Table 2 Significantly independent pretreatment variables associated with sustained virological response to triple combination therapy

Variable	Regression coefficient	Odds ratio	95% CI	*P*-value
IL28B rs8099917 1. TG/GG 2. TT	3.110	22.42	4.98–100.82	5.04×10^{-5}
Pre-existence of cirrhosis 1. Presence 2. Absence	2.079	8.00	2.09–30.68	2.42×10^{-3}
Previous treatment 1. NVR 2. Naïve/relapse	2.026	7.58	2.07–27.76	2.22×10^{-3}

Value of constant was –9.778.
CI, confidence interval; NVR, non-virological response.

Table 3 Significantly independent pretreatment and on-treatment variables associated with sustained virological response to triple combination therapy

Viriable	Regression coefficient	Odds ratio	95% CI	*P*-value
IL28B rs8099917 1. TG/GG 2. TT	3.091	22.01	4.80–100.88	6.90×10^{-5}
Pre-existence of cirrhosis 1. Presence 2. Absence	2.035	7.65	1.92–30.58	3.99×10^{-3}
Previous treatment 1. NVR 2. Naïve/relapse	1.742	5.71	1.45–22.43	0.0126
RVR 1. Failure 2. Achievement	1.570	4.81	1.23–18.78	0.0239

Value of constant was –11.868.
CI, confidence interval; NVR, non-virological response; RVR, rapid virological response.

($P = 0.0126$), and RVR (failure *vs* achievement, $P = 0.0239$). eRVR was excluded from the final step because all of the patients with RVR achieved eRVR (Table 1). On both the multivariable analyses, *IL28B* SNP rs8099917 genotype was the strongest contributor to SVR. Using the SNP genotype alone as a predictor of treatment outcome, sensitivity and specificity were 75.0% and 88.0%. Positive and negative predictive values and predictive accuracy were 96.6%, 44.0%, and 77.4%, respectively.

Each significant, independent factor associated with SVR. Of 87 patients with rs8099917 genotype TT, 84 (97%) achieved SVR (Fig. 1a). By contrast, 28 of 50 (56%) patients with genotype TG/GG had SVR ($P = 3.29 \times 10^{-9}$). As for pre-existence of cirrhosis (Fig. 1b), 89 of 100 (89%) patients without cirrhosis and 23 of 37 (62%) patients with cirrhosis achieved SVR ($P = 3.05 \times 10^{-4}$). Concerning prior treatment response, 53 of 60 (88%) naïve patients achieved SVR (Fig. 1c). In this study, all of prior relapsers and NVRs had previously received combination therapy with peg-IFN alfa-2a or -2b/RBV for 48 or 72 weeks. Fifty of 54 (93%) prior relapsers showed SVR. When prior NVRs were further divided into prior partial and null responders, 9 of 13 (69%) prior partial responders achieved SVR (Fig. 1c). None of 10 (0%) prior null responders showed SVR. Regarding attainment of RVR, 96 of 108 (89%) RVR patients and 16 of 29 (55%) non-RVR patients showed SVR (Fig. 1d, $P = 2.99 \times 10^{-5}$).

Impact of IL28B SNP on subgroup category. As described earlier, *IL28B* SNP rs8099917 genotype was the strongest among significantly independent factors. The SNP had an impact on each category of significantly independent contributors to SVR: pre-existence of cirrhosis, prior treatment response, and RVR (Table 4). Except for prior relapsers and prior partial responders, most of categories in these contributors were significantly influenced by the *IL28B* SNP. Specifically, the impact on prior null responders was remarkable (Table 4).

HCV-related factors. Among viral variables analyzed in this study, only core 70 was significantly associated with SVR in bivariate analysis (Table 1), although it was excluded from the final multivariable analysis. In 60 naïve patients, 37 of 42 (88%) with wild core 70 and 16 of 18 (89%) with mutant core 70 achieved SVR ($P = 0.651$). In 54 prior relapsers, 36 of 37 (97%) with wild core 70 and 14 of 17 (82%) with mutant core 70 achieved SVR ($P = 0.0871$).

Discussion

Aged and/or female patients generally have a susceptibility to treatment-induced anemia and have poor response to peg-IFN/RBV therapy.[18,19] In this study, median patient age was around 60 years in both SVRs and non-SVRs, indicating that over-60s accounted for nearly one-half of the community-based patient cohort in Japan. Female frequently achieved SVR with the addition of telaprevir comparable with male. This study regimen differed from phase 3 trials[9,10] in that reduction and modification of telaprevir dose was approved, probably leading to reduction of the discontinuation rate and increase of the SVR rate. This study showed that close monitoring and proper management, including dose modifications, make it possible for aged and/or female patients to safely receive telaprevir-based triple therapy, with further improvement of the SVR rate.

Response of HCV genotype 1 patients to peg-IFN/RBV therapy is strongly associated with host genetic variations, such as SNPs rs12979860 and rs8099917 nearby the *IL28B* gene.[17,21,22] The frequencies of rs12979860 C-allele and the favorable genotype CC in this cohort were 80% and 64%, indicating no selection bias of study population.[19] The frequencies of rs12979860 genotype CC and rs8099917 TT are closely correlated with ethnicity and the highest in East Asians among diverse ethnic groups.[17] Nevertheless, peg-IFN/RBV therapy yielded the SVR rates of only 45% in the overall East Asian cohort and 60% in the favorable genotype subgroup[19] compared with 69–82% in Caucasian and 48–53% in African American with the same genotype.[17,26] The racial differences suggest that any factors other than the *IL28B* SNPs could

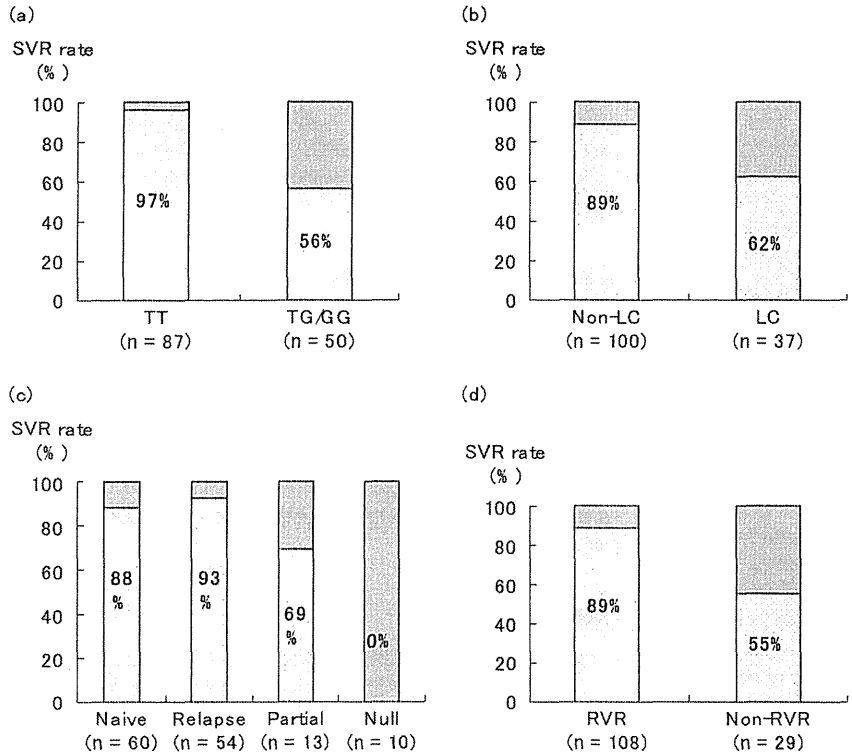

Figure 1 Sustained virological response (SVR) rates in each significantly independent factor. (a) *IL28B* single nucleotide polymorphism rs8099917 genotype ($P = 3.29 \times 10^{-9}$). (b) Absence versus pre-existence of liver cirrhosis (LC; $P = 3.05 \times 10^{-4}$). (c) Treatment-naïve patients/prior relapsers versus prior partial responders/null responders ($P = 3.43 \times 10^{-7}$). (d) Achievement of rapid virological response (RVR) versus non-RVR ($P = 2.99 \times 10^{-5}$). Light-gray and dark-gray prismatic columns represent the SVR rates and non-SVR rates, respectively.

Table 4 Distribution of *IL28B* SNP rs8099917 and sustained virological response rates in each subgroup

IL28B rs8099917	Non-LC ($n = 100$)	LC ($n = 37$)	RVR ($n = 108$)	Non-RVR ($n = 29$)	Naïve ($n = 60$)	Relapse ($n = 54$)	Partial ($n = 13$)	Null ($n = 10$)
TT ($n = 87$)	100% (65/65)	86% (19/22)	97% (71/73)	93% (13/14)	98% (42/43)	97% (37/38)	83% (5/6)	– (0/0)
TG/GG ($n = 50$)	69% (24/35)	27% (4/15)	71% (25/35)	20% (3/15)	65% (11/17)	81% (13/16)	57% (4/7)	0% (0/10)
P-value	2.95×10^{-6}	4.14×10^{-4}	1.84×10^{-4}	1.16×10^{-4}	1.43×10^{-3}	0.073	0.343	–

P-values denote the impact of rs8099917 genotype on each subgroup category.
–, not calculable; LC, liver cirrhosis; RVR, rapid virological response; SNP, single nucleotide polymorphism.

influence the treatment outcome. It remains unclear how the addition of telaprevir alters the value of *IL28B* SNPs in different races and/or ethnicity.

