



図8 IL28B SNPによって認められるHCV各アミノ酸配列の違い(N=85)

形成すると考えられるが、ヒトのIL28B遺伝子多型がウイルス感染によって変化するとは考えにくいことから、ウイルスのコア70番アミノ酸配列が宿主遺伝子によって規定されている可能性が強く示唆された。

#### 多変量解析による治療効果と 関連する宿主・ウイルス因子の抽出

HCV全長解析を用いた解析により、PEG-IFN/RBV治療においてISDR, コア, IRRDRなどの各ウイルス因子は治療反応性と関連する重要な領域であることが示されたが、これらの因子に加えて、従来より治療効果と関連するとして知られている年齢、線維化、血小板などの臨床因子を含め、最終治療効果と関連する因子について、さらに多くの症例を対象に単変量・多変量解析を行った(表1)。単変量では年齢、BMI、F因子、 $\gamma$ GTP、血小板、alpha-fetoprotein (AFP)、ウイルス量、ISDR、IRRDR、コア70番、IL28Bなど多数の因子が関連する因子として示される。しかしこれらのなかで、多変量解析において、年齢、ISDR変異数、IRRDR変異数、IL28B多型が治療効果に独立して関連する因子として最終的に抽出された。特に最も高いオッズ比を示す因子として、IL28BとIRRDRの2つが最も治療

効果と関連することが示された。

#### おわりに

C型慢性肝炎の治療成績は来年以降のプロテアーゼ阻害薬の市場導入によって、現在のPEG-IFN/RBV時代からさらに大幅に改善されることが期待される。一方で、新しい治療では、貧血、皮疹など副作用の増加も懸念され、現行治療の効果を正確に予測し、どのような症例に新しい治療を導入するのかを決定していくことが臨床の場において非常に重要な問題である。

今回われわれはゲノタイプ1bのC型慢性肝炎にてHCV全翻訳領域解析を用い、PEG-IFN/RBV治療においてISDR, コア, IRRDRがそれぞれ異なるウイルス反応性を呈する集団のサブ解析にて抽出されることを明らかとした。これらの領域は治療効果と関連する部位としてそれぞれが報告されていたが<sup>2)7)8)</sup>、治療反応性のどの部分に重要なのか不明であった。本解析によってそのことが明らかになり、一方で近年報告されたIL28BSNPとの強い関連性も示され、宿主とウイルスの重要な相互関係が明らかとなった。これらの事実は、臨床上治療効果を予測する上で非常に重要であるが、のみならずウイルス増殖のメカニズム解明の上で基礎的に重要な事実を含

表 1 PEG-IFN+RBV療法(標準投与期間)のSVRに寄与する因子(n=292)

		単変量解析			多変量ロジスティック回帰分析		
		odds比	95%CI	P	odds比	95%CI	P
年齢	<60/≥60	2.25	1.38~3.68	0.0012	10.3	2.2~47.6	0.0030
性別	M/F	0.647	0.404~1.038	0.0709			
BMI	<23/≥23	2.019	1.161~3.509	0.0127			
F因子	0~1/2~4	0.371	0.216~0.639	0.0003			
A因子	0~1/2~3	0.600	0.348~1.034	0.0657			
Alb	<4.1/≥4.1	1.017	0.598~1.730	0.9503			
γGTP	<40/≥40	0.476	0.282~0.805	0.0056			
ALT	<62/≥62	1.448	0.879~2.386	0.1459			
AST	<56/≥56	0.649	0.391~1.078	0.0951			
T. Chol	<170/≥170	1.222	0.728~2.050	0.4477			
血糖	<97/≥97	0.944	0.528~1.686	0.8445			
HbA1c	<5.2/≥5.2	1.087	0.568~2.080	0.8005			
白血球数	<4700/≥4700	1.168	0.704~1.938	0.5409			
好中球数	<2300/≥2300	1.239	0.698~2.200	0.4642			
Hb	<14/≥14	1.311	0.784~2.192	0.3027			
血小板数	<15/≥15	2.476	1.476~4.153	0.0006			
AFP	<4.8/≥4.8	0.448	0.251~0.799	0.0065			
HCV RNA(logIU/ml)	<6.0/≥6.0	0.231	0.113~0.475	<0.0001			
ISDR変異数	0~1/2~	2.501	1.535~4.075	0.0002	7.989	1.651~38.663	0.0098
IRRDR変異数	≤3/≥4	7.411	2.972~18.480	<0.0001	18.626	3.367~103.050	0.0008
Core aa70	Q/R	2.469	1.219~5.003	0.0121			
Core aa91	L/M	0.591	0.293~1.189	0.1403			
IL28B(rs8099917)	GG+TG/TT	7.794	2.451~24.779	0.0005	20.240	3.091~132.553	0.0017
ITPA(rs1127354)	CC/AA+AC	1.181	0.473~2.949	0.7209			

