These results suggest that male patients who are infected with HCV genotype 1 and have coagulation disorders will have a higher sustained virological response than patients without coagulation disorders, if the coagulation disorder patients do not discontinue treatment. However, these results do not account for the differences in age. Therefore, male, age-matched patients infected with HCV genotype 1 were evaluated. The characteristics that differed between patients with and without coagulation disorders were body weight, BMI and baseline Hb levels.

In male, age-matched patients infected with HCV genotype 1, the sustained virological response rate based on both intention-to-treat and per-protocol analyses was not different between patients with and without coagulation disorders.

Using a multivariate analysis, whether patients had coagulation disorders was not associated significantly with a sustained virological response. Only BMI and GGT were identified as factors associated with a sustained virological response to combination therapy in male, age-matched patients infected with HCV genotype 1. A previous report showed that GGT levels may represent a surrogate marker of tumor necrosis factor-alpha expression in the liver and explain the importance of serum analyses to in predict the treatment outcome [Taliani et al., 2002]. Several studies revealed that GGT is one predictor of a sustained virological response [Taliani et al., 2002, 2006; Villela-Nogueira et al., 2005]. In western countries, obesity and a high BMI are associated with the absence of a sustained virological response to combination therapy of pegIFN or IFN with ribavirin [Bressler et al., 2003; Camma et al., 2004]. However, in Japan, most of the patients who are treated with combination therapy are not obese and have lower BMIs than patients in western countries. In this population, the mean BMI was 22.7 ± 2.8. In this low BMI population, a higher BMI would be associated with a sustained virological response. However, the reason why a low BMI is associated with the absence of a sustained virological response has not elucidated.

Adverse effects are thought to increase in patients with coagulation disorders; however, there was not a significant difference in adverse effects necessitating discontinuation of pegIFN and ribavirin between patients with and without coagulation disorders (13.0% vs. 9.4%). In addition, severe adverse effects and bleeding adverse effects were not associated with coagulation disorders. A previous report showed that IFN and ribavirin combination therapy may reduce the use of clotting factors in hemophilia patients with chronic hepatitis C [Honda et al., 2005; Yamamoto et al., 2006]. Ribavirin may reduce the side effect of bleeding during combination therapy. In this study, patients with coagulation disorders did not experience an adverse effect of bleeding.

In conclusion, treatment of chronic hepatitis C with combination therapy was effective comparably between patients with and without coagulation disorders and there were no adverse effects of bleeding.

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Prevalence of Hepatitis C Virus Genotype 1a in Japan and Correlation of Mutations in the NS5A Region and Single-Nucleotide Polymorphism of Interleukin-28B With the Response to Combination Therapy With Pegylated-Interferon-Alpha 2b and Ribavirin

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Hepatitis C virus (HCV) genotype 1a is rare in Japanese patients and the clinical characteristics of this genotype remain unclear. The interferon (IFN) sensitivity-determining region (ISDR) and single-nucleotide polymorphisms (SNPs) of interleukin-28B (IL28B) among patients with HCV genotype 1b are associated with IFN response, but associations among patients with genotype 1a are largely unknown. This study investigated the clinical characteristics of genotype 1a and examined whether genomic heterogeneity of the ISDR and SNPs of IL28B among patients with HCV genotype 1a affects response to combination therapy with pegylated-IFN-α2b and ribavirin. Subjects comprised 977 patients infected with HCV genotype 1, including 574 men and 412 women (mean age, 55.2 ± 10.6 years). HCV was genotyped by direct sequencing of the 5′-untranslated region and/or core regions and confirmed by direct sequencing of the NS5A region. HCV genotypes 1a (n = 32) and 1b (n = 945) were detected. Twenty-three (71.9%) of the 32 patients with genotype 1a were patients with hemophilia who had received imported clotting factors. Prevalence of genotype 1a after excluding patients with hemophilia was thus 0.9%. Of the 23 patients with genotype 1a who completed IFN therapy, 11 (47.8%) were defined as achieving sustained virological response. Factors related to sustained virological response by univariate analysis were IL28B and ISDR. In conclusion, HCV genotype 1a is rare in Japan. The presence of IL28B genotype TT, and more than two mutations, in the ISDR are associated with a good response to IFN therapy in patients with HCV genotype 1a. ***J. Med. Virol. 84:438–444, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: hepatitis C virus; genotype 1a; NS5A; IL 28B; interferon

INTRODUCTION

Hepatitis C virus (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma [Seeff, 2002]. HCV infection is a significant global health problem, affecting 170 million individuals worldwide. HCV can be divided into six genotypes and several subtypes according to genomic heterogeneity [Simmonds et al., 2005]. Each genotype shows a unique distribution and clinical characteristics such

All authors have nothing to disclose.

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as interferon (IFN) responsiveness [Ghany et al., 2009]. HCV genotypes 1b, 2a, and 2b are the major types encountered in Japan [Enomoto et al., 1990; Hayashi et al., 2003]. Genotype 1a is common worldwide, but is rare in Japan except among individuals with hemophilia who have received imported clotting factors [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. The prevalence and clinical characteristics, including IFN responsiveness, of Japanese patients with HCV genotype 1a are unclear. HCV NS5A protein reportedly includes a domain associated with IFN response. This domain, located in the NS5A region of HCV genotype 1b, is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR) [Enomoto et al., 1996]. IFN acts to inhibit viral replication by inducing double-stranded RNA-dependent protein kinase (PKR). The ISDR is located at the 5′ end of the PKR-binding domain and is inhibited by PKR in vitro [Gale et al., 1998]. ISDR heterogeneity of genotype 1b is thus an important factor that may affect response to IFN [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. Several studies have reported a relationship between ISDR and IFN responsiveness among patients with HCV genotype 1a [Hofgärtner et al., 1997; Zeuzem et al., 1997; Kumthip et al., 2011; Yahoo et al., 2011]. However, this remains controversial for genotype 1a, and the utility of ISDR sequences for predicting IFN responsiveness has not been investigated for HCV genotype 1a in Japan due to the rarity of this genotype. Both genetic heterogeneity of the HCV genome and host genetics contribute to IFN responsiveness. Several genome-wide association studies have thus been performed to clarify host factors associated with IFN responsiveness, revealing that interleukin-28B (IL28B) polymorphisms are strongly associated with response to IFN therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009]. Combined use of the single-nucleotide polymorphisms (SNPs) of IL28B and amino acid substitutions in the core region and ISDR could thus improve the prediction of response to IFN in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. However, the effects of a combined evaluation of the SNPs of IL28B and amino acid substitutions in the ISDR in patients with HCV genotype 1a on IFN response are unclear. The aim of the present study was to determine whether genomic heterogeneity of the ISDR and SNPs of IL28B among patients with HCV genotype 1a affect response to combination therapy with pegylated-IFN-α2b and ribavirin.

PATIENTS AND METHODS

A total of 977 patients (569 men, 408 women) with chronic hepatitis C genotype 1 and high viral load (<100 KIU/ml) who were treated at Nagoya University Hospital and affiliated hospitals were enrolled in this study. Mean age of patients was 55.1 ± 12.2 years (range: 18–75 years). None of the patients had a history of chronic alcohol abuse, autoimmune disease, or metabolic disease. Patients with active intravenous drug use and immigrants were excluded from this study. The core region (aa 30–110) and ISDR (aa 2,209–2,248) of HCV were examined by direct sequencing. SNPs of IL28B (rs8099917) were identified using a real-time polymerase chain reaction (PCR) system. Patients received subcutaneous injections of pegylated-IFN-α2b (1.5 μg/kg) once each week along with oral ribavirin (600 mg/day for patients <60 kg, 800 mg/day for 60–80 kg, 1,000 mg/day for >80 kg) for 48 weeks. Patients who became negative for HCV-RNA between 16 and 36 weeks after initiating IFN treatment had the IFN treatment extended to 72 weeks, in accordance with Japanese guidelines [Kumada et al., 2010]. HCV-RNA levels in serum samples were examined at 12 weeks, at the end of IFN therapy, and at 6 months after the end of treatment. Serum was stored at −80°C for virological examination at pretreatment. Early virological response was defined as HCV-negative status at 12 weeks. Patients who were persistently negative for serum HCV-RNA at 24 weeks after withdrawal of IFN treatment were considered to show sustained virological response. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological Analysis

