. The mean age was 48 years. The mean BMI was 25.1. Patients with hypertension accounted for 13.0% and patients with T2DM accounted for 8.2%. The eGFR level was 74.6±11.9 mL/min/1.73 m^2. The mean follow-up period was 5.5 years.

Incidence of CKD in Patients with NAFLD

Of 5.561 NAFLD patients, 263 developed CKD. Figure 1 shows that the cumulative development rate of CKD was 3.1% at the 5th year and 12.2% at the 10th year in all patients with NAFLD. Cox proportional hazards analysis showed that CKD development in NAFLD patients occurred when patient had eGFR of 60-75 mL/min/1.73 m^2 [hazard ratio:2.75; 95% confidence interval (CI) =1.93-3.94; p< 0.001], age of $\geq$50 years (hazard ratio:2.67; 95% CI =2.06-3.46; p<0.001), T2DM (hazard ratio:1.92; 95% CI=1.45-2.54; p<0.001), hypertension (hazard ratio:1.69; 95% CI= 1.25-2.29; p<0.001), and elevated serum GGT (hazard ratio: 1.35; 95% CI=1.02-1.78; p=0.038) at the initiation of follow up (Table 2).

Figure 2 shows the cumulative development rate of CKD based on the difference of age and eGFR level at the starting time of follow-up. Figure 3 shows the cumulative development rate of CKD based on the difference of FPG, blood pressure, and serum GGT at the starting time of follow-up. On the difference of serum GGT level, the cumulative rate of CKD at 10th year in NAFLD was 11.3% in patients with

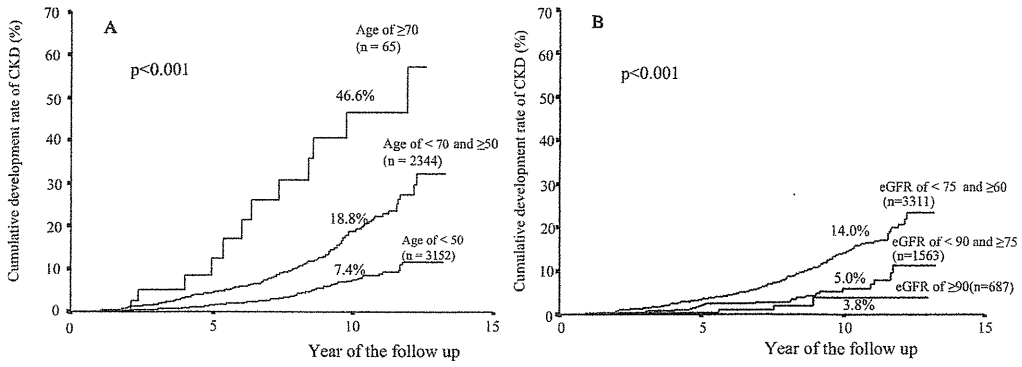

Figure 2. Cumulative development rate of CKD in NAFLD patients. Panel A: Cumulative development rate of CKD based on the difference of age at the starting time of follow-up, Panel B: Cumulative development rate of CKD based on the difference of eGFR level at the starting time of follow-up

