to DAAs can exist at low frequency prior to antiviral treatment with DAAs. Enrichment of variants during therapy has been reported, although monitoring changes in variant frequency using ultra-deep sequencing is not commonly performed. HCV is an error-prone RNA virus in which substitutions frequently occur throughout the HCV genome [27,28], and drug-resistant variants are sometimes present as minor populations in patients who have never been exposed to DAAs [29].

In this study, ultra-deep sequence analysis detected DCV-resistant variants in 5 (50%) patients before treatment. In recent Japanese studies, the prevalence of NS3/4A protease inhibitor- and NS5A inhibitor-resistant variants in HCV genotype 1b-infected patients was reported to be approximately 4.9% and 11–23%, respectively, by direct sequence analysis, [16,17,29].

Patients with no ASV-resistant variants but with NS5A L31M/S and a high frequency of Y93H variants (32.4% and 99.4%) resulted in the development of double resistance variants, indicating that pre-existence of a high frequency of Y93H variants might be associated with relapse or viral breakthrough with ASV and DCV combination

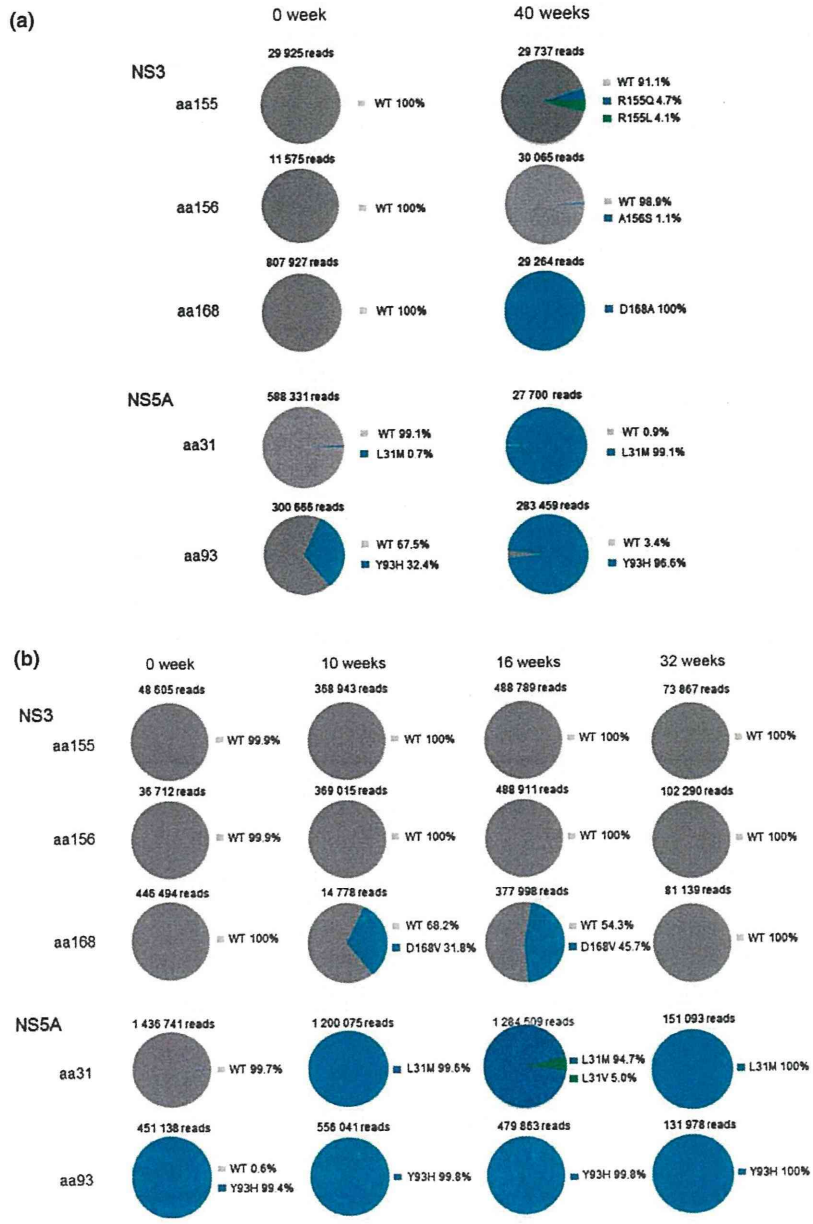


Fig. 2 Time courses of the amino acid frequencies at R155, A156 and D168 in the NS3 region and at L31 and Y93 in the NS5A region by ultra-deep sequencing in Case 9 (a) and Case 10 (b).

treatment. However, Case 3 achieved SVR despite a pre-existing NS5A L31V variant and a high frequency (99.4%) Y93H variant. These results suggest that pre-existing DCV-resistant variants might be associated with viral breakthrough for DCV and ASV combination treatment; however, identifying pre-existing resistant variants by ultra-deep sequence seems to have limited utility in predicting the outcome of therapy.

Karino *et al.* [17] reported a relationship between pre-existing drug-resistant variants by direct sequencing analysis and clinical antiviral responses to DCV and ASV combination treatment. McPhee *et al.* [30] also reported that six of seven genotype 1a HCV-infected patients treated with ASV and DCV developed viral breakthrough even though no resistance variants were detected at baseline by population sequencing analysis. Ultra-deep sequence analysis may permit more detailed analysis of resistance variants.

In Case 9, although ASV-resistant variants had not been detected before treatment, the frequency of D168A had reached 100% by 16 weeks after cessation of treatment, indicating that the wild type amino acid had been completely replaced. Similarly, in Case 10, the frequency of the D168V variant had already reached 31.8% by week 10, and the frequency increased to 45.7% by week 16. In both patients, NS5A aa31 and 93 were predominantly replaced by DCV-resistant variants. In Case 10, NS3 aa168 had completely returned to wild type 16 weeks after cessation of the treatment, while NS5A aa31 was completely replaced by the DCV-resistant variant. We previously reported that TVR-resistant variants have reduced replication capacity and are easily replaced by wild type when TVR is not present [25]. Karino *et al.* reported that DCV-resistant substitutions persisted through 48 weeks post-treatment, whereas ASV-resistant substitutions were no longer detectable by direct sequence analysis in viral breakthrough patients treated with DCV and ASV. Long-term follow-up of these variants by ultra-deep sequence analysis is required to fully understand their fitness *vs* wild type sequence. The analysis of a larger number of patients is now ongoing.

In conclusion, 10 patients with HCV genotype 1b infection were treated with ASV and DCV combination treatment. This treatment is expected to improve the SVR rate greatly, but viral breakthrough might develop in some patients with the emergence of ASV- and DCV-resistant variants. Patients with a high frequency of pre-existing DCV-resistant variants might be more susceptible to viral breakthrough during combination therapy, although it remains to be seen whether ultra-deep sequencing analysis of resistance variants prior to treatment can effectively predict treatment outcome.

ACKNOWLEDGEMENTS

The authors thank Rie Akiyama and Yoko Matsumoto for their expert technical help. This study was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Labor, Health and Welfare.

FINANCIAL SUPPORT

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

REFERENCES

1 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; 12: 671–675.
2 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; 28: 1687–1695.
3 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peg-interferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; 360: 1827–1838.
4 Zeuzem S, Andreone P, Pol S *et al.* Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; 364: 2417–2428.
5 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for patients with chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.
6 Hoofnagle JH, Ghany MG, Kleiner DE *et al.* Maintenance therapy with ribavirin in patients with chronic hepatitis C who fail to respond to combination therapy with interferon alfa and ribavirin. *Hepatology* 2003; 38: 66–74.
7 Jacobson IM, McHutchison JG, Dusheiko G *et al.* Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; 364: 2405–2416.
8 Hézode C, Forestier N, Dusheiko G *et al.* Telaprevir and peg-interferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; 360: 1839–1850.
9 Kumada H, Toyota J, Okanoue T *et al.* Telaprevir with peg-interferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; 56: 78–84.
10 Hayashi N, Okanoue T, Tsubouchi H *et al.* Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. *J Viral Hepat* 2012; 19: e134–e142.
11 Gao M, Nettles RE, Belema M *et al.* Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010; 465: 96–100.
12 Arima N, Kao CY, Licht T *et al.* Modulation of cell growth by the hepatitis C virus nonstructural protein NS5A. *J Biol Chem* 2012; 276: 12675–12684.