HCV-RNA quantitative viremia load was determined by PCR. HCV was genotyped by direct sequencing of the 5′-untranslated region and/or core regions as described previously and confirmed by direct sequencing of the NS5A region [Otagiri et al., 2002; Dal Pero et al., 2007; Hayashi et al., 2011a]. Genotypes were classified according to the nomenclature proposed by Simmonds et al. [2005]. Direct sequencing of the core and NS5A-ISDR regions was performed as reported previously [Dal Pero et al., 2007; Hayashi et al., 2011a]. In brief, RNA was extracted from 140 μl of serum using a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA) and dissolved in 50 μl of diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligos and random hexamer primers with a commercial kit (iScript cDNA Synthesis Kit; Bio-Rad, Hercules, CA). The HCV core region and NS5A-ISDR were amplified by nested PCR. In brief, each 50-μl PCR reaction mixture contained 100 nM of each primer, 1 ng of template cDNA, 5 μl of GeneAmp 10× PCR buffer, 2 μl of dNTPs, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primers for the core region were: sense, 5′-GGGAGGTCTCGTAGACCGTGCACCATG-3′ and antisense, 5′-GAGMGGKATRTACCCCATGAGRTCGGC-3′. Primers for the NS5A-ISDR were: sense, 5′-GCCTGGAGCCCTTGTAGTC-3′ and

TABLE I. Clinical Characteristic of Patients With HCV Genotype 1a

	N = 32
Age (y.o.)	36.4 ± 2.2
Sex: male/female	28/4
AST (IU/L)	48.8 ± 33.6
ALT (IU/L)	64.6 ± 57.8
Platelet (10^4/μl)	18.8 ± 6.0
HCV RNA level (KIU/ml)	2607.4 ± 3072.2
Source (clotting factor/BTF/unknown)	23/2/7

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

antisense, 5′-CTGCGTGAAGTGGTGGAATAC-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was performed using the same reaction buffer with the first-round PCR product as template, and the following sets of primers: for the core region, sense primer 5′-AGACCGTGCACCATGAGCAC-3′ and antisense 5′-TACGCCGGGGGTCAKTRGGGCCCCA-3′; and for the NS5A-ISDR, sense 5′-TGTTTCCCCCACGCACTAC-3′ and antisense 5′-TGATGGGCAGTTTTTGTTCTTC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers using a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genotyping Analysis

Detection of SNPs for IL28B (rs8099917) was conducted using a real-time PCR system. In brief, genomic DNA was extracted from 150 μl of whole blood with a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μl of diethylpyrocarbonate-treated water. DNA (10 ng) was used for PCR and genotyping of IL28B SNP (rs8099917) was performed by TaqMan allelic discrimination (ABI-Prism 7300 SDS software; Applied Biosystems) with TaqMan SNP Genotyping Assays provided by Applied Biosystems (C__11710096_10).

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The paired *t*-test was used to analyze differences in variables. A value of $P < 0.05$ was considered statistically significant. Statview 5.0 software (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Thirty-two of the 977 patients (3.3%) were infected by genotype 1a. Clinical characteristics of patients with genotype 1a are summarized in Table I. Twenty-three cases involved patients with hemophilia who had received imported clotting factors. The prevalence of genotype 1a after excluding patients with hemophilia was 0.9%. A comparison of clinical characteristics according to hemophilia status is shown in Table II. No significant differences were apparent among the two groups. Differences in clinical characteristics between genotypes 1a and 1b are shown in Table III. Males were more frequent among patients with genotype 1a (87.5%) than among those with genotype 1b (57.2%), as the majority of patients with genotype 1a were young male patients with hemophilia. Sequence alignments of the core region at codons 71 and 90 showed arginine and cysteine, respectively, in all patients. The HCV core region of genotype 1a was thus well-conserved, with no significant mutations at codons 71 or 90. This is not similar to previous findings for genotype 1b [Akuta et al., 2005, 2011; Hayashi et al., 2011a,b; Kurosaki et al., 2011]. Alignment of the amino acid sequence for NS5A-ISDR is shown in Figure 1. The sequence of the HCV-1 strain was defined as the consensus sequence of genotype 1a, and the number of mutations to the chosen consensus sequence in ISDR was used to analyze the ISDR system. Sequences of the HCV-1 strain and HCV-1 strain with only one amino acid substitution were defined as wild-type, while ISDR sequences with more than two amino acid substitutions were defined as mutant-type. Twenty-seven strains were defined as wild-type and 5 strains were defined as mutant-type. IL28B genotypes could be obtained for 25 patients, and IL28B alleles were TT (n = 14) and TG (n = 11). Twenty-three patients received pegylated-IFN-α2b plus ribavirin therapy. Twenty patients were treated for 48 weeks, and 1 patient was treated for 72 weeks. Two patients were withdrawn at 24 weeks due to a

TABLE II. Clinical Characteristic According to Hemophilia

	Patients with hemophilia (N = 23)	Patients without hemophilia (N = 9)	*P*-value
Age (y.o.)	37.1 ± 9.2	37.1 ± 16.3	0.9966
Sex: male/female	22/1	6/3	0.0572
AST (IU/L)	51.2 ± 34.8	41.9 ± 30.9	0.5072
ALT (IU/L)	68.2 ± 55.8	54.0 ± 66.1	0.5566
Platelet (10^4/μl)	18.4 ± 6.8	19.8 ± 3.0	0.5602
HCV levels (KIU/ml)	2599.6 ± 3108.0	2630.0 ± 3176.5	0.9812

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

TABLE III. Clinical Characteristic According to Genotypes

	Genotype 1a (N = 32)	Genotype 1b (N = 945)	*P*-value
Age (y.o.)	36.4 ± 2.2	55.9 ± 11.6	0.0001
Sex: male/female	28/4	546/408	0.0004
Patients with hemophilia	23	4	0.0001
AST (IU/L)	48.8 ± 33.6	59.9 ± 45.0	0.1745
ALT (IU/L)	64.6 ± 57.8	64.6 ± 57.8	0.9894
Platelet (10^4/μl)	18.8 ± 6.0	17.2 ± 6.0	0.0918
HCV levels (KIU/ml)	2607.4 ± 3072.2	2011.5 ± 1453.8	0.0642

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus.

lack of response to IFN therapy. Frequency of early virological response, characterized by undetectable HCV at 12 weeks, was 30.4% (7/23). Virological response rate at the end of treatment was 47.8% (11/23). Finally, 11 of 23 patients (47.8%) achieved sustained virological response. Clinical characteristics were compared between patients who achieved sustained virological response and patients who did not (Table IV), revealing significant differences in two factors on univariate analysis: IL28B and ISDR.

DISCUSSION

The present study investigated 977 patients with genotype 1 using direct sequencing of core and NS5A regions, revealing that genotype 1a is rare (3.3%) in Japan. Of the 33 patients with genotype 1a, 23 (71.9%) were patients with hemophilia, confirming that the majority of cases with genotype 1a involve patients with hemophilia who have received imported clotting factors, as previously reported [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. Analysis after excluding patients with hemophilia revealed the prevalence of genotype 1a in Japan was 0.9% (9/954). Recently, the distributions of HBV genotypes have been changing in Japan due to international exchange [Hayashi et al., 2007; Matsuura et al., 2009]. However, prevalences of HCV genotypes have remained stable because of the different modes of infection involved. The present study revealed that 11 (47.8%) of 23 patients achieved sustained virological response. The IFN responsiveness of HCV genotype 1a in Japanese patients was reported in 1999 from Okinawa, a far southern island in Japan [Sakugawa et al., 1997]. That study reported that the rate of sustained virological response tended to be higher in patients with genotype 1a than in those with genotype 1b, but no significant differences were identified because of the small number of patients with genotype 1a. Low virological response rates in both genotypes 1a and 1b were confirmed in the present Japanese patients, as in Caucasian patients [Manns et al., 2001; McHutchison et al., 2009]. No significant differences in sustained virological response rate were seen between genotypes 1a and 1b. Discriminating between genotypes 1a and 1b thus seems to have little clinical relevance in terms of IFN responsiveness. Viral factors associated with sustained virological response, including HCV genotype, have been studied most frequently studied and mutations in the core and NS5A regions of HCV genotype 1b have been associated with response to IFN therapy [Akuta et al., 2005, 2010, 2011; Okanoue et al., 2009; Nakagawa et al., 2010; Toyoda et al., 2010; Hayashi et al., 2011a; Hayes et al., 2011; Kumthip et al., 2011; Kurosaki et al., 2011]. These viral factors could improve prediction of sustained virological response for genotype 1a, as in 1b. Amino acid substitutions at positions 70 and 91 of the HCV core region in genotype 1b have been related to IFN responsiveness, liver steatosis, hepatic oxidative stress, insulin resistance, and carcinogenesis [Akuta et al., 2005, 2007, 2009; Tachi et al., 2010]. These substitutions may have substantial impacts on