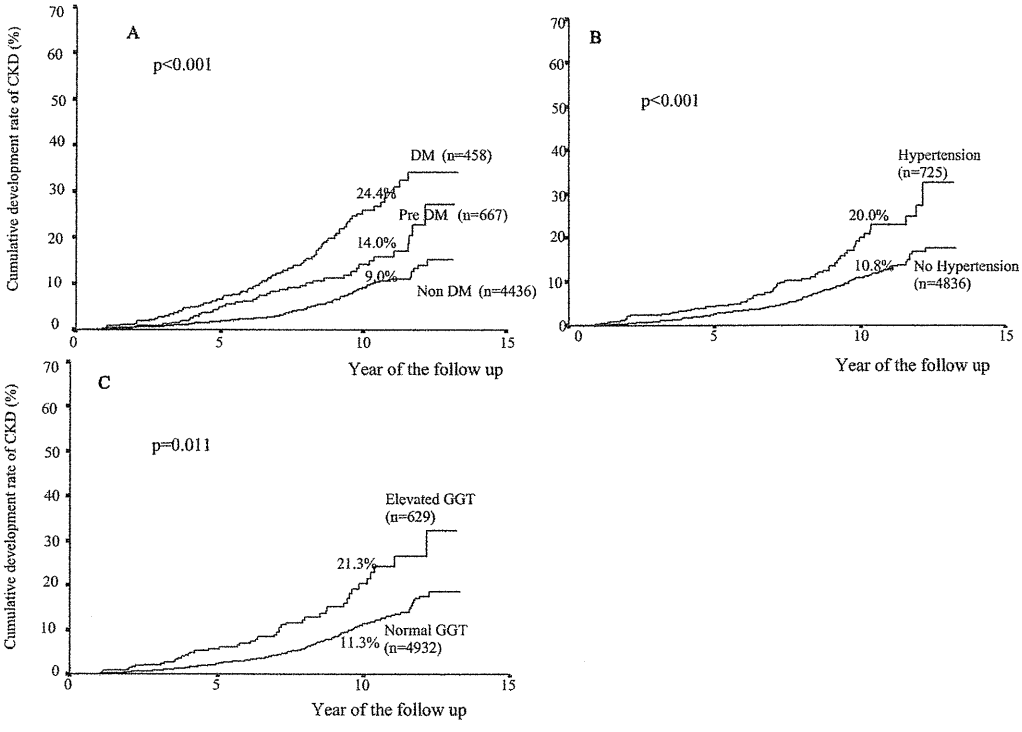

Figure 3. Cumulative development rate of CKD in NAFLD patients. Panel A: Cumulative development rate of CKD based on the difference of glucose level at the starting time of follow-up, Panel B: Cumulative development rate of CKD in patients with hypertension or without hypertension at the starting time of follow-up, Panel C: Cumulative development rate of CKD based on difference of serum GGT level at the starting time of follow-up

normal GGT level and 21.3% in those with elevated GGT level.

Impact of GGT on the incidence of CKD

In addition to elevated level of serum GGT, the four factors of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, and T2DM, hypertension were high risk factors of developing CKD with statistical significance. Figure 4 shows the cumulative development of CKD based on the difference of serum GGT in NAFLD patients with each risk factor of age of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, T2DM, or hypertension. Elevated serum GGT enhances the new development of CKD with statistically significant differences in NAFLD patients with each risk factor of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, or hypertension. In NAFLD patients with T2DM, elevated serum GGT tended to facilitate the new development of CKD (p=0.068).

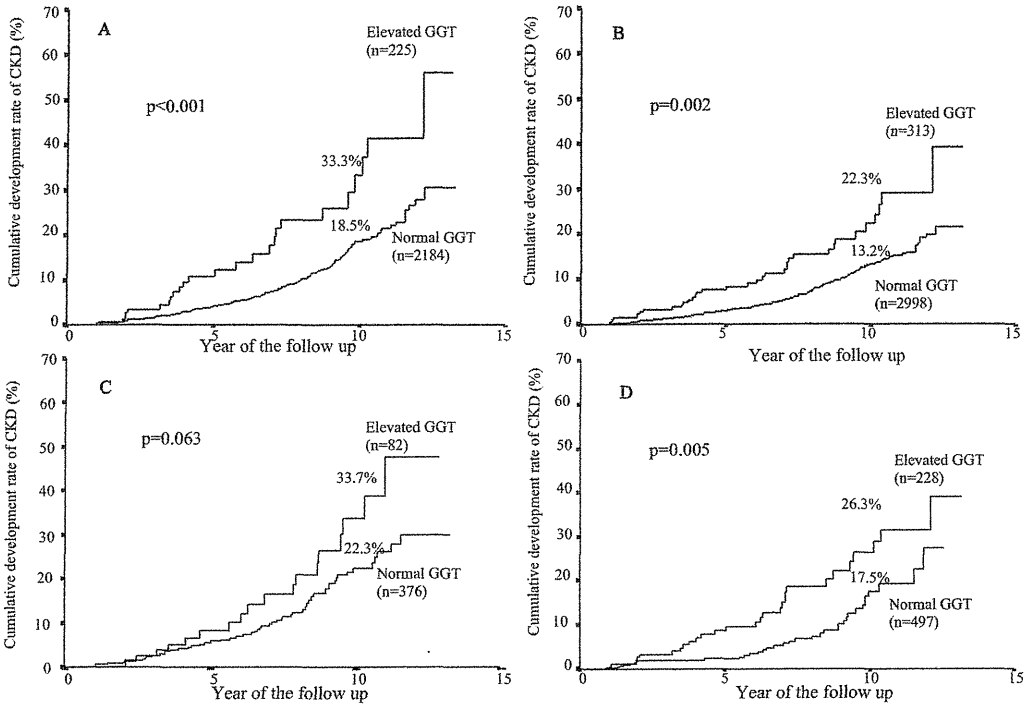

Figure 4. Cumulative development rate of CKD in NAFLD patients. Panel A: Cumulative development rate of CKD based on the difference of serum GGT level at the starting time of follow-up in NAFLD patients aged ≥50 years, Panel B: Cumulative development rate of CKD based on the difference of serum GGT level at the starting time of follow-up in NAFLD patients with eGFR of 60-75 mL/min/1.73 m^2 and absence of dipstick proteinuria (≥+1), Panel C: Cumulative development rate of CKD based on the difference of serum GGT level at the starting time of follow-up in NAFLD patients with T2DM, Panel D: Cumulative development rate of CKD based on the difference of GGT levels at the starting time of follow-up in NAFLD patients with hypertension