13 McPhee F, Sheaffer AK, Friborg J *et al.* Preclinical profile and characterization of the hepatitis C virus NS3 protease inhibitor asunaprevir (BMS-650032). *Antimicrob Agents Chemother* 2012; 56: 5387–5396.
14 Soriano V, Peters MG, Zeuzem S. New therapies for hepatitis C virus infection. *Clin Infect Dis* 2009; 48: 313–320.
15 Chayama K, Takahashi S, Toyota J *et al.* Dual therapy with the NS5A inhibitor, daclatasvir, and the NS3 protease inhibitor, asunaprevir, in HCV genotype 1b-infected null responders. *Hepatology* 2012; 55: 742–748.
16 Suzuki Y, Ikeda K, Suzuki F *et al.* Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options. *J Hepatol* 2013; 58: 655–662.
17 Karino Y, Toyota J, Ikeda K *et al.* Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir. *J Hepatol* 2013; 58: 646–654.
18 Nasu A, Marusawa H, Ueda Y *et al.* Genetic heterogeneity of hepatitis C virus in association with antiviral therapy determined by ultra-deep sequencing. *PLoS ONE* 2011; 6: e24907.
19 Ninomiya M, Ueno Y, Funayama R *et al.* Use of illumina deep sequencing technology to differentiate hepatitis C virus variants. *J Clin Microbiol* 2012; 50: 857–866.
20 Lauck M, Alvarado-Mora MV, Becker EA *et al.* Analysis of hepatitis C virus intrahost diversity across the coding region by ultradeep pyrosequencing. *J Virol* 2012; 86: 3952–3960.
21 Akuta N, Suzuki F, Seko Y *et al.* Emergence of telaprevir-resistant variants detected by ultra-deep sequencing after triple therapy in patients infected with HCV genotype 1. *J Med Virol* 2013; 85: 1028–1036.
22 Bronowicki JP, Pol S, Thuluvath PJ *et al.* BMS-650032, an NS3 inhibitor, in combination with peginterferon alfa-2a and ribavirin in treatment-naive subjects with genotype 1 chronic hepatitis C infection. *J Hepatol* 2011; 54: S472.
23 Mori N, Imamura M, Kawakami Y *et al.* Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region. *J Med Virol* 2009; 81: 640–649.
24 Abe H, Ochi H, Maekawa T *et al.* Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* 2010; 53: 439–443.
25 Hiraga N, Imamura M, Abe H *et al.* Rapid emergence of telaprevir resistant hepatitis C virus strain from wildtype clone in vivo. *Hepatology* 2011; 54: 781–788.
26 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009; 25: 1754–1760.
27 Kuntzen T, Timm J, Berical A *et al.* Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. *Hepatology* 2008; 48: 1769–1778.
28 Lu L, Mo H, Pilot-Matias TJ *et al.* Evolution of resistant M414T mutants among hepatitis C virus replicon cells treated with polymerase inhibitor A-782759. *Antimicrob Agents Chemother* 2007; 51: 1889–1896.
29 Suzuki F, Sezaki H, Akuta N *et al.* Prevalence of hepatitis C virus variants resistant to NS3 protease inhibitors or the NS5A inhibitor (BMS-790052) in hepatitis patients with genotype 1b. *J Clin Virol* 2012; 54: 352–354.
30 McPhee F, Hernandez D, Yu F *et al.* Resistance analysis of hepatitis C virus genotype 1 prior treatment null responders receiving daclatasvir and asunaprevir. *Hepatology* 2013; 58: 902–911.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1: Relationship between coverage and frequency of nucleotide substitutions in deep sequencing target regions in the control plasmid.

Table S1: Nucleotide frequencies from the plasmid control sequence.

Table S2: Codon frequencies from the plasmid control sequence.

Table S3: Codon frequencies in patients prior to therapy.

Table S4: Amino acid frequencies in patients prior to therapy.

JOURNAL OF VIRAL HEPATITIS JVH

Journal of Viral Hepatitis, 2014 doi:10.1111/jvh.12275

Ribavirin dose reduction during telaprevir/ribavirin/peg-interferon therapy overcomes the effect of the ITPA gene polymorphism

S. Akamatsu,[1,2,3,*] C. N. Hayes,[1,2,*] M. Tsuge,[1,2,4] E. Murakami,[1,2] N. Hiraga,[1,2] H. Abe,[1,2] D. Miki,[1,2,3] M. Imamura,[1,2] H. Ochi[1,2,3] and K. Chayama[1,2,3] Hiroshima Liver Study Group

[1]*Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima, Japan;* [2]*Liver Research Project Center, Hiroshima University, Hiroshima, Japan;* [3]*Laboratory for Digestive Diseases, SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Hiroshima, Japan; and* [4]*Natural Science Center for Basic Research and Development, Hiroshima University, Hiroshima, Japan*

Received January 2014; accepted for publication May 2014

SUMMARY. Treatment success of chronic hepatitis C virus genotype 1 infection has improved with the advent of telaprevir plus peg-interferon/ribavirin triple combination therapy. However, the effect of inosine triphosphatase (ITPA) polymorphism on dose reduction during triple therapy, especially during the postmarketing phase, has not been sufficiently evaluated. We analysed 273 patients with genotype 1 infection who were treated with triple therapy and assessed the effect of the ITPA polymorphism on dose reduction. ITPA and IFNL4 SNP genotypes were determined by the Invader assay. A stepwise multivariate regression analysis was performed to identify factors associated with outcome of the therapy. The overall sustained viral response (SVR) rate 12 weeks after the end of therapy was 80.2% (219/273). Decline of haemoglobin was significantly faster, and ribavirin was more extensively reduced in patients with ITPA SNP rs1127354 genotype CC than CA/AA. Extensive reduction of ribavirin resulted in mild reduction of telaprevir and peg-interferon, but no significant increase in viral breakthrough. Although the amount of telaprevir given was slightly higher in CA/AA patients, the total dose of peg-interferon and the SVR rate did not differ between the two groups. Multivariate analysis showed that IFNL4 but not ITPA SNP genotype, platelet count and peg-interferon adherence were significantly associated with outcome of therapy. Postmarketing-phase triple therapy resulted in a high SVR rate in spite of extensive ribavirin dose reduction in a diverse patient population, indicating the importance of treatment continuation and appropriate management of adverse events.

Keywords: anaemia, dose reduction, haemoglobin, hepatitis C virus, sustained viral response.

Hepatitis C virus (HCV) infection is a serious global health problem affecting more than 200 million people [1]. With the introduction of protease inhibitors, the sustained viral response (SVR) rate with peg-interferon, ribavirin and telaprevir/boceprevir triple therapy has improved from 50% to 70% for genotype 1 [2]. While response to therapy varies depending upon prior treatment history, increases in each group have been observed, including up to 92% in treatment-naive patients [3], 86% in prior relapsers, 57% in prior partial responders and 31% in prior null responders [2,4].

Although the eradication rate has improved substantially, a number of issues remain, including premature termination due to anaemia [5–8], skin rash [6,9–11] and renal damage [12–14]. However, some potentially difficult-to-treat patients might respond successfully to triple therapy. For example, patients with advanced fibrosis were successfully treated with a relatively high eradication rate [15], although in other studies, the safety profile was poor, and patients with a platelet count ≤100 000/mm [12] and serum albumin <35 g/L should not be treated with triple therapy [7].

A fixed, three times daily 750 mg telaprevir dose without reduction or interruption is recommended [16]. However, the recommended dose was determined based on

Abbreviations: HCV, Hepatitis C virus; ITPA, inosine triphosphatase; SVR, sustained viral response.

Correspondence: Kazuaki Chayama, Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima-shi, Hiroshima 734-8551, Japan.
E-mail: chayama@hiroshima-u.ac.jp
*Authors contributed equally.

studies conducted in the USA and Europe [17], whereas Japanese participants in telaprevir clinical trials tended to have body weights 15–30 kg lower than their American and European counterparts, resulting in severe side effects at this dosage [18]. To avoid treatment discontinuation, Suzuki *et al.* [19] reduced the dose of telaprevir from 2250 mg/day to 1500 mg/day and did not observe a worsening of the sustained viral response (SVR) rate. We also compared 2250 mg and 1500 mg telaprevir doses in a randomized clinical trial and showed that telaprevir discontinuation was significantly reduced with the smaller dose even though the SVR rate (92%) and viral dynamics did not differ significantly during treatment [20]. Therefore, mild telaprevir dose reduction might help to avoid strong side effects without compromising the SVR rate in patients with lower body weight.

Ribavirin was reduced extensively in the above studies to avoid premature termination, but ribavirin remains necessary to prevent viral breakthrough, so a safe balance must be determined. Several single-nucleotide polymorphisms in the inosine triphosphatase (ITPA) locus (rs7270101 and rs1127354) have been reported to influence the incidence of anaemia [7,15,21–24]. While the rs7270101 A allele is fixed in Asian populations (HapMap JPT, HCB = 1.000), Japanese patients with the rs1127354 *CC genotype are prone to developing severe anaemia* during therapy [22,23]. In this study, we assessed the influence of ITPA polymorphism only in patients who began triple therapy after telaprevir was approved. This restriction is important because pre-approval clinical trials included mainly younger and nonanaemic patients, but patients in Japan tend to be on average 10 years older than in western countries and include a higher proportion of women, for whom ribavirin-induced anaemia is of particular concern [25]. Findings from this study may apply to other countries with older patients awaiting a safer treatment regimen.

METHODS

Patients

From December 2011 to May 2013, 273 patients with chronic genotype 1 infection who were HCV RNA positive for more than 6 months with an HCV RNA titre higher than 5 log copy mL-1, as determined by the Roche HCV Amplicor assay, were enrolled in the study. Patients positive for active hepatitis B virus or human immunodeficiency virus infection markers were excluded. Basic characteristics of the patients are shown in Table 1. All patients provided written informed consent. The experimental protocol met the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the Hiroshima University Ethical Committee.

Therapeutic regimen and evaluation of the therapy

Peg-interferon alpha 2b (Peg-Intron, MSD, Tokyo, Japan) and ribavirin (Rebetol, MSD, Tokyo, Japan) were administered according to patient body weight for 24 weeks, as described previously [18]. Telaprevir (Telavic, MSD, Tokyo, Japan) was given three times daily at an initial dose of 750 mg or 500 mg based on sex and baseline haemoglobin levels, and all drugs were reduced as necessary in accordance with dose reduction guidelines [18]. Briefly, for female patients with baseline haemoglobin levels <14 g/dL or male patients with baseline haemoglobin levels <13 g/dL, ribavirin dosage was reduced by 200 mg and telaprevir dosage were reduced to 1500 mg. Haemoglobin levels were closely monitored, and in the case of anaemia, ribavirin dosage was reduced based on both the absolute value of the haemoglobin levels as well as the amount of haemoglobin reduction. Patients who remained HCV RNA negative 12 weeks after the end of treatment were considered to have achieved SVR.

SNP genotyping

SNP genotyping for interferon lambda 4 (rs8099917 and ss469415590) and ITPA (rs1127354) was determined by the Invader assay as described previously [22].