んでおり、最終的に新しい薬剤開発の可能性も含め、C型肝炎治療において重要な足がかりとなることが期待される。

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**Analysis of the Complete Open Reading Frame of Hepatitis C Virus in Genotype  
2a Infection Reveals Critical Sites Influencing the Response to Peginterferon and  
Ribavirin Therapy.**

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**Short title:** PEG-IFN/RBV response in HCV-2a

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## Abbreviations

EVR: Early Virological response

IFN: Interferon

IRF-1: Interferon Regulatory Factor 1

IRRDR: Interferon Ribavirin Resistance Determinant Region

ISDR: Interferon Sensitivity Determinant Region

ORF: Open Reading Frame

PEG-IFN: Pegylated-Interferon

PePHD: PKR -eIF2 Phosphorylation Homology Domain

PKR-BD: Double-Stranded RNA-activated Protein Kinase Binding Domain

RBV: Ribavirin

RVR: Rapid Virological Response

SVR: Sustained Virological Response

## ABSTRACT

**Purpose:** A proportion of patients infected with genotype 2a hepatitis C virus (HCV) cannot achieve a sustained virological response (SVR) to pegylated-interferon plus ribavirin therapy (PEG-IFN/RBV) but the reason remains unclear. The present study aimed to clarify the possible correlation between viral sequence variations and final outcome.

**Methods:** The pretreatment complete open reading frame (ORF) sequences of genotype 2a HCV were determined by direct sequencing for two independent groups of patients (43 patients as test; group 1 and 35 as validation; group 2), and the correlation with the final outcome was explored.

**Results:** Patients with SVR (n=58) and with non-SVR (n=20) differed significantly in pretreatment HCV RNA level ( $p=0.002$ ), fibrosis score ( $p=0.047$ ), and cumulative ribavirin dosage ( $p=0.003$ ). By comparison of all amino acid positions in the complete HCV ORFs, threonine at amino acid (aa) 110 in the core region was remarkably frequent in SVR ( $p=0.01$  for group 1,  $p=0.004$  for group 2, and  $p=5E-05$  for combined). A sliding window analysis revealed that the total numbers of amino acid variations within the NS5A aa 2258 to 2306 region were significantly high in SVR compared to non-SVR patients ( $p=0.01$  for group 1,  $p=0.006$  for group 2, and  $p=0.0006$  for combined). Multivariate analyses revealed that core aa 110 ( $p=0.02$ ), NS5A aa 2258-2306 ( $p=0.03$ ), and cumulative ribavirin dosage ( $p=0.02$ ) were identified as independent variables associated with the final outcome.

**Conclusions:** The outcome of PEG-IFN/RBV therapy is significantly influenced by variation in the core and NS5A regions in genotype 2a HCV infection.

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## INTRODUCTION

Worldwide, 180 million of people are estimated to be infected with hepatitis C virus (HCV), and HCV is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (1). In HCV-infected patients with chronic hepatitis, treatment with interferon (IFN) can result in viral clearance and biochemical and histological improvements(2). The response to the therapy varies according to HCV genotype and pretreatment HCV RNA level (3-4).