Discussion

We have described the incidence of development of CKD in NAFLD patients. The present study indicates that the annual incidence of CKD for a prolonged follow-up among NAFLD patients is about 1.2% based on a follow-up of 10 years. The present study was limited by a retrospective cohort trial. A blood sample and a urine sample were taken for routine analyses during follow-up. Next limitation of the study was that patients were treated with different types of exercise and diet for the NAFLD during follow-up. Moreover, although the NAFLD can be categorized into simple steatosis and steatohepatitis, the present study was undertaken without histological differentiation of simple steatosis and steatohepatitis. Next, prescribed agents during the follow-up were not considered in the present study. Finally, the interval of follow-up was different for each patient. This heterogeneity makes it slight difficult to precisely interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up with a large numbers of patients included.

The present study shows several findings with regard to development of CKD in NAFLD patients. First, the CKD development rate in NAFLD patients with an elevated level of GGT was higher than that in those with a normal level of GGT. The fact that elevated GGT enhanced the onset of CKD is in accordance with the data reported by Chang et al (28), Ryu et al (29), and Fraser et al (30). Though the role of elevated GGT in the pathogenesis of CKD remains speculative, the following possible mechanism have been reported, 1) GGT is related to T2DM and/or insulin resistance by meta-analysis; insulin resistance may be associated with an increased risk for CKD (31-33). 2) GGT is linked with systemic low-grade inflammation; low grade inflammation may cause a change in kidney function (34). 3) GGT has been proposed as a sensitive marker of oxidative stress; oxidative stress plays an important role in renal damage (35).

Second, in addition to the elevation of GGT, the present study suggests that aging, eGFR of 60-75 mL/min/1.73 m^2, T2DM, and hypertension enhanced the development of CKD in NAFLD patients. The present findings of factors of metabolic syndrome such as T2DM and hypertension, which enhanced the new development of CKD is in accordance with the data reported by Chen et al (36), and Luk et al (37). Moreover, when GGT was elevated in NAFLD patients with each factor of ≥50 years, eGFR of 60-75 mL/min/1.73 m^2, or hypertension, the cumulative development rate of CKD increased with significant difference compared to those with a normal GGT level. In NAFLD patients with T2DM, an

Table 2. **Predictive Factors for CKD Development Based on the Clinical Data at the Starting Time of Follow-up**

Variables	Univariate analysis		Cox-regression	
	HR (95%CI)	p	HR (95%CI)	p
Age (years, ≥50/<50)	2.92(2.27-3.75)	< .001	2.67(2.06-3.46)	< .001
Gender (female/male)	1.08(0.73-1.60)	.706		
BMI (≥25/<25)	1.15(0.90-1.46)	.270		
Hypertension (+/-)	2.04(1.55-2.69)	< .001	1.69(1.25-2.29)	<.001
Smoking (+/-)	1.19(0.63-2.24)	.588		
AST(IU/L, ≥34/<34)	1.25(0.95-1.65)	.113		
ALT(IU/L, ≥43/<43)	1.06(0.82-1.38)	.640		
GGT (IU/L, ≥109/<109)	1.43(1.09-1.88)	.011	1.35(1.02-1.78)	.038
Diabetes (+/-)	2.42(1.85-3.17)	< .001	1.92(1.45-2.54)	< .001
WBC ($\times 10^3/mm^3$, <5.0/≥5.0)	1.04(0.80-1.35)	.770		
Hemoglobin (g/dL, <15/≥15)	1.08(0.84-1.39)	.552		
Platelet ($\times 10^4/mm^3$, <25/≥25)	1.04(0.80-1.34)	.770		
Total protein(g/dL, ≥7.5/<7.5)	0.84(0.45-1.50)	.588		
Triglyceride(mg/dL, ≥150/<150)	1.58(1.24-2.00)	< .001	1.32(0.99-1.76)	.059
Total Cholesterol (mg/dL, ≥220/<220)	1.17(0.87-1.57)	.314		
HDL Cholesterol (mg/dL, <40/≥40)	0.94(0.73-1.23)	.693		
Uric acid (mg/dL, ≥7/<7)	1.15(0.86-1.53)	.330		
eGFR (≥60 and <75 /≥75)	2.73(1.92-3.88)	< .001	2.75(1.93-3.94)	< .001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; HR, hazards ratio

elevated GGT indicated tendency to increase the cumulative development rate of CKD compared to those with normal GGT level.

Thus, the present results indicate that T2DM, hypertension, and elevated GGT enhance the new development of CKD in NAFLD patients. This means that in addition to the improvement of glucose level and hypertension, normalization of serum GGT could reduce the aggravation of kidney function.