Recently, 48-week telaprevir-based triple therapy was reported to attenuate the predictive value of *IL28B* SNP in predominantly Caucasian cohort with previous treatment failure.[20] In this East Asian cohort treated with 24-week course, the SVR rate of patients with rs8099917 genotype TT was 97%. The SNP genotype remained a significantly independent factor associated with SVR and had an impact on majority of categories within other independent contributors. This study population consisted of naïve patients and prior treatment failures. Certainly, rs8099917 genotype was not significantly related to SVR in prior relapsers and partial responders. T12PR24 appears to yield the SVR rate of approximately 90% for Japanese prior relapsers with genotype 1b.[8,10,27] It was unclear whether rs8099917 genotype may influence treatment response of prior null responders because all of them had minor rs8099917 genotype TG/GG and the numbers were very small. By contrast, *IL28B* SNPs had a significant impact on naïve patients: rs12979860 genotype ($P = 9.42 \times 10^{-4}$), rs8099917 genotype ($P = 1.42 \times 10^{-3}$), RVR ($P = 5.73 \times 10^{-3}$), and pre-existing cirrhosis ($P = 0.0451$) in bivariate comparisons (data not shown). The limited and non-significant impact of *IL28B* SNP in prior treatment failures was consistent with the retrospective study.[20] When more potent antiviral treatment is available, not only *IL28B* SNP but also other factors may have little or no predictive value. Taken together, *IL28B* SNP genotyping appears to be most useful especially in shortened treatment regimen for naïve patients. However, careful interpretation should be required because the distribution of *IL28B* genotypes and treatment regimens differed considerably between different studies. Further studies are also required to identify factors associated with SVR in patients with unfavorable *IL28B* genotypes.

Prior treatment response is an important predictor of SVR or on-treatment virological failure.[4,6,8,10,28] Prior relapsers appear to be stable for telaprevir-based combination therapy because of their

high SVR rates of around 90%.[6,8,10,27,28] In this cohort, the distribution of *IL28B* SNP genotypes was similar between naïve patients and prior relapsers. Nevertheless, among patients with unfavorable genotype TG/GG, the SVR rate was higher in prior relapsers than in naïve patients, although not statistically significant. These findings suggest that prior relapsers have favorable conditions, including any unidentified factors other than *IL28B* SNP, for achieving SVR. When prior NVRs were divided into prior partial and null responders, the SVR rates were 69% and 0%, respectively. In previous trials, T12PR48 with/without lead-in increased SVR rates to 54–59% in prior partial responders and to 29–33% in prior null responders.[6] Approximately one-half of prior null responders had on-treatment virological failure.[28] Although it is unclear why the SVR rates differed considerably between different studies, prior null-responders had better await another treatment including next-generation DAAs. T12PR may be a retreatment strategy of reasonable promise for prior partial responders.

From the era of conventional IFN monotherapy, numerous studies have reported that patients with advanced fibrosis have unsatisfactory SVR rates compared with those with less severe fibrosis. Also in this study for T12PR24, pre-existing of cirrhosis was an independent factor with a negative impact on SVR. T12PR24/48 for naïve patients yielded SVR rates of 63% in advanced fibrosis and 75% in less severe fibrosis.[7] Among patients with advanced fibrosis who achieved eRVR, even T12PR24 generated a high SVR rate of 82%. In another T12PR regimen, SVR rates decreased from 81% to 62% with advancing stage of fibrosis.[5] Meanwhile, SVR rates remained almost flat, irrespective of liver fibrosis, in T12/PR24 for previously treated patients.[4] T12/PR48 produced a high SVR rate (84%) for prior relapsers with advanced fibrosis compared with those of partial responders (44%) and null responders (28%).[6] This study suggested that cirrhotic patients with positive factors, such as favorable *IL28B* SNP, prior relapse and RVR/eRVR, may be candidates with an increased likelihood of SVR.

RVR is highly predictive of SVR and thus a strong independent on-treatment predictor.[29,30] Response-guided therapy based on RVR has been also utilized in telaprevir-based triple combination therapy.[5,7] In this study, RVR was relatively less significant compared with other independent contributors, partly because the SVR rates of patients with favorable *IL28* SNP genotype were constantly high across independent contributors. Alternatively, this study cohort consisted of a mixed population of naïve and previously treated patients or included cirrhotic patients. Because the SVR rates of patients with unfavorable genotype differed considerably between RVR and non-RVR, predictive algorithm by combining with other factors may heighten the importance as a more stable milestone in the response-guided treatment.

The size of this study population was small. Specifically, the number of non-SVRs was small because the SVR rate was higher than expected. However, this is the first report to clarify the value of *IL28B* SNP in stratified subgroups of East Asian patients with HCV genotype 1b, who received 24-week telaprevir-based triple therapy. Further investigation including a randomized, controlled trial is required in a larger and multinational scale or stratified subgroups according to closely intertwined factors to improve the predictive precision and to develop personalized treatment strategies.

In conclusion, 12-week telaprevir combined with 24-week peg-IFN alpha-2b plus RBV yielded high SVR rates even in the community-based East Asian patients infected with HCV genotype 1b. The *IL28B* SNP still remained informative as a predictor of SVR to 24-week telaprevir-based triple therapy. The findings in this study will be helpful in making an algorithmic decision on telaprevir-based treatment and in developing the individual tailoring and optimization of therapeutics, including the next-generation DAAs.

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Serum Lipoprotein Profiles and Response to Pegylated Interferon Plus Ribavirin Combination Therapy in Patients With Chronic HCV Genotype 1b Infection

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ABSTRACT

Background: Abnormal serum lipid profiles have been noted in patients with chronic hepatitis C virus (HCV) infection. Moreover, many reports suggest that serum lipoprotein profiles are more profoundly distorted in patients with HCV G1b infection who have an unfavorable response to pegylated interferon (peg-IFN) plus ribavirin (RBV) combination therapy. However, after the discovery of single nucleotide polymorphisms near the IL28B gene (rs8099917 and rs12979860) as potent predictive factors affecting the response to peg-IFN plus RBV, lipid factors are thought to be confounding factors.

Objectives: To re-examine the significance of lipoprotein profiles on virological response to peg-IFN plus RBV combination therapy in patients with chronic HCV G1b infection, we examined cholesterol and triglyceride concentrations in each lipoprotein fraction separated by high performance liquid chromatography.

Patients and Methods: Lipoprotein profiles were examined using fasting sera from 108 patients infected with HCV G1b who had chronic hepatitis, as determined by liver biopsy. Results of lipoprotein profiles and clinical data, including IL28B genotype and amino acid substitution at aa70 of HCV G1b, were compared between patients with a sustained virological response (SVR) and non-SVR or a non-virological response (NVR) and virological responses other than NVR (non-NVR). In addition, significant predictive factors independently associated with virological response to peg-IFNα-2b plus RBV were determined by logistic regression analysis.

Results: An increased ratio of cholesterol/triglyceride in very low-density lipoprotein (odds ratio (OR) 3.03; 95% confidence interval (CI) 1.01-9.44) along with a major genotype of rs8099917 (OR 9.09; 95% CI 2.94-33.33), were independent predictive factors for SVR. In contrast, lipid factors were not elucidated as independent predictive factors for NVR.

Conclusions: Examination of the fasting lipid profile has clinical importance in predicting the efficacy of peg-IFN-α-2b plus RBV combination therapy for patients with HCV G1b even after the discovery of the IL28 genotype as a potent predictive factor.

Keywords: Hepatitis C; Ribavirin; Lipoproteins; Lipoproteins VLDL; Chromatography, High Pressure Liquid

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▸Implication for health policy/practice/research/medical education:
Examination of serum lipid profile in detail is important for understanding the biology of HCV and predicting virological response to pegylated interferon plus ribavirin combination therapy for chronic hepatitis C caused by HCV G1b infection

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1. Background

Hepatitis C virus (HCV) is a unique virus targeted towards liver cells; this virus closely interacts with host lipoprotein metabolism (1, 2). Very low-density lipoproteins (VLDL) synthesized and secreted from liver cells play a critical role in the generation and secretion of HCV (3). HCV particles are composed of HCV-lipoprotein complexes, referred to as lipoviral particles (LVP), in peripheral blood (4, 5). HCV particles in the plasma of patients with chronic HCV infection are thought to be associated with different classes of lipoproteins showing different buoyant density. The infectivity of HCV is stronger with low buoyant density LVP than with high buoyant density LVP, suggesting the significance of VLDL as a component of LVP on the HCV lifecycle (6). Lipoprotein profiles in the sera of patients with chronic HCV infection have been studied with special attention to the association of HCV genotype and the response to interferon (IFN)-based antiviral therapy (7-10). Abnormal lipoprotein profiles are more prominent in HCV genotype 3 (G3) than in HCV genotype 1 (G1) infection (11). Apolipoprotein B (Apo B)-related cholesterol is lower in patients with a non-favorable response to pegylated IFN (peg-IFN) plus ribavirin (RBV) therapy than in patients with a favorable response who are infected by HCV G1 (12). These findings suggest that investigation of lipoprotein profiles in patients with chronic HCV infection may be helpful in understanding the interaction of HCV and host lipoprotein metabolism. Lipoproteins in peripheral blood are usually examined using conventional simplified methods. High-density lipoprotein cholesterol (HDL-C) is usually determined by the precipitation method, whereas low-density lipoprotein cholesterol (LDL-C) is estimated indirectly by the Friedwald equation (13) or direct methods. However, it is difficult to measure VLDL cholesterol (VLDL-C) directly by routine laboratory tests. To determine serum lipid profiles more precisely, one of the most reliable methods is density gradient ultracentrifugation. However, ultracentrifugation requires a relatively large sample volume and a lot of time; thus it is not suitable for general use in clinical settings. Polyacrylamide gel electrophoresis or nuclear magnetic resonance is another method to assess lipid profiles (14, 15). In addition, high-performance liquid chromatography (HPLC) could be applied to differentiate lipoproteins based on the differences in particle diameter (16, 17).

2. Objectives

In the present study, we used a computer-assisted online dual detection method by HPLC that allows simultaneous determination of cholesterol and triglyceride (TG) profiles from a single injection of sample. The characteristics of lipoprotein profiles participating in the response to peg-IFNα-2b plus RBV combination therapy in patients with chronic HCV G1b infection were analyzed to re-examine the significance of lipoprotein profiles on the virological response to this treatment regimen.