Statistical analysis

Statistical analysis was performed using the R statistical package version 3.0.2 and SPSS statistics 19.0 (IBM SPSS Inc., Chicago, IL, USA). Continuous variables are reported using the median and range and were analysed using the nonparametric Mann–Whitney U-test. Count data was analysed using the chi-square test. Multivariate logistic regression was performed to identify factors associated with SVR using forward stepwise variable selection. Kaplan–Meier analyses were performed to estimate the cumulative incidence of dose reduction for ribavirin, telaprevir and peg-interferon.

RESULTS

Patients ranged in age from 24 to 79, and 49.5% were female (Table 1). Of the 273 patients who underwent triple therapy, 219 (80.2%) achieved SVR, 4.8% relapsed during follow-up, 5.5% experienced viral breakthrough during treatment, and 9.5% failed to respond to the therapy. A total of 61.1% of patients with fibrosis ≥3 achieved SVR, and 69.7% of patients with fibrosis ≥2 achieved SVR. Only 26% of patients were treatment-naive, but 78% of these patients achieved SVR. Among patients who had previously undergone interferon therapy, 79 (41.4%) were nonresponders and 110 (57.6%) relapsed under prior therapy. A total of 90.1% of treatment-experienced patients

Table 1 Baseline patient characteristics

	Total (n = 273)	SVR (219)	non-SVR (54)
Sex (M/F)	138/135	110/109	28/26
Age	62 (24–79)	61 (24–79)	63 (27–79)
Body height (cm)	160 (134.5–181)	160.5 (134.5–181)	158.5 (142.7–179.5)
Body weight (kg)	60 (36.3–100.1)	60 (36.3–100.1)	60.4 (43–81.3)
HCV genotype (1b/1a/ND)	243/1/29	192/1/26	51/0/3
IL28B (TT/TG/GG/ ND)	165/78/3/27	152/40/0/27	13/38/3/0
ITPA (CC/CA/AA/ ND)	185/59/2/27	144/46/2/27	41/13/0/0
IFNλ4(TT/TT,TT/⊿G,⊿G/⊿G,ND)	146/77/3/47	136/40/0/43	10/37/3/4
HCV core70 (wild/mix/mutant/ND)	81/5/64/123	73/4/39/103	8/1/25/20
WBC ($10^3/\mu L$)	4890 (2400–11830)	4880 (2400–11830)	4900 (2400–7804)
Plt ($10^4/\mu L$)	16.3 (5.2–40.4)	16.8 (5.2–40.4)	12.4 (5.4–24.8)
Hb (g/dL)	13.9 (10–18.1)	14 (10–18.1)	13.5 (10.8–17)
AST (IU/L)	38 (5–200)	36 (5–200)	45 (16–145)
ALT (IU/L)	38 (10–286)	37 (10–286)	41 (13–204)
γGTP (IU/L)	31 (1–669)	30 (1–669)	43 (11–442)
HCV RNA (Log IU/mL)	6.6 (0–7.8)	6.6 (0–7.8)	6.6 (5–7.5)

ND, not determined. Continuous values are reported as median (range).

achieved SVR. A total of 53% of the prior nonresponders achieved SVR under triple therapy, whereas 95% of the prior relapsers achieved SVR. While 12 (15%) prior nonresponders experienced viral breakthrough during telaprevir treatment, only 1 (0.9%) prior relapser experienced viral breakthrough. Substitution at core amino acid 70 (core70) of the HCV core protein was strongly associated with viral breakthrough (P = 2.6E-17), and no viral breakthrough occurred in patients with wild-type core70. Viral breakthrough was also associated with baseline platelet count and adherence to telaprevir, peg-interferon and ribavirin in univariate analysis, although only IFNL4 genotype was significant (P = 0.028) when all factors were considered simultaneously.

Haemoglobin decline during triple therapy by ITPA SNP genotype and reduction of drugs

Patients with the anaemia-prone ITPA rs1127354 CC genotype showed a significantly more rapid decline in haemoglobin levels compared to patients with CA or AA genotypes (Fig. 1). Haemoglobin levels were significantly lower in CC patients than CA/AA patients at each time point for the first 4 weeks of therapy (Fig. 1). In response to haemoglobin decline, ribavirin, telaprevir and peg-interferon dosages were reduced as necessary according to treatment guidelines. The period without reduction of ribavirin was significantly shorter in patients with the rs1127354 CC genotype compared to CT/TT patients (P = 0.028; Fig. 2).

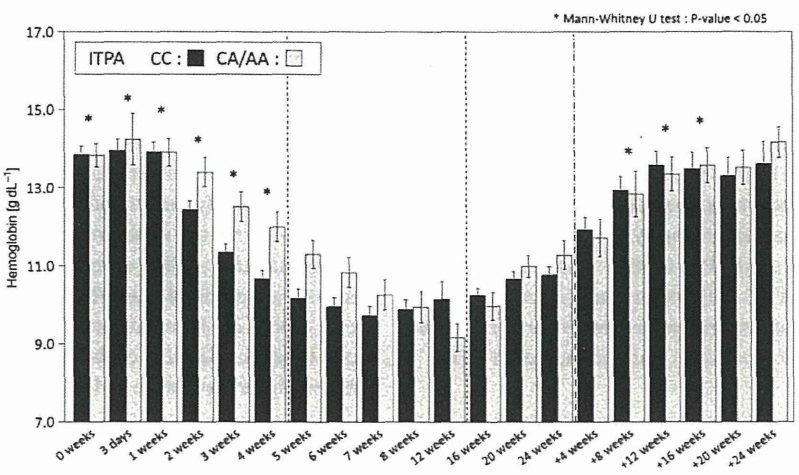


Fig. 1 Haemoglobin levels by ITPA rs1127354 genotype during 24 weeks of therapy and 24 weeks of follow-up. Dashed lines indicate treatment at 4 weeks, 8 weeks and end of treatment at 24 weeks.

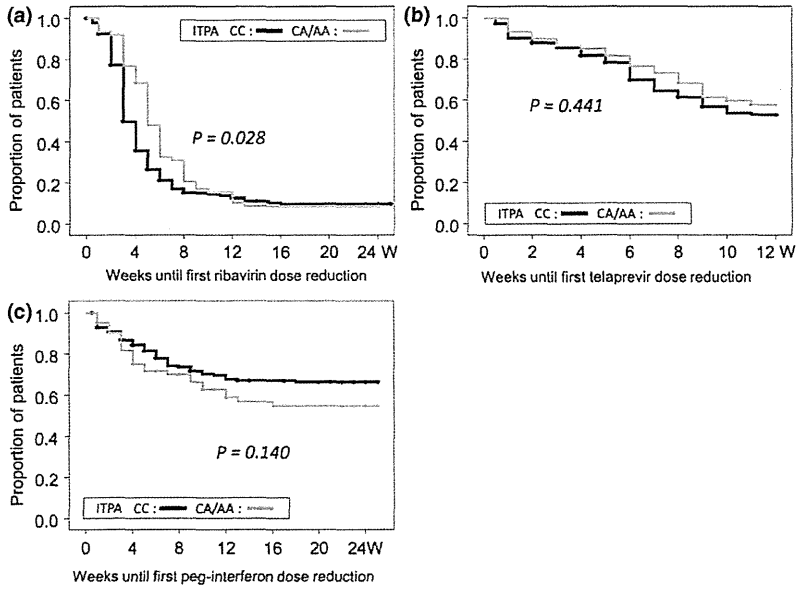


Fig. 2 Time until dose reduction by ITPA rs1127354 genotype. The difference in the number of weeks until the first dose reduction in rs1127354 *CC vs* CA/AA patients was determined by the log-rank test for (**a**) ribavirin, (**b**) telaprevir and (c) peg-interferon.

In contrast, the period without reduction for telaprevir ($P = 0.441$) and peg-interferon (0.140) was not significantly different among these patients, although it tended to be shorter in rs1127354 CC patients (Fig. 2). Consequently, the total amount of ribavirin given to each patient was significantly lower in rs1127354 CC patients (51470 mg) compared with CA/AA patients (63950 mg) ($P = 0.016$; Fig. 3). The difference in total amount of telaprevir given was less pronounced in CC (122030 mg) *vs* CA/AA (136170 mg) patients, but the difference was still statistically significant ($P = 0.024$), whereas there was no significant difference in total peg-interferon dosage (CC: 1699 mg *vs* CA/AA: 1781 mg; $P = 0.61$).

Effect of ITPA SNP genotype on total dosage of the drugs and outcome of therapy

Patients who achieved SVR received a significantly greater total amount of each of the three drugs (Fig. 3). However, when we assessed the effect of ITPA genotype on outcome of therapy, the SVR ratio between rs1127354 CC and CA/AA patients was not significantly different (144/185 [77.8%] *vs* 48/61[78.7%]; $P = 0.889$; Table 2). Among prior relapsers, 93% of CC patients and all CA/AA patients achieved SVR, whereas among prior nonresponders, 54% of CC patients and 44% of CA/AA patients achieved SVR.

Effect of adherence and duration of each drug on outcome of therapy

We further assessed the effect of adherence of each drug (defined as the amount of drug taken relative to the amount planned for each patient) with respect to outcome of therapy (Figure S1). All of the patients who received at least 90% of the planned dose of ribavirin achieved SVR, although the number of such patients was quite small ($n = 18$) and included 7 prior relapsers and 2 prior nonresponders. Notably, more than 80% of the patients who took at least 40% of the planned ribavirin dose achieved SVR. Most patients took ribavirin for the full 24 weeks, resulting in an SVR rate of 89.7% in these patients. A similar trend was observed for telaprevir, where ingestion of at least 60% of the total planned amount of the drug and a minimum of 8 weeks of administration was necessary to achieve an 80% SVR rate. For peg-interferon, at least 80% of the planned amount of the drug and continuation over the full 24 weeks of therapy was necessary to achieve an 80% SVR rate.