NAFLD that is considered to be a risk factor for developing CKD is emerging into a new significant health problem in many countries. In addition, the life span in Japan has recently become long. In the near future, a large number of patients with NAFLD will be >60 years of age. CKD occurs more frequently in elderly patients than in young patients. Thus, it is reasonable to conclude that CKD will be increasing in NAFLD patients. CKD often progresses to ESRD with its accompanying complications. Medical physicians regarding the daily management of patients with NAFLD should check on the development of CKD in addition to the aggravation of liver function.

In conclusion, our retrospective study indicates that the annual incidence of CKD in Japanese patients with NAFLD is about 1.2%. The following five factors enhance the risk of development of CKD: low eGFR level, aging, type 2 diabetes, hypertension, and elevated GGT.

The authors state that they have no Conflict of Interest (COI).

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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

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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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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 1 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).

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).

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, × 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.

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-

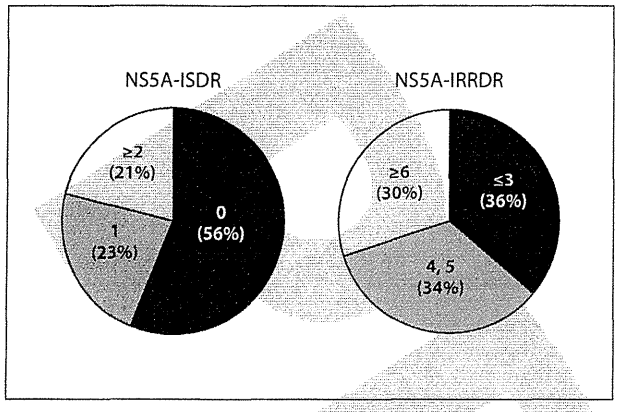

Fig. 1. The number of aa substitutions in NS5A-ISDR and NS5A-IRRDR. The percentage of patients with ≤1 aa substitution in ISDR (WT) was 79%.

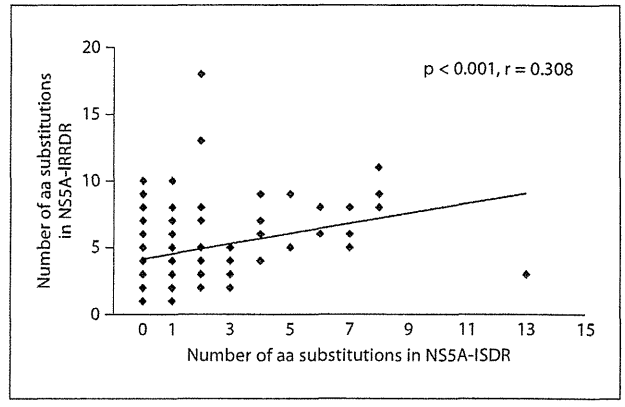

Fig. 2. Correlation between NS5A-ISDR and NS5A-IRRDR. There was a significant positive correlation between the number of aa substitutions in ISDR and that in IRRDR (r = 0.308, p < 0.001).

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.

