Amino Acid Substitution in HCV Core Region and Genetic Variation near IL28B Gene Predict Viral Response to Telaprevir with Peginterferon and Ribavirin

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Genetic variation near IL28B gene and substitution of amino acid (aa) 70 and 91 in the core region of HCV genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the predictive factors of sustained virological response to 12- or 24-week regimen of triple therapy in 72 of 81 Japanese adults infected with HCV genotype 1. Overall, sustained virological response and end-of-treatment response were achieved by 61% and 89%, respectively. Especially, sustained virological response was achieved by 45% and 67% in 12- and 24-week regimen, respectively. Multivariate analysis identified rs8099917 near IL28B gene (genotype TT) and substitution at aa 70 (Arg70) as significant determinants of sustained virological response. Prediction of response to therapy based on combination of these factors had high sensitivity, specificity, positive and negative predictive values. Efficacy of triple therapy was high in the patients with genotype TT who accomplished sustained virological response (84%), irrespective of substitution of core aa 70. In the patients having genotype non-TT, those of Arg70 gained high sustained virological response (50%), and sustained virological response (12%) were the worst in patients who possessed both of genotype non-TT and Gln70(His70). In conclusions, this study identified genetic variation near IL28B gene and aa substitution of the core region as predictors of sustained virological response to triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV genotype 1b.

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Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).[1,2] At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 KIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.[3] Such background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads.[4,5] Recently, a new strategy was introduced in the treatment of chronic HCV infection by means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection.[6] Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity.[7,8] Specifically, HCV RNA is suppressed below the limits of detection in the blood, in almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin.[9] However, treatment resistant patients who do not achieve sustained virological response by the triple therapy, have been reported.[9-11] The underlying mechanism of the response to the treatment is still not clear.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy,[12-14] and also affect

clinical outcome, including hepatocarcinogenesis.[15,16] Furthermore, recent report showed that aa substitutions in the core region can be also used before therapy to predict very early dynamics (within 48 hours) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin.[17] However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict sustained virological response to triple therapy.

Recent reports showed that genetic variations near IL28B gene (rs8099917, rs12979860) on chromosome 19 as host-related factor, which encodes IFN-λ-3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,[18-21] and also affect clinical outcome, including spontaneous clearance of HCV.[22] However, it is not clear at this stage whether genetic variation near IL28B gene can be used before therapy to predict sustained virological response to triple therapy.

The present study included 81 patients with HCV-1b and high viral loads, who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict sustained virological response, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near IL28B gene).

Patients and Methods

Study Population. Between May 2008 and September 2009, 81 patients infected with HCV were recruited to this study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. 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 an informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN and ribavirin] (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

All of 81 patients met the following inclusion and exclusion criteria: 1) Diagnosis of chronic hepatitis C. 2) HCV-1 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). 4) Japanese (Mongoloid) ethnicity. 5) Age at study entry of 20-65 years. 6) Body weight ≥35 kg and ≤120 kg at the time of registration. 7) Lack of decompensated liver cirrhosis. 8) Negativity for hepatitis B surface antigen (HBsAg) in serum. 9) Negative history of HCC. 10) No previous treatment for malignancy. 11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. 12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension,

chronic renal dysfunction or creatinine clearance of ≤50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of ≥110 mg/dL, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. 13) Hemoglobin level of ≥12 g/dl, neutrophil count ≥1,500/mm^3, and platelet count of ≥100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. Furthermore, 72 of 81 patients were followed-up for at least 24 weeks after the completion of triple therapy. The treatment efficacy was evaluated by HCV-RNA negative at the end of treatment (end-of-treatment response) and 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 mg or 500 mg three times a day at an 8-h (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, NJ) was injected subcutaneously at a median dose 1.5 μg/kg (range: 1.3-2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200-600 mg twice a day after breakfast and dinner (daily dose: 600-1000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1500/mm^3, neutrophil count below 750/mm^3 or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these

counts decreased below 1000/mm^3, 500/mm^3 or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400 mg, from 800 to 600 mg and 1000 mg to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 81 patients at the commencement of treatment. They included 44 males and 37 females, aged 23 to 65 years (median, 55 years).

