Association of Amino Acid Substitution Pattern in Core Protein of Hepatitis C Virus Genotype 2a High Viral Load and Virological Response to Interferon-Ribavirin Combination Therapy

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Key Words

Hepatitis C virus • Genotype 2a • Core region • Interferon • Ribavirin • Rapid response

Abstract

Background: Substitution of amino acids (aa) 70 and 91 in the core region of HCV genotype 1b is a useful pretreatment predictor of poor response to interferon + ribavirin combination therapy, but the impacts of aa substitutions in the core region of HCV genotype 2a are still not clear. **Methods:** 154 consecutive Japanese adults with a high viral load (≥ 100 kIU/ml) of genotype 2a who could complete combination therapy for 24 weeks were evaluated. To examine the differences in virological characteristics between non-sustained virological response (non-SVR) and rapid responder (SVR patients who could achieve a HCV-RNA-negative status within 8 weeks), 86 patients could be analyzed by pretreatment substitution patterns of the core region. **Results:** SVR was achieved in 127 of 154 patients (83%), and rapid response in 113 of 127 (90%). In all 154 patients, multivariate analysis identified younger age, lower level of viremia, and higher level of albumin as significant determinants of SVR. As sig-

nificant determinants of rapid response in 86 patients, multivariate analysis identified substitution of aa 4 (non-asparagine) in addition to the significant determinants of SVR. **Conclusions:** Our results suggest that the aa substitution pattern of the core region in patients with a high titer of genotype 2a may partly affect the virological response to combination therapy.

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Introduction

The response to interferon (IFN)-related therapy varies according to hepatitis C virus (HCV) genotype [1, 2]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b, and about 25% with genotype 2a [3]. Sustained virological response (SVR) to 48-week IFN + ribavirin combination therapy is about 50% in genotype 1b infection, and SVR to 24-week combination therapy is more than 80% in genotype 2 infection [4–9].

IFN + ribavirin combination therapy carries potential serious side effects and is costly, especially when used

long enough to achieve a high SVR. For these reasons, especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (≤ 16 weeks) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. Furthermore, we also sometimes encounter treatment-resistant patients infected with genotype 2a [3, 10]. The underlying mechanism(s) of the different virological responses to treatment in patients infected with genotype 2a is still unclear. Hence, the pretreatment predictors of the efficacy of IFN + ribavirin combination therapy were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of infected patients with genotype 1b and a high viral load (≥ 100 kIU/ml) are predictors of poor virological response to 48- and 72-week pegylated IFN (PEG-IFN) + ribavirin combination therapies [11–15], and also affect clinical outcomes, including insulin resistance and hepatocarcinogenesis [16–18]. However, it is unknown whether the aa substitutions of the core region in patients infected with genotype 2a who have high viral load might also be as useful as the pretreatment virological predictive factors apart from the genotype and viral load.

The present study included 154 Japanese adults with genotype 2a and a high viral load, who received combination therapy for 24 weeks. The aim of the study was to investigate the treatment efficacy and pretreatment predictive factors including virological features.

Materials and Methods

Study Population

A total of 190 HCV genotype 2a-infected Japanese adult patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFN α -2b or IFN α -2b) + ribavirin for 24 weeks between March 2002 and February 2008 at Toranomon Hospital, Tokyo, Japan. Among these, 154 patients, who could complete a total of 24 weeks of combination therapy, were enrolled in this retrospective study and fulfilled the following criteria: (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV-RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Calif., USA). (2) They were naive to ribavirin therapy. (3) They were infected with HCV genotype 2a alone. (4) Each had a high viral load (≥ 100 kIU/ml) by quantitative analysis of HCV-RNA with PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc., Pleasanton, Calif., USA) within the preceding 2 months of enrolment. (5) They had no hepatocellular carcinoma. (6) Their body weight was >40 kg. (7) All

were free of coinfection with human immunodeficiency virus. (8) None had been treated with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (9) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (10) None had other forms of hepatitis, such as hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (11) None of the females were pregnant or lactating mothers. (12) All patients had completed a 24-week follow-up program after cessation of treatment, and SVR could be evaluated. (13) Each signed a consent form of the study protocol that had been approved by the Human Ethics Review Committee.