3. Patients and Methods

3.1. Patients

Among patients with chronic HCV G1b infection diagnosed as chronic hepatitis by liver biopsy and treated with Peg-IFNα-2b plus RBV combination at Katsushika Medical Center and/or Kashiwa Hospital, the Jikei University School of Medicine, and Shinmatsudo Central General Hospital between April 2008 and November 2010, 120 Japanese patients were randomly asked to participate into this observational cohort study designed to evaluate the significance of host lipids on the response to anti-viral therapy, and 119 patients accepted our proposal. HCV genotype was confirmed using the conventional polymerase chain reaction (PCR)-based method. Patients with HBV co-infection, with cirrhosis or hepatocellular carcinoma, or co-infected with HIV were excluded. In addition, patients with diabetes mellitus or treated with a lipid-lowering drug were excluded. All enrolled patients were treated according to the treatment protocol based on response-guided therapy (18). Patients were treated with standard peg-IFN and ribavirin therapy according to the American Association for the Study of the Liver Diseases (AASLD) guidelines (19). Briefly, patients with chronic HCV G1b infection received subcutaneous peg-IFNα-2b (Peg-Intron®; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg once weekly, and oral RBV (Rebetol®; MSD) at a dose of 600–1000 mg twice daily, adjusted according to body weight (600 mg for weight of 60 kg or less, 800 mg for weight of 60–80 kg or less, and 1000 mg for weight above 80 kg). The standard treatment duration lasted 48 weeks, although among patients with detectable HCV RNA at week 12, the treatment period was extended to 72 weeks. When serum HCV RNA levels were not decreased more than 2 logs at week 12 or HCV RNA was detectable at week 24 of therapy, treatment was discontinued prematurely and defined as a non-virological response (NVR). A virological response (VR) was defined as undetectable serum HCV RNA. Sustained virological response (SVR) was defined as undetectable serum HCV, 24 weeks post-treatment. Transient viral response (TVR) was defined as VR during treatment, but reappearance of serum HCV RNA during the follow-up period. Adherence to more than 80% of the scheduled doses during the first 12 weeks was required for inclusion in this study. Moreover, patients who discontinued treatment within 24 weeks of treatment for reasons other than virological failure were excluded. Finally, 108 out of the 119 patients were suitable for the inclusion criteria. Liver biopsy specimens were reviewed using the METAVIR scoring system for staging of fibrosis and grading of necro-inflammation activity (20). The serum HCV RNA was assessed using a quantitative PCR assay (COBAS TaqMan HCV test, Roche Diagnostics, Tokyo, Japan). Clinical and laboratory data were assessed

at the time of the liver biopsy, before treatment, and fasting sera (taken early in the morning after at least 8 hours of fasting) were collected and used for detection of lipoprotein profiles. This study protocol was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Institutional Review Board of each participating facility. Informed consent was obtained from all patients. Genotype of IL28B (rs8099917) was examined by our previously described method in 101 out of 108 HCV G1b patients (21). The major (TT) genotype of IL28B was noted in 69 patients and the minor (TG or GG) genotype was noted in 32 patients. Pre-treatment amino acid (aa) substitution at aa70 in the core region of HCV G1b, a viral factor affecting the therapeutic outcome, was analyzed by the method of Akuta et al. in 98 patients (8). Wild type (arginine, Arg70) was noted in 62 patients and non-wild type (glutamine/histidine, Gln70/His70) was noted in 36 patients. Both IL28B genotype and aa substitution at 70 were noted in 98 patients.

3.2. Analysis of Lipoprotein Profiles

Fasting serum lipoprotein profiles were analyzed by HPLC with online enzymatic dual detection of cholesterol and TG (Skylight Biotech, Inc., Akita, Japan) as described previously (16, 17). Briefly, a 10 µL whole serum sample was injected into two connected columns (300 × 7.8 mm) of TSKgel LipopropakXL (Tosoh, Tokyo, Japan) and eluted by TSKeluent Lp-1 (Tosoh). The effluent from the columns was divided by micro splitter and continuously monitored at 550 nm after an online enzymatic reaction with a commercial kit, Determiner L TC (Kyowa Medex Co. Limited, Tokyo, Japan) and Determiner L TG (Kyowa Medex Co. Limited). Then, the cholesterol and TG concentrations were calculated by a computer program. Lipoprotein particles fractionated into four major lipoproteins according to particle diameter were as follows: ≥80 nm was clarified as chylomicrons, from 30 nm ≤ to <80 nm as VLDL, from 16 nm ≤ to <30 nm as LDL, and from 8 nm ≤ to <16 nm as HDL. The concentrations of cholesterol and TG were measured in each major lipoprotein fraction and the cholesterol/TG (C/T) concentration ratio was calculated. The typical pattern of serum lipoprotein fractionated by this HPLC system is shown in *Figure 1*.

3.3. Statistical Analysis

Continuous variables are given as mean ± SD and categorical variables are given as numbers. Differences between two groups were evaluated using the Mann-Whitney U-test or the $\chi 2$ test with Yates' correction. All *P* values less than 0.05 with a two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) were entered into multivariate logistic regression analysis to identify independent predictive factors. The odds ratios (OR) and 95% confidence intervals (CI) were also calculated. STATISTICA version 8 (StatSoft Japan Inc., Tokyo, Japan) and Mintab 16 (Kozo Keikaku Engineering Inc., Tokyo, Japan) were used for statistical calculations. The *P* value was calculated to three decimal places and $P <$ 0.001 was used for lower values.

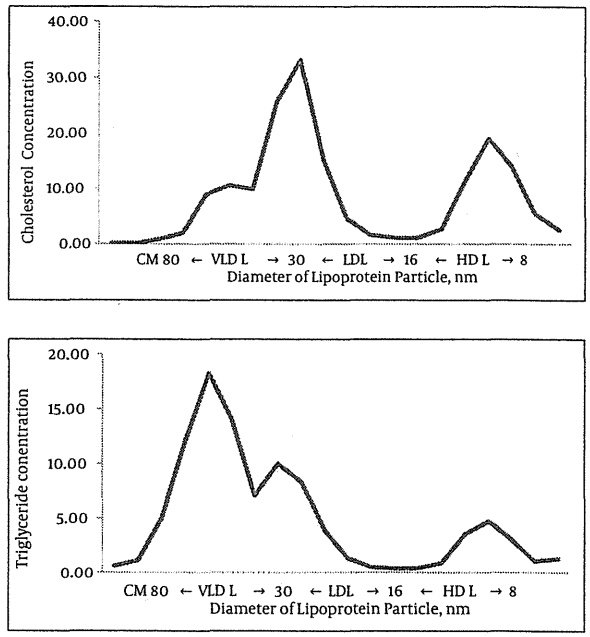

Figure 1. Pattern of Serum Lipoprotein Fractionated by HPLC with Online Enzymatic Dual Detection of Cholesterol and Triglyceride

4. Results

4.1. Lipoprotein Profiles and Clinical Data

Fasting serum lipoprotein profiles examined by online dual detection HPLC and clinical data of 108 patients are summarized in *Table 1*. The C/T ratio was low in TG rich lipoprotein (VLDL) fraction, and similar in LDL and HDL fractions.

4.2. Responses to Peg-IFN-α-2b Plus RBV Therapy, Distribution of aa 70 Substitution, and IL28B Genotype

Fifty-six patients were classified as SVR, 22 were TVR, and 30 were NVR. The SVR rate was 51.9% and the NVR rate was 27.8%. In terms of aa substitution at 70 in the HCV G1b core region, among patients with SVR, 39 were Arg70 and 14 were Gln70/His70. In TVR patients, 14 were Arg70 and 5 were Gln70/His70, and in NVR patients, 9 were Arg70 and 17 were Gln70/His70. In terms of the IL28B (rs8099917) genotype defined as a single nucleotide polymorphism, among patients with NVR, only 4 were defined as the major genotype and 23 were the minor genotype. In patients with TVR, 16 were the major genotype and 4 were the minor genotype, and in patients with SVR, 49 were the major genotype and 5 were the minor genotype.

Table 1. Clinical Features and Lipoprotein Profiles

Discrete Traits	n = 108
Sex, No. (%)	
Males	44 (41)
Females	66 (59)
METAVIR Fibrosis Stage, No. (%)	
1	56 (52)
2	34 (31)
3	18 (17)
METAVIR Activity Grade, No. (%)	
1	53 (49)
2	50 (46)
3	5 (5)
Quantitative Traits	
Age, mean ± SD, y[a]	58.1 ± 11.0
HCV RNA, mean ± SD, log IU/mL	6.4 ± 0.6
ALT, mean ± SD, IU/L	59.1 ± 42.5
AST, mean ± SD, IU/L	53.8 ± 37.6
Albumin, mean ± SD, g/dL	4.2 ± 0.3
WBC, mean ± SD, X102/mm3	52.3 ± 17.2
Hb, mean ± SD, g/dL	13.4 ± 1.8
Platelet, mean ± SD, X104/mm3	18.1 ± 6.0
BMI, mean ± SD, kg/m3	23.3 ± 2.1
Lipids, mean ± SD	
Total Cholesterol, mg/dL	164.3 ± 30.6
Triglycerides, mg/dL	90.6 ± 30.1
VLDL, mean ± SD	
Cholesterol, mg/dL	34.0 ± 10.6
Triglycerides, mg/dL	41.3 ± 18.0
C/T ratio	0.94 ± 0.47
LDL, mean ± SD	
Cholesterol, mg/dL	78.4 ± 19.6
Triglycerides, mg/dL	26.0 ± 7.8
C/T ratio	3.2 ± 0.9
HDL, mean ± SD	
Cholesterol, mg/dL	48.9 ± 14.1
Triglycerides, mg/dL	17.3 ± 6.2
C/T ratio	3.1 ± 1.1

[a] Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; C/T ratio, cholesterol/triglyceride ratio; Hb, hemoglobin; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; WBC, white blood cell; y, year

4.3. Factors Contributing to SVR in Chronic HCV G1b Patients

When pre-treatment clinical data (age, sex, alanine aminotransferase (ALT) level, albumin, quantity of HCV RNA, and platelet count), histological stage, activity score, substitution at aa70 in the core region of HCV, and IL28Bgenotype other than lipoprotein profiles, including those obtained by HPLC, were compared between patients with SVR and non-SVR (TVR plus NVR), a significant difference was found in distribution of the IL28B genotype, distribution of the aa70 substitution, serum ALT level, and the C/T ratio in VLDL (*Table 2*).