Measurement of HCV RNA. The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment 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 Amino Acid Substitutions in Core, and NS5A Regions of HCV-1b. In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and

MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by PCR using the following primers: (a) Nucleotide sequences of the core region: The first-round PCR was performed with CE1 (sense, 5'-GTC TGC GGA ACC GGT GAG TA-3', nucleotides: 134-153) and CE2 (antisense, 5'-GAC GTG GCG TCG TAT TGT CG-3', nucleotides: 1096-1115) primers, and the second-round PCR with CC9 (sense, 5'-ACT GCT AGC CGA GTA GTG TT-3', nucleotides: 234-253) and CE6 (antisense, 5'-GGA GCA GTC GTT CGT GAC AT-3', nucleotides: 934-953) primers. (b) Nucleotide sequences of NS5A-ISDR: The first-round PCR was performed with ISDR1 (sense, 5'-ATG CCC ATG CCA GGT TCC AG-3', nucleotides: 6662-6681) and ISDR2 (antisense, 5'-AGC TCC GCC AAG GCA GAA GA-3', nucleotides: 7350-7369) primers, and the second-round PCR with ISDR3 (sense, 5'-ACC GGA TGT GGC AGT GCT CA-3', nucleotides: 6824-6843) and ISDR4 (antisense, 5'-GTA ATC CGG GCG TGC CCA TA-3', nucleotides: 7189-7208) primers. ([a,b]; nested PCR.). All samples were initially denatured at 95ºC for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95ºC, annealing of primers for 30 sec at 55ºC, and extension for 1 min at 72ºC with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo).

With the use of HCV-J (accession no. D90208) as a reference,[23] the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 81 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91).[12] The sequence of 2209-2248 aa in the NS5A of HCV-1b (IFN-sensitivity determining region [ISDR]) reported by Enomoto and coworkers[24] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non wild-type (≥2).

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.[25,26]

In this study, genetic variations near IL28B gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome,[18-22] were investigated.

Statistical Analysis. Non-parametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to sustained virological response. The odds ratios (OR) and 95% confidence intervals (95%CI) were also calculated. All p values less than 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 univariate and multivariate analyses. The potential pretreatment factors associated with sustained virological response 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, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near IL28B gene, and amino acid substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of predictors of the response to therapy.

Results

Virological Response to Therapy. Sustained virological response was achieved by 44 of 72 (61.1%) patients. 64 of 72 (88.9%) patients were considered end-of-treatment response. According to treatment regimen, sustained virological response were achieved by 45.0% (9 of 20 patients) and 67.3% (35 of 52 patients), in the T12PR12 group and the T12PR24 group, respectively. Of 8 patients, who could not achieve end-of-treatment response, 6 (75.0%) patients resulted in re-elevation of viral loads regardless of HCV-RNA temporary negative, and the other 2 patients (25.0%) did not achieve HCV-RNA negative during treatment.

Especially, in the T12PR24 group, according to the past history of treatment, sustained virological response were achieved by 76.4% (13 of 17 patients), 86.4% (19 of 22 patients), and 23.1% (3 of 13 patients), in treatment-naive, relapsers to previous treatment, and non-responders to previous treatment, respectively.

Sustained Virological Response According to Amino Acid Substitutions in Core, and NS5A Regions. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions (74.4%) showed sustained virological response than that of patients who showed Gln70(His70) (41.4%) (Figure 1, P=0.007). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 (65.0%) and Met91 (56.3%) (Figure 1). Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between

wild-type (56.3%) and non wild-type (66.7%) (Figure 1). Thus, sustained virological response was influenced by the substitution of core aa 70.

Sustained Virological Response According to Genetic Variation near IL28B Gene. According to the genetic variation in rs8099917, sustained virological response was achieved by 83.8% (31 of 37 patients), 29.6% (8 of 27 patients), and 0% (0 of 2 patients), in patients with genotype TT, TG, and GG, respectively. Thus, a significantly higher proportion of patients with genotype TT (83.8%) showed sustained virological response than that of patients who showed genotype non-TT (27.6%) (Figure 2, P<0.001) (Table 2).