The currently recommended treatment for patients infected genotype 2a HCV with high viral load is pegylated-interferon (PEG-IFN) plus ribavirin (RBV) for 24 weeks (1). Approximately 80% of patients infected with genotype 2a HCV can achieve a sustained virological response (SVR) with this regimen (5-6), although much lower percentages of patients infected with other genotypes can achieve SVR, especially with genotype 1(1). Because of its high response rate, shorter treatment duration was suggested by some studies, although an agreement has not been reached yet (7-8). On the other hand, about 20% of patients infected with this genotype cannot achieve SVR and it remains elusive which patients show poor responses.

Previous studies have reported that amino acid variations in the NS5A-ISDR (9), NS5A-IRRDR(10), NS5B(11), PKR-eIF2 phosphorylation homology domain (PePHD) of E2(12), and core (13-14) correlate with clinical outcome of IFN-based therapy, including PEG-IFN/RBV therapy in patients infected with genotype 1b HCV. Recent full HCV open reading frame analysis for genotype 1 also have reported that core, NS3, and NS5A were associated with early viral response and the outcome in PEG-IFN/RBV therapy (15-16). However, in genotype 2a infection, only a few studies have investigated the association between HCV sequence variation and treatment

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2 response (17-19), and the role of viral factors has not been established yet, especially in  
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4 the era of PEG-IFN/RBV therapy. Moreover, these previous studies investigated only  
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6 several isolated HCV genomic regions, and comprehensive analysis of the full HCV  
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8 open reading frame (ORF) has not been undertaken so far.  
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12 In the present study, to assess comprehensively the influence of viral variations  
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14 on response to the PEG-IFN/RBV therapy in genotype 2a HCV infection, we  
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16 determined the complete pretreatment HCV ORFs from Japanese patients and  
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18 investigated viral amino acid variation and their correlation with the response to the  
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20 combination therapy of PEG-IFN plus RBV.  
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## PATIENTS & METHODS

### Study Population

A total of 103 adult Japanese patients infected with genotype 2a HCV, who received the combination therapy with PEG-IFN (PEGINTRON<sup>®</sup>, Schering-Plough, Tokyo, Japan) plus RBV (REBETOL<sup>®</sup>, Schering-Plough) between 2005 and 2008 at University of Yamanashi, Tokyo Medical and Dental University, and related institutions were first included in the study. They all fulfilled following criteria: (1) negative for hepatitis B surface antigen, (2) high viral load ( $\geq 100$  KIU/ml), (3) absence of hepatocellular carcinoma, (4) no other form of hepatitis, such as primary biliary cirrhosis, autoimmune liver disease, or alcoholic liver disease, (5) free of co-infection with human immunodeficiency virus. Informed consent was obtained from each patient. The study was approved by the ethics committees of all the participating universities and hospitals. The therapy was performed according to the standard treatment protocol of PEG-IFN/RBV therapy for Japanese patients established by a hepatitis study group of the Ministry of Health, Labour, and Welfare, Japan (PEG-IFN $\alpha$ -2b 1.5  $\mu$ g/kg body weight, once weekly subcutaneously, and RBV 600-800 mg daily per os for 24 weeks). To clearly disclose the non-SVR viral characteristics, we have considered those patients who achieved total drug administration of 60% or more for both PEG-IFN and RBV, with the completion of the standard treatment duration. Moreover, although we excluded the patients with extended therapy to make the studied population uniform, we have included non-SVR patients with extended therapy to clarify the specific characteristics of non-SVR patients, a minor population group. As a result, 25 patients were excluded for the following reasons: 4 patients received insufficient dose, 8 patients were discontinued from the therapy within 12 weeks, and 13 SVR patients received