Table 2. Difference in Clinical Data Between Patients with Sustained Virological Response and Non-Sustained Virological Response

	SVR[a], n = 56	Non-SVR, n = 52, No. (%)	*P* value
Discrete Traits			
Sex, No. (%)			0.067
Males	28 (50)	16 (31)	
Females	28 (50)	36 (69)	
METAVIR Fibrosis Stage, No. (%)			0.547
1	30 (53)	26 (50)	
2	15 (27)	19 (37)	
3	11 (20)	7 (13)	
METAVIR Activity, No. (%)			0.445
1	25 (45)	28 (54)	
2	28 (50)	22 (42)	
3	3 (5)	2 (4)	
HCV Core aa70 (n = 98), No. (%)			0.037
Arg70	39 (74)	23 (51)	
Gln70 or His70	14 (26)	22 (49)	
IL28B Genotype (n = 101), No. (%)			< 0.001
major	49 (91)	20 (43)	
minor	5 (9)	27 (57)	
Quantitative Traits, mean ± SD			
Age, y	58.0 ± 11.3	58.4 ± 10.9	0.795
HCV RNA, log IU/mL	6.38 ± 0.60	6.40 ± 0.46	0.594
ALT, IU/L	68.4 ± 51.4	49.3 ± 25.9	0.042
Albmin, g/dL	4.19 ± 0.31	4.14 ± 0.29	0.772
Platelet, X104/mm3	18.54 ± 5.83	17.66 ± 6.23	0.241
Lipid, mean ± SD, mg/dL			
Total Cholesterol	167.2 ± 28.1	161.2 ± 33.1	0.498
Triglycerides	87.8 ± 27.5	93.5 ± 32.6	0.658
VLDL			
Cholesterol, mean ± SD, mg/dL	35.7 ± 10.5	32.1 ± 10.6	0.056
Triglycerides, mean ± SD, mg/dL	42.5 ± 14.5	48.3 ± 21.0	0.241
C/T ratio	0.92 ± 0.40	0.75 ± 0.35	0.011
LDL, mean ± SD			
Cholesterol, mg/dL	80.1 ± 19.2	77.4 ± 20.2	0.448
Triglycerides, mg/dL	26.1 ± 7.1	25.9 ± 8.5	0.510
C/T ratio	3.15 ± 1.06	3.13 ± 0.86	0.894
HDL, mean ± SD			
Cholesterol, mg/dL	48.9 ± 11.8	49.7 ± 16.3	0.969
Triglycerides, mg/dL	16.9 ± 6.8	17.7 ± 5.6	0.242
C/T ratio	3.15 ± 1.06	2.99 ± 1.15	0.573

[a] Abbreviations: aa, amino acid; ALT, alanine aminotransferase; Arg, arginine; AST, aspartate aminotrasferase; C/T ratio, cholesterol/triglyceride ratio; Gln, glutamine; HCV, hepatitis C virus; His, histidine; HDL, high-density lipoprotein; IFN, interferon; LDL, low-density lipoprotein; SVR, sustained virological response; VLDL, very low-density lipoprotein

Multivariate logistic model was applied for the four variables that were significantly different between SVR and non-SVR to determine independent predictive factors. Among the 98 patients in whom both aa substitution of

the HCV core 70 and IL28B genotype were examined, the IL28B major genotype (OR 9.09; 95% CI 2.94-33.33) and the C/T ratio of VLDL (OR 3.03; 95% CI 1.01-9.44) were elucidated as independent factors contributing to SVR. However, substitution at aa70 and serum ALT levels were not independent factors contributing to SVR (*Table 3*).

Table 3. Multivariate Logistic Regression Analysis of Factors Predicting Sustained Virological Response in Patients with Hepatitis C Virus G1b (n = 98)

Variable	Odds Ratio	95% CI[a]	*P*value
ALT, IU/L	1.02	1.00-1.03	0.058
Arg70/Gln70 or His70	2.27	0.79-6.67	0.129
Major/Minor IL28B Genotypes	9.09	2.94-33.33	< 0.001
C/T Ratio in VLDL	3.03	1.01-9.44	0.048

[a] Abbreviations: ALT, alanine aminotransferase; Arg, arginine; CI, confidence interval; C/T ratio, cholesterol/triglyceride ratio; Gln, glutamine; His, histidine; VLDL, very low-density lipoprotein

4.4. Factors Affecting NVR

When the study population, was divided into 30 patients with NVR and 78 patients with non-NVR (TVR plus SVR), a significant difference was found in the level of VLDL-C (28.8 ± 10.1 mg/dL vs 35.8 ± 10.3 mg/dL, $P = 0.012$), the C/T ratio of VLDL (0.82 ± 0.44 vs 0.99 ± 0.47; $P = 0.045$), distribution of IL28B genotype (major/minor, 4/23 vs. 65/9; $P < 0.001$), and substitution at aa70 (Arg70:Gln70/His70, 9:17 vs. 53:19; $P = 0.001$). However, the difference in LDL-C level was not significant (72.5 ± 21.4 mg/dL vs 80.5 ± 18.6 mg/dL; $P = 0.158$). After multivariate logistic analysis for extracting independent predictive variables in 98 patients who were determined to have both the genotype of IL28B and substitution at aa70, the minor IL28B genotype (OR 50; 95% CI 10-100) and Gln70/His70 in the core region of HCV (OR 6.25; 95% CI 1.52-25) were shown to be independent factors predicting NVR, but VLDL-C and the C/T ratio of VLDL were not (*Table 4*).

Table 4. Multivariate Logistic Regression Analysis of Factors Predicting Non-Virological Response in Patients with Hepatitis C Virus G1b (n = 98)

Variable	Odds Ratio	95% CI	*P*value
Gln70 or His70/Arg70	6.25	1.52-25	0.011
Minor/Major IL28B Genotypes	50	10-100	< 0.001
VLDL Cholesterol, mg/dL	0.97	0.88-1.06	0.487
C/T Ratio in VLDL	0.57	0.06-5.26	0.626

Abbreviations: CI, confidence interval; Arg, arginine; Gln, glutamine; His, histidine; VLDL, very low-density lipoprotein; C/T ratio, cholesterol/triglyceride ratio

5. Discussion

Disturbance of serum lipoprotein profiles in peripheral blood of patients with chronic HCV infection may play a critical role in cell entry of HCV and affect the efficacy of IFN-based antiviral therapy (22). In addition, the importance of lipid factors on treatment outcome of antiviral therapy may continue in the era of direct acting antivirals (DAA). DAA should be used in combination with IFN plus RBV because monotherapy with a newly developed DAA (protease inhibitor; boceprevir or telaprevir) causes rapid emergence of resistant HCV (23, 24). Therefore, factors affecting IFN plus RBV combination therapy such as lipid profiles may also influence the efficacy of triple therapy (DAA, IFN, and RBV). In the present study, we fractionated serum lipoprotein particles into chylromicrons, VLDL, LDL, and HDL using HPCL. This HPLC-based method required a small sample size and a relatively short time (about 25 minutes) for fractionation. Therefore, this HPLC-based method may be superior to other methods for detailed examination of lipoproteins in many patients. Furthermore, this HPLC system offers precise information on TG concentrations along with cholesterol levels in each fraction simultaneously (25). In a study on HCV G1, distortion of lipoprotein metabolism was more profound in patients who were non-favorable responders, and decreases of LDL-C, HDL-C, and TG were reported using conventional measurement technologies (26). However, after the discovery of rs8099917 and rs12979860 near the human IL28B gene as a strong host factor affecting the response to peg-IFN plus RBV therapy (27, 28), the significance of lipid profiles on predicting SVR was thought to be doubtful because the power of the IL28B genotype for predicting the efficacy of peg-IFN plus RBV therapy was so strong (29, 30). In the present study, we found that the C/T ratio in fasting serum VLDL was independently associated with SVR along with the IL28B genotype in patients with chronic HCV G1b who were treated with peg-IFNα-2b plus RBV. However, significant differences were not observed in other lipids including LDL-C. Using a similar method, Mawatari et al. showed that the cholesterol levels in the 44.5-nm and 36.8-nm subfraction of VLDL, in addition to the 25.5-nm subfraction of LDL, were significantly higher in patients with SVR than in non-SVR who were infected with HCV G1b and treated with peg-IFN plus RBV (31). However, they examined a relatively small number of patients without information on IL28B genotype. In our study, we did not subdivide the VLDL and/or LDL fraction because the VLDL and/or LDL fraction was not clearly separated into subfractions by the HPLC method as shown in *Figure 1*. It is known that there are at least two subtypes of VLDL: large, TG-rich VLDL1 and small VLDL2 (32). Therefore, increases of the C/T ratio in VLDL may indicate a decrease in VLDL1 and/or an increase in VLDL2. Based on the report of Mawatari et al., increases in cholesterol in the 44.5-nm and 36.8-nm subfractions of VLDL were associated with a favorable

response to combination therapy (31). This finding may indicate an increase in the specific subtype of VLDL sized 36.8-44.5 nm. As shown in their report, TG levels of the 44.5-nm subfraction were concomitantly increased in patients with SVR. Because of the relative increase of cholesterol compared with TG, the C/T ratio of this subfraction was higher in patients with SVR than in patients with non-SVR. Thus, our finding of increased C/T ratio in VLDL does not conflict with the results of previous reports, and we assume that the increase of C/T ratio in VLDL fraction in patients with SVR was not merely due to increases or decreases in specific subtypes of VLDL but also to relative increases of cholesterol components in the VLDL particle having a similar diameter. Our finding that the difference of biochemical nature of VLDL may affect the response to peg-IFN plus RBV therapy is an independent phenomenon apart from the IL28B genotype. This issue has not been mentioned till now and we believe that our literature is the first report to refer to the significance of the biochemical nature of VLDL. The reason why the increase of C/T ratio in the VLDL fraction could be related to a favorable response to peg-IFN plus RBV is not fully understood. We assume that one of the reasons may be modulation of the immune function of dendritic cells by modified lipoproteins (33). In the present study, we also examined factors associated with NVR. However, no lipid factor was elucidated as an independent factor. In terms of predicting NVR, the IL28B genotype is an extraordinary strong factor, and aa substitution at 70 in the core region of HCV G1b is also a strong predictor. These factors may also affect serum lipid profiles, as described in our previous study (21). Therefore, lipid factors were not independent but thought to be confounding factors of the IL28B genotype and/or aa70 substitution for predicting NVR. In conclusion, the nature of VLDL expressed as C/T ratio is an independent factor in predicting a favorable response to peg-IFN plus RBV combination therapy in patients with chronic HCV genotype 1b infection.