According to the genetic variation in rs12979860, sustained virological response was achieved by 83.8% (31 of 37 patients), 34.5% (10 of 29 patients), and 0% (0 of 2 patients), in patients with genotype CC, CT, and TT, respectively. Thus, a significantly higher proportion of patients with genotype CC (83.8%) showed sustained virological response than that of patients who showed genotype non-CC (32.3%) (Figure 2, P<0.001) (Table 2).

Predictive Factors Associated with Sustained Virological Response. Univariate analysis identified 3 parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 4.12, P=0.007), and genetic variation in rs8099917 (genotype TT; OR 13.6, P<0.001) and rs12979860 (genotype CC; OR 10.8, P<0.001). Two factors were identified by multivariate analysis as independent parameters that significantly influenced sustained virological response

[(rs8099917 genotype TT; OR 10.6, P<0.001) and (Arg70; OR 3.69, P=0.040)] (Table 3).

Assessment of Amino Acid Substitutions in Core Region and Genetic Variation near IL28B Gene as Predictors of Sustained Virological Response. The ability to predict sustained virological response by substitution of core aa 70 and rs8099917 genotype near IL28B gene was evaluated. The sustained virological response rates of patients with a combination of Arg70 or rs8099917 genotype TT were defined as PPV (prediction of sustained virological response). The non-sustained virological response rates of patients with a combination of Gln70(His70) or rs8099917 genotype non-TT were defined as NPV (prediction of non-sustained virological response).

In patients with rs8099917 genotype TT, the sensitivity, specificity, PPV, and NPV for sustained virological response were 79.5, 77.8, 83.8, and 72.4%, respectively. Thus, genotype TT has high sensitivity, specificity, and PPV for prediction of sustained virological response. In patients with Arg70, the sensitivity, specificity, PPV, and NPV were 76.9, 63.0, 75.0, and 65.4%, respectively. Thus, Arg70 has high sensitivity and PPV in predicting sustained virological response. Furthermore, when both predictors were used, the sensitivity, specificity, PPV, and NPV were 61.5, 85.2, 85.7, and 60.5%, respectively. When one or more of the two predictors were used, the sensitivity, specificity, PPV, and NPV were 94.9, 55.6, 75.5, and 88.2%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response (Table 4).

Predicting Sustained Virological Response by Amino Acid Substitutions in Core Region in Combination with Genetic Variation near IL28B Gene. Sustained virological response by core aa 70 in combination with rs8099917 genotype was shown in Figure 3. In patients with rs8099917 genotype TT, sustained virological response was not different between Arg70 (85.7%) and Gln70(His70) (77.8%). In contrast, in patients with rs8099917 genotype TG and GG, a significantly higher proportion of patients with Arg70 (50.0%) showed sustained virological response than that of patients with Gln70(His70) (11.8%) (P=0.038).

Based on a strong power of substitution of core aa 70 and rs8099917 genotype in predicting sustained virological response (Table 3), it was evaluated how they increase the predictive value when they were combined. The results are schematically depicted in Figures 3, respectively. Together they demonstrate three points: (1) efficacy of triple therapy was high in the patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70; (2) in the patients having genotype TG and GG, those of Arg70 gained high sustained virological response (50.0%); and (3) sustained virological response (11.8%) were the worst in patients who possessed both of genotype TG and GG, and Gln70(His70).

Discussion

Two previous studies (PROVE1 in USA, and PROVE2 in Europe) showed that the T12PR12 and T12PR24 group of telaprevir, PEG-IFN and ribavirin could achieve sustained virological response rates of 35-60% and 61-69%, respectively.[10,11] In the present Japanese study, sustained virological response rates were 45% and 67% in the T12PR12 and T12PR24 group, respectively, like two previous studies. There were the differences at the three points between the present study and two previous studies: (1) PEG-IFN in two previous studies was used at a fixed dose of PEG-IFNα-2a, but that of the present study was a body weight-adjusted dose of PEG-IFNα-2b; (2) Body mass index of our patients (median; 23 kg/m^2) was much lower than that of the participants of the previous study by McHutchison et al (median; >25 kg/m^2); and (3) The present study was performed based on the Japanese patients infected with HCV-1b, except for only one patient of HCV-1a. Especially, in PROVE-1, viral breakthrough rate was higher in HCV-1a subjects compared to HCV-1b, and one of the reasons might be due to the low genetic barrier to the emergence of the R155K variant in HCV-1a.[10,27] Further studies of larger number of patients matched for background, including genotype, race, body mass index, treatment regimen, and past history of IFN therapy, are required to investigate the rate of the sustained virological response by triple therapy.