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2 extended therapy. Finally, 78 patients were considered as eligible for the study. During  
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4 the combination therapy, blood samples were obtained at least once every month before,  
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6 during and after treatment and were analyzed for blood count, ALT and HCV RNA  
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8 levels. Liver biopsy specimens were obtained from most of the patients.  
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11 The 78 patients belonging to the different institutions were separately analyzed:  
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13 43 patients registered in Y-PERS (Yamanashi Pegintron Ribavirin Study Group) were  
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15 included in group 1 (test group), and the 35 patients from Tokyo Medical and Dental  
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17 University and related institutions (Ochanomizu Liver Conference Group) were  
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19 included in group 2 (validation group). We divided the patients into these two groups in  
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21 order to exclude the false positives (type I errors) which might arise in successive  
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23 HCV-ORF study. Since genotype-2a HCV contains as many as 3033 amino acids, it was  
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25 possible that incorrect amino acids to be judged as significant in full HCV-ORF  
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27 comparison study as a result of type I errors. Therefore, to guard against false positives,  
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29 HCV-ORF comparison study was undertaken in group 1, group 2, and combined group.  
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#### 40 **Complete HCV-ORF Sequence Determination by Direct Sequencing from** 41 42 **Pretreatment Sera**

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44 HCV RNA was extracted from pretreatment serum samples by the AGPC  
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46 method using Isogen (Wako, Osaka, Japan) according to the manufacturer's protocol.  
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48 Complementary DNA was synthesized with Superscript II (Invitrogen, Tokyo, Japan)  
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50 using random primers (Invitrogen) and then amplified by two-step nested PCR using the  
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52 primers newly designed for this study. All samples were initially denatured at 95°C for 7  
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54 min., followed by 40 cycles with denaturation at 95°C for 15 seconds, annealing at 55°C  
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2 for 15 seconds, and extension at 72°C for 45 seconds with BD Advantage™ 2 PCR  
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4 Enzyme System (BD Biosciences Clontech, CA, USA).  
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7 PCR amplicons were sequenced directly by Big Dye Terminator Version 3.1  
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9 (ABI, Tokyo, Japan) with universal M13 forward / M13 reverse primers using an ABI  
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11 prism 3130 sequencer (ABI). Generated sequence files were assembled using Vector  
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13 NTI software (Invitrogen) and base-calling errors were corrected following inspection  
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15 of the chromatogram.  
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### 21 **Sliding Window Analysis**

22 A sliding window analysis was introduced to search through HCV amino acid  
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24 “regions”, rather than single amino acid positions, related to the final outcome of  
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26 PEG-IFN/RBV therapy. Briefly, the total number of amino acid substitutions compared  
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28 to the consensus sequence within a given amino acid length were counted in each amino  
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30 acid position in each HCV sequence. Then the relation of substitution numbers and the  
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32 final outcome was compared statistically between the SVR and non-SVR groups by  
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34 Mann-Whitney's U test for each amino acid position. In this study, we changed the  
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36 window length from 1 to 50 to search for those HCV regions. To visualize the result,  
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38 significantly lower p-values were colored in red and non-significant p-values were  
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40 colored in green to generate a “heat map” appearance using Microsoft Excel software.  
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48 In the present study, p-value of 1/1000 or lower was colored in the maximum red.  
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### 55 **Statistical Analysis**

56 Statistical differences in the parameters, including all available patients’  
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2 demographic, biochemical, hematological, and virological data such as sequence  
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4 variation factors, was determined between the various groups by Student t test or  
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6 Mann-Whitney's U test for numerical variables and Fisher's exact probability test for  
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8 categorical variables. To evaluate the optimal threshold of variations for SVR  
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10 prediction, the receiver operating characteristic curve was constructed. Variables that  
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12 achieved statistical significance ( $p < 0.05$ ) in univariate analysis were entered into  
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14 multiple logistic regression analysis to identify significant independent factors. We also  
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16 calculated the odds ratios and 95% confidence intervals. All p values of  $< 0.05$  by the  
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18 two-tailed test were considered significant.  
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## RESULTS