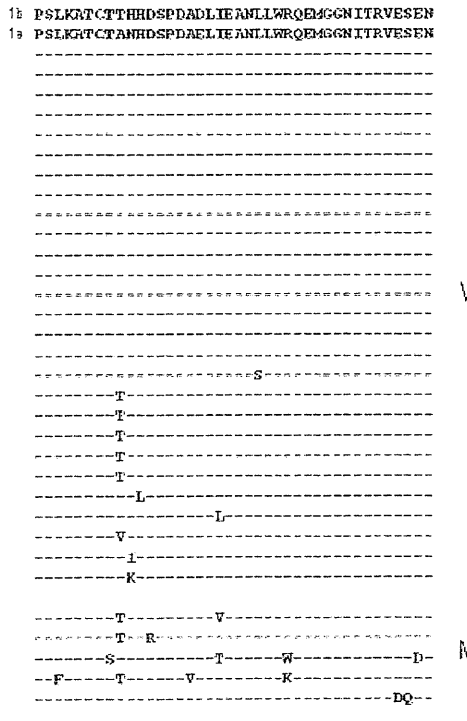

Fig. 1. Alignment of the amino acid sequence for the NS5A-ISDR. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCV1. Sequences of the HCV1 strain and HCV1 strains with one-nucleotide substitutions were defined as wild-type ISDR, and all other strains were defined as mutant-type ISDR. ISDR, interferon sensitivity-determining region.

TABLE IV. Univariate Analysis: Factors Predictive of Sustained Virologic Response

Factors	Sustained virologic response (n = 11)	Non-sustained virologic response (n = 12)	*P*-value
Age (y.o.)	37.9 ± 10.9	39.8 ± 11.3	0.6958
Gender: male/female	10/1	10/2	0.9999
ALT (IU/L)	78.2 ± 50.8	62.6 ± 68.1	0.5435
AST (IU/L)	51.4.4 ± 29.2	48.8 ± 40.4	0.8616
PLT ($\times 10^4$/mm^3)	19.0 ± 5.4	19.3 ± 5.7	0.8870
HCV RNA level (KIU/ml)	1323.1 ± 1077.3	2567.0 ± 2940.8	0.2481
ISDR: wild/mutant	7/4	12/0	0.0373
IL28B:TT/TG	9/1	4/8	0.0115

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; IL28B, interleukin 28B.

the pathogenesis of HCV genotype 1a infection. However, the HCV core region of genotype 1a is well-conserved and no significant mutations were seen in the core region, which is associated with IFN responsiveness. Several reports have also found that the HCV core region, including positions 70 and 91, of HCV genotype 1a is highly conserved [Alestig et al., 2011; Kumthip et al., 2011]. Mutations in the core region of genotype 1a would be rare, so this region might be unsuitable for routine clinical use, unlike in genotype 1b. However, the number of patients in this study was small, and large studies including from other countries are needed to clarify these issues. The ISDR in the NS5A region of HCV genotype 1b is closely associated with response to IFN therapy. ISDR mutations of genotype 1b are well known to be more important in predicting sustained virological response in Japanese patients than European patients [Hofgärtner et al., 1997; Zeuzem et al., 1997; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. European studies have failed to detect the specific amino acid substitutions in ISDR of genotype 1a associated with IFN responsiveness [Hofgärtner et al., 1997; Zeuzem et al., 1997]. In this study, sustained virological response was achieved in 36.8% of patients with wild-type ISDR and 100% of patients with mutant-type ($P = 0.0373$). The present analysis showed a close relationship between ISDR of genotype 1a and sustained virological response, as in genotype 1b. Recent investigations in Thailand and Iran have failed to identify the usefulness of ISDR for HCV genotype 1a in predicting sustained virological response [Kumthip et al., 2011; Yahoo et al., 2011]. The high virological response rate and low prevalence of patients with mutations in the ISDR do not favor the use of ISDR analysis in predicting IFN responsiveness [Herion and Hoofnagle, 1997; Yokozaki et al., 2011]. Rates of sustained virological response among these studies were much higher than those in the present study (68.4% and 75% vs. 47.8%). The mean number of mutations in patients who achieved sustained virological response in the studies by Kumthip et al. [2011] and Yahoo et al. [2011], and the present group were 1.4, 1.4, and 1.6, respectively. Differences in sustained virological response and the number of mutations to the ISDR might underpin this discrepancy in the evaluation of ISDR. Although the sample size in the present study was small, the results indicate that ISDR represents a strong indicator of progression to sustained virological response for patients with HCV genotype 1a. Amino acid substitutions in the ISDR of genotype 1a thus also play an important role in predicting sustained virological response in Japanese patients compared to patients from other countries. IL28B polymorphisms such as host genetics, as well as mutations in the HCV genome, contribute to IFN treatment outcomes. Rates of sustained virological response in patients in this study with TT and TG were 69.2% and 11.1%, respectively. The TG allele of the IL28B genotype was significantly associated with poor response to IFN therapy ($P = 0.0115$). SNPs of IL28B would regulate the expression of IFN-stimulated genes and affect IFN responsiveness. IL28B and ISDR thus exert independent effects on IFN responsiveness and both host and viral factors impacting IFN responsiveness would improve the prediction of sustained virological response. Several studies have thus reported that both the SNP of IL28B and mutations in the ISDR were associated with sustained virological response in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. In the present study of HCV genotype 1a, among the 9 patients who had simultaneously the TG allele for IL28B and wild-type ISDR, only 1 achieved sustained virological response (11.1%). The best-sustained virological response was achieved in patients with mutant-type ISDR and the T allele (100%). The combination of SNPs for IL28B and mutations in ISDR may thus predict response to IFN therapy in patients with HCV genotype 1a as well as genotype 1b. Given the small sample size in this investigation, larger cohorts are needed to confirm the present results. Furthermore, infection with genotype 1a in Japanese patients is rare, making large-scale studies difficult to perform.

In conclusion, the prevalence of HCV genotype 1a is rare in Japan and the majority of cases involve patients with hemophilia. The TG genotype of IL28B is associated with poor response, while mutant-type ISDR is associated with good response to combination therapy with pegylated-IFN-α2b and ribavirin in patients with HCV genotype 1a. Combined use of both IL28B and ISDR could improve the prediction of IFN response.

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Predictive Value of Early Viral Dynamics During Peginterferon and Ribavirin Combination Therapy Based on Genetic Polymorphisms Near the *IL28B* Gene in Patients Infected With HCV Genotype 1b

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A study was carried out to determine whether early viral dynamics retain prediction of the outcome of peginterferon (PEG-IFN) and ribavirin combination therapy based on different genetic polymorphisms near the *IL28B* gene, the strongest baseline predictor of response to this therapy. A total of 272 patients infected with hepatitis C virus (HCV) genotype 1b were grouped according to genetic polymorphisms near the *IL28B* gene (rs8099917). The ability of reduced HCV RNA levels at 4 and 12 weeks after starting therapy to predict a sustained virologic response was evaluated based on these genotypes. Among patients with the TT genotype for rs8099917 (associated with a favorable response), the rates of sustained virologic response were higher in patients with a $\geq$3 $\log_{10}$ reduction in serum HCV RNA levels at 4 weeks after starting therapy ($P < 0.0001$). In contrast, among patients with the TG/GG genotype (associated with an unfavorable response), there were no differences in this rate based on the reduction in HCV RNA levels at 4 weeks. Early viral dynamics at 4 weeks after starting therapy retains its predictive value for sustained virologic response in patients with the TT genotype for rs8099917, but not in patients with the TG/GG genotype. Patients who are likely to achieve sustained virologic response despite unfavorable TG/GG genotype cannot be identified based on early viral dynamics during therapy. In contrast, lack of early virologic response at 12 weeks retains a strong predictive value for the failure of sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy. ***J. Med. Virol. 84:61–70, 2012.*** © 2011 Wiley Periodicals, Inc.