IL28A, IL28B, and IL29 (IFN-λ-2, -3, and -1, respectively) are novel IFNs identified recently.[28,29] They are similar to type 1 IFNs in terms of biological activities and mechanism of action, in contrast to their differences in structure and genetics.[30] The

anti-viral effects of IFN-λ against hepatitis B virus and HCV have been already reported.[31] Furthermore, α and λ IFNs act synergistically against HCV.[32-34] Recent reports showed that genetic variation near IL28B gene (rs8099917, rs12979860) are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,[18-21] and also affect clinical outcome, including spontaneous clearance of HCV.[22] At AASLD 2009, Thompson et al reported that genetic variation near IL28B gene also affected the viral suppression in the first 2 to 4 weeks of PEG-IFN plus ribavirin, and this phenomenon probably explains much of the differences in treatment response rate.[35] The present study is the first to report that genetic variation near IL28B gene significantly also affect sustained virological response by triple therapy. This results should be interpreted with caution since the races other than Japanese populations were not included. Any generalization of the results should await confirmation by studies of patients of other races to explore the relationship between genetic variation near IL28B gene and the response to triple therapy.

The present study indicated that the use of the combination of aa substitution of the core region and genetic variation near IL28B gene had high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response. Efficacy of triple therapy was high in the patients with TT, irrespective of substitution of core aa 70. In the patients having non-TT, those of Arg70 gained high sustained virological response, and sustained virological response was the worst in patients who possessed both of non-TT, and Gln70(His70). Along with a high sustained virological response, combined PEG-IFN and ribavirin accompany severe side effects and entail high costs.

Hence, the patients who do not achieve sustained virological response need to be identified, as early as possible, in order to free them of unnecessary side effects and high costs. The present study is the first to report that the combination of aa substitution of the core region and genetic variation near IL28B gene is very useful as pretreatment predictors of sustained virological response by triple therapy, and further studies based on the larger number of patients are necessary to investigate the present results.

Another limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR),[36] were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation,[37] were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both *in vivo* and *in vitro* studies.[38,39] Thus, viral factors before and during triple therapy should be investigated in future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, triple therapy with telaprevir, PEG-IFN and ribavirin in Japanese patients infected with HCV-1 and high viral load achieved high sustained virological response rates. Furthermore, the aa substitution pattern of the core region and genetic variation near IL28B gene seem to affect treatment efficacy. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple 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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Figure legends

Figure 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) (P=0.007). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 and Met91. Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wild-type and non wild-type.

Figure 2. According to the genetic variation in rs8099917 or rs12979860 near IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively (P<0.001 or P<0.001, respectively).

Figure 3. Predicting sustained virological response by aa substitution in core region in combination with genetic variation near IL28B gene. Efficacy of triple therapy was high in the patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70. In the patients having genotype TG and GG, those of Arg70 gained high sustained virological response (50.0%), and sustained virological response (11.8%) were the worst in patients who possessed both of genotype TG and GG, and Gln70(His70).