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Authors' Contributions

Study concept and design: Aizawa, and Abe. Analysis and interpretation of data: Aizawa, Shimada, Tsubota, Seki, Ika, Kato and Ishiguro. Drafting of the manuscript: Aida. Critical revision of the manuscript for important intellectual content: Aizawa, Tsubota, Shimada, Abe, Seki, Aida, and Ishiguro. Statistical analysis: Aizawa, Shimada and Abe.

Financial Disclosure

Dr Aizawa reported receiving research grants and consulting fees for speaking from the MSD and Mitsubishi Tanabe Pharma Corporation, receiving research grants from Chugai Pharmaceutical Co. Ltd. Dr Abe reported receiving consulting fees for speaking from MSD. Dr Shimada reported receiving consulting fees for speaking from MSD and Mitsubishi Tanabe Pharma Corporation. Dr Tsubota reported receiving consulting fees for speaking from MSD. Multifaceted roles for lipids in viral infection.

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Serum Apolipoprotein B-100 Concentration Predicts the Virological Response to Pegylated Interferon Plus Ribavirin Combination Therapy in Patients Infected With Chronic Hepatitis C Virus Genotype 1b

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Host lipoprotein metabolism is associated closely with the life cycle of hepatitis C virus (HCV), and serum lipid profiles have been linked to the response to pegylated interferon (Peg-IFN) plus ribavirin (RBV) therapy. Polymorphisms in the human IL28B gene and amino acid substitutions in the core and interferon sensitivity-determining region (ISDR) in NS5A of HCV genotype 1b (G1b) were also shown to strongly affect the outcome of Peg-IFN plus RBV therapy. In this study, an observational cohort study was performed in 247 HCV G1b-infected patients to investigate whether the response to Peg-IFN and RBV combination therapy in these patients is independently associated with the level of lipid factors, especially apolipoprotein B-100 (apoB-100), an obligatory structural component of very low density lipoprotein and low density lipoprotein. The multivariate logistic analysis subsequently identified apoB-100 (odds ratio (OR), 1.602; 95% confidence interval (CI), 1.046–2.456), alpha-fetoprotein (OR, 0.764; 95% CI, 0.610–0.958), non-wild-type ISDR (OR, 5.617; 95% CI, 1.274–24.754), and the rs8099917 major genotype (OR, 34.188; 95% CI, 10.225–114.308) as independent factors affecting rapid initial virological response (decline in HCV RNA levels by $\geq$3-$\log_{10}$ at week 4). While lipid factors were not independent predictors of complete early or sustained virological response, the serum apoB-100 level was an independent factor for sustained virological response in patients carrying the rs8099917 hetero/minor genotype. Together, we conclude that serum apoB-100 concentrations could predict virological response to Peg-IFN plus RBV combination therapy in patients infected with HCV G1b, especially in those with the rs8099917 hetero/minor genotype. ***J. Med. Virol. 85:1180–1190, 2013.***

KEY WORDS: hepatitis C virus (HCV); lipid metabolism; pegylated interferon (Peg-IFN); ribavirin (RBV)

INTRODUCTION

The life cycle of hepatitis C virus (HCV) is closely associated with human lipoprotein metabolism [Popescu and Dubuisson, 2009; Syed et al., 2010; Targett-Adams et al., 2010]. In particular, assembly of HCV particles is closely related to the formation of lipid droplets in hepatic cells, which may serve as an assembly platform [Targett-Adams et al., 2010; Alvisi et al., 2011]. In addition, the production and secretion of HCV particles is linked closely to the generation of very low density lipoprotein (VLDL), as well as the

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secretion pathway [Huang et al., 2007; Gastaminza et al., 2008].

During chronic HCV infection, some HCV particles circulate in the blood and form lipo-viral particles (LVP) that are rich in triglycerides (TG), apolipoprotein B-100 (apoB-100), and apolipoprotein E (apoE). These LVP particles are similar physiochemically to VLDL particles and are highly infectious [Nielsen et al., 2006; Sabahi et al., 2010]. Studies have suggested that Claudin-1 and CD81, both of which directly bind to HCV E2 protein, may participate in the later steps of HCV entry into hepatocytes [Meredith et al., 2012], whereas low density lipoprotein (LDL) receptors (the ligand, apoB-100) or remnant receptors (the ligand, apoE) may participate in binding to LVPs [Maillard et al., 2011]. ApoB-100 is an indicator of the total number of VLDL, intermediate-density lipoprotein (IDL), and LDL particles in the blood, because each of these particles contains an apoB-100 molecule as an obligatory structural component. Therefore, apoB-100 has been considered as a good marker of host lipid metabolism.

HCV infects up to 180 million people worldwide and is a cause of chronic liver diseases, cirrhosis, and hepatocellular carcinoma [Shepard et al., 2005; Williams, 2006]. The combination therapy of pegylated interferon (Peg-IFN) and ribavirin (RBV) has been found to lead to a sustained virological response in 40–50% of patients infected with HCV genotype 1b (G1b) [Fried et al., 2002]. Although Peg-IFN plus RBV combination has been a useful treatment, cost, adverse events, and a relatively low sustained virological response rate are still problems that remain to be solved. To select patients who could attain a virological cure from HCV with the use of Peg-IFN and RBV, it is necessary to predict the response before therapy [Backus et al., 2007]. In place of Peg-IFN and RBV combination therapy, triple therapy with the addition of telaprevir (a protease inhibitor) may become a standard of care for chronic HCV G1b infection [Lin et al., 2006]. However, even with such triple therapy, the efficacy is still strongly dependent on the response to Peg-IFN and RBV [Welsch et al., 2012].

Factors that may be used to predict a poor response to Peg-IFN and RBV combination therapy include older age, advanced stage of fibrosis, HCV genotype 1, high viral load [Kurosaki et al., 2011], high body mass index (BMI), increased homeostasis model assessment of insulin resistance (HOMA-IR) score [Dai et al., 2009], and serum lipid profiles [Akuta et al., 2007; Economou et al., 2008; Sheridan et al., 2009]. In addition, host genetic variation near the IL28B gene (single nucleotide polymorphism (SNP) rs8099917 or rs12979860) on chromosome 19 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009], amino acid (aa) substitution at position 70 and/or 91 in the HCV core [Akuta et al., 2007], as well as the number of aa substitutions in the interferon sensitivity-determining region (ISDR) located in HCV G1b NS5A [Enomoto et al., 1996], are also strongly associated with the response to chronic hepatitis C treatment.

The aim of this study is to investigate whether host serum lipid profiles, especially serum apoB-100 levels, can serve as predictors of the response to Peg-IFN alpha 2b and RBV combination therapy in patients infected chronically with HCV G1b.

PATIENTS AND METHODS

Study Subjects

Japanese patients with chronic HCV G1b infection were recruited for an observational cohort study designed to evaluate basal factors, including host lipid factors that influence the response to antiviral therapy. A total of 308 consecutive patients infected with HCV G1b were recruited and treated at Jikei University Katsushika Medical Center and Shinmatsudo Central General Hospital between April 2008 and April 2011. Among these patients, 247 of them were selected based on the following criteria: (1) the absence of decompensated cirrhosis or hepatocellular carcinoma; (2) no previous IFN-based treatment during the 6 months before enrollment; (3) more than 48 weeks of previous treatment, excepted for the early stopping due to non-virological response; (4) >80% of total Peg-IFN and RBV adherence during the first 12 weeks; and (5) completion of 24-week observation for evaluating sustained virological response after cessation of treatment. Patients with the following conditions were excluded: (1) hepatitis B or human immunodeficiency virus infection, (2) alcohol consumption, (3) any other causes of significant liver diseases, (4) history of familial hyperlipidemia, (5) drug abuse, and (6) use of medications that can modify lipid levels. Patients who stopped Peg-IFN and RBV treatment due to adverse events arising from the therapy (n = 30) and patients with total Peg-IFN and RBV adherence during the first 12 weeks less than 80% (n = 31) were also excluded. Written informed consent was obtained from each patient. The study protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the Review Board of Jikei University School of Medicine and Shinmatsudo Central General Hospital.

Laboratory Tests

Basal factors, except SNP rs8099917, were evaluated just before treatment. For patients who started treatment between April 2008 and March 2010, SNP rs8099917 was determined after April 2010, whereas for those who started after April 2010, SNP rs8099917 was determined before treatment. Serum aspartate: 2-oxoglutarate aminotransferase (AST), alanine: 2-oxoglutarate aminotransferase (ALT), albumin, alpha fetoprotein (AFP), gamma-glutamyl transferase (GGT), white blood cell (WBC) and platelet count, and hemoglobin were measured by standard

laboratory procedures. Insulin resistance (HOMA-IR) was investigated using the standard formula: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mg/dl)/405. HCV RNA concentrations in plasma were determined using the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland).