Table 1 summarizes the profiles and data of the 154 patients at the commencement of combination therapy with IFN + ribavirin. The study included 92 men and 62 women, aged 20–70 years (median 53). In all patients, the total duration of treatment was 24 weeks. In 43 of the 154 (27.9%) patients, the dose of ribavirin was reduced during treatment due to a fall in Hb concentration.

With regard to the treatment protocol, 104 (67.5%) patients received PEG-IFN α -2b + ribavirin for 24 weeks, and the remaining 50 (32.5%) patients received IFN α -2b + ribavirin for 24 weeks. They received PEG-IFN α -2b at a median dose of 1.5 μ g/kg (range 1.0–1.8) subcutaneously each week, or IFN α -2b at a median dose of 6 million units (range 3–6) intramuscularly each day (7 times per week for the initial 2 weeks, followed by 3 times per week for 22 weeks). They also received oral ribavirin at a median dose of 11.2 mg/kg (range 5.4–14.1) daily.

The treatment efficacy was evaluated by HCV-RNA positivity based on qualitative PCR analysis at the end of treatment (non-virological response; NVR), and by HCV-RNA negativity based on qualitative PCR analysis at 24 weeks after the completion of therapy (SVR). Furthermore, rapid responders were defined as SVR patients who could achieve a negative status within 8 weeks after the start of treatment, based on qualitative PCR analysis.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase (ALT) and HCV-RNA levels. The serum samples were frozen at -80° within 4 h of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region [19]. HCV-RNA levels were measured by quantitative PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5–5,000 kIU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA (<5 kIU/ml) were checked by qualitative PCR (Amplicor HCV v2.0, Roche Molecular Systems, Inc.), which has a higher sensitivity than quantitative analysis, and the results were expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens

Table 1. Profile and laboratory data of 154 patients infected with HCV genotype 2a

Demographic data	
Number of patients	154
Sex, M/F	92/62
Age, years*	53 (20–70)
History of blood transfusion	43 (27.9%)
Family history of liver disease	26 (16.9%)
Body mass index*	22.7 (17.9–31.8)
Laboratory data*	
Serum aspartate aminotransferase, IU/l	43 (7–404)
Serum alanine aminotransferase, IU/l	54 (8–651)
Serum albumin, g/dl	3.9 (3.2–4.7)
γ -Glutamyl transpeptidase, IU/l	38 (9–406)
Leukocytes, /mm ³	4,800 (2,200–9,000)
Hemoglobin, g/dl	14.4 (9.9–17.8)
Platelet count, $\times 10^4$ /mm ³	17.9 (6.1–32.9)
Indocyanine green retention rate at 15 min, %	13 (4–35)
Serum iron, μ g/dl	136 (22–336)
Serum ferritin, μ g/l	124 (<10–820)
Level of viremia, kIU/ml	720 (5–>5,000)
α -Fetoprotein, μ g/l	4 (2–103)
Total cholesterol, mg/dl	174 (107–275)
High-density lipoprotein cholesterol, mg/dl	47 (15–109)
Low-density lipoprotein cholesterol, mg/dl	105 (48–201)
Triglycerides, mg/dl	98 (36–449)
Uric acid, mg/dl	5.6 (2.5–9.4)
Fasting plasma glucose, mg/dl	93 (75–187)
Histological findings	
Stage of fibrosis (F1/F2/F3/ND)	58/23/16/57
Grade of activity (A1/A2/ND)	57/40/57
Hepatocyte steatosis (<5% (absent)/ $\geq$ 5% (present)/ND)	35/52/67
Treatment	
PEG-IFN α -2b/IFN α -2b	104/50
Ribavirin dose, mg/kg*	11.2 (5.4–14.1)
Total duration of treatment, weeks	24
Past history of IFN monotherapy	56 (36.4%)

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

for examinations contained ≥ 6 portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H. K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histological assessment according to the scoring system of Desmet et al. [20].