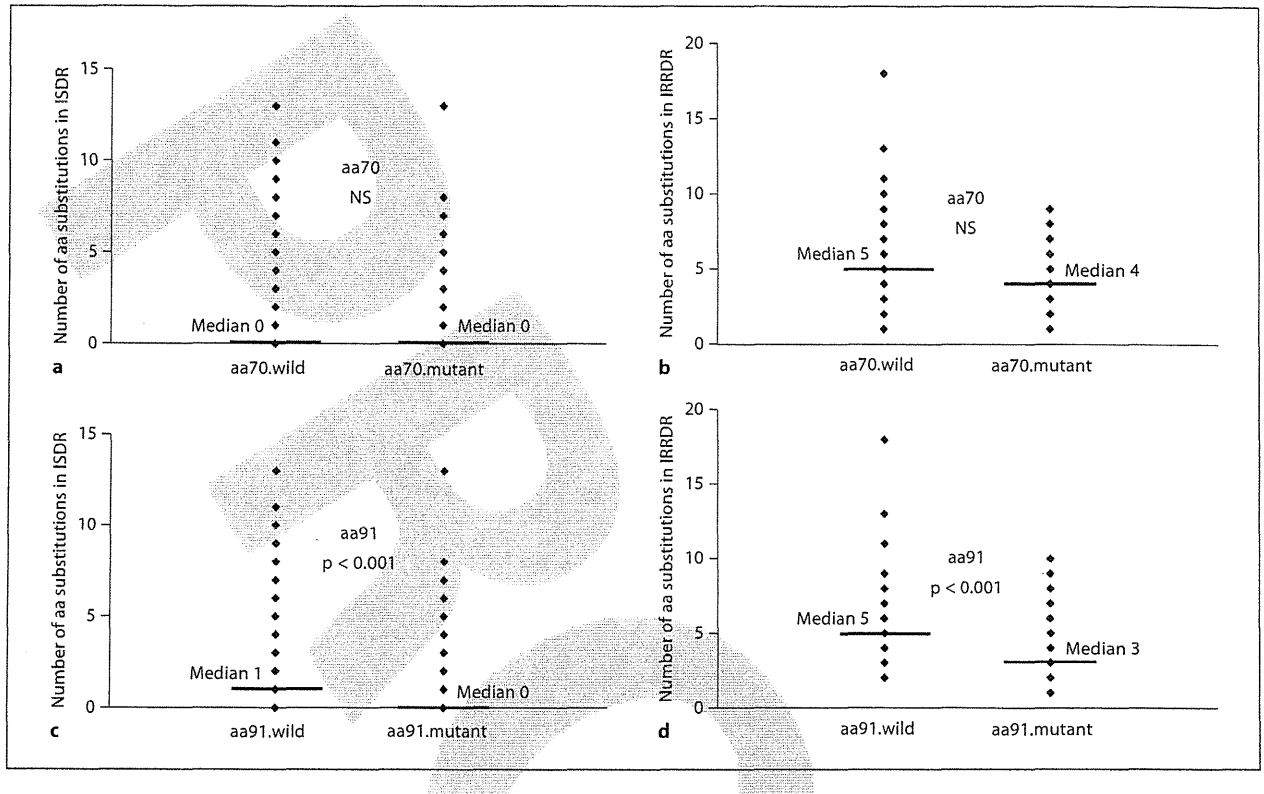

Fig. 3. aa substitutions in the core region and NS5A-ISDR/IRRDR. **a**, **b** Concerning the substitution of core aa 70, the number of aa substitutions in ISDR/IRRDR of patients with Arg70 was not significantly different from that of patients with Gln70 (His70). **c**, **d** Concerning the substitution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