KEY WORDS: chronic hepatitis C; early viral dynamics; genetic polymorphisms near the *IL28B* gene; peginterferon; response-guided therapy; ribavirin

INTRODUCTION

The current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin [Ghany et al., 2009]. Although this treatment regimen has increased markedly the number of patients with a sustained virologic response, i.e., the eradication of hepatitis C virus (HCV), only 50% of patients infected with HCV genotype 1 achieved a sustained virologic response approximately.

Many investigators have examined factors that predict the treatment outcome of PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1. In addition to the baseline factors, the response of HCV during combination therapy, i.e., the changes in serum HCV RNA levels after starting therapy, has been shown to be an important predictor of the treatment outcome [Zeuzem et al., 2001; Buti

Conflict of interest: None.

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et al., 2002; Berg et al., 2003], with the emphasis on "response-guided therapy" [Lee and Ferenci, 2008; Marcellin and Rizzetto, 2008]. Recent reports have emphasized the importance of evaluating the viral dynamics at 4 weeks after starting therapy to predict a sustained virologic response. A rapid virologic response, in which serum HCV RNA is undetectable at 4 weeks after starting therapy, has been the strongest predictive factor of a sustained virologic response reportedly [Martinez-Bauer et al., 2006; Poordad et al., 2008; de Segadas-Soares et al., 2009; Martinot-Peignoux et al., 2009]. In addition, the predictive value of reduced serum HCV RNA levels at 4 weeks after starting therapy has been clarified further, and a ≥ 3 $\log_{10}$ reduction in HCV RNA levels at 4 weeks after starting therapy has high predictive value that a patient will achieve a sustained virologic response as a final outcome, even in the absence of a rapid virologic response [Toyoda et al., 2011].

In contrast, the lack of an early virologic response, defined as either undetectable serum HCV RNA or HCV RNA levels decreased by >2.0 $\log_{10}$ from the pretreatment level at 12 weeks after starting therapy, has been the most important predictor for the failure of a sustained virologic response in patients infected with HCV genotype 1 reportedly [Fried et al., 2002; Davis et al., 2003]. Therefore, treatment may be discontinued in patients without an early virologic response at 12 weeks of treatment, according to the recommendation in the AASLD guidelines [Ghany et al., 2009].

More recently, several studies reported that genetic polymorphisms near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 affect the virologic response to PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; McCarthy et al., 2010; Rauch et al., 2010]. Furthermore, genetic polymorphisms near the *IL28B* gene are the strongest baseline predictive factor of the final outcome of combination therapy. An additional report showed the effects of genetic polymorphisms near the *IL28B* gene on HCV viral dynamics during PEG-IFN and ribavirin combination therapy [Thompson et al., 2010].

Although early HCV viral dynamics during therapy was shown originally to have a high predictive value for a sustained virologic response in HCV genotype 1-infected patients before genetic polymorphisms near the *IL28B* gene were linked to a therapeutic response, it is not clear whether early viral dynamics retain their predictive value in light of this additional information. The purpose of the present study was to investigate whether response-guided therapy based on viral dynamics at 4 or 12 weeks after initiating therapy retains its ability to predict the final outcome of PEG-IFN and ribavirin combination therapy after accounting for genetic polymorphisms near the *IL28B* gene.

MATERIALS AND METHODS

Patients and Treatment

Between January 2007 and June 2008, a total of 402 patients with chronic hepatitis C received antiviral combination therapy with PEG-IFN and ribavirin for HCV infection at the Ogaki Municipal Hospital or the Nagoya University Hospital. Among these patients, 272 were infected with HCV genotype 1b and had pretreatment HCV RNA levels >5.0 $\log_{10}$ IU/ml based on a quantitative real-time PCR-based method for HCV (HCV COBAS AmpliPrep/COBAS TaqMan System; Roche Molecular Systems, Pleasanton, CA; Lower limit of quantification, 1.7 $\log_{10}$ IU/ml: Lower limit of detection, 1.0 $\log_{10}$ IU/ml) [Colucci et al., 2007; Pittaluga et al., 2008]. This study did not include any patients infected with HCV genotype 1a because this genotype is not found in the general Japanese population.

All patients were given PEG-IFN alpha-2b (Pegintron, Schering-Plough, Tokyo, Japan) weekly and ribavirin (Rebetol, Schering-Plough, Kenilworth, NJ) daily. The PEG-IFN and ribavirin doses were adjusted based on the patient's body weight. Patients weighing $\leq$45 kg were given 60 μg of PEG-IFN alpha-2b once a week, those weighing >45 and $\leq$60 kg were given 80 μg, those weighing >60 and $\leq$75 kg were given 100 μg, those weighing >75 and $\leq$90 kg were given 120 μg, and those weighing >90 kg were given 150 μg. Patients weighing $\leq$60 kg were administered 600 mg of ribavirin per day, those weighing >60 and $\leq$80 kg were given 800 mg per day, and those weighing >80 kg were administered 1000 mg per day. The PEG-IFN and ribavirin doses were modified based on the manufacturer's recommendations. All patients were scheduled to undergo 48 weeks of treatment. The treatment duration was extended up to 72 weeks in some patients. In addition, treatment was discontinued before 48 weeks in some patients who had a low likelihood of achieving an eradication of HCV due to the presence of serum HCV RNA at 24 weeks after starting therapy.

A sustained virologic response was defined as undetectable serum HCV RNA at 24 weeks after ending the therapy. A patient was considered to have relapsed when serum HCV RNA was detectable between the end of treatment and 24 weeks after completing treatment, although serum HCV RNA was undetectable during and at the end of therapy. Patients were considered to have non-response if serum HCV RNA was detectable at 24 weeks after initiating therapy (i.e., null response or partial response according to the American guidelines [Ghany et al., 2009]). Patients were considered to have a rapid virologic response if they had undetectable serum HCV RNA at 4 weeks after starting therapy. An early virologic response was defined as the disappearance or decrease in serum HCV RNA levels by at least 2 $\log_{10}$ at 12 weeks after starting therapy. Patients were considered to have a complete early virologic response if serum HCV RNA was undetectable at 12 weeks after starting therapy and a partial early virologic response if the serum

HCV RNA levels had decreased by at least 2 $\log_{10}$ at 12 weeks after initiating therapy. Patients were considered not to have an early virologic response if their HCV RNA levels did not decrease by more than 2 $\log_{10}$ at 12 weeks compared to the pretreatment levels. Patients were considered to have a slow virologic response if the serum HCV RNA became undetectable between 12 and 24 weeks.

The study protocol was in compliance with the Helsinki Declaration and was approved by the ethics committee of the Ogaki Municipal Hospital and the Nagoya University School of Medicine. Prior to initiating the study, each patient provided written informed consent to use the laboratory data, analyze genetic polymorphisms near the *IL28B* gene, and test stored serum samples.

Assessments of Serum HCV RNA Levels and Genetic Polymorphisms Near the *IL28B* Gene

After a patient provided informed consent, serum samples were obtained at the patient's regular hospital visits, just prior to initiating treatment, every 4 weeks during the treatment period, and during the 24-week follow-up period after treatment. Serum samples were stored at −80°C until further use. The HCV RNA levels were measured using a quantitative real-time PCR-based method for HCV (HCV COBAS AmpliPrep/COBAS TaqMan System).

Genotyping of rs 8099917 polymorphisms near the *IL28B* gene was performed using the TaqMan SNP assay (Applied Biosystems, Foster City, California) according to the manufacturer's guidelines. A predesigned and functionally tested probe was used for rs8099917 (C__11710096_10, Applied Biosystems).