Multivariate analysis of factors associated with SVR

We assessed factors associated with SVR by logistic regression analysis. In univariate analysis, the following factors were significantly associated with SVR, telaprevir, ribavirin and peg-interferon adherence; age, baseline platelet, haemoglobin, AST, γGPT levels, total cholesterol and HDL; and the genotype of the IFNλ4 locus and core70 substitutions (Table 2). In a multivariate analysis of these factors, only baseline platelet levels, genotype of the IFNλ4 locus and peg-interferon were independent predictors for SVR.

Treatment discontinuation due to adverse events

The treatment was discontinued in 60 of the 273 patients (22.0%) due to anaemia (35%), nonvirological response

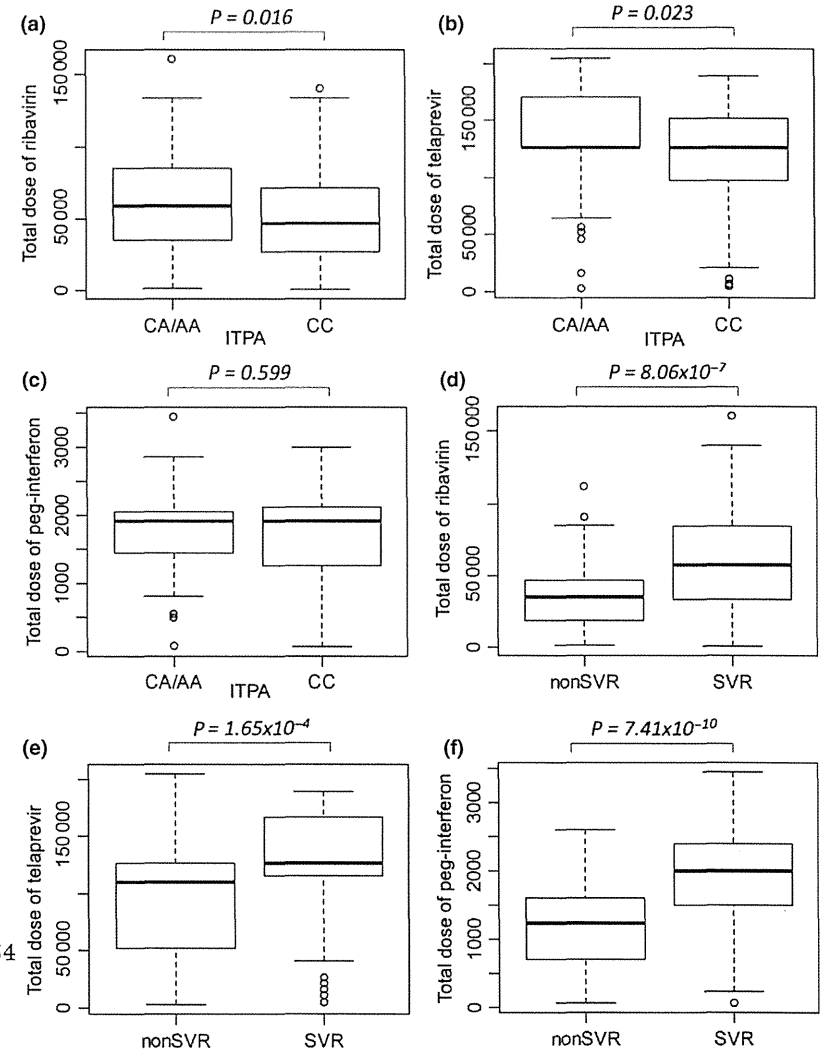


Fig. 3 Total dose of each drug by ITPA genotype and sustained viral response. Total dose of (**a**) ribavirin, (**b**) telaprevir and (**c**) peg-interferon by ITPA rs1127354 genotype. Total dose of (**d**) ribavirin, (**e**) telaprevir and (**f**) peg-interferon by sustained viral response (SVR).

(15%), malaise (13%), renal damage (12%), anorexia (8%), skin rash (5%), insomnia (5%), transient ischaemic attack (2%), pneumonia (2%), hypotension (2%) and retinopathy (2%).

DISCUSSION

This study examined the effect of dose reduction on outcome of telaprevir triple therapy in genotype 1 patients in the postmarketing phase in Japan. We found a high overall 80.2% SVR rate in this diverse group of patients in spite of frequent dose reduction. When we analysed the duration of ribavirin administration, the majority of patients took ribavirin for the full 24 weeks, resulting in an SVR rate of 89.7% in these patients, suggesting that continuous administration of ribavirin is important to achieve SVR even when accompanied by substantial dose reduction. Patients with ITPA rs1127354 CC genotype were more vulnerable to anaemia and experienced significantly faster haemoglobin decline than CA/AA patients, especially during the first 4 weeks of therapy. Ribavirin dose was reduced in response to haemoglobin decline, and in some cases, telaprevir and peg-interferon doses were reduced as well. Cumulatively, CC genotype patients therefore received less total ribavirin and telaprevir; nonetheless, there was no significant difference in SVR rate. Despite receiving a smaller effective dose of ribavirin, which is still required to prevent emergence of telaprevir-resistant mutants [26], genotype CC patients were not more likely to encounter viral breakthrough ($P = 0.88$). Viral breakthrough was instead associated with the presence of core70 substitution, which is related to IFNλ4 genotype, both of which have been reported to be associated with triple therapy [27]. Although both telaprevir and ribavirin doses were

Table 2 *Predictive factors associated with sustained viral response in patients with chronic infection with hepatitis C genotype 1 determined by multivariate logistic regression analysis with forward/backward stepwise selection*

	Simple			Multiple			
Variable	*N*	OR	*P*	*N*	OR	(95% CI)	*P*
Sex	270	1.13	0.691				
Age	268	0.67	0.019*	234	0.62	(0.33–1.15)	0.126
BMI	233	0.9	0.65				
IFNλ4 TT/TT	244	0.075	6.40E-15***	234	0.0032	(0.00046–0.022)	5.00E-09***
ITPA C/C	244	1.03	0.93				
Core70 mutant	149	0.16	2.40E-05***				
Fibrosis	137	0.22	0.00017***				
Activity	131	0.52	0.123				
Treatment-naïve	260	2.48	0.033*				
Telaprevir adherence	263	2.23	0.00012***				
Peg-IFN adherence	262	3.78	8.70E-10***	234	16.27	(5.57–47.56)	3.40E-07***
Ribavirin adherence	261	4.29	1.40E-07***				
α-Fetoprotein	246	0.72	2.50E-07***				
Fasting blood sugar	216	0.87	0.163				
WBC	266	1.2	0.571				
Neutrophil percentage	247	1.23	0.287				
HB	267	1.66	0.021*	234	2.01	(0.93–4.35)	0.076.
Plt	266	4.1	8.80E-08***	234	5.03	(2.10–12.04)	0.00029***
AST	269	0.73	0.018*				
ALT	269	0.93	0.33				
γGPT	257	0.78	8.30E-05***				
Creatinine	265	0.76	0.316				
Uric acid	211	0.91	0.755				
eGFR	182	1.12	0.532				
Total cholesterol	237	1.38	0.044*				
Triglycerides	211	0.83	0.228				
HDL	189	2.12	0.015*				
HCV RNA	255	0.86	0.581				

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

significantly associated with SVR, only peg-interferon dose adherence was an independent predictor of SVR in multivariate analysis. These results suggest that dose reductions in ribavirin and telaprevir can help alleviate or prevent side effects without severely compromising efficacy, while dose reductions of peg-interferon below 80% should be avoided. Given further study, it may also be possible to set a lower initial dose of the drugs when ITPA and IFNλ4 SNP genotypes are known beforehand, in a step towards more personalized medicine.

Results of phase II and III trials garnered high expectations for a substantial improvement in the SVR rate for treatment of HCV genotype 1 with triple therapy, even among difficult-to-treat patients [28]. The risk is that results of clinical trials might overestimate treatment success in clinical practice due to the focus on well-defined patient populations with stringent inclusion criteria and strict adherence protocols that exclude a number of patients who will require treatment; therefore, postmarketing evaluation is needed to compare treatment efficacy in clinical practice to expectations [29]. While the anticipated improvements in SVR rate have largely been met [30], in a retrospective study, adoption of telaprevir/boceprevir triple therapy was found to be lower than expected at only 18.7% of eligible patients during the year following FDA approval, due in part to concerns over safety and anticipation of alternative therapies currently under development [31]. Other studies have confirmed results of clinical trials in other demographics and patient populations, such as liver transplant recipients [32]. However, the greater variability among patients in a clinical setting has also revealed new risks, including drug interaction risks in transplant recipients and the greater risk of infection and hepatic decompensation in cirrhotic patients, a group that was not well represented during clinical trials [33]. An adverse relationship between renal function and ribavirin metabolism associated with telaprevir was not detected until after approval of triple therapy in Japan [13]. As the

drug enters wider use, future studies may uncover additional side effects and risk factors, including changes in resistance patterns, but with careful management, the therapy appears safe and effective for the majority of patients [18].

Interferon and ribavirin-free DAA combination therapies are also near at hand. Dual therapy with asunaprevir, a second-wave first-generation protease inhibitor, and daclatasvir, an NS5A inhibitor, achieves high SVR rates even in difficult-to-treat patients with fewer side effects and a relatively high barrier to resistance, especially in Japan, where genotype 1b is common [34,35]. Oral therapy with sofosbuvir, an NS5B polymerase inhibitor, with or without ribavirin or in combination with other antivirals, is another promising interferon-free therapy [36]. Many new DAAs have pan-genotypic efficacy and may be approved for use against HCV genotypes other than genotype 1. Nonetheless, removal of peg-interferon has revealed unexpected differences in treatment outcome between genotypes 2 and 3, perhaps related to the viral steatosis phenotype associated with genotype 3 [37]. The role of telaprevir in the new era of multiple DAA therapies is unclear, but while telaprevir may be a short-lived drug to do strong side effects, it will likely remain on the market due to different resistance profiles in second-generation protease inhibitors [38].