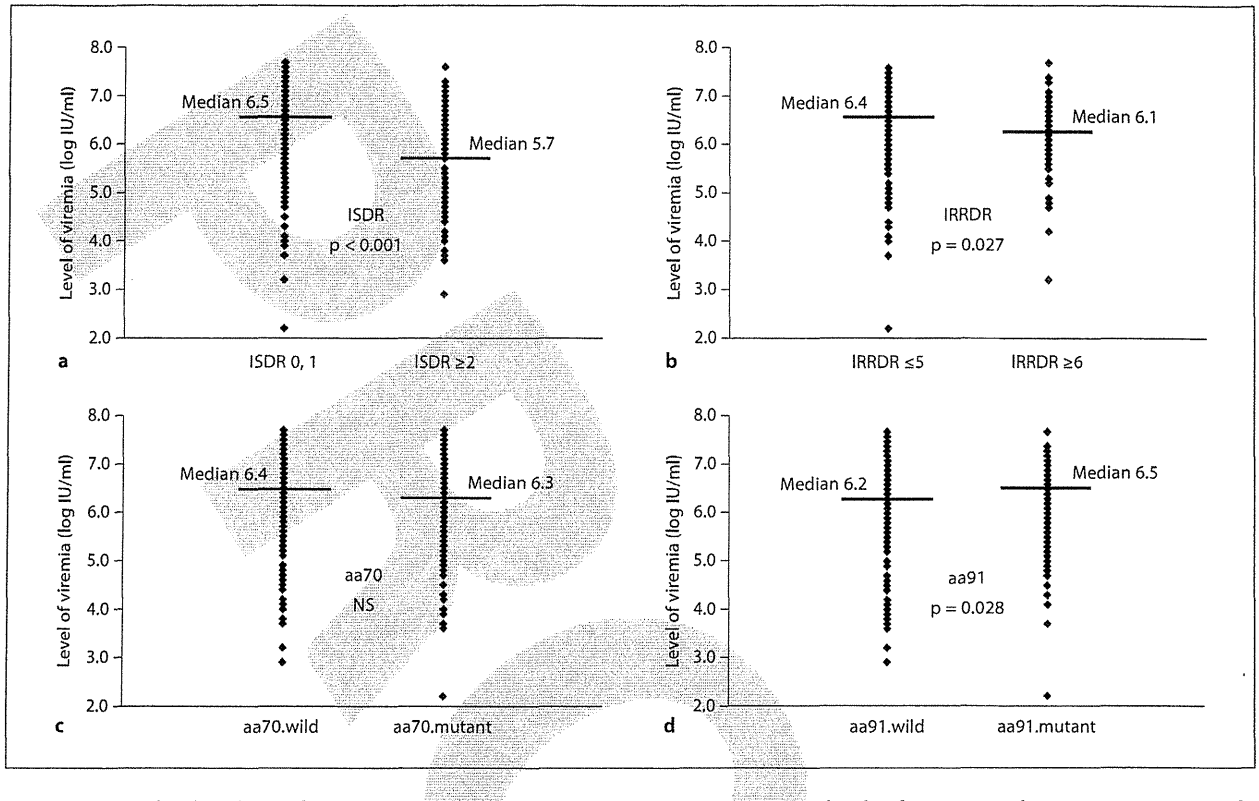

Fig. 4. Viremia level and aa substitutions in core region/ISDR/IRRDR. **a** Concerning the number of substitutions in ISDR, viremia levels of patients with WT were significantly higher than those of patients with non-WT ($p < 0.001$). **b** Concerning the number of substitutions in IRRDR, viremia levels of patients with ≤5 aa substitutions were significantly higher levels than those of patients with ≥6 ($p = 0.027$). **c** Concerning the substitution of core aa 70, viremia levels of patients with Arg70 were not significantly different from those of patients with Gln70 (His70). **d** Concerning the substitution of core aa 91, viremia levels of patients with Met91 were significantly higher than those of patients with Leu91 ($p = 0.028$). Thus, levels of viremia might be influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

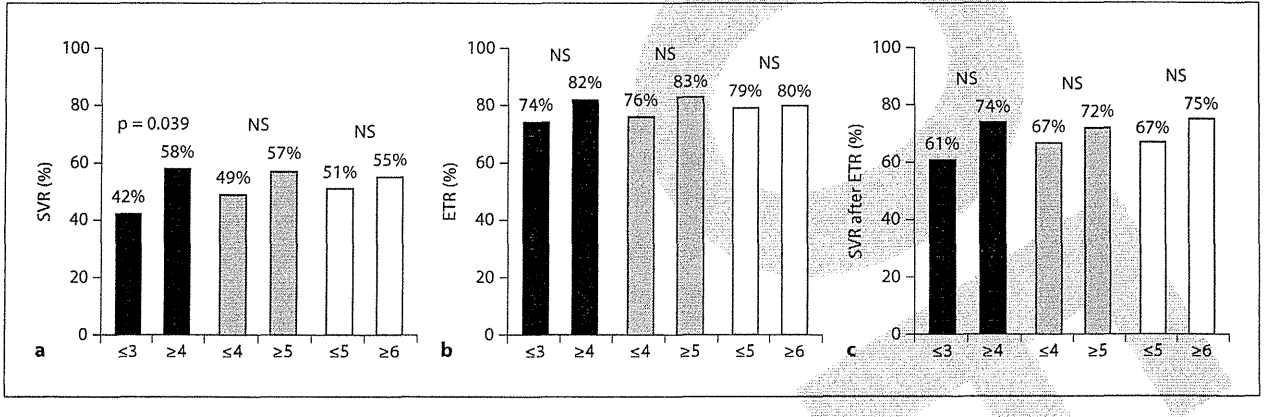

Fig. 5. Treatment response according to the number of aa substitutions in NS5A-IRRDR. **a** A significantly higher proportion of patients with ≥4 (58%) aa substitutions showed SVR compared to patients with ≤3 (42%) ($p = 0.039$), and it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. **b**, **c** ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