Nucleotide Sequencing of the Core Region

We determined the sequences of aa 1–191 in the core region by the direct sequencing method using pretreatment sera of patients who could be analyzed due to adequate serum samples obtained at the start of combination treatment. These sequences were compared with the consensus sequences of genotype 2a, which were determined by comparing the sequences obtained in this study and prototype sequence (HCV J6) [21]. HCV-RNA was extracted from serum samples at the start of treatment and reverse tran-

scribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by nested PCR using the following primers. Nucleotide sequences of the core region: the first-round PCR was performed with 2ACF5 (sense, 5'-GCA AGA CTG CTA GCC GAG TA-3') and 2ACR6 (antisense, 5'-ATC TGA GCT GCG AGC ATC AC-3') primers, and the second-round PCR with 2ACF3N (sense, 5'-CCT TGT GGT ACT GCC TGA TA-3') and 2ACR8 (antisense, 5'-CCA GGT GAT GCT GTC ATT AG-3') primers. All samples were initially denatured at 95° for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 s at 95°, annealing of primers for 30 s at 55°, and extension for 1 min at 72° with an additional 7 min for extension. Then 1 μ l 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 prim-

Table 2. Factors associated with sustained virological response to combination therapy with IFN + ribavirin for 24 weeks in 154 patients infected with HCV genotype 2a, identified by multivariate analysis

Factor	Category	Odds ratio (95% CI)	p
Age, years	1: ≥50	1	0.005
	2: <50	6.37 (1.76–23.3)	
Serum albumin, g/dl	1: <3.9	1	0.024
	2: ≥3.9	3.19 (1.17–8.73)	
Level of viremia, kIU/ml	1: ≥1,000	1	0.030
	2: <1,000	2.86 (1.11–7.41)	

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

ers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo, Japan).

To avoid false-positive results, the procedures recommended by Kwok and Higuchi [22] to prevent contamination were strictly applied to these PCR assays. No false-positive results were observed in this study.

Statistical Analysis

Non-parametric tests were used to analyze the aa substitutions in HCV core between the groups, including the Mann-Whitney U test, χ^2 test and Fisher's exact probability test. Uni- and multivariate logistic regression analyses were used to determine the factors that significantly contributed to SVR and rapid response. We also calculated the odds ratios and 95% confidence intervals (CI). All p values < 0.05 calculated by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with SVR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), ALT, albumin, γ -glutamyl transpeptidase (γ GTP), leukocyte count, hemoglobin, platelets, indocyanine green retention rate at 15 min (ICG R15), iron, ferritin, level of viremia, α -fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting blood glucose, type of IFN, ribavirin dose/body weight, and past history of IFN monotherapy. Furthermore, in addition to potential predictive factors associated with SVR, potential predictive factors associated with a rapid response also included aa substitution in the core region. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, Ill., USA).

Table 3. Amino acid substitutions in the core region in non-SVR and rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a

	Non-SVR (n = 25)	Rapid response (n = 61)	p*
Presence of substitution site			
aa 4	1 (4.0%)	15 (24.6%)	0.032
aa 23	2 (8.0%)	0 (0%)	0.082
aa 70	1 (4.0%)	0 (0%)	NS
aa 91	0 (0%)	4 (6.6%)	NS
aa 110	11 (44.0%)	34 (55.7%)	NS

* Non-SVR vs. rapid response (Fisher's exact probability test; statistical significance ($p < 0.05$), marginal significance ($p < 0.10$)). aa = Amino acid; SVR = sustained virological response; NS = not significant.

Results

Virological Response Rates to Combination Therapy

The virological response could be evaluated in all 154 patients. SVR was achieved in 127 of 154 (82.5%) patients, and rapid response in 113 of 127 (90.0%). Only 5 of 154 (3.2%) patients were considered NVR.