Detection of Serum Lipids and apoB-100

Fasting serum apoB-100 concentrations were determined as follows. Briefly, ApoB was measured by immunonephelometry using an apoB detection kit (Sekisui Medical, Ibaraki, Japan), and ApoB-48 was measured using a chemiluminescence enzyme immunoassay [Kinoshita et al., 2005] with an apoB-48 CLEIA kit (Fujirebio, Tokyo, Japan). Serum apoB-100 was then calculated as apoB minus apoB-48. Fasting total cholesterol (TC) and TG were determined using standard laboratory tests. Serum LDL cholesterol (LDL-C) was measured using a direct assay with the LDL-Cholesterol Kit (Sekisui Medical, Tokyo, Japan), the results of which show an extremely high correlation with those measured indirectly with the Friedewald equation [Friedewald et al., 1972] in healthy people whose serum TG levels were lower than 400 mg/dl (unpublished data).

Genotyping of rs8099917

Genomic DNA was extracted from whole blood using a DNA isolation kit on a MagNA Pure LC instrument (Roche Diagnostics, Basel, Switzerland). SNP rs8099917 was subsequently determined by real-time polymerase chain reaction using the TaqMan SNP genotyping assay on the 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA). Results were confirmed by performing experiments in duplicate, and the rs8099917 genotype was classified as either the major genotype (T/T) or the hetero/minor genotype (T/G or G/G).

Detection of Amino Acid Substitutions in the Core and NS5A-ISDR Regions of HCV G1b

RNA was extracted from patient sera, and nucleotide sequences of the core and NS5A-ISDR regions of HCV were analyzed by nested PCR and then direct sequencing, according to the methods described by Enomoto et al. [1996] and Akuta et al. [2007]. In the core region, aa substitutions at residue 70 (arginine, Arg70; or glutamine/histidine, Gln70/His70) and residue 91 (leucine, Leu91; or methionine, Met91) were determined. The NS5A region containing aa 2209–2248 (ISDR) was also sequenced and classified as either wild type or non-wild type based on the number of aa substitutions observed (wild type, 0 or 1; non-wild type, ≥2).

Liver Histology

Of 247 patients, 231 agreed to a pretreatment liver biopsy. The Metavir classification was used to evaluate the degree of histological activity (grades A0, A1, A2, and A3) and fibrosis (Stages F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, septal fibrosis; and F4, cirrhosis).

Pegylated Interferon Plus Ribavirin Combination Therapy

The therapy protocol combined Peg-IFNα2b (1.5 μg/kg/week) and RBV (600–1200 mg/day). Dose modifications of both drugs were based on the patient's body weight, as well as the manufacturer's recommendations. The standard duration of this combination therapy was set at 48 weeks. For patients whose HCV RNA did not disappear after 12 weeks but did disappear by 24 weeks, an extended therapy duration of up to 72 weeks was recommended. In addition, discontinuation of treatment was recommended before 48 weeks in patients with less than 2-$\log_{10}$ drop in HCV RNA after 12 weeks or patients with detectable HCV RNA after 24 weeks, because the likelihood of these patients achieving elimination of HCV RNA was very low, even if combination therapy was continued [Ghany et al., 2009]. A reduction in the dose of Peg-IFN/RBV may lead to a reduced possibility of a virological cure; therefore, only patients with >80% compliance during the first 12 weeks were included in this study.

Definitions of Response to Therapy

A sustained virological response was defined as undetectable serum HCV RNA at 24 weeks after the therapy; in contrast, a non-viral response was defined as detectable serum HCV RNA throughout the therapy. Less than 2-$\log_{10}$ drops in HCV RNA after 12 weeks of therapy or detectable HCV RNA at 24 weeks with the prematurely stopping therapy was also classified as non-viral response. Other virological responses examined in the present study included rapid initial virological response (decline in serum HCV RNA levels by at least 3-$\log_{10}$ 4 weeks after starting therapy) and complete early virological response (undetectable HCV RNA at week 12 of therapy). All patients were assessed for achievement of rapid initial virological response, complete early virological response, and sustained virological response.

Statistical Analysis

Baseline data were expressed as mean ± SD. Virological response was evaluated using a per protocol analysis. Differences between two groups were evaluated using the Mann–Whitney *U*-test or chi-square test. All *P* values less than 0.05 with a 2-tailed test were considered significant. To identify independent predictive factors by stepwise selection, variables that achieved statistical significance ($P < 0.05$) were subjected to the multiple logistic regression analysis. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated.

Potential pretreatment factors associated with virological response included the following variables: sex, age, body mass index (BMI), AST, ALT, albumin, GGT, WBC count, hemoglobin, platelet count, HCV RNA level, AFP, TC, TG, LDL-C, apoB-100, HOMA-IR, histological activity and fibrosis, SNP of rs8099917, aa substitutions at residues 70 and 91 in the core region, and the type of NS5A-ISDR. All statistical analyses were performed using STATISTICA 06J (StatSoft Japan, Tokyo, Japan).

RESULTS

Characteristics of Patients and their Virological Response to Peg-IFN Plus RBV Combination Therapy

The basic clinical and laboratory data, as well as the mean administered dose of Peg-IFN and RBV in 247 patients, are summarized in Table I. Females were enrolled more frequently than males in this study, and the mean age at enrollment was 59.3 years old (range, 20–80). The number of obese patients was relatively small (mean BMI and HOMA-IR, 23.6 and 2.7, respectively). HCV core Arg70 was found in 147 patients, whereas Gln70/His70 was seen in 93 patients. Wild-type ISDR was identified in 26 patients, with non-wild-type in 215 patients. The rs8099917 major genotype was found in 156 patients, and the hetero/minor genotype was seen in 79 patients. The doses of Peg-IFN and RBV administered were 1.53 ± 0.47 μg/kg/week and 11.4 ± 1.9 mg/kg/day, respectively.

TABLE I. Patient Profile and Laboratory Data at Commencement of Therapy

Factor	Mean ± SD or n
N	247
Age (years)	59.3 ± 11.5
Sex (male/female)	111/136
BMI (kg/m^2)	23.6 ± 3.6
History of IFN treatment (treatment-naive/experienced)	180/67
White blood cells (×10^2/mm^3)	51.8 ± 16.1
Hemoglobin (g/dl)	13.6 ± 1.5
Platelets (×10^4/mm^3)	17.3 ± 6.1
AST (IU/L)	57.1 ± 40.8
ALT (IU/L)	66.5 ± 54.8
GGT (IU/L)	59.3 ± 65.8
Albumin (g/dl)	4.0 ± 0.4
Total cholesterol (mg/dl)	169.4 ± 33.0
LDL cholesterol (mg/dl)	96.4 ± 26.5
Triglyceride (mg/dl)	94.5 ± 43.5
Apolipoprotein B-100 (mg/dl)	73.6 ± 19.4
HOMA-IR	2.7 ± 3.1
HCV RNA (log IU/ml)	6.2 ± 0.8
AFP (ng/ml)	13.0 ± 27.6
Histology	
Fibrosis: 1/2/3/4/ND	84/56/38/53/16
Activity: 1/2/3/ND	114/105/12/16
Amino acid substitutions	
Core aa 70 (arginine/glutamine or histidine/ND)	147/93/7
Core aa 91 (leucine/methionine/ND)	147/93/7
ISDR in NS5A (wild-type/non-wild-type/ND)	26/215/6
rs8099917 genotype (TT/TG/GG/ND)	156/74/5/12
Peg-IFN dose (μg/kg/week)	1.53 ± 0.47
Ribavirin dose (mg/kg/day)	11.4 ± 1.9

SD, standard deviation; BMI, body mass index; IFN, interferon; AST, aspartate: 2-oxoglutarate aminotransferase; ALT, alanine: 2-oxoglutarate aminotransferase; GGT, gamma-glutamyl transferase; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HCV, hepatitis C virus; AFP, alpha fetoprotein; ISDR, interferon sensitivity-determining region; ND, not determined.

Among these 247 patients, rapid initial virological response and complete early virological response were attained in 117 (47.4%) and 95 (38.5%) patients, respectively (Tables II and III), and 170 (68.8%) patients had undetectable HCV RNA at the end of therapy. While sustained virological response was achieved in 123 (49.8%) patients, 47 (19.0%) patients relapsed and 77 (31.2%) patients showed non-viral response (Table IV). Moreover, 92 out of 117 (78.6%) patients with rapid initial virological response and 84 out of 95 (88.4%) patients with complete early virological response achieved sustained virological response. Of the 75 patients who did not attain complete early virological response but showed the elimination of serum HCV RNA at 24 weeks of therapy, 39 (52.0%) patients achieved sustained virological response. Among these 75 patients, 62 underwent 72 weeks of therapy, and 38 (61.3%) of which achieved sustained virological response. Of the 13 patients who refused the recommendation for 72-week therapy and just underwent 48 weeks, only one patient (7.7%) attained sustained virological response. The progression of patients in this study is shown in Figure 1.

Univariate and Multivariate Analyses of Factors Affecting Rapid Initial Virological Response

As indicated by the univariate analysis results shown in Table II, the degree of fibrosis, GGT, albumin, TC, LDL-C, apoB-100, AFP, hemoglobin, platelets, ISDR of NS5A, aa substitution at residue 70 in the HCV core, and rs8099917 genotype were factors significantly associated with rapid initial virological response. After further multivariate logistic regression analysis using the stepwise selection method, serum apoB-100, as well as ISDR of NS5A, AFP, and rs8099917 genotype, was identified as significant independent factors; however, TC, LDL-C, histological fibrosis, and serum albumin levels were not.

Factors Associated With Complete Early Virological Response and Sustained Virological Response

Based on the univariate analysis results shown in Table III, age, sex, ALT, albumin, TC, LDL-C, apoB-100, AFP, hemoglobin, platelets, HCV RNA, ISDR of NS5A, aa substitution at residue 70 in the HCV core, and rs8099917 genotype were factors significantly associated with complete early virological response.