Statistical analyses. Quantitative values are reported as the mean ± SD. In between-group differences were analyzed by the chi-square test. Univariate and multivariate analyses using a logistic regression model were performed to identify factors that predict a sustained virologic response, including age, sex, body weight, serum alanine aminotransferase activity, serum aspartate aminotransferase activity, serum gamma-glutamyl transpeptidase levels, serum alkaline phosphatase values, serum albumin levels, total serum bilirubin values, white blood cell counts, hemoglobin, platelet counts, hepatitis activity grade (A0 and A1 vs. A2 and A3), liver fibrosis grade (F0 and F1 vs. F2 and F3), pretreatment HCV RNA levels ($\geq$6.5 $\log_{10}$ vs. <6.5 $\log_{10}$), reduction in peginterferon dose and ribavirin dose, reduction in HCV RNA levels at 4 weeks after starting therapy ($\geq$3 $\log_{10}$ vs. <3 $\log_{10}$), and the type of an early virologic response. All *P*-values are two-tailed, and $P < 0.05$ was considered significant statistically.

RESULTS

The characteristics of the patients examined in this study are shown in Table I. Liver histology was evaluated according to the METAVIR score [The French METAVIR Cooperative Study Group, 1994]. Although some patients had a reduction in their PEG-IFN and ribavirin doses during therapy, respectively, all patients except for those who discontinued the therapy had more than 80% adhesion to both the PEG-IFN and ribavirin regimens. No patients discontinued the therapy because of adverse effects. The treatment duration was extended up to 72 weeks in 51 of 71 patients (71.8%) who exhibited a slow virologic response. As a final outcome, 118 patients (43.4%) achieved a sustained virologic response, 84 patients (30.9%) relapsed, and the remaining 70 patients (25.7%) had no response.

TABLE I. Characteristics of all Study Patients (n = 272)

Age (years)	56.0 ± 10.9
Sex (female/male)	139 (51.1)/133 (48.9)
Body weight (kg)	57.8 ± 10.5
Alanine aminotransferase (IU/L)	64.6 ± 56.4
Aspartate aminotransferase (IU/L)	53.9 ± 42.7
Gamma-glutamyl transpeptidase (IU)	48.5 ± 43.9
Alkaline phosphatase (IU/L)	267.9 ± 101.3
Albumin (g/dl)	4.04 ± 0.37
Total bilirubin (mg/dl)	0.79 ± 0.30
White blood cell count (/μl)	4892 ± 1333
Hemoglobin (g/dl)	14.0 ± 1.3
Platelet count ($\times 10^3$/μl)	163 ± 51
Liver histology-activity (A0/A1/A2/A3)*	3 (1.2)/136 (55.3)/ 92 (37.4)/15 (6.1)
Liver histology-fibrosis (F0/F1/F2/F3)*	27 (11.0)/114 (46.3)/ 70 (28.5)/35 (14.2)
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.35 ± 0.79
Reduction in the peginterferon dose	81 (29.8)
Reduction in the ribavirin dose	130 (47.8)
Final outcomes (sustained virologic response /relapse/ no response)	118 (43.4)/ 84 (30.9)/70 (25.7)

HCV, hepatitis C virus.
Percentages are shown in parentheses.
*Liver biopsy was not performed in 26 patients.

Reduction in Serum HCV RNA Levels at 4 Weeks after Starting Therapy and Treatment Outcome According to Genetic Polymorphisms Near the *IL28B* Gene

An analysis of genetic polymorphisms at rs8099917 near the *IL28B* gene indicated that 207 patients (76.1%) had a TT genotype, 3 patients had a GG genotype (1.1%), and the remaining 62 patients were TG heterozygote (22.8%). Table II shows the comparison of the background characteristics between patients with the favorable TT genotype and those with the unfavorable TG/GG genotype. As reported previously [Abe et al., 2010], gamma-glutamyl transpeptidase level was higher significantly in patients with the TG/GG genotype. As a final outcome, the rate of a sustained virologic response was higher significantly in patients with the TT genotype. Among 207 patients with the TT genotype, serum HCV RNA became undetectable in 19 patients (9.2%) at 4 weeks after starting therapy (a rapid virologic response). In the remaining 188 patients, the decrease in serum HCV RNA levels at 4 weeks after starting therapy ranged from 0.12

TABLE II. Characteristics of Study Patients According to the Genetic Polymorphisms Near the *IL28B* Gene

	Patients with TT genotype of rs8099917 (n = 207)	Patients with TG/GG genotype of rs8099917 (n = 65)	*P*-value
Age (years)	56.5 ± 10.4	54.4 ± 12.4	0.4112
Sex (female/male)	107 (51.7)/100 (48.3)	32 (49.2)/33 (50.8)	0.8384
Body weight (kg)	57.8 ± 10.9	57.8 ± 9.4	0.8361
Alanine aminotransferase (IU/L)	65.1 ± 53.3	62.8 ± 65.6	0.2548
Aspartate aminotransferase (IU/L)	53.6 ± 34.8	54.7 ± 62.0	0.3339
Gamma-glutamyl transpeptidase (IU)	44.2 ± 37.1	62.3 ± 59.0	0.0003
Alkaline phosphatase (IU/L)	263.1 ± 90.3	282.8 ± 129.9	0.3875
Albumin (g/dl)	4.04 ± 0.36	4.05 ± 0.43	0.8020
Total bilirubin (mg/dl)	0.79 ± 0.30	0.76 ± 0.32	0.3010
White blood cell count (/μl)	4826 ± 1333	5100 ± 1320	0.1608
Hemoglobin (g/dl)	13.9 ± 1.3	14.1 ± 1.4	0.3339
Platelet count ($\times 10^3$/μl)	161 ± 49	169 ± 57	0.3871
Liver histology-activity (A0/A1/A2/A3)*	2 (1.1)/98 (52.4)/ 74 (39.6)/13 (6.9)	1 (1.7)/38 (64.4)/ 18 (30.5)/2 (3.4)	0.3241
Liver histology-fibrosis (F0/F1/F2/F3)*	21 (11.2)/83 (44.4)/ 57 (30.5)/26 (13.9)	6 (10.2)/31 (52.5)/ 13 (22.0)/9 (15.3)	0.6401
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.37 ± 0.85	6.29 ± 0.55	0.0582
Reduction in the peginterferon dose	61 (29.5)	20 (30.8)	0.9644
Reduction in the ribavirin dose	101 (48.8)	29 (44.6)	0.5565
Final outcomes (sustained virologic response /relapse/ no response)	106 (51.2)/ 69 (33.3)/32 (15.5)	12 (18.4)/15 (23.1)/ 38 (58.5)	<0.0001

HCV, hepatitis C virus.
Percentages are shown in parentheses.
*Liver biopsy was not performed in 26 patients.

$\log_{10}$ to 5.71 $\log_{10}$ (mean, 3.12 $\log_{10}$). The reduction in serum HCV RNA levels was ≥3 $\log_{10}$ in 98 patients (47.3%), <3 $\log_{10}$ and ≥2 $\log_{10}$ in 52 patients (25.1%), <2 $\log_{10}$ and ≥1 $\log_{10}$ in 23 patients (11.1%), and <1 $\log_{10}$ in 15 patients (7.3%). Figure 1A shows the rate of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy in patients with the TT genotype. The rates were higher significantly in patients who achieved a rapid virologic response or had a ≥3 $\log_{10}$ decrease in