Predictive Factors Associated with SVR in Multivariate Analysis

We then analyzed the data of all 154 patients to determine those factors that could predict SVR. Univariate analysis identified 5 parameters associated with SVR that achieved statistical significance or marginal significance. These included age (< 50 years; $p < 0.001$), serum albumin (≥ 3.9 g/dl; $p = 0.003$), level of viremia ($< 1,000$ kIU/ml; $p = 0.049$), history of blood transfusion (absent; $p = 0.064$), and ALT (≥ 30 IU/l; $p = 0.088$).

Multivariate analysis identified 3 parameters that independently influenced SVR, including age (< 50 years; $p = 0.005$), serum albumin (≥ 3.9 g/dl; $p = 0.024$), and level of viremia ($< 1,000$ kIU/ml; $p = 0.030$) (table 2).

Fig. 1. Sequences of aa 1–30 and aa 61–110 in the core region at the commencement of combination therapy in 86 patients infected with high HCV viral load genotype 2a. Dashes indicate aa identical to the consensus sequence of genotype 2a, and substituted aa are shown by standard single-letter codes. The aa patterns at positions that are probably associated with sensitivity to therapy are shown in boldface characters. NSR = Non-SVR; RR = rapid response.

Consensus	10	20	30	70	80	90	100	110	Efficacy
HCJ6	MSTNPKPQRK	TKRNTNRRPQ	DVKFPGGGQI//RRQPIPKDRR	STGKSWGKFG	YPWPLYGNEG	LGWAGWLLSP	RGSRPSWGPT		
Case 1									
2									NSR
3									NSR
4									NSR
5									NSR
6									NSR
7	T			R-H					NSR
8									NSR
9			R						NSR
10				H			H		NSR
11									NSR
12									NSR
13									NSR
14									NSR
15									NSR
16									NSR
17			R						NSR
18									NSR
19									NSR
20		SN							NSR
21									NSR
22	Q	I		H	P		Q		NSR
23									NSR
24									NSR
25									NSR
26									NSR
27									NSR
28	IS	H							NSR
29									NSR
30									NSR
31	Q	Q			R				NSR
32									NSR
33	V								NSR
34	I				T				NSR
35									NSR
36									NSR
37				SQ	S-R				NSR
38									NSR
39									NSR
40	D			H					NSR
41									NSR
42	I	I							NSR
43									NSR
44		I							NSR
45				H	P				NSR
46									NSR
47					R-R				NSR
48	Y	Q			P-R				NSR
49	I			RV		F		G	NSR
50									NSR
51									NSR
52			R						NSR
53									NSR
54									NSR
55	D								NSR
56		I					C	Y	NSR
57									NSR
58		H							NSR
59									NSR
60								N	NSR
61	I			R					NSR
62									NSR
63									NSR
64	T	I	Y	N	P				NSR
65									NSR
66	D	I							NSR
67									NSR
68					R				NSR
69									NSR
70									NSR
71									NSR
72									NSR
73	I								NSR
74		Q		H					NSR
75									NSR
76	D								NSR
77								N	NSR
78									NSR
79									NSR
80	I				R-R				NSR
81							C		NSR
82	I				P				NSR
83		H		Q					NSR
84									NSR
85					A				NSR
86	T						C		NSR

Table 4. Patient profile and laboratory data of non-SVR and rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a, who could be analyzed by the nucleotide sequences of the core region