TABLE II. Factors Aassociated With Rapid Initial Virological Response (RIVR) to Pegylated Interferon Plus Ribavirin Therapy

Factor	RIVR (n = 117)	Non-RIVR (n = 130)	*P* value
(a) Univariate analysis			
Age (years)	58.8 ± 11.3	60.5 ± 11.4	0.15
Male (%)	55/117 (47)	56/130 (43)	0.54
Histological fibrosis: 0–2/3–4	76/32	64/59	0.004
Histological activity: 0–1/2–3	53/55	61/62	0.94
BMI (kg/m^2)	23.8 ± 3.6	23.5 ± 3.6	0.44
AST (IU/L)	54.8 ± 35.7	57.3 ± 34.9	0.13
ALT (IU/L)	69.9 ± 60.4	59.9 ± 39.1	0.82
GGT (IU/L)	53.7 ± 66.9	62.3 ± 62.6	0.002
Albumin (g/dl)	4.1 ± 0.3	3.9 ± 0.4	<0.0001
Total cholesterol (mg/dl)	181.7 ± 33.1	158.3 ± 30.8	<0.0001
Triglyceride (mg/dl)	96.1 ± 46.3	93.1 ± 41.4	0.94
LDL cholesterol (mg/dl)	108.8 ± 26.7	85.8 ± 22.4	<0.0001
Apolipoprotein B-100 (mg/dl)	83.5 ± 18.6	65.2 ± 15.7	<0.0001
HOMA-IR	2.4 ± 2.8	3.0 ± 3.4	0.15
AFP (ng/ml)	6.9 ± 8.0	18.0 ± 32.5	<0.0001
White blood cells ($\times 10^2/mm^3$)	53.2 ± 15.9	50.2 ± 16.3	0.08
Hemoglobin (g/dl)	13.9 ± 1.6	13.3 ± 1.5	0.006
Platelets ($\times 10^4/mm^3$)	18.3 ± 6.2	16.3 ± 5.9	0.003
HCV RNA (log IU/ml)	6.2 ± 0.9	6.2 ± 0.7	0.4
Peg-IFN dose (μg/kg/week)	1.6 ± 0.7	1.5 ± 0.2	0.12
Ribavirin dose (mg/kg/day)	11.5 ± 1.7	11.2 ± 2.1	0.25
ISDR in NS5A wild-type (%)	94/114 (82)	121/127 (95)	0.002
Substitution at aa 70 Arg (%)	84/112 (75)	63/128 (49)	<0.0001
Substitution at aa 91 Leu (%)	71/112 (63)	76/128 (59)	0.52
rs8099917 TT genotype (%)	107/112 (96)	49/123 (40)	<0.0001

Factor	Category	Odds ratio (95% CI)	*P* value
(b) Multivariate analysis			
Apolipoprotein B-100	By 10 (mg/dl)	1.602 (1.046–2.456)	0.03
ISDR of NS5A	1: wild type	1	0.02
	2: non-wild type	5.617 (1.274–24.754)	0.02
AFP	By 5 (ng/ml)	0.764 (0.610–0.958)	
rs8099917 genotype	1: TG or GG	1	<0.0001
	2: TT	34.188 (10.225–114.308)	

Peg-IFN, pegylated interferon; CI, confidence interval; BMI, body mass index; AST, aspartate: 2-oxoglutarate aminotransferase; ALT, alanine: 2-oxoglutarate aminotransferase; GGT, gamma-glutamyl transferase; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; AFP, alpha fetoprotein; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

Among them, substitution at Arg70, ALT, sex, age, HCV RNA, and rs8099917 genotype, but not lipid factors, were characterized as significant independent factors by further multivariate analysis. On the other hand, as shown in Table IV, age, albumin, TC, LDL-C, apoB-100, AFP, hemoglobin, platelets, ISDR of NS5A, substitution at Arg70 in the HCV core, and rs8099917 genotype were factors significantly associated with sustained virological response. The multivariate logistic regression analysis of the variables described above identified the following four parameters that independently influenced the sustained virological response: hemoglobin, platelets, ISDR of NS5A, and rs8099917 genotype. In contrast, lipid factors were not independent factors associated with sustained virological response.

Factors Associated With Sustained Virological Response in Patients With the rs8099917 Hetero/minor Genotype

Of the 79 patients identified to have the hetero/minor genotype of rs8099917, only 7 (8.9%) achieved both complete early virological response and sustained virological response. Of the 72 patients who did not achieve complete early virological response, 51 (70.8%) showed non-viral response, and the remaining 21 underwent 72 weeks of therapy. Nine out of these 21 (42.9%) patients later achieved sustained virological response. Therefore, in total, only 16 out of 79 (20.3%) patients achieved sustained virological response.

As illustrated in Table V, age, albumin, apoB-100, AFP, platelet counts, and HCV RNA levels were factors significantly associated with sustained virological response in patients with the rs8099917 hetero/minor genotype, whereas lipid factors other than apoB-100 were not. Further multivariate logistic regression analysis of the variables described above identified apoB-100 and HCV RNA load as two parameters that influenced independently a sustained virological response.

DISCUSSION

Lipid factors have been considered as candidates for predicting the outcome of Peg-IFN plus RBV

TABLE III. Factors Associated With Complete Early Virological Response (cEVR) to Pegylated Interferon Plus Ribavirin Therapy

Factor	cEVR (n = 95)	Non-cEVR (n = 152)	*P* value
(a) Univariate analysis			
Age (years)	56.6 ± 12.3	61.6 ± 10.3	0.002
Male (%)	55/95 (58)	56/152 (37)	<0.0001
Histological fibrosis: 0–2/3–4	57/32	83/59	0.40
Histological activity: 0–1/2–3	39/50	75/67	0.18
BMI (kg/m^2)	24.0 ± 3.4	23.4 ± 3.7	0.08
AST (IU/L)	58.8 ± 37.5	54.4 ± 33.8	0.62
ALT (IU/L)	79.6 ± 64.4	55.3 ± 36.6	0.002
GGT (IU/L)	65.8 ± 74.1	53.6 ± 57.8	0.09
Albumin (g/dl)	4.1 ± 0.3	3.9 ± 0.4	<0.0001
Total cholesterol (mg/dl)	177.7 ± 33.4	164.2 ± 33.3	0.003
Triglyceride (mg/dl)	98.1 ± 49.3	92.2 ± 39.9	0.41
LDL cholesterol (mg/dl)	105.3 ± 25.7	91.3 ± 26.6	<0.0001
Apolipoprotein B-100 (mg/dl)	81.1 ± 19.1	69.4 ± 18.2	<0.0001
HOMA-IR	2.7 ± 3.3	2.7 ± 3.0	0.94
AFP (ng/ml)	7.5 ± 7.4	15.9 ± 30.6	0.02
White blood cells (×10^2/mm^3)	53.7 ± 15.7	50.3 ± 16.4	0.07
hemoglobin (g/dl)	14.0 ± 1.5	13.3 ± 1.5	0.0002
Platelets (×10^4/mm^3)	18.6 ± 6.3	16.3 ± 5.8	0.006
HCV RNA (log IU/ml)	6.0 ± 0.9	6.3 ± 0.7	0.02
Peg-IFN dose (μg/kg/week)	1.6 ± 0.7	1.5 ± 0.2	0.62
Ribavirin dose (mg/kg/day)	11.4 ± 1.7	11.3 ± 2.0	0.70
ISDR in NS5A wild-type (%)	18/91 (20)	8/150 (5)	0.0004
Substitution at aa 70 Arg (%)	70/90 (78)	77/150 (51)	<0.0001
Substitution at aa 91 Leu (%)	59/90 (66)	88/150 (59)	0.29
rs8099917 TT genotype (%)	82/89 (92)	74/146 (51)	<0.0001

Factor	Category	Odds ratio (95% CI)	*P* value
(b) Multivariate analysis			
Substitution at aa 70	1: Gln70/His70	1	0.04
	2: Arg70	2.358 (1.023–5.432)	
ALT	By 10 (IU/L)	1.149 (1.011–1.305)	0.03
Sex	1: female	1	0.027
	2: male	3.124 (1.143–8.538)	
Age	By 10 years	0.618 (0.413–0.924)	0.019
HCV RNA	By 1 (log IU/ml)	0.301 (0.161–0.565)	0.0002
rs8099917 genotype	1: TG or GG	1	<0.0001
	2: TT	17.743 (5.331–59.050)	

Peg-IFN, pegylated interferon; CI, confidence interval; BMI, body mass index; AST, aspartate: 2-oxoglutarate aminotransferase; ALT, alanine: 2-oxoglutarate aminotransferase; GGT, gamma-glutamyl transferase; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; AFP, alpha fetoprotein; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

combination therapy, especially in patients with chronic HCV genotype 1 infection [Akuta et al., 2007; Economou et al., 2008; Sheridan et al., 2009]. However, after the discovery of host genetic factors (SNPs near the IL28B gene) as an extraordinarily potent predictor of the response to Peg-IFN plus RBV combination therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009] the significance of lipid factors has been questioned [Li et al., 2010]. In this observational cohort study, we confirmed the significance of SNP rs8099917 near the IL28B gene in sustained virological response in chronic HCV G1b-infected patients treated with Peg-IFN plus RBV combination therapy. Recent studies have also reported an extraordinarily close correlation between the rs8099917 genotype and SNPs rs12979860 and/or rs12980275 in Japanese patients, because of very strong linkage disequilibrium [Ochi et al., 2011; Kobayashi et al., 2012]; therefore, SNP rs8099917 was used here to represent the IL28B genotype.