Fig. 1

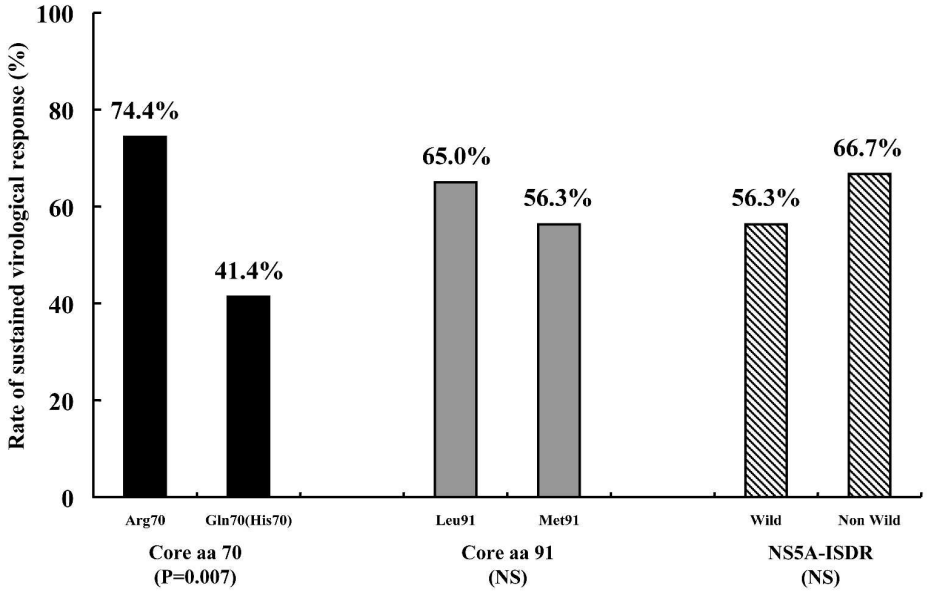

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Fig. 2

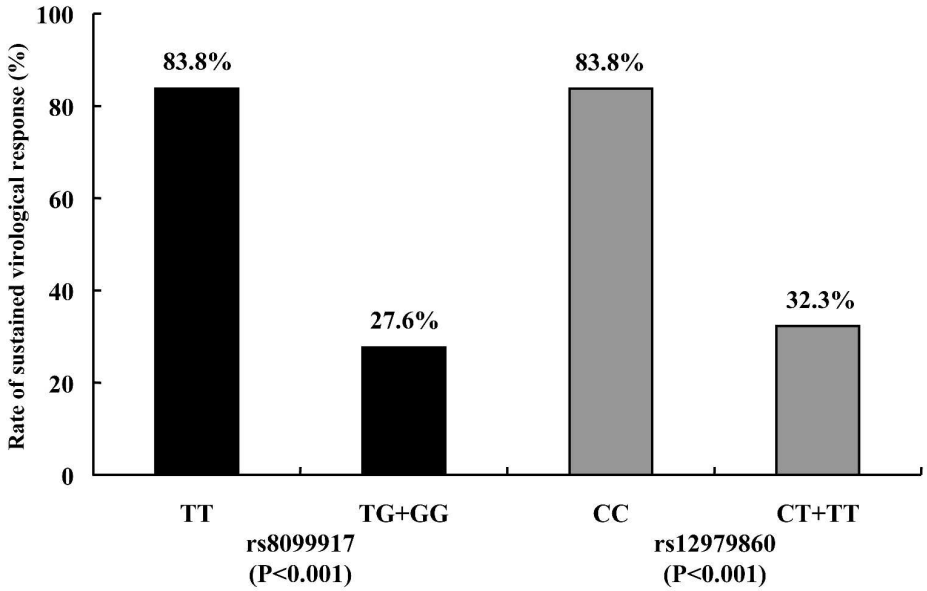

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Fig. 3

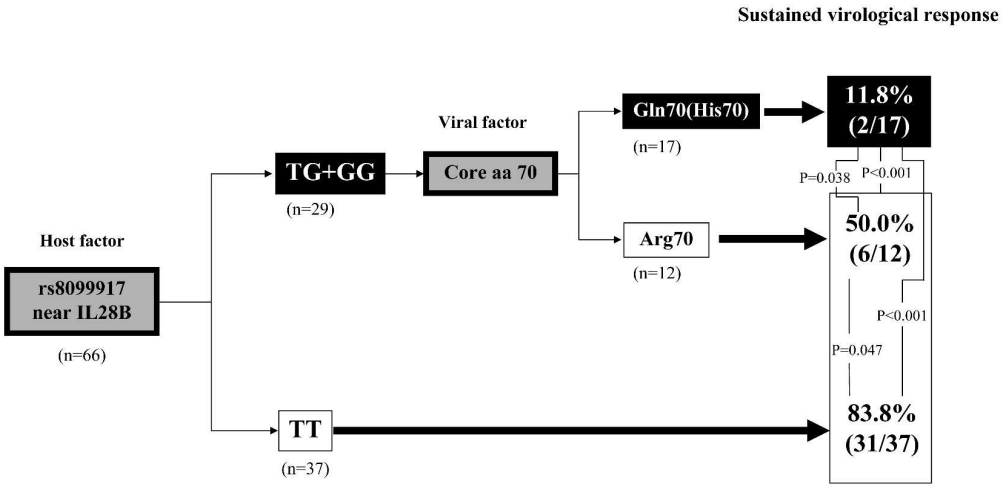

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Table 1. Profile and laboratory data at commencement of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV genotype 1

Demographic data	
Number of patients	81
Sex (M/F)	44 / 37
Age (years)*	55 (23-65)
History of blood transfusion	24 (29.6%)
Family history of liver disease	13 (16.0%)
Body mass index (kg/m^2)*	22.5 (13.2-32.4)
Laboratory data*	
HCV genotype (1a / 1b)	1 / 80
Level of viremia (log IU/ml)	6.7 (5.1-7.6)
Serum aspartate aminotransferase (IU/l)	34 (15-137)
Serum alanine aminotransferase (IU/l)	42 (12-175)
Serum albumin (g/dl)	3.9 (3.2-4.6)
Gamma-glutamyl transpeptidase (IU/l)	36 (9-229)
Leukocyte count ($/mm^3$)	4,800 (2,800-8,100)
Hemoglobin (g/dl)	14.3 (11.7-16.8)
Platelet count ($\times 10^4/mm^3$)	17.1 (9.1-33.8)
Alpha-fetoprotein (μg/l)	4 (2-39)
Total cholesterol (mg/dl)	180 (110-276)
Fasting plasma glucose (mg/dl)	92 (64-125)
Treatment	
PEG-IFNα-2b dose (μg/kg)*	1.5 (1.3-2.0)
Ribavirin dose (mg/kg)*	11.7 (7.2-18.4)
Telaprevir dose (1,500 / 2,250 mg/day)	10 / 71
Treatment regimen (T12PR12 group / T12PR24 group)	20 / 61
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine / glutamine (histidine) / ND)	47 / 33 / 1
Core aa 91 (leucine / methionine / ND)	43 / 37 / 1
ISDR of NS5A (wild-type / non wild-type / ND)	76 / 4 / 1
Genetic variation near IL28B gene	
rs8099917 genotype (TT / TG / GG / ND)	42 / 30 / 2 / 7
rs12979860 genotype (CC / CT / TT / ND)	42 / 32 / 2 / 5
Past history of IFN therapy	
Treatment-naive / Relapsers to previous treatment / Non-responders to previous treatment	27 / 33 / 21

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values. ND: Not determined.

Table 2. According to genetic variation near IL28B gene, background at commencement of triple therapy and treatment efficacy