### Characteristics of the patients studied

Of the patients analyzed, the SVR rate was 78.3% (58/74) with the standard therapy (four non-SVR patients received an extended therapy). The baseline characteristics of the patients (group 1, group2, and combined) classified according to SVR achievement are shown in Table 1. Fibrosis score ( $p=0.047$ ) and HCV RNA levels ( $p=0.002$ ) were significantly higher in non-SVR patients, but the cumulative ribavirin dose  $\geq 80\%$  ( $p=0.003$ ) and rapid virological response (RVR) rate ( $p=0.011$ ) were significantly higher in SVR patients. In addition, patients with non-SVR had a tendency to be older ( $p=0.058$ ). Achievement of RVR reached 61.5% when all patients were included, and this rate was extremely high compared to achievement of RVR in patients with genotype 1b infection ( $\sim 10\%$ ) observed in Yamanashi University Hospital (data not shown). The early virological response (EVR) rate was equally high in the SVR (100%) and non-SVR (89%) groups, showing that relapse to be the characteristic feature of the non-SVR patients with genotype 2a HCV. Actually, 18 patients in non-SVR were relapser, while two patients were null responder.

### Comparison of amino acid variations between the SVR and non-SVR in the complete HCV polyprotein and each HCV protein

To determine whether the sequence variations differed between the SVR and non-SVR groups, we first compared amino acid variations that were unique, relative to a population consensus, to either the SVR or non-SVR patients for the complete HCV polyprotein and each HCV protein. The number of amino acid variations in the

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2 sequences from the SVR patients was significantly higher than in those from the  
3 non-SVR patients, when the entire HCV polyprotein was analyzed (Fig.1, left). These  
4 differences were especially significant in E1 and NS3 (Fig.1, right). This result  
5 demonstrated that HCV sequences from patients with SVR comprised a heterogeneous  
6 population, while HCV sequences from patients with non-SVR comprised a rather  
7 homogeneous population, indicating the existence of unique non-responsive HCV  
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#### 22 **Comparison of HCV sequence variation between the SVR and non-SVR patients at** 23 **each amino acid position** 24

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26 Next, each amino acid position in the HCV ORF was compared to detect any  
27 differences between the SVR and non-SVR patients after determination of the  
28 consensus sequence from all 78 patients. In Fig.2a, the final differences of the two  
29 independent studies combined are shown as dots demonstrating  $-\log P$  values. As shown  
30 in the figure, amino acid usage at amino acid 110 in the core region differed strikingly  
31 between the two groups ( $p=5E-05$ ). The site was detected in group 1 ( $p=0.01$ ) and was  
32 validated in group 2 ( $p=0.004$ ) (Table 2a), and the final p-value became remarkably  
33 high, making the p-value at this site most significantly low. Variations of aa 773 in p7,  
34 aa 2099 in the NS5A, and aa 3013 in NS5B were also shown to differ significantly  
35 between the SVR and the non-SVR patients when the two studies were combined;  
36 however, they were not confirmed by one of the studies (Table 2a). Fig.2b shows the  
37 aligned sequences of amino acids 1-120 of the core region. Substitutions at aa 110 from  
38 non-T (N/S) to T were significantly more frequent in SVR (32/58, 55.2%) than in  
39 non-SVR (1/20, 3.6%,  $p=5E-05$ ). Amino acid 4, the site reported recently to vary  
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2 according to the viral response in genotype 2a infection, did not differ significantly in  
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4 our study. Amino acid 70 and 91, which have been reported to vary according to viral  
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6 response to PEG-IFN/RBV therapy in genotype 1b infection, were conserved  
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9 irrespective of the outcome.  
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#### 11 12 13 14 **Comparison of amino acid variation between the SVR and non-SVR patients** 15 **across HCV “regions” using sliding window analysis** 16 17