In the present study, the factors affecting rapid initial virological response were studied, which is thought to be one of the most significant predictors of sustained virological response during Peg-IFN plus RBV combination therapy [Poordad et al., 2008; Ghany et al., 2009]. In addition, Marcellin et al. [2012] also reported that patients with over 3-$\log_{10}$ drops in HCV RNA at week 4 (rapid initial virological response) had a high probability of achieving sustained virological response. Consistently, in this cohort study we found that 78.6% of patients who attained rapid initial virological response achieved sustained virological response. Subsequently, high serum apoB-100 levels, low AFP levels, and mutant ISDR, along with the major genotype of rs8099917, were identified as basic independent pretreatment factors. Although many other factors also displayed significant differences between patients who achieved rapid initial virological response and those who did not, these factors

TABLE IV. Factors Associated With Sustained Virological Response (SVR) to Pegylated Interferon Plus Ribavirin Therapy

Factor	SVR (n = 123)	Non-SVR (n = 124)	*P* value
(a) Univariate analysis			
Age (years)	58.0 ± 12.3	61.4 ± 10.2	0.04
Male (%)	62/123 (50)	49/124 (40)	0.09
Histological fibrosis: 0–2/3–4	77/39	63/52	0.07
Histological activity: 0–1/2–3	59/57	55/60	0.64
BMI (kg/m^2)	23.8 ± 3.8	23.5 ± 3.5	0.83
AST (IU/L)	55.3 ± 35.5	56.9 ± 35.1	0.46
ALT (IU/L)	70.9 ± 57.5	58.4 ± 41.7	0.07
GGT (IU/L)	55.5 ± 65.3	61.0 ± 64.2	0.06
Albumin (g/dl)	4.1 ± 0.3	3.9 ± 0.4	0.0002
Total cholesterol (mg/dl)	175.2 ± 32.9	163.6 ± 34.0	0.03
Triglyceride (mg/dl)	93.3 ± 46.3	95.7 ± 41.2	0.30
LDL cholesterol (mg/dl)	104.2 ± 25.9	89.3 ± 26.2	<0.0001
Apolipoprotein B-100 (mg/dl)	79.4 ± 19.6	68.5 ± 17.7	0.001
HOMA-IR	2.7 ± 3.1	2.7 ± 3.2	0.83
AFP (ng/ml)	7.3 ± 7.1	18.0 ± 33.4	0.0003
White blood cells ($\times 10^2/mm^3$)	53.4 ± 15.7	49.8 ± 16.4	0.08
Hemoglobin (g/dl)	13.8 ± 1.5	13.3 ± 1.5	0.02
Platelets ($\times 10^4/mm^3$)	18.8 ± 6.0	15.6 ± 5.7	0.0002
HCV RNA (log IU/ml)	6.1 ± 0.9	6.3 ± 0.7	0.28
Peg-IFN dose (μg/kg/week)	1.6 ± 0.7	1.5 ± 0.2	0.06
Ribavirin dose (mg/kg/day)	11.4 ± 1.9	11.2 ± 2.0	0.43
ISDR in NS5A wild-type (%)	20/118 (17)	6/123 (5)	0.002
Substitution at aa 70 Arg (%)	80/117 (68)	67/123 (54)	0.03
Substitution at aa 91 Leu (%)	73/117 (62)	74/123 (60)	0.72
rs8099917 TT genotype (%)	100/116 (86)	56/119 (47)	<0.0001

Factor	Category	Odds ratio (95% CI)	*P* value
(b) Multivariate analysis			
Hemoglobin	By 1 (g/dl)	1.304 (1.014–1.679)	0.04
Platelets	By $5 \times 10^4/mm^3$	1.436 (1.014–2.033)	0.04
ISDR in NS5A	1: wild type	1	0.03
	2: non-wild type	3.637 (1.111–11.902)	
rs8099917 genotype	1: TG or GG	1	<0.0001
	2: TT	10.363 (4.269–10.363)	

Peg-IFN, pegylated interferon; CI, confidence interval; BMI, body mass index; AST, aspartate: 2-oxoglutarate aminotransferase; ALT, alanine: 2-oxoglutarate aminotransferase; GGT, gamma-glutamyl transferase; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; AFP, alpha fetoprotein; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

were not identified as independent factors by the multivariate logistic model.

In the present study, it was observed that fasting serum levels of lipid factors, including TC, LDL-C, and apoB-100, were significantly different between patients who attained a rapid initial virological response and those who did not. While many lipid factors are thought to be significantly associated with virological response to Peg-IFN plus RBV therapy in chronic HCV genotype 1-infected patients [Akuta et al., 2007; Economou et al., 2008; Sheridan et al., 2009], only the serum apoB-100 level was identified as an independent lipid factor in this cohort. Compared to apoB, which was reported not to be associated with the response to Peg-IFN plus RBV therapy [Petit et al., 2010], measurement of serum apoB-100 in this study was distinctive. In addition, ApoB-48, an apoB-100 splice variant that is derived from the small intestine and is associated with chylomicrons, was excluded. Therefore, the apoB-100 levels represented the total number of serum VLDL, LDL, and IDL particles, since each of these particles contains one apoB-100 molecule [Young, 1990]. Because IDL was barely detectable in peripheral blood, serum apoB-100 levels essentially indicated the total number of LDL plus VLDL particles. In addition, since the dynamics of serum VLDL is rarely monitored by currently used routine laboratory tests, serum apoB-100 is considered as a unique marker for determining the number of VLDL/LDL particles and for partly reflecting the dynamics of VLDL. Moreover, although we observed correlations between apoB-100 and other lipid factors, specifically TC and LDL-C ($r^2 = 0.4867$ and 0.7006, respectively; Table VI), apoB-100 was not equal to LDL-C. Overall, apoB-100 may be more discriminating than other lipid factors as a unique factor representing the dynamics of serum VLD/LDL particles.

The precise mechanisms of how the serum apoB-100 level independently affects rapid initial virological response in chronic HCV G1b-infected patients still remain to be determined. However, such a finding suggests that the number of serum LDL and/or VLDL particles may affect the HCV life cycle, because the secretion of HCV particles is associated closely with the secretion of VLDL, and that the

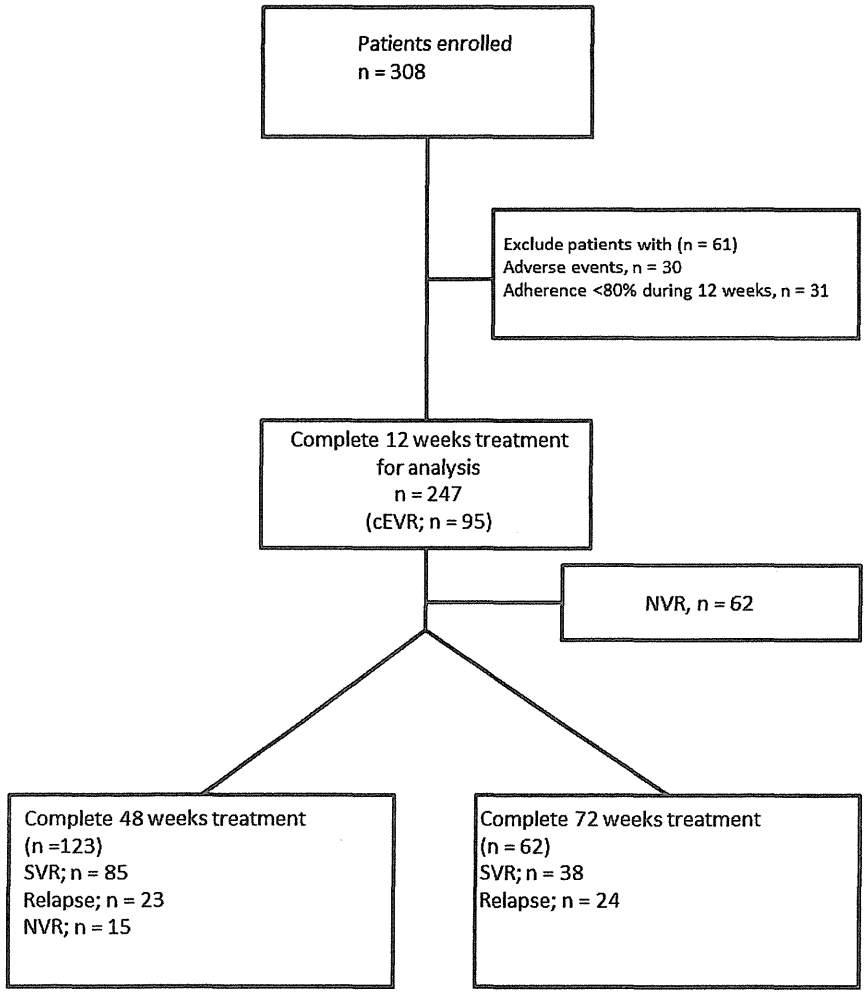

Fig. 1. Flow of patient enrolment. cEVR, complete early virological response; NVR, non-viral response; SVR, sustained virological response.

uptake of HCV by hepatocytes may also be affected by dynamics of serum VDLD/LDL particles [Huang et al., 2007; Gastaminza et al., 2008]. In other words, serum apoB-100 could influence the dynamics of HCV during the initial phase of antiviral treatment.

The results indicate that factors, including TC, LDL-C, and apoB-100, are associated with complete early and sustained virological responses. However, none of lipid factors were identified as independent predictors of these virological responses in this study. These findings suggest that serum lipid factors, including apoB-100, may not be directly related to the complete elimination of serum HCV or eradication of HCV, presumably because lipid factors do not affect directly on the abolishment of HCV RNA in hepatocytes.

Interestingly, Arg70 in the HCV core region, high ALT level, male sex, younger age, low HCV RNA titer, and the rs8099917 major type were identified as independent factors affecting complete early virological response, whereas high hemoglobin level, high platelet count, mutant ISDR, and the rs8099917 major type were identified as independent factors affecting sustained virological response. Our finding that hemoglobin, instead of well-known predictors such as age or HCV RNA load, was identified as a factor affecting sustained virological response may be partly due to the relatively high frequency of older female patients with slight anemia in this study. Studies have reported that older HCV G1b-infected female patients were difficult to cure [Sezaki et al., 2009; Chayama et al., 2010], presumably because of reduced drug dosage due to progressive anemia.

One of the most interesting findings in this study is that the serum apoB-100 level is an independent factor affecting sustained virological response in patients with the IL28B hetero/minor genotype. It is still debatable as to how to care for IL28B-hetero/minor patients who are infected chronically with HCV genotype 1. Even with recently approved triple