Despite the prospect of upcoming interferon and ribavirin-free therapies, triple therapy offers the best current chance of eradication and far exceeds the success rate of the previous standard of care [18]. However, prevention of side effects, which may be severe in some patients, requires close monitoring and appropriate dose reduction as necessary, especially in anaemia-prone rs1127354 CC patients. Results of this study suggest that, even with rapid reduction of ribavirin, continuous treatment with all three drugs is necessary to achieve high SVR rates, and reduction of peg-interferon should be avoided.

ACKNOWLEDGMENTS AND DISCLOSURES

The authors thank Rie Akiyama for her excellent technical assistance and Akemi Sada and Emi Nishio for clerical assistance. This work was carried out at the Research Center for Molecular Medicine, Faculty of Medicine, Hiroshima University and the Analysis Center of Life Science, Hiroshima University. Hiroshima Liver Study Group: Members of the Hiroshima Liver Study Group (listed in alphabetical order) include Shiomi Aimitsu (Hiroshima General Hospital of West Japan Railway Company, Hiroshima, Japan), Yasuyuki Aisaka (Hiroshima Red Cross Hospital, Hiroshima, Japan), Hajime Amano (Onomichi General Hospital, Hiroshima, Japan), Tatsuya Amimoto (Amimoto Clinic, Hiroshima, Japan), Keiko Arataki (Hiroshimakinen Hospital, Hiroshima, Japan), Nobuyuki Asada (Asada Clinic, Hiroshima, Japan), Takahiro Azakami (Hiroshima Kinen Hospital, Hiroshima, Japan), Kunio Ishida (Hiroshima General Hospital, Hiroshima, Japan), Hiroto Ishihara (Hiroshima-Nishi Medical Center, Hiroshima, Japan), Tomokazu Ishitobi (Hiroshima Chuden Hospital, Hiroshima, Japan), Hiroyuki Ito (Saiseikai Kure Hospital, Hiroshima, Japan), Koji Kamada (Shobara Red Cross Hospital, Hiroshima, Japan), Masaya Kamiyasu (Kamiyasu Clinic, Hiroshima, Japan), Hiroiku Kawakami (Kawakami Clinic, Hiroshima, Japan), Shinsuke Kira (Saiseikai Hiroshima Hospital, Hiroshima, Japan), Mikiya Kitamoto (Hiroshima Prefectural Hospital, Hiroshima, Japan), Hideaki Kodama (Miyoshi Cyuo Hospital, Hiroshima, Japan), Hiroshi Kohno (Kure Medical Center, Hiroshima, Japan), Hirotaka Kohno (Kure Medical Center, Hiroshima, Japan), Toshiyuki Masanaga (Masanaga Clinic, Hiroshima, Japan), Toshio Miura (Akitsu Prefectural Hospital, Hiroshima, Japan), Nami Mori (Hiroshima Red Cross Hospital, Hiroshima, Japan), Takashi Moriya (Chugoku Rousai Hospital, Hiroshima, Japan), Yutaka Nabeshima (Chuden Hospital, Hiroshima, Japan), Yuko Nagaoki (Higashihiroshima Medical Center, Hiroshima, Japan), Toshio Nakamura (Nakamura Clinic, Hiroshima, Japan), Toshio Nakanishi (Miyoshi Cyuo Hospital, Hiroshima, Japan), Ryo Nakashio (Nakashio Clinic, Hiroshima, Japan), Michihiro Nonaka (Hiroshima General Hospital, Hiroshima, Japan), Makoto Ohbayashi (Onomichi General Hospital, Hiroshima, Japan), Shintaro Takaki (Hiroshima Red Cross Hospital, Hiroshima, Japan), Eichi Takesaki (Higashihiroshima Medical Center, Hiroshima, Japan), Toru Tamura (Mazda Hospital, Hiroshima, Japan), Keiji Tsuji (Hiroshima City Asa Hospital, Hiroshima, Japan), Koji Waki (Hiroshima City Asa Hospital, Hiroshima, Japan), Masashi Watanabe (Kuchiwa Clinic, Hiroshima, Japan), Syuji Yamaguchi (Kure Kyosai Hospital, Hiroshima, Japan) and Keitaro Yamashina (Hiroshima General Hospital of West Japan Railway Company, Hiroshima, Japan). None of the authors has a personal interest to declare. This study was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Health, Labour and Welfare.

REFERENCES

1 Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009; 29(Suppl 1): 74–81.
2 Barritt ASt, Fried MW. Maximizing opportunities and avoiding mistakes in triple therapy for hepatitis C virus. *Gastroenterology* 2012; 142 (6): 1314–1323 e1.
3 Sherman KE, Flamm SL, Afdhal NH *et al.* Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; 365(11): 1014–1024.
4 Zeuzem S, Andreone P, Pol S *et al.* Telaprevir for retreatment of HCV

infection. *N Engl J Med* 2011; 364 (25): 2417–2428.
5 Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; 56(1): 78–84.
6 Jacobson IM, McHutchison JG, Dusheiko G *et al.* Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; 364(25): 2405–2416.
7 Ogawa E, Furusyo N, Nakamuta M *et al.* Clinical milestones for the prediction of severe anemia by chronic hepatitis C patients receiving telaprevir-based triple therapy. *J Hepatol* 2013; 59(4): 667–674.
8 Belperio PS, Hwang EW, Thomas IC, Mole LA, Cheung RC, Backus LI. Early virologic responses and hematologic safety of direct-acting antiviral therapies in veterans with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2013; 11(8): 1021–1027.
9 Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H. Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. *J Viral Hepatitis* 2012; 19(2): e134–e142.
10 Hara T, Akuta N, Suzuki F *et al.* A pilot study of triple therapy with telaprevir, peginterferon and ribavirin for elderly patients with genotype 1 chronic hepatitis C. *J Med Virol* 2013; 85(10): 1746–1753.
11 Jesudian AB, Jacobson IM. Optimal treatment with telaprevir for chronic HCV infection. *Liver Int* 2013; 33(Suppl 1): 3–13.
12 Fukuda K, Imai Y, Hiramatsu N *et al.* Renal impairment during the treatment of telaprevir with peginterferon and ribavirin in patients with chronic hepatitis C. *Hepatol Res* 2013; doi: 10.1111/hepr.12229.
13 Karino Y, Ozeki I, Hige S *et al.* Telaprevir impairs renal function and increases blood ribavirin concentration during telaprevir/pegylated interferon/ribavirin therapy for chronic hepatitis C. *J Viral Hepatitis* 2014; 21(5): 341–347.
14 Romero-Gomez M, Berenguer M, Molina E, Calleja JL. Management of anemia induced by triple therapy in patients with chronic hepatitis C: challenges, opportunities and recommendations. *J Hepatol* 2013; 59 (6): 1323–1330.
15 Ogawa E, Furusyo N, Nakamuta M *et al.* Telaprevir-based triple therapy for chronic hepatitis C patients with advanced fibrosis: a prospective clinical study. *Aliment Pharmacol Ther* 2013; 38(9): 1076–1085.
16 Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; 54(4): 1433–1444.
17 Reesink HW, Zeuzem S, Weegink CJ *et al.* Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. *Gastroenterology* 2006; 131(4): 997–1002.
18 Chayama K, Hayes CN, Ohishi W, Kawakami Y. Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines. *J Gastroenterol* 2013; 48(1): 1–12.
19 Suzuki F, Suzuki Y, Sezaki H *et al.* Exploratory study on telaprevir given every 8 h at 500 mg or 750 mg with peginterferon-alpha-2b and ribavirin in hepatitis C patients. *Hepatol Res* 2013; 43(7): 691–701.
20 Kawakami Y, Suzuki F, Karino Y, Toyota J, Kumada H, Chayama K. Telaprevir is effective given every 12 hours at 750 mg with peginterferon-alfa-2b and ribavirin to Japanese patients with HCV-1b IL28B rs8099917 TT. *Antivir Ther* 2013; doi: 10.3851/IMP2706 [Epub ahead of print].
21 Fellay J, Thompson AJ, Ge D *et al.* ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010; 464(7287): 405–408.
22 Ochi H, Maekawa T, Abe H *et al.* ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy–a genome-wide study of Japanese HCV virus patients. *Gastroenterology* 2010; 139(4): 1190–1197.
23 Suzuki F, Suzuki Y, Akuta N *et al.* Influence of ITPA polymorphisms on decreases of hemoglobin during treatment with pegylated interferon, ribavirin, and telaprevir. *Hepatology* 2011; 53(2): 415–421.
24 D'Avolio A, De Nicolo A, Cusato J *et al.* Association of ITPA poly morphisms rs6051702/rs1127354 instead of rs7270101/rs1127354 as predictor of ribavirin-associated anemia in chronic hepatitis C treated patients. *Antiviral Res* 2013; 100(1): 114–119.
25 Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. *Intervirology* 2006; 49(1–2): 7–17.
26 Hezode C, Forestier N, Dusheiko G *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; 360(18): 1839–1850.
27 Akuta N, Suzuki F, Hirakawa M *et al.* Amino Acid Substitution in HCV Core Region and Genetic Variation near the IL28B Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment. *Intervirology* 2012; 55(6): 417–425.
28 Fowell AJ, Nash KL. Telaprevir: a new hope in the treatment of chronic hepatitis C? *Adv Ther* 2010; 27(8): 512–522.
29 D'Ambrosio R, Colombo M. Safety of direct antiviral agents in real life. *Dig Liver Dis* 2013; 45(Suppl 5): S363–S366.
30 Yang D, Liang HJ, Li D, Wei X, Ma L, Jia Z. The efficacy and safety of telaprevir-based regimens for treating chronic hepatitis C virus genotype 1 infection: a meta-analysis of randomized trials. *Intern Med* 2013; 52(6): 653–660.
31 Chen EY, Sclair SN, Czul F *et al.* A small percentage of patients with hepatitis C receive triple therapy with boceprevir or telaprevir. *Clin Gastroenterol Hepatol* 2013; 11(8): 1014–1020 e1-2.
32 Coilly A, Roche B, Dumortier J *et al.* Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: a multicenter experience. *J Hepatol* 2014; 60(1): 78–86.