	Non-SVR	Rapid response	p ^a
Demographic data			
Number of patients	25	61	
Sex, M/F	12/13	39/22	NS
Age, years*	58 (34–64)	51 (20–66)	0.006
History of blood transfusion	10 (40.0%)	15 (24.6%)	NS
Family history of liver disease	4 (16.0%)	10 (16.4%)	NS
Body mass index*	23.1 (19.5–30.0)	22.7 (17.9–31.1)	NS
Laboratory data*			
Serum aspartate aminotransferase, IU/l	31 (19–200)	42 (7–125)	NS
Serum alanine aminotransferase, IU/l	44 (14–357)	53 (8–280)	NS
Serum albumin, g/dl	3.8 (3.2–4.2)	3.9 (3.2–4.5)	0.005
γ-Glutamyl transpeptidase, IU/l	38 (14–141)	39 (10–406)	NS
Leukocytes, /mm ³	4,600 (3,100–8,000)	4,800 (2,400–9,000)	NS
Hemoglobin, g/dl	14.2 (12.5–17.8)	14.7 (11.1–17.2)	NS
Platelet count, × 10 ⁴ /mm ³	16.3 (8.0–32.9)	18.0 (10.6–30.6)	NS
Indocyanine green retention rate at 15 min, %	16 (5–26)	12 (6–35)	NS
Serum iron, μg/dl	152 (30–284)	144 (26–304)	NS
Serum ferritin, μg/l	133 (16–756)	123 (10–820)	NS
Level of viremia, kIU/ml	1,200 (93–>5,000)	680 (5–4,600)	0.053
α-Fetoprotein, μg/l	5 (2–103)	5 (2–48)	NS
Total cholesterol, mg/dl	172 (117–236)	184 (137–264)	NS
High-density lipoprotein cholesterol, mg/dl	49 (27–82)	49 (15–101)	NS
Low-density lipoprotein cholesterol, mg/dl	102 (48–150)	109 (73–198)	NS
Triglycerides, mg/dl	108 (55–276)	97 (39–418)	NS
Uric acid, mg/dl	5.2 (3.2–8.7)	5.7 (2.5–8.7)	NS
Fasting blood glucose, mg/dl	92 (80–126)	93 (77–109)	NS
Treatment			
Ribavirin dose, mg/kg*	11.1 (8.0–12.9)	11.2 (7.3–14.0)	NS
Past history of IFN monotherapy	9 (36.0%)	19 (31.1%)	NS

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

^a Non-SVR vs. rapid responder (Mann-Whitney U test or χ^2 test; statistical significance ($p < 0.05$), marginal significance ($p < 0.10$)). SVR = Sustained virological response; NS = not significant.

Treatment Efficacy according to Substitution Patterns in Amino Acids of the HCV Core Region

To examine the differences in virological characteristics between non-SVR and rapid response, 86 patients (25 of 27 non-SVR patients and 61 of 113 rapid responders) could be analyzed by the nucleotide sequences of HCV core region due to adequate serum samples obtained at the start of combination treatment. Figure 1 shows the sequences of aa 1–30 and aa 61–110 of the core region in 86 patients at the commencement of combination therapy. Substitutions at aa 4 (non-asparagine) of HCV core were significantly more frequent in rapid response ($n = 15$, 24.6%) than non-SVR ($n = 1$, 4.0%) patients ($p = 0.032$).

Inversely, substitutions at aa 23 (non-lysine) were more frequent in non-SVR ($n = 2$, 8.0%) than rapid response ($n = 0$, 0%) patients ($p = 0.082$). There were no significant differences in the other substitution sites, including aa 70, aa 91, and aa 110 of the previous report [11], concerning the treatment efficacy of rapid response and non-SVR (table 3).

Predictive Factors Associated with Rapid Response in Multivariate Analysis

We then evaluated the data of all 86 patients who could be analyzed by the nucleotide sequences of core region to determine those factors that could predict rapid re-

Table 5. Factors associated with rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a, identified by multivariate analysis

Factor	Category	Odds ratio (95% CI)	p
Age, years	1: ≥ 50	1	0.006
	2: < 50	7.46 (1.79–31.3)	
Level of viremia kIU/ml	1: $\geq 1,000$	1	0.013
	2: $< 1,000$	4.33 (1.36–13.9)	
Substitution of aa 4	1: asparagine	1	0.039
	2: non-asparagine	9.97 (1.12–89.0)	

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

sponse. Univariate analysis identified 5 parameters associated with rapid response that achieved statistical significance or marginal significance. As potential predictors of rapid response, table 4 indicates age (< 50 years; $p = 0.006$), serum albumin (≥ 3.9 g/dl; $p = 0.005$), and level of viremia ($< 1,000$ kIU/ml; $p = 0.053$). Furthermore, table 3 shows aa substitution of the core region in the pre-treatment sample (substitution of aa 4 [non-asparagine], $p = 0.032$, and aa 23 [lysine], $p = 0.082$).