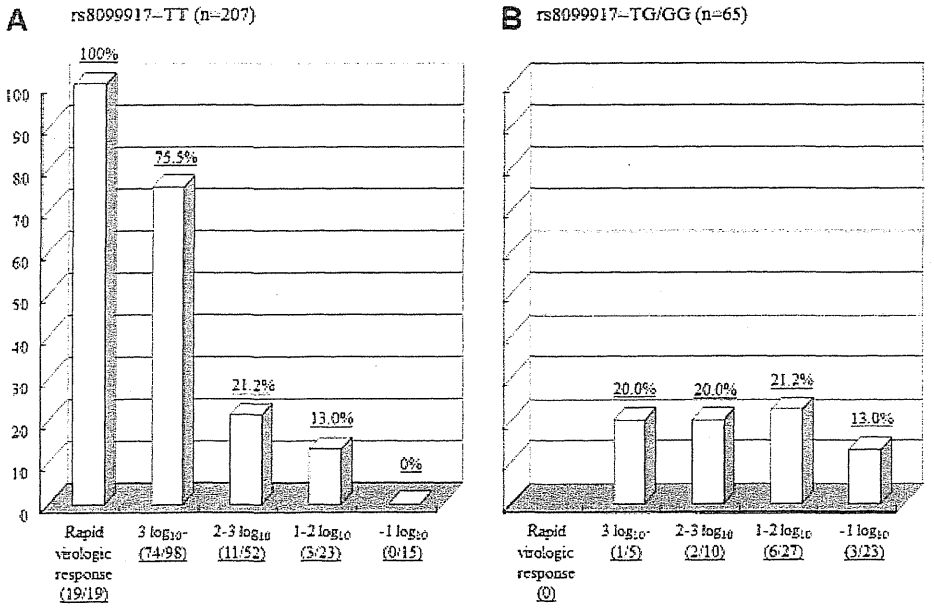

Fig. 1. The rate of sustained virologic responses (%) based on the reduction in serum HCV RNA levels at 4 weeks after starting therapy. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

serum HCV RNA levels at 4 weeks compared to those with a <3 $\log_{10}$ decrease in serum HCV RNA levels ($P < 0.0001$). When a 3 $\log_{10}$ decrease in serum HCV RNA levels was defined as the cut-off point, 56.5% of patients were considered to have a ≥3 $\log_{10}$ decrease in serum HCV RNA levels. The sensitivity, specificity, positive predictive value, and negative predictive value for a sustained virologic response were 86.8, 75.2, 78.6, and 84.4%, respectively.

Among the 65 patients who had the TG/GG genotype, no patient achieved a rapid virologic response at 4 weeks after initiating therapy. The decrease in serum HCV RNA levels at 4 weeks after starting therapy ranged from 0.11 $\log_{10}$ to 4.75 $\log_{10}$ (mean, 1.66 $\log_{10}$). The reduction in serum HCV RNA levels at 4 weeks after starting the therapy were smaller in patients with the TG/GG genotype than those with the TT genotype (1.66 ± 1.02 $\log_{10}$ in patients with the TG/GG genotype vs. 3.12 ± 1.37 $\log_{10}$ in patients with TT genotype excluding RVR, $P < 0.0001$). The reduction in serum HCV RNA levels was ≥3 $\log_{10}$ in five patients (7.7%), <3 $\log_{10}$ and ≥2 $\log_{10}$ in 10 patients (15.4%), <2 $\log_{10}$ and ≥1 $\log_{10}$ in 27 patients (41.5%), and <1 $\log_{10}$ in 23 patients (35.4%). Figure 1B shows the rates of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy in patients with the TG/GG genotype. There were no differences in the rate of a sustained virologic response based on the reduction in HCV RNA levels at 4 weeks after starting therapy; the rate of a sustained virologic response remained at 20% approximately regardless of the reduction in HCV RNA levels in 42 patients with a ≥1 $\log_{10}$ reduction in serum HCV RNA levels.

Association Between an Early Virologic Response at 12 Weeks and Treatment Outcome Based on Genetic Polymorphisms Near the *IL28B* Gene

Figure 2 shows the rate of patients with the TT genotype or TG/GG genotype for rs8099917 who achieved a complete early virologic response, a partial early virologic response, and those who did not achieve early virologic response at 12 weeks after starting therapy based on the reduction in serum HCV RNA level at 4 weeks after initiating therapy. Nearly 75% of patients with the TT genotype whose HCV RNA levels were reduced by ≥3 $\log_{10}$ at 4 weeks after starting the therapy achieved a complete early virologic response. In contrast, 80% of patients with the TG/GG genotype whose HCV RNA levels were reduced by ≥3 $\log_{10}$ at 4 weeks after starting the therapy showed a partial early virologic response. The majority of patients with the TT or TG/GG genotypes achieved a partial early virologic response when their reduction in HCV RNA levels was <3 $\log_{10}$ and ≥2 $\log_{10}$ or <2 $\log_{10}$ and ≥1 $\log_{10}$.

Figure 3 shows the rates of a sustained virologic response according to the type of early virologic response in patients with the TT genotype (Fig. 3A) and TG/GG genotype (Fig. 3B). Among patients with the TT genotype, the rate of sustained virologic response was significantly higher in patients with a complete early virologic response than in those with a partial early virologic response ($P < 0.0001$). In contrast, there was no difference in the rate of a sustained virologic response between patients with a complete early virologic response and those with a partial early virologic response ($P = 0.8917$) among patients with

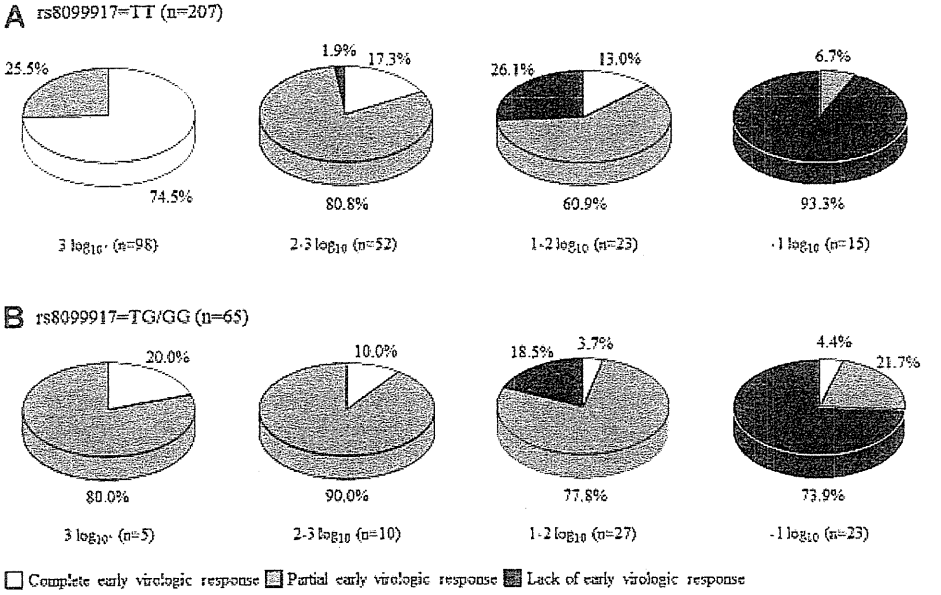

Fig. 2. The association between the virologic responses at 12 weeks after starting therapy and the reduction in serum HCV RNA levels at 4 weeks after starting therapy. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

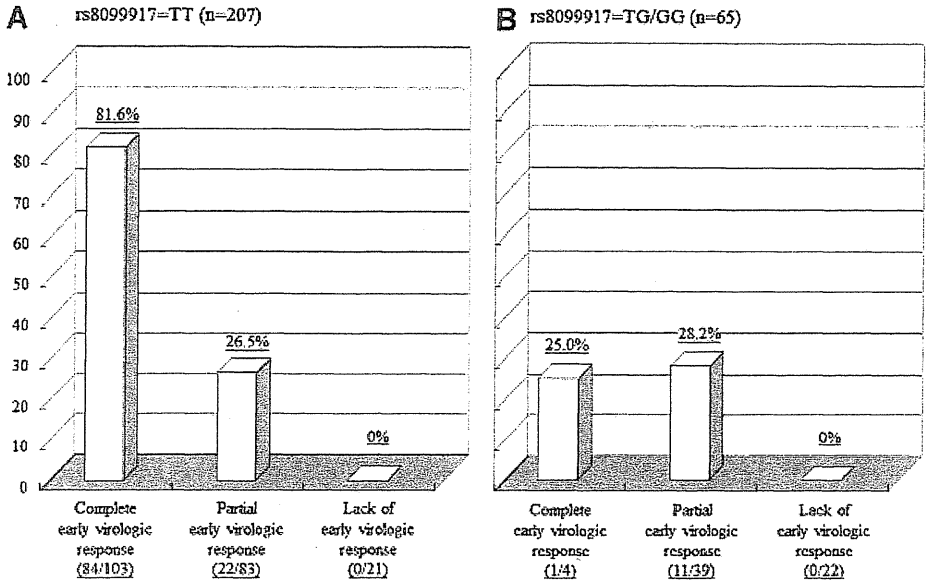

Fig. 3. The rate of sustained virologic responses based on the type of early virologic response. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

the TG/GG genotype. None of the patients with the TT genotype or TG/GG genotype who yielded a lack of an early virologic response reached a sustained virologic response.