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19 Fig.2c shows the combined result of sliding window analysis for study groups  
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21 1 and 2, this approach was used to detect differing HCV amino acid “regions”, rather  
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23 than single amino acid positions, between the SVR and the non-SVR patients.  
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25 According to the result, four regions were notably associated with the final outcome  
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27 (p-values less than 1/1000). Core aa 110, detected as a single amino acid position  
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29 discriminating between the SVR and the non-SVR patients, was also identified as one  
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31 of these regions. Because core aa 110 was already known for its strong correlation with  
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33 the response as above, the region was excluded from further analysis. Among the other  
34  
35 three regions, only NS5A aa 2258-2306 showed significant differences in the two  
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37 independent study groups (Table 2b). Interestingly, the NS5A region overlapped the  
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39 PKR-binding domain, which includes the interferon sensitivity determining region  
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41 (ISDR). Fig.2d shows the aligned sequences of amino acids around 2258-2306 of HCV  
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43 NS5A. As with previous studies, variations in the ISDR were also significantly more  
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45 frequent in SVR patients.  
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#### 55 **Multivariate analysis to detect independent factors contributing to the SVR** 56

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58 Multivariate analysis revealed that variation of core aa 110, the total number of  
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2 substitutions within NS5A aa 2258-2306, and total ribavirin dose  $\geq 80\%$  were finally  
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5 identified as the independent variables influencing the final outcome (odds ratio 24.7,  
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7 11.5 and 16.0;  $p = 0.02, 0.03$  and  $0.02$ , Table 3).  
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#### 10 11 **Biological relevance of variation in core and NS5A in this study group** 12 13 14

15 To determine biological relevance of core aa110 and NS5A aa2258-2306, we  
16  
17 investigated their relationship with clinical background factors. Multiple variations in  
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19 the NS5A region aa 2258-2306 were significantly related to pretreatment HCV RNA  
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21 titer ( $p=9E-05$ , Fig. 3 and Table 4a). Interestingly, variation of the core aa110 was  
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23 significantly associated with the patients' age ( $p=0.03$ , Table 4b).  
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## DISCUSSION

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In this study, based on analysis of complete HCV ORF sequences and comparison of SVR and non-SVR patients in two independent study groups, we have shown that amino acid variations in the core and NS5A correlate most significantly with the final outcome in the treatment for genotype 2a chronic hepatitis C. The study is unique in that the patients studied were all Japanese, excluding any affect of racial differences and providing a clearer analysis of the viral differences.

From the analysis of the characteristics of patients infected with genotype 2a HCV, it was clear that most non-SVR patients responded to the PEG-IFN/RBV therapy at least transiently, given that most of these non-SVR patients (89%) achieved EVR. This result demonstrated that most non-SVR patients were relapser, but were not null-responders as observed frequently among genotype 1b patients treated with PEG-IFN/RBV therapy. Therefore, we compared the different viral responses according to the final outcome of SVR or non-SVR.

Variation of core aa 110 was identified as the single amino acid residue most significantly related to the final outcome ( $p=5E-05$ ). In recent studies of treatment of genotype 1b infection with PEG-IFN/RBV, amino acid variation in the core region was reported to be associated with response. It is interesting that the core region was also identified as an HCV gene associated with the response to PEG-IFN/RBV therapy of genotype 2a infection, although the amino acid residues of core in genotype 1b were different, being aa 70 and aa 91. It is also interesting that amino acids aa 70 and aa 91 are conserved as arginine and leucine, respectively, in genotype 2a, as reported to be associated with favorable PEG-IFN/RBV responses in genotype 1b infection, consistent with the association with a high SVR rate in genotype 2a infection. Very recently, a