33 Hezode C, Fontaine H, Dorival C *et al.* Triple therapy in treatment-experienced patients with HCV-cirrhosis in a multicentre cohort of the French Early Access Programme (ANRS CO20-CUPIC) - NCT01514890. *J Hepatol* 2013; 59(3): 434–441.
34 Suzuki Y, Ikeda K, Suzuki F *et al.* Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options. *J Hepatol* 2013; 58(4): 655–662.
35 Lok AS, Gardiner DF, Lawitz E *et al.* Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012; 366(3): 216–224.
36 Rodriguez-Torres M. Sofosbuvir (GS-7977), a pan-genotype, direct-acting antiviral for hepatitis C virus infection. *Expert Rev Anti Infect Ther* 2013; 11(12): 1269–1279.
37 Jacobson IM, Gordon SC, Kowdley KV *et al.* Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; 368(20): 1867–1877.
38 Akuta N, Suzuki F, Seko Y *et al.* Emergence of telaprevir-resistant variants detected by ultra-deep sequencing after triple therapy in patients infected with HCV genotype 1. *J Med Virol* 2013; 85(6): 1028–1036.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Drug adherence and duration of treatment with respect to SVR. Adherence (upper) was defined as the percentage ratio of the actual total dose to the planned total dose based on the package insert. Duration of therapy (lower) was compared between SVR and non-SVR patients. The full duration of therapy was 24 weeks. SVR: sustained viral response.

Journal of General Virology (2014), 95, 1297–1306 DOI 10.1099/vir.0.060103-0

IFNL4/IL-28B haplotype structure and its impact on susceptibility to hepatitis C virus and treatment response in the Japanese population

Hidenori Ochi,[1,2,3] Daiki Miki,[1,2,3] C. Nelson Hayes,[1,2,3] Hiromi Abe,[1,2,3] Yasufumi Hayashida,[1] Michiaki Kubo[4] and Kazuaki Chayama[1,2,3]

Correspondence
Kazuaki Chayama
chayama@hiroshima-u.ac.jp

[1]Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

[2]Laboratory for Digestive Diseases, Center for Integrative Medical Sciences, RIKEN, Hiroshima, Japan

[3]Liver Research Project Center, Hiroshima University, Hiroshima, Japan

[4]Laboratory for Genotyping Development, Center for Integrative Medical Sciences, RIKEN, Yokohama, Japan

A new type III interferon, IFN lambda 4 (IFNL4), and its single-nucleotide polymorphism (SNP) ss469415590 causing a frame shift have been recently reported strongly to affect antiviral therapy for chronic hepatitis C virus (HCV) infection in African and Caucasian populations compared to previously reported IL-28B SNPs rs12979860 and rs8099917. To compare the predictability for treatment outcome among those polymorphisms, we estimated haplotype structure of IFNL4/IL-28B consisting of the three SNPs in 4630 Japanese chronic hepatitis C patients and 1122 healthy controls and then compared their impact on response to pegylated-IFN (PEG-IFN) plus ribavirin (RBV) combined therapy in 903 HCV-1b-infected patients. A total of five haplotypes were identified, although two major haplotypes accounted for >99 % of the variation. The SNPs were tightly linked but not in absolute linkage disequilibrium. We could not find any difference in the predictive impact of any of these three SNPs with regard to susceptibility to HCV and treatment response. However, patients with favourable rs8099917 TT, linked to unfavourable genotypes of ss469415590 and rs12979860, showed poor initial viral response compared with those with all favourable genotypes (P=0.0022). These findings suggest that, in part, ss469415590 and rs12979860 may have better predictive impact on response to PEG-IFN plus RBV therapy in the Japanese population, especially in patients with any of the minor haplotypes consisting of these SNPs.

Received 4 October 2013
Accepted 18 March 2014

INTRODUCTION

Hepatitis C virus (HCV) infection is the major cause of chronic liver disease, liver cirrhosis and hepatocellular carcinoma. There are more than 180 million HCV chronic carriers worldwide (Chevaliez & Pawlotsky, 2007; Shepard *et al.*, 2005). The current standard of care for the treatment of chronic hepatitis C (CHC) is pegylated-IFN (PEG-IFN) with ribavirin (RBV). However, less than half of patients with HCV genotype 1 achieve a sustained viral response (SVR) with this therapy (Hadziyannis *et al.*, 2004). The addition of direct-acting antiviral (DAA) protease inhibitors, such as telaprevir and boceprevir, to the current standard of care regimen improves the rate of SVR to 65–75 % (Morgan & O'Brien, 2011), while entailing increased risk of side effects, including anaemia and rash. Therefore it would be helpful to be able to identify patients who will respond to the current standard of care without DAA agents.

A number of pretreatment predictors of SVR have been reported. HCV genotype, baseline viral load, liver fibrosis, age, sex, obesity, insulin resistance, low-density lipoprotein cholesterol levels and γ-glutamyl transpeptidase (γ-GTP) levels have been reported to be associated with the outcome of PEG-IFN plus RBV therapy (Bergmann *et al.*, 2007; Charlton *et al.*, 2006; Gao *et al.*, 2004; Gopal *et al.*, 2006; Romero-Gómez *et al.*, 2005; Zeuzem *et al.*, 1996, 2000).

In addition, both host and viral genetic factors have been implicated in treatment response. Substitutions within the HCV IFN sensitivity determining region (ISDR) (Akuta *et al.*, 2009; Yen *et al.*, 2008) and the IFN/RBV resistance determining region (IRRDR) (El-Shamy *et al.*,

2008) and a substitution at amino acid 70 of the HCV core protein (Akuta *et al.*, 2006) have also been reported to affect PEG-IFN plus RBV combination therapy. With respect to host genetic factors, recent genome-wide association studies (GWAS) have reported a set of common single-nucleotide polymorphisms (SNPs) near the IL-28B locus on chromosome 19 that are strong predictors of SVR (Ge *et al.*, 2009; Suppiah *et al.*, 2009; Tanaka *et al.*, 2009) as well as spontaneous viral clearance (Thomas *et al.*, 2009).

Despite recent research efforts, the true causal variant at the IL-28B locus and the mechanism by which it modulates the IFN response remain unclear. In our previous study using Japanese subjects, we resequenced the region surrounding the locus and found that several known and newly discovered SNPs were associated with virological response (Ochi *et al.*, 2011). One of these, a dinucleotide polymorphism that introduces a frame shift, was recently reported to affect the expression of IFN lambda 4 (IFNL4), a newly identified type III IFN, by causing a frame shift (Prokunina-Olsson *et al.*, 2013). Furthermore, this polymorphism was found to be more strongly associated with the outcome and early viral dynamics of PEG-IFN and RBV therapy than the major IL-28B SNPs, especially in African populations (Prokunina-Olsson *et al.*, 2013).

The aim of this study was to examine how well the IFNL4 polymorphism (ss469415590) can predict viral response in Japanese patients infected with HCV, as compared to IL-28B polymorphisms (rs8099917 and rs12979860) conventionally used for prediction.

RESULTS

Allelic and haplotype frequencies in the Japanese population

No significant deviation from Hardy–Weinberg equilibrium (HWE) was observed either in CHC patients or in healthy controls for any of the SNPs, thus, selection bias or genotyping error was unlikely (Hosking *et al.*, 2004; Salanti *et al.*, 2005). Allelic frequencies of the three SNPs in CHC and control groups are shown in Table 1. The unfavourable allele frequencies of rs12979860, ss469415590 and rs8099917 in CHC patients were higher than those in controls for each SNP (P=0.001, P=0.0007, P=0.002, respectively). However, an integrated discrimination improvement (IDI) test showed that there was no significant difference in the strength of association with CHC (i.e. difference in P value) between any two of the three SNPs. When stratified by HCV genotype, significant differences in favourable allele frequency between HCV-1b patients and healthy controls were found (Fig. 1). By contrast, in the HCV-2a and HCV-2b subgroup, favourable genotype frequency was similar to that of control subjects (Fig. 1).

Linkage disequilibrium among the three SNPs in the studied population is shown in Fig. 2. These SNPs were in strong but not absolute linkage disequilibrium (r^2=1.0). Haplotype frequencies in control subjects and CHC patients were estimated and compared using the Haploview program. A total of five haplotypes including two major haplotypes were identified (Table 1). The two major haplotypes accounted for >99 % of the variation in both groups. There were significant differences in haplotype frequencies between

Table 1. IFNL4/IL-28B polymorphisms and haplotypes in CHC patients and controls

	Allele	Frequency (%)		*P* value*	*P* value†
		Healthy controls (n=1122)	CHC patients (n=4630)		
rs12979860					
	C	89.8	87.3	0.001	0.021
	T	10.2	12.7		
ss469415590					
	TT	89.9	87.3	0.0007	0.022
	ΔG	10.1	12.7		
rs8099917					
	T	90.2	87.9	0.002	0.021
	G	9.8	12.1		
Haplotype‡					
	C-TT-T	89.8	87.3	0.0008	
	T-ΔG-G	9.7	12.1	0.0013	
	T-ΔG-T	0.40	0.56	0.35	
	G-TT-C	0.04	0.011	0.35	
	G-TT-T	0.08	0.0	0.04	

*Chi-squared test under allelic model.

†Adjusted by age and sex.

‡Each haplotype represents allele information of the three adjacent SNPs in sequence, i.e. rs12979860-ss469415590-rs8099917.