Multivariate analysis identified 3 parameters that independently influenced rapid response, including age (< 50 years; $p = 0.006$), level of viremia ($< 1,000$ kIU/ml; $p = 0.013$), and substitution of aa 4 (non-asparagine; $p = 0.039$) (table 5).

Discussion

Previous reports indicated that viral factors (e.g., viral load, aa substitutions in the NS5A region, early viral kinetics, and periods from the start of treatment to initial point of undetectable HCV-RNA) and host factors (e.g., body mass index, fibrosis stage, and level of soluble interleukin-2 receptor) might be important predictors of treatment response to 24-week IFN + ribavirin combination therapy in patients infected with HCV genotype 2a, in addition to treatment-related factors (e.g., treatment duration and ribavirin dose) [6–9, 23–28]. Using multivariate analysis, the present study identified viral- (viral load and substitution of aa 4) and host-related factors (age and serum albumin levels as surrogate markers of liver fibrosis [3, 11]) that influenced the virological response to 24-week combination therapy in patients with genotype 2a

infection and a high viral load. IFN + ribavirin combination therapy carries potential serious side effects and is costly, especially when used long enough to achieve a high SVR. For these reasons, especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (≤ 16 weeks) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. Identification of these viral and host factors before the start of combination therapy should help design better therapeutic regimens.

Amino acid substitutions at position 70 and/or 91 in the core region of patients with genotype 1b infection and a high viral load are predictors of poor virological response to 48- and 72-week PEG-IFN + ribavirin combination therapy [11–15] and also affect clinical outcomes, including insulin resistance and hepatocarcinogenesis [16–18]. This study, based on 24-week combination therapy in patients with genotype 2a infection and a high viral load, identified substitution of aa 4 in the core region as the significant determinant of treatment efficacy, but did not identify substitutions of aa 70, aa 91, and aa 110. These discrepant findings might be due to the difference of genotype and treatment duration. Other mechanisms could be also explained by the small number of NVR patients with genotype 2a (only 3%), compared with about 25% of patients infected with genotype 1b [11]. Previous studies reported that the core region might be associated with resistance to IFN monotherapy involving the Jak-STAT signaling cascade [29–32]. The present result could also be interpreted to mean that aa substitutions in the core region are associated with those proteins involved in resistance to IFN monotherapy, such as SOCS proteins, which are known to inhibit IFN- α -induced activation of the Jak-STAT pathway and expression of the antiviral proteins 2',5'-OAS and MxA [33]. Furthermore, this result also indicates that aa substitutions in the core region might serve as surrogate markers for other proteins associated with resistance to the antiviral actions of IFN. One limitation of this study based on the small number of patients was that only the nucleotide sequences of rapid response within SVR patients were analyzed, although all of SVR patients should have been investigated (e.g., possible type II error). Further large-scale studies that examine the structural and functional impacts of aa substitutions during combination therapy should be conducted to confirm the above findings.

In conclusion, our results suggest that the aa substitution pattern in the core region in patients with a high viral load of HCV genotype 2a may partly affect the viro-

logical response to combination therapy. The limitations of this study were that it did not investigate other viral factors, such as the number of substitutions in aa 2193–2228 (the region corresponding to the IFN sensitivity-determining region [ISDR] of genotype 1b [34–36]) or aa 2163–2228 of NS5A in genotype 2a [10, 26, 37], the geographic diversities of the genotype 2a core region (distribution of consensus sequence), and other races apart from Asians in Japan. Further prospective studies, matched for aa substitutions of the core region and large

groups of patients of different races, are required to determine the virological response to 24-week IFN + ribavirin combination therapy in patients with a high viral load of HCV genotype 2a.

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