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2 correlation was reported between amino acid variations in the core region and viral  
3 responses of genotype 2a HCV infection (20). Though the result seems discrepant from  
4 our study, we suspect the inconsistent results were at least partially attributable to the  
5 different groups used in comparison: we compared the difference between non-SVR  
6 patients and SVR patients while they compared the difference between non-SVR and  
7 RVR patients.  
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11 In systemic searching for the viral “regions” associated with the treatment  
12 outcome, NS5A aa2258-2306 was identified by two independent studies. Interestingly,  
13 the region overlaps the PKR-binding domain (PKR-BD), including the ISDR, in which  
14 the number of amino acid substitutions is known to be related to the response to  
15 IFN-based therapy in genotype 1b, and also in genotype 2a (17-18). Therefore, we also  
16 confirmed that total number of substitutions in the ISDR and PKR-BD is significantly  
17 associated with the final outcome in this group of patients when the two studies were  
18 combined.  
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22 Some viral regions other than core and NS5A also showed the potential  
23 association with the final outcome. Viral single amino acid substitutions of aa 773 in p7,  
24 aa 2099 in the NS5A, and aa 3013 in NS5B, or viral regions in E1 aa 400-403 and in E2  
25 aa 724-744 were more frequent in SVR. However, because these were not extracted as  
26 significant in one of the two studies when analyzed separately, additional studies are  
27 needed to confirm the association with the final outcome. On the other hand, we could  
28 not find an association with the final outcome and the PePHD or IRRDR, including the  
29 V3 regions (data not shown) reported 1b HCV infection (21-22).  
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33 It is interesting that the variation of the core region showed the clear  
34 association with age. The younger patients with core aa 110T showing favorable  
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1 responses while the older patients with core aa 110 non-T showed unfavorable  
2 responses. It is possible that different response rates according to the patients' ages in  
3 genotype 2a infection might have been related to the core substitutions, although further  
4 study is needed. In NS5A, it was reported that the variations within the PKR-binding  
5 region, including those within the ISDR, can disrupt the NS5A-PKR interaction,  
6 possibly rendering HCV sensitive to the antiviral effects of interferon (23). Clinically,  
7 the number of substitutions within the region has been reported to correlate with the  
8 serum HCV RNA level (12). We also confirmed that the number of substitutions within  
9 the NS5A aa 2258-2306 was significantly associated with the pretreatment HCV RNA  
10 titers.  
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26 Multivariate analysis of the combined group of patients showed that variation  
27 of core aa 110, NS5A aa 2258-2306, and total ribavirin dose  $\geq 80\%$  were independent  
28 variables associated with the final outcome (Table. 3). The association of ribavirin dose  
29 and HCV relapse rate was reported previously (24) and that result was confirmed in this  
30 study. On the other hand, the total PEG-IFN dosage was not identified when it was  
31 administered at greater than 60% of the initially scheduled amount. Indeed, when the  
32 drug dosage is excluded, the strongest association was seen in the viral elements of core  
33 and NS5A, revealing the importance of these two regions in the treatment of genotype  
34 2a HCV infection with PEG-IFN/RBV therapy.  
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49 On the other hand, our study still has some limitations. In recent studies, IL28B  
50 single nucleotide polymorphisms were reported to be correlated significantly with the  
51 treatment response in genotype 1b HCV infections (25-26). In genotype 2a HCV  
52 infection, a correlation was also reported to exist between the IL28B SNP and the  
53 treatment response (27). However, we could not investigate the association of the IL28B  
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2 single nucleotide polymorphisms in the treatment response in genotype 2a HCV  
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5 infections. In addition, the number of analyzed patients was rather small, especially in  
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7 non-SVR patients.  
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10 In conclusion, by comprehensive investigation of the complete HCV ORF in  
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12 patients showing different responses to PEG-IFN/RBV therapy, we have demonstrated  
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14 that amino acid variation in the core and NS5A are significantly associated with the  
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16 final outcome of treatment of genotype 2a chronic hepatitis C. Considering this result,  
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18 determination of those HCV regions before treatment might provide further benefits for  
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20 the patients infected with genotype 2a HCV.  
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