Univariate and Multivariate Analyses for Factors Associated With a Sustained Virologic Response to Peginterferon and Ribavirin Combination Therapy in Patients With the TT and the TG/GG Genotype for the rs8099917

Univariate and multivariate analyses were conducted for factors associated with a sustained virologic response based on different genetic polymorphisms near the *IL28B* gene. In patients with the TT genotype, the factors that were associated with a sustained virologic response included serum alkaline phosphatase levels, serum albumin, platelet counts, hepatitis activity grade, liver fibrosis grade, reduction in HCV RNA levels at 4 weeks after starting therapy, and a complete early virologic response based on a univariate analysis (Table IIIA). In a multivariate analysis, the serum albumin levels, reduction in HCV RNA levels 4 weeks after starting therapy, and a complete early virologic response were independent factors that were significantly associated with a sustained virologic response (Table IIIB). A reduction in HCV RNA levels 4 weeks after starting therapy was the strongest factor that affected a sustained virologic response. In patients with the TG/GG genotype, the factors that were associated with a sustained virologic response included patient age, platelet counts, and pretreatment HCV RNA levels based on a univariate analysis (Table IIIA). A reduction in the HCV RNA levels at 4 weeks after starting therapy was not associated with a sustained virologic response. In a multivariate analysis, patient age and pretreatment HCV RNA levels were independent factors that were significantly associated with a sustained virologic response (Table IIIC).

Characteristics of Patients who Achieved a Sustained Virologic Response to the Combination Therapy Despite the Unfavorable TG/GG Genotype Near the *IL28B* Gene

Table IV shows the characteristics of 12 patients who achieved a sustained virologic response despite having the unfavorable TG/GG genotype for rs8099917 near the *IL28B* gene. All but one patient was under 60 years old and had liver fibrosis not more than grade 2 (one patient did not undergo a liver biopsy). Except for one patient, the reduction in the serum HCV RNA levels at 4 weeks after starting therapy was less than 3 $\log_{10}$ and all but one patient showed a partial early virologic response at 12 weeks after starting the therapy. In all 11 patients with a partial early virologic response, the serum HCV RNA was undetectable up to 24 weeks after starting the therapy. All but one patient extended the treatment duration from 48 to 72 weeks (two patients discontinued therapy at 60 weeks during the extended treatment period). When the characteristics of patients who achieved a sustained virologic response were compared between those with the unfavorable TG/GG genotype and those with the favorable TT genotype, patients with the TG/GG genotype were younger (41.8 ± 14.4 years vs. 55.1 ± 10.4 years, $P = 0.0023$) and had lower pretreatment HCV RNA levels (5.91 ± 0.44 $\log_{10}$ IU/ml vs. 6.21 ± 1.05 $\log_{10}$ IU/ml, $P = 0.0199$).

TABLE III. Univariate and Multivariate Analyses for Factors Associated With a Sustained Virologic Response to Peginterferon and Ribavirin Combination Therapy in Patients With the TT and the TG/GG Genotype for the rs8099917

(A) Univariate analyses	*P*-value	
	Patients with TT genotype of rs8099917 (n = 207)	Patients with TG/GG genotype of rs8099917 (n = 65)
Age (years)	0.0505	0.0007
Sex (female/male)	0.1830	0.2296
Body weight (kg)	0.6891	0.2456
Alanine aminotransferase (IU/L)	0.7988	0.4032
Aspartate aminotransferase (IU/L)	0.5021	0.1705
Gamma-glutamyl transpeptidase (IU)	0.6340	0.6648
Alkaline phosphatase (IU/L)	0.0315	0.0599
Albumin (g/dl)	0.0002	0.6594
Total bilirubin (mg/dl)	0.2929	0.7130
White blood cell count (/μl)	0.2508	0.5549
Hemoglobin (g/dl)	0.0847	0.2289
Platelet count ($\times 10^3$/μl)	0.0454	0.0411
Liver histology-activity (A0–1/A2–3)	0.0445	0.1117
Liver histology-fibrosis (F0–1/F2–3)	0.0002	0.2283
Pretreatment HCV RNA concentration ($\geq$6.5 $\log_{10}$ vs. <6.5 $\log_{10}$)	0.5279	0.0379
Reduction in the peginterferon dose	0.4316	0.5563
Reduction in the ribavirin dose	0.1823	0.4272
Reduction in HCV RNA levels at 4 weeks after starting the therapy ($\geq$3 $\log_{10}$ vs. < 3 $\log_{10}$)	<0.0001	0.9265
Early virologic response (complete vs. partial)	<0.0001	0.9777
Early virologic response (partial vs. non)	0.8632	0.0686

(B) Multivariate analyses: Patients with TT genotype of rs8099917	*P*-value	Odds ratio (95% confidence interval)
Alkaline phosphatase (IU/L)	0.2617	
Albumin (g/dl)	0.0365	28.287 (1.4107–755.41)
Platelet count ($\times 10^3$/μl)	0.2599	
Liver histology-activity (A0–1/A2–3)	0.6678	
Liver histology-fibrosis (F0–1/F2–3)	0.2307	
Reduction in HCV RNA levels at 4 weeks after starting the therapy ($\geq$3 $\log_{10}$ vs. <3 $\log_{10}$)	<0.0001	16.029 (6.8593–40.406)
Early virologic response (complete vs. partial)	0.0224	0.3685 (0.1557–0.8749)

(C) Multivariate analyses: Patients with TG/GG genotype of rs8099917	*P*-value	Odds ratio (95% confidence interval)
Age (years)	0.0022	0.0034 (0.0000–0.0840)
Platelet count ($\times 10^3$/μl)	0.3344	
Pretreatment HCV RNA concentration ($\geq$6.5 $\log_{10}$ vs. <6.5 $\log_{10}$)	0.0304	0.0548 (0.0020–0.4950)

HCV, hepatitis C virus.

DISCUSSION

Several previous studies reported that patients who achieved a rapid virologic response, in which serum HCV RNA become undetectable at 4 weeks after starting therapy, had a high likelihood of achieving a sustained virologic response [Martinez-Bauer et al., 2006; Poordad et al., 2008; de Segadas-Soares et al., 2009; Martinot-Peignoux et al., 2009]. In addition, several recent studies reported the predictive value of the degree of reduction in serum HCV RNA levels at 4 weeks after starting therapy [Yu et al., 2007; Huang et al., 2010; Toyoda et al., 2011]. Therefore, the viral dynamics of HCV at 4 as well as 12 weeks after starting therapy is important for response-guided therapy.

Genetic polymorphisms near the *IL28B* gene have emerged as the strongest predictive factor of a sustained virologic response in patients infected with HCV genotype 1 [Hayes et al., 2011; Kurosaki et al., 2011]. In addition, Thompson et al. [2010 reported that genetic polymorphisms near the *IL28B* gene were associated strongly with early viral dynamics during PEG-IFN and ribavirin combination therapy. These findings raised an important issue of whether response-guided therapy, based on the reduction in serum HCV RNA levels at 4 or 12 weeks after starting

TABLE IV. Patients who Achieved a Sustained Virologic Response Despite the TG/GG Genotype for the rs8099917

	Age (years)	Sex	Liver histology	Pretreatment HCV RNA level (log_{10} IU/ml)	HCV RNA reduction at 4 weeks	Response at 12 weeks	HCV RNA became undetectable (weeks)	Treatment duration (weeks)
1.	31	Female	A1/F1	6.13	2.19	partial EVR	20	48
2.	55	Male	A1/F1	5.80	1.77	partial EVR	16	72
3.	57	Female	A1/F1	5.58	3.01	partial EVR	16	72
4.	57	Female	A1/F1	6.21	1.81	partial EVR	20	72
5.	62	Male	N.D.	6.23	1.13	partial EVR	24	72
6.	21	Male	A1/F2	6.04	1.83	partial EVR	24	72
7.	42	Male	A1/F1	6.27	0.57	partial EVR	24	72
8.	29	Female	A1/F2	5.83	1.83	partial EVR	20	60
9.	52	Male	A1/F0	5.91	2.12	complete EVR	12	48
10.	40	Male	A2/F1	5.84	1.34	partial EVR	20	72
11.	27	Male	N.D.	5.63	0.42	partial EVR	24	72
12.	28	Male	A1/F0	6.59	0.76	partial EVR	20	60

N.D., not done; HCV, hepatitis C virus; EVR, early virologic response.

therapy, retains a predictive value when considering genetic polymorphisms near the *IL28B* gene.