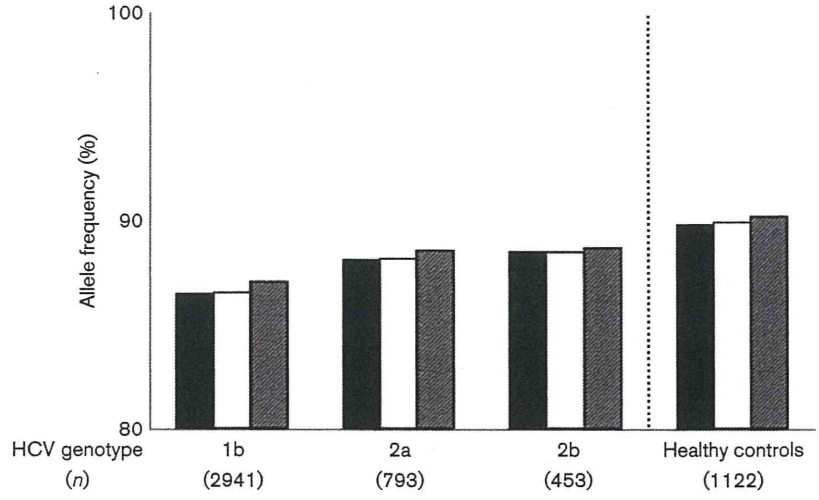


Fig. 1. Allele frequencies of IFNL4/IL-28B SNPs in CHC patients stratified by HCV genotype. Black bars, rs12979860; white bars, ss469415590; grey bars, rs8099917. Favourable allele frequency of each SNP in HCV-1b was significantly lower than in healthy controls (rs12979860, $P=4.6\times10^{-5}$, odds ratio (OR) 0.72; ss469415590 $P=4.1\times10^{-5}$, OR 0.72; rs8099917, $P=1.3\times10^{-4}$, OR 0.74); there was no significant difference for HCV-2a or HCV-2b.

CHC and control subjects for the two major haplotypes, however, which were similar to results from the single-marker analysis (Table 1).

Association with treatment outcome of PEG-IFN plus RBV therapy for HCV-1b patients

Among HCV-1b patients completing a full treatment course (n=903), allelic frequencies of the IFNL4/IL-28B SNPs in SVR and non-SVR groups are shown in Table 2. The unfavourable allelic frequencies of rs12979860, ss469515590 and rs8099917 in non-SVR patients were higher than those in SVR patients with each SNP ($P=1.2\times10^{-16}$, $P=1.2\times10^{-16}$ and $P=2.1\times10^{-16}$, respectively). However, an IDI test showed that there was no significant difference in the strength of association with SVR (i.e. difference in P value) between any two of the three SNPs.

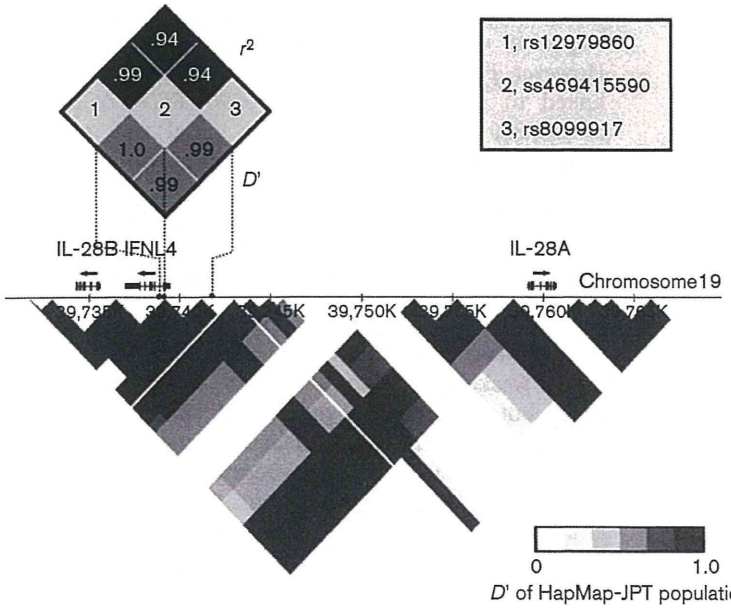


Fig. 2. Linkage disequilibrium among IFNL4/IL-28B SNPs in a Japanese population. The upper panel shows linkage disequilibrium of the studied population among the IFNL4/IL-28B SNPs. Each value in the box represents D' or r^2 calculated by the Haploview program. The lower panel depicts the haplotype structure of IFNL4/IL-28B loci on chromosome 19 from Phase II HapMap Japanese in Tokyo (JPT) genotype data.

Table 2. IFNL4/IL-28B polymorphisms and haplotypes in SVR and non-SVR groups

	Allele	Frequency (%)		*P* value*
		SVR (n=547)	Non-SVR (n=356)	
rs12979860				
	C	93.1	80.1	1.2×10^{-16}
	T	6.9	19.9	
ss469415590				
	TT	93.1	80.1	1.2×10^{-16}
	ΔG	6.9	19.9	
rs8099917				
	T	93.5	80.8	2.1×10^{-16}
	G	6.5	19.2	
Haplotype†				
	C-TT-T	93.0	80.1	1.3×10^{-16}
	T-ΔG-G	6.5	19.2	1.2×10^{-16}
	T-ΔG-T	0.5	0.7	0.49
	C-TT-G	0.09	0.0	1
	T-TT-G	0.0	0.0	–

*Chi-squared test under allelic model.
†Each haplotype represents allele information of the three adjacent SNPs in sequence, i.e. rs12979860-ss469415590-rs8099917.

Early virological response in patients with minor haplotypes

IL-28B SNPs have been reported to be associated with early viral kinetics as well as SVR (Thompson *et al.*, 2010). Further, IFNL4 polymorphism ss469415590 has recently been reported to affect HCV RNA decline after 28 days of treatment more strongly than rs12979860 in the African-American population (Prokunina-Olsson *et al.*, 2013). We examined whether there was any difference in the effect of genotype on early viral kinetics among the three SNPs. As shown in Fig. 3, with respect to median HCV RNA decline of the three genotype groups at weeks 2 and 4 of treatment, very similar patterns were observed, and there were no significant differences in the impact of genotypes on early viral decline among the three SNPs.

Next, we focused on patients with minor haplotypes, because these subgroups may provide valuable insights by highlighting differences in the genotype effect on therapeutic response among the SNPs. Among HCV-1b patients having at least one minor haplotype and treated with PEG-IFN plus RBV therapy, including patients who stopped treatment prematurely, 17 patients could be analysed for correlations between viral load change at week 4 and genotypes for each SNP. As shown in Fig. 4, among patients with minor haplotypes, viral load changes in patients with favourable rs8099917 TT linked to unfavourable genotypes of the other SNPs were significantly less than those in patients with favourable genotypes in all the three SNPs (P=0.0022). Likewise, similar correlation coefficients were observed for rs12979860 (r=0.50) and ss469415590 (r=0.50) compared to that of patients with major haplotypes (r=0.46), whereas a negative correlation was observed for rs8099917 (r=-0.32). These findings suggest that, in the Japanese population, rs12979860 and ss469415590 may provide better predictive ability than rs8099917, especially in patients with minor haplotypes.

DISCUSSION

In this study, we showed that IFNL4 polymorphism ss469415590 and IL-28B polymorphisms (rs12979860 and rs8099917) were in strong linkage disequilibrium with one another in the Japanese population but were not in absolute linkage disequilibrium. We could not find any differences in the overall predictive impact of any of these three SNPs with respect to susceptibility to HCV and treatment response. However, HCV-1b patients with favourable rs8099917 TT, linked to unfavourable ss469415590 TTΔG/ΔGΔG and rs12979860 CT/TT, showed poor initial viral reduction compared with those with all favourable genotypes.

Recent GWAS from several laboratories (Ge *et al.*, 2009; Suppiah *et al.*, 2009; Tanaka *et al.*, 2009) reported that genetic variants within the IL-28B locus were associated with the efficacy of PEG-IFN and RBV combined therapy in patients infected with HCV genotype 1. Subsequently their findings have been replicated in HCV genotypes 2 albeit with a weaker effect (Kawaoka *et al.*, 2011; Mangia *et al.*, 2010) but not in HCV genotype 3 (Bucci *et al.*, 2013; Moghaddam *et al.*, 2011). Further, spontaneous resolution of acute HCV infection has also been shown to be associated with IL-28B polymorphism (Thomas *et al.*, 2009; Tillmann *et al.*, 2010). A number of clinical phenotypes have been found to be associated with IL-28B variants, e.g. necro-inflammatory activity (Abe *et al.*, 2010), fibrosis, steatosis