	rs8099917 genotype			rs12979860 genotype		
	TT (n=42)	TG+GG (n=32)	TT vs. TG+GG P	CC (n=42)	CT+TT (n=34)	CC vs. CT+TT P
Demographic data						
Sex (M/F)	22 / 20	18 / 14	NS	22 / 20	19 / 15	NS
Age (years)*	54 (23-65)	56 (36-65)		54 (23-65)	55 (36-65)	NS
History of blood transfusion	15 (35.7%)	9 (28.3%)	NS	15 (35.7%)	9 (26.5%)	NS
Family history of liver disease	6 (14.3%)	6 (18.8%)	NS	6 (14.3%)	6 (17.6%)	NS
Body mass index (kg/m^2)*	22.1 (13.2-32.4)	22.4 (18.7-26.5)	NS	22.1 (13.2-32.4)	22.3 (18.7-26.5)	NS
Laboratory data*						
HCV genotype (1a / 1b)	0 / 42	1 / 31	NS	0 / 42	1 / 33	NS
Level of viremia (log IU/ml)	6.9 (5.4-7.5)	6.6 (5.1-7.4)	NS	6.9 (5.4-7.5)	6.5 (5.1-7.4)	NS
Serum aspartate aminotransferase (IU/l)	38 (15-118)	31 (20-137)	0.036	38 (15-118)	31 (20-137)	0.031
Serum alanine aminotransferase (IU/l)	50 (12-175)	36 (17-136)	0.029	50 (12-175)	35 (17-136)	0.014
Serum albumin (g/dl)	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS
Gamma-glutamyl transpeptidase (IU/l)	29 (9-194)	53 (9-154)	0.008	29 (9-194)	53 (9-229)	0.004
Leukocyte count ($/mm^3$)	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS
Hemoglobin (g/dl)	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS
Platelet count ($\times 10^4/mm^3$)	16.8 (9.9-33.8)	17.1 (9.1-24.8)	NS	16.8 (9.9-33.8)	17.8 (9.1-28.8)	NS
Alpha-fetoprotein (μ g/l)	4 (2-39)	5 (2-38)	NS	4 (2-39)	5 (2-38)	NS
Total cholesterol (mg/dl)	184 (112-276)	178 (110-263)	NS	184 (112-276)	178 (110-263)	NS
Fasting plasma glucose (mg/dl)	97 (80-125)	90 (66-111)	0.038	97 (80-125)	91 (66-111)	0.030
Treatment regimen						
T12PR12 group / T12PR24 group	12 / 30	7 / 25	NS	12 / 30	7 / 27	NS
Amino acid substitutions in the HCV genotype 1b						
Core aa 70 (arginine / glutamine (histidine))	30 / 12	13 / 18	0.016	30 / 12	13 / 20	0.009
Core aa 91 (leucine / methionine)	25 / 17	13 / 18	NS	25 / 17	14 / 19	NS
ISDR of NS5A (wild-type / non wild-type)	39 / 3	30 / 1	NS	39 / 3	32 / 1	NS
Past history of IFN therapy						
Treatment-naive / Relapsers to previous treatment / Non-responders to previous treatment	16 / 24 / 2	7 / 6 / 19	<0.001	16 / 24 / 2	8 / 7 / 19	<0.001
Treatment efficacy**						
End-of-treatment response (%)	35 (94.6%)	23 (79.3%)	NS	35 (94.6%)	25 (80.6%)	NS
Sustained virological response (%)	31 (83.8%)	8 (27.6%)	<0.001	31 (83.8%)	10 (32.3%)	<0.001

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.
** Treatment efficacy according to rs8099917 genotype was evaluated in 66 patients, and that according to rs12979860 genotype were done in 68 patients.

Table 3. Multivariate analysis of factors associated with sustained virological response of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV genotype 1.

Factor	Category	Odds ratio (95% CI)	P
rs8099917 genotype	1: TG+GG	1	
	2: TT	10.6 (3.07-36.5)	≤0.001
Substitution of aa 70	1: Gln70 (His70)	1	
	2: Arg70	3.69 (1.06-12.8)	0.040

Only variables that achieved statistical significance ($p<0.05$) on multivariate logistic regression analysis are shown.
95% CI: 95% confidence interval

Table 4

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for sustained virological response, according to substitution of core aa 70 and genetic variation near IL28B gene

	% (Number)			
	Sensitivity	Specificity	PPV*	NPV**
(A) rs8099917 genotype TT	79.5 (31/39)	77.8 (21/27)	83.8 (31/37)	72.4 (21/29)
(B) Substitution at aa 70 of arginine (Arg70)	76.9 (30/39)	63.0 (17/27)	75.0 (30/40)	65.4 (17/26)
(A) and (B)	61.5 (24/39)	85.2 (23/27)	85.7 (24/28)	60.5 (23/38)
(A) and/or (B)	94.9 (37/39)	55.6 (15/27)	75.5 (37/49)	88.2 (15/17)

*PPV; Sustained virological response rates for patients with a combination of Arg70 or rs8099917 genotype TT (prediction of sustained virological response).
**NPV; Non-sustained virological response rates for patients with a combination of Gln70(His70) or rs8099917 genotype non-TT (prediction of non-sustained virological response).