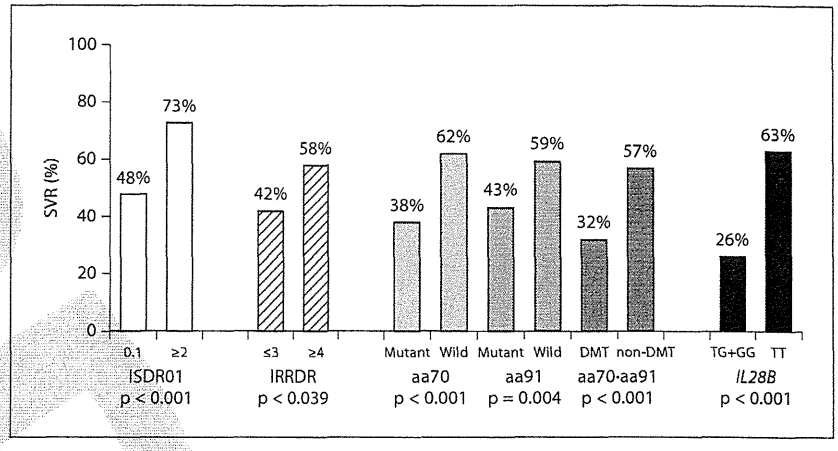

Fig. 6. SVR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

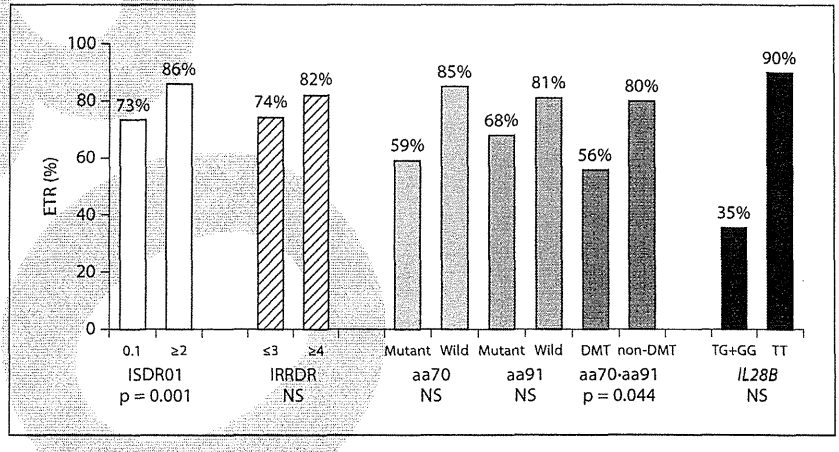

Fig. 7. ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Table 2. Factors associated with SVR to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 16.7 (4.54–61.3)	<0.001
Gender	1: Female 2: Male	1 10.5 (3.47–32.3)	<0.001
ISDR of NS5A	1: WT 2: Non-WT	1 5.68 (1.22–26.3)	0.027

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Factors associated with ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 18.2 (6.29–52.6)	<0.001
Level of viremia log IU/ml	1: ≥6.0 2: <6.0	1 9.20 (2.59–32.6)	0.001
Core aa 70	1: Gln70 (His70) 2: Arg70	1 4.68 (1.65–13.3)	0.004
Serum albumin g/dl	1: <3.9 2: ≥3.9	1 3.08 (1.11–8.47)	0.030

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

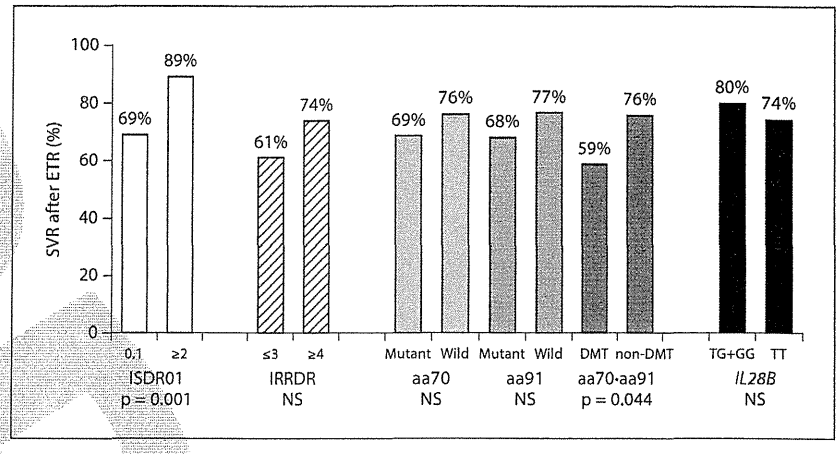

Fig. 8. SVR after ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 4 parameters that independently influenced ETR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.001$), substitution of aa 70 (Arg70; $p = 0.004$), and albumin (≥3.9 g/dl; $p = 0.030$) (table 3).

Predictors of SVR after ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 11 parameters that influenced SVR after ETR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.025$), leukocyte count (≥4,500/mm^3; $p = 0.033$), hemoglobin (≥14.0 g/dl; $p = 0.025$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.020$), total cholesterol (<170 mg/dl; $p = 0.017$), α-fetoprotein (<10 μg/l; $p = 0.004$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.044$), and the number of aa substitutions in ISDR (non-WT; $p = 0.001$). Figure 8 shows the SVR after ETR 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 6 parameters that independently influenced the SVR after ETR: gender (male sex; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.002$), the number of aa substitutions in ISDR (non-WT; $p = 0.012$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.023$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.033$), and α-fetoprotein (<10 μg/l; $p = 0.042$) (table 4).

Comparison of Factors Associated with Treatment Efficacy Identified by Multivariate Analysis

Table 5 shows the variables that achieved statistical significance on multivariate logistic regression for each evaluation of treatment efficacy. Rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core region was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. Level of viremia was an important predictor of ETR. Thus, genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia) were important predictors of treatment efficacy. Furthermore, gender, α-fetoprotein, albumin, and platelet count were also identified as other important predictors of treatment efficacy, in addition to genetic variation near *IL28B* and viral factors.