In the present study, the predictive value of the decrease in serum HCV RNA levels was evaluated at 4 and 12 weeks after starting therapy in Japanese patients infected with HCV genotype 1b based on genetic polymorphisms near the *IL28B* gene. Consistent with previous reports, patients with the TG/GG genotype for rs8099917 had a smaller reduction in serum HCV RNA levels at 4 weeks after starting treatment ($P < 0.0001$), which indicates an unfavorable response to the combination therapy. Patients with the TT genotype for rs8099917, which is associated with a favorable response to the combination therapy, exhibited a significant difference in the rate of a sustained virologic response based on the reduction in serum HCV RNA levels at 4 weeks after initiating the therapy. Patients with a rapid virologic response or with a ≥ 3 log_{10} reduction in HCV RNA levels had a higher likelihood of achieving a sustained virologic response.

In contrast, these factors did not have any predictive value in patients with the TG/GG genotype. Only 18.5% of patients achieved a sustained virologic response (12 of 65 patients), and it was difficult to identify these patients based on the reduction in HCV RNA levels at 4 weeks or the type of an early virologic response at 12 weeks after starting therapy. Patients who achieved a sustained virologic response, despite the TG/GG genotype for rs8099917, were identified among those with a <2 log_{10} and ≥ 1 log_{10} or even <1 log_{10} reduction in HCV RNA levels at 4 weeks after starting therapy. Interestingly and paradoxically, the possibility of a sustained virologic response can be expected in patients with a <1 log_{10} reduction in HCV RNA levels at 4 weeks after starting therapy only when they have the unfavorable TG/GG genotype.

In the evaluation at 12 weeks after starting therapy, patients with the TT genotype who achieved a complete early virologic response had a higher rate of a sustained virologic response significantly than patients who achieved a partial early virologic response, whereas this difference was not found in patients with the TG/GG genotype. No patients who failed to achieve an early virologic response achieved a sustained virologic response regardless of the genetic polymorphisms near the *IL28B* gene. Thus, the lack of an early virologic response retained a strong predictive value for the failure of achieving a sustained virologic response. This result supports the recommendation in the AASLD guidelines, in which treatment may be discontinued in patients without an early virologic response at 12 weeks of treatment.

The characteristics of patients who achieved a sustained virologic response despite the unfavorable TG/GG genotype were younger in age and lower pretreatment HCV RNA levels. Most patients with the TG/GG genotype who achieved a sustained virologic response showed a partial early virologic response and extended the treatment duration. It was difficult to identify these patients according to viral dynamics at 4 or 12 weeks after starting therapy.

There are several limitations in this study. Some patients with a slow virologic response did not have their treatment period extended from 48 to 72 weeks. This is because the effectiveness of a 72-week combination therapy regimen in patients with HCV genotype 1 with a slow virologic response [Berg et al., 2006; Pearlman et al., 2007] had not been established in Japan in the earlier part of this study. This fact might have influenced the treatment outcome especially in patients with the unfavorable TG/GG genotype. Another limitation is a smaller sample size of patients with the TG/GG genotype in comparison to that of patients with the TT genotype. This sample size could have caused the lack of statistical significance in the rate of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy or according to the type of an early virologic response in patients with the TG/GG genotype. In addition, the data were based on Japanese patients infected with HCV genotype 1b. Therefore, these results should be confirmed in other ethnicities and patients infected with HCV genotype 1a.

In conclusion, among patients infected with HCV genotype 1b with the TT genotype for rs8099917, a rapid virologic response or a ≥ 3 $\log_{10}$ reduction in HCV RNA levels at 4 weeks after starting therapy, or a complete early virologic response indicate strongly that these patients will achieve a sustained virologic response as a final outcome for PEG-IFN and ribavirin combination therapy. Early viral dynamics retain the predictive value in this patient subpopulation. A reduction in HCV RNA levels at 4 weeks after starting therapy or the type of an early virologic response does not predict the likelihood that patients with the TG/GG genotype will achieve a sustained virologic response. In contrast, the lack of an early virologic response retains a strong predictive value for the failure to achieve a sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy.

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HEPATOLOGY

Clinical impact of HFE mutations in Japanese patients with chronic hepatitis C

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Abbreviations
CHC, chronic hepatitis C; HCV, hepatitis C virus; HH, hereditary hemochromatosis; IFN, interferon; IL28B, interleukin 28B; PCR, polymerase chain reaction; PEG-IFN, pegylated-interferon-alpha 2b; RBV, ribavirin; SNP, single-nucleotide polymorphism; SVR, sustained virological response.

Abstract

Background and Aim: HFE mutations, a common cause of hereditary hemochromatosis (HH), are reportedly associated with hepatic iron overload, severe liver fibrosis, and good response to interferon treatment in European patients with chronic hepatitis C (CHC). HH shows ethnicity-based differences and little is known about the effects of HH mutations on CHC in the Japanese. Thus, the aim of this study was to clarify the clinical influence of HFE mutations in Japanese CHC patients.

Methods: In a total of 251 patients with CHC, we analyzed the frequencies of H63D and S65C mutations in the HFE gene, and the influence of these mutations on clinical parameters and response to pegylated-interferon-alpha 2b (PEG-IFN) plus ribavirin therapy.

Results: Fourteen patients (5.6%) carried the H63D mutation; all were heterozygotes. No S65C mutations were found. Only hemoglobin levels in the H63D heterozygotes were higher than in wild-type patients. Eleven of 14 H63D heterozygotes achieved sustained virological response (SVR). On univariate analysis, factors associated with SVR were interleukin 28B (IL28B) polymorphism, age, hepatitis C virus (HCV) genotype, HCV viral load, white blood cell count, stage of fibrosis and H63D mutation. All patients with both TT genotype in IL28B (rs8099917) and H63D mutation in HFE ($n = 10$) achieved SVR.

Conclusions: The H63D mutation has little impact on the clinical characteristics of CHC, but is related to favorable response to PEG-IFN plus ribavirin therapy, particularly in patients with the TT allele in IL28B.

Introduction

Hepatitis C virus (HCV) infection is a significant global health problem, affecting 170 million individuals worldwide. HCV infection causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma. Elevated hepatic iron concentration has often been found in patients with chronic hepatitis C (CHC),[1] and this excess iron increases oxidative stress, which can accelerate the progression of fibrosis[2] and may promote hepatic carcinogenesis.[3] Moreover, hepatic iron accumulation is thought to lower the response rate to interferon (IFN)-based therapy in patients with CHC.[4–8]

HFE mutations are the major gene variations in hereditary hemochromatosis (HH),[9] which is a common autosomal recessive disorder associated with iron overload in Caucasians.[10] Therefore, there has been much interest in the roles of HFE mutations in patients with HCV infection. Several studies have been performed in order to assess the correlations among HFE mutations, hepatic iron overload and disease progression in CHC. However, the effects of HFE mutations on hepatic iron concentration and disease severity remain controversial.[11–18] On the other hand, the presence of HFE mutations was reported to be associated with good response to IFN therapy.[11,19] As the prevalence of HFE mutations is lower in Asian populations than in Caucasian populations,[20] most studies on HFE mutations have been conducted in Western countries, with only one small study being conducted in an Asian country.[21] Clarifying the effects of these mutations on iron loading and clinical features in Asian patients may help to further understand the role of HFE mutations in HCV-infected patients.

The aim of this study was to examine the influence of HFE gene variants on iron overload and clinical characteristics, and to investigate whether HFE mutations affect response to pegylated-interferon-alpha 2b (PEG-IFN) plus ribavirin (RBV) therapy in Japanese CHC patients.

Methods

Patients. A total of 251 Japanese patients infected with HCV and being treated at Nagoya University Hospital were enrolled in this retrospective study. Patients included 143 men and 108 women with a mean age of 53.8 ± 12.3 years. Exclusion criteria