Discussion

Using multivariate analysis, the present study identified viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene) that influenced treatment efficacy to 48-week IFN/ribavirin combination therapy, which is in agreement with recent findings [22, 23]. Identification of these viral and host factors before the start of IFN/ribavirin combination therapy should help to select better therapeutic regimens, including triple therapy of telaprevir/PEG-IFN/ribavirin [24–26], for those patients who are less likely to achieve SVR.

According to the number of substitutions in ISDR, a previous report showed that levels of viremia were sig-

Table 4. Factors associated with SVR in patients who achieved ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
Gender	1: Female 2: Male	1 4.27 (2.15–8.55)	<0.001
Ribavirin dose, mg/kg	1: <11.0 2: ≥11.0	1 2.95 (1.48–5.86)	0.002
ISDR of NS5A	1: WT 2: Non-WT	1 4.00 (1.35–11.8)	0.012
Core aa 70 and 91	1: Gln70 (His70) and Met91 2: Arg70 and/or Leu91	1 2.96 (1.16–7.52)	0.023
Platelet count $\times 10^4/mm^3$	1: <15.0 2: ≥15.0	1 2.19 (1.07–4.50)	0.033
α-Fetoprotein μg/l	1: ≥10 2: <10	1 2.66 (1.04–6.80)	0.042

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 5. Comparison of factors associated with efficacy of 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	ETR response (at 48 weeks)	SVR after ETR response	SVR
IL28B	rs8099917 p < 0.001, 18.2 (6.29–52.6)[a]		rs8099917 p < 0.001, 16.7 (4.54–61.3)[a]
Virus	Core aa 70 p = 0.004, 4.68 (1.65–13.3)[a]	Core aa 70 and 91 p = 0.023, 2.96 (1.16–7.52)[a]	
	Level of viremia p = 0.001, 9.20 (2.59–32.6)[a]	ISDR p = 0.012, 4.00 (1.35–11.8)[a]	ISDR p = 0.027, 5.68 (1.22–26.3)[a]
Others	Albumin p = 0.030, 3.08 (1.11–8.47)[a]	α-Fetoprotein p = 0.042, 2.66 (1.04–6.80)[a]	
		Platelet count p = 0.033, 2.19 (1.07–4.50)[a]	
		Gender p < 0.001, 4.27 (2.15–8.55)[a]	Gender p < 0.001, 10.5 (3.47–32.3)[a]
		Ribavirin dose p = 0.002, 2.95 (1.48–5.86)[a]	

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.
[a] OR (95% CI).

nificantly lower in patients with non-WT of ISDR than in those with WT [8]. The present study indicated that substitution of IRRDR and core aa 91, in addition to substitution of ISDR, also significantly influenced levels of viremia. Furthermore, there was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR, and the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. To our knowledge, this is the first report of the relationship between viremia levels and aa substitutions in core region/ISDR/IRRDR. This result might be interpreted to mean

that core aa 91/ISDR/IRRDR might be associated with viremia levels involved in resistance to combination therapy. Further studies that examine the functional impact of aa substitutions to combination therapy should be conducted to confirm the above finding.

The present results showed that α-fetoprotein, albumin, platelet count, and gender were predictors of virological response to IFN/ribavirin combination therapy. Previous data indicated that absence of advanced liver fibrosis was a positive predictor of SVR to IFN monotherapy and IFN/ribavirin combination therapy [2, 3, 13, 27–29], and that advanced liver fibrosis was usually associated with higher levels of α-fetoprotein, and lower levels of albumin and platelet count [1, 3, 30–32]. Furthermore, gender is also a predictor of treatment response to IFN/ribavirin combination therapy [2, 3, 14]. In the present study based on a large number of patients, histopathological changes in the liver and gender were identified as independent predictors of virological response, in addition to genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia).

In a previous study, multivariate analysis identified core region, gender, and stage of liver fibrosis as parameters that independently influenced the SVR of patients who achieved early virological response, but ISDR was not entered into uni- and multivariate analysis [3]. To our knowledge, the present study based on multivariate analysis is the first report to identify ISDR as pretreatment predictor of SVR after ETR to combination therapy. Interestingly, ISDR was not a predictor of ETR, but was a significant predictor of SVR to combination therapy. Thus, the underlying mechanisms of failure to develop SVR in those patients who achieve HCV-RNA negativity remain unclear. Further studies that examine the impact of aa substitutions of ISDR to combination therapy should be conducted to confirm the above finding.

One limitation of the present study was that aa substitutions in areas other than the core region and NS5A-ISDR/IRRDR of the HCV genome were not examined. Other limitations were differences in host factors including race [24, 33, 34] and differences in viral factors, such as the distribution of HCV-1a or -1b, and geographic diversities of HCV-1b [35]. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of 48-week IFN/ribavirin combination therapy, and further understanding of the complex interaction between virus- and host- related factors should facilitate the development of more effective therapeutic regimens.

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