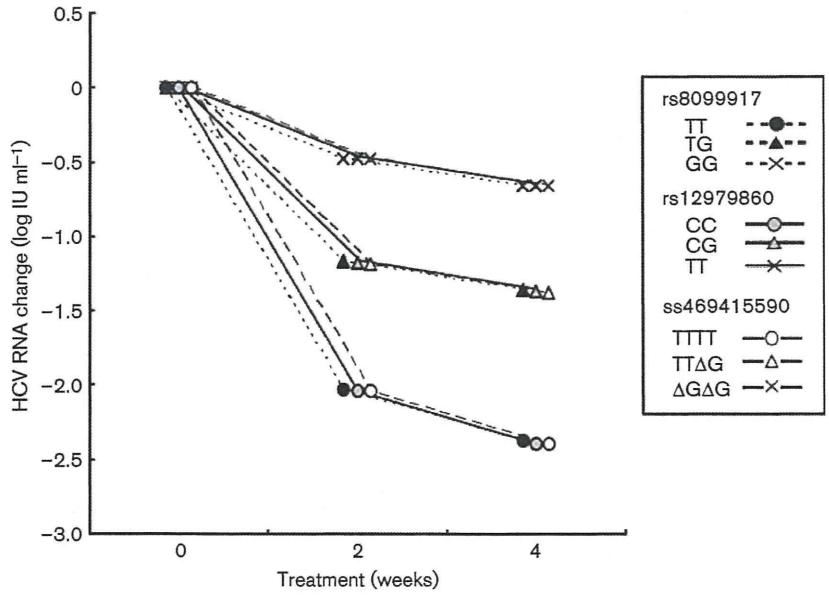
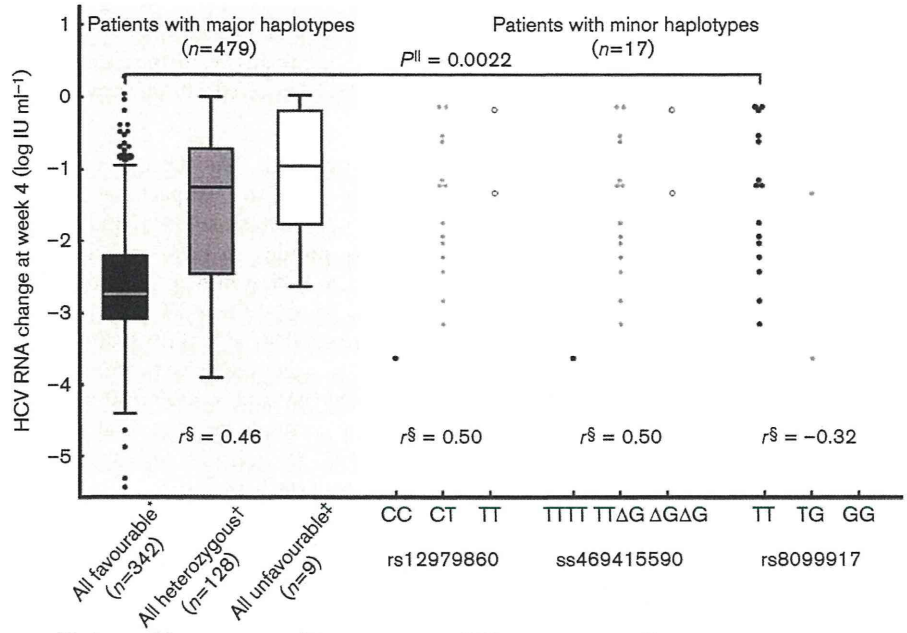


Fig. 3. Early viral kinetics and IFNL4/IL-28B SNPs. Median HCV RNA decline, compared to the baseline value, at weeks 2 and 4 of PEG-IFN plus RBV treatment are plotted for each of the three IFNL4/IL-28B SNP genotype groups. There were no significant differences in the impact of genotypes on early viral decline among the SNPs.



*Patients with rs12979860CC ss469415590TTTT and rs8099917TT
†Patients with rs12979860CT ss469415590TTΔG and rs8099917TG
‡Patients with rs12979860TT ss469415590ΔGΔG and rs8099917GG
§Pearson's correlation coefficient
‖Mann–Whitney U test

Fig. 4. Comparison of viral load change at week 4 and IFNL4/IL-28B SNPs in patients with minor haplotypes. In 17 patients with minor haplotypes, initial viral load changes at week 4 for each genotype with respect to rs12979860, ss469415590 and rs8099917 are plotted compared with patients with all major haplotypes. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. Whiskers denote the lowest and highest values within 1.5 × IQR and the dots represent the outliers.

(Ohnishi *et al.*, 2012; Tillmann *et al.*, 2011), γ-GTP levels (Abe *et al.*, 2010), baseline viral titre (Ge *et al.*, 2009; Ochi *et al.*, 2011), HCV core amino acid 70 substitution (Akuta *et al.*, 2010; Hayes *et al.*, 2011), and hepatic IFN-stimulated gene (ISG) expression (Abe *et al.*, 2011; Honda *et al.*, 2010; Urban *et al.*, 2010). Although great efforts have been made, the functional mechanism underlying the influence of the IL-28B polymorphism on HCV viral response remains unclear.

In our previous study, we performed intensive resequencing and fine-mapping around the IL-28B locus in the Japanese population and found that the two IL-28B SNPs generally used for predicting treatment response were in strong linkage disequilibrium with several novel SNPs including a dinucleotide polymorphism (Ochi *et al.*, 2011), which was recently reported by Prokunina-Olsson *et al.* (2013) to cause a frame shift within the newly identified type III IFN, IFNL4. They showed that the SNP was more strongly associated with HCV clearance in populations of African ancestry compared to rs12979860. On the other hand, they also speculated that the SNP may provide no more information in Asians on the grounds of the haplotype structure in which the IFNL4/IL-28B SNPs were tightly linked to each other. The present observations are consistent with their speculation. Bibert *et al.* (2013) reported that TT/-G polymorphism (ss469415590) is a better predictor of spontaneous HCV clearance than rs12979860 and that inductions of IL-28B and IFN gamma-induced protein 10 (IP-10) relies on TT/-G but not rs12979860 in Caucasian patients. Thus, ss469415590 seems to show the strongest association consistently between studies suggesting that the SNP may be a true causal variant across populations. By contrast, the other SNPs did not, which could be partly explained by different linkage disequilibrium patterns among the studied populations.

When comparing allele frequencies between CHC patients and controls, we could not find any significant differences between these SNPs by the IDI test (Table 1). Although we have not examined patients with self-limiting infection, our findings of the deviation of allele frequencies in CHC patients from controls appears to support previous findings that IL-28B variants are associated with spontaneous clearance of HCV infection (Thomas *et al.*, 2009).

In a subgroup that includes patients who possessed only one of the two major haplotypes (i.e. C-TT-T or T-ΔG-G), the predictive impact could logically be the same for each of the IFNL4/IL-28B SNPs. Most of the HCV patients in this study possessed one of the three diplotypes (n=4579, 98.9 %), which may explain why the differences in predictability for treatment outcome were insignificant among the IFNL4/IL-28B SNPs in Japanese CHC patients. However, from a medical point of view, patients carrying minor haplotypes cannot be disregarded in spite of their low frequency in the Japanese population. Moreover, with respect to early viral response in patients with minor haplotypes, patients with favourable rs8099917 TT, linked to unfavourable genotypes of ss469415590 and rs12979860, showed poor initial viral response compared with those with all favourable genotypes (P=0.0022) (Fig. 4). IL-28B variants have also been reported to be associated with early viral kinetics during treatment with PEG-IFN plus PBV in HCV genotype 1 patients (Thompson *et al.*, 2010), which is, in turn, a strong predictor of the eventual response to therapy (Davis *et al.*, 2003). Taken together, these findings suggest that ss469415590 and rs12979860 might be a better predictor of treatment outcome than rs8099917, especially in patients with minor haplotypes.

Several studies, including ours, have reported that the expression levels of IFN-stimulated genes in human hepatocytes infected with HCV vary by genotype in IL-28B polymorphisms (Abe *et al.*, 2011; Honda *et al.*, 2010; Urban *et al.*, 2010). On the other hand, no correlation has been demonstrated between IL-28B polymorphism genotypes and expression of IL-28B in hepatocytes (Dill *et al.*, 2011; Honda *et al.*, 2010; Urban *et al.*, 2010). Prokunina-Olsson *et al.* (2013) showed that endogenous IFNL4 upregulated ISGs through phosphorylation of signal transducers and activators of transcription STAT1 and STAT2 without signalling through an external receptor in stimulated primary human hepatocytes carrying the unfavourable ss469415590 ΔG allele. Bibert *et al.* (2013) showed that ss469415590 is located within a CpG island and is associated with surrounding CpG methylation, as well as with IL-28B and/or ISG expression. Although the molecular basis remains elusive, further studies are awaited to determine how IFNL4 affects HCV clearance in conjunction with other IFNs.

In conclusion, we found no significant difference in overall predictive impact of any of the IFNL4/IL-28B SNPs, rs12979860, ss469415590 and rs8099917, with regard to susceptibility to HCV and treatment-induced clearance. However, taking into account the finding that patients with favourable rs8099917 TT, linked to unfavourable genotypes of ss469415590 and rs12979860, showed poor initial viral response compared with those with all favourable genotypes, ss469415590 and rs12979860 may have better predictive impact on treatment response in the Japanese population, especially in patients with any of the minor haplotypes consisting of these SNPs.

METHODS

Study subjects and design. A total of 4630 CHC patients who were outpatients of Hiroshima University Hospital and Hiroshima University-affiliated hospitals were included in the study; 1122 healthy control subjects were also included. All patients had elevated serum alanine transaminase levels for more than 6 months and were positive for both anti-HCV antibody and serum HCV RNA. All patients were negative for hepatitis B surface antigen, had no evidence of other liver diseases, and had not received immunosuppressive therapy before enrolment in the study. Fibrosis stage and activity were diagnosed by pathologists at each hospital according to the criteria of Desmet *et al.* (1994). Subjects received weekly injections of PEG-IFN-α-2b at 1.5 μg kg^{-1} body mass and oral administration of RBV

for 48 weeks. The dose of RBV was adjusted based on body mass (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients with less than 75 % compliance with prescribed doses of PEG-IFN and RBV were excluded from the analysis of association with treatment outcome. Patients were divided into SVR and non-SVR groups based on treatment outcome. SVR was defined as undetectable serum HCV RNA at 24 weeks after completion of therapy, whereas non-SVR patients were still viraemic at this time including transient responders and non-responders. Table 3 lists the demographic features of the subjects. All subjects received a detailed explanation and all gave written informed consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the ethical committee of each participating medical centre, Hiroshima University, and the SNP Research Center, the Institute of Physical and Chemical Research (RIKEN), Yokohama.

The study design is shown in Fig. 5. In both CHC patients and controls, haplotypes of the three IFNL4/IL-28B SNPs (rs12979860, ss469415590 and rs8099917) were estimated. Of these patients, 1151 infected with HCV-1b were treated with PEG-IFN plus RBV. Among those, early viral dynamics data (serum HCV RNA levels at baseline and at week 4 of therapy) were available in 479 patients. A total of 903 patients with HCV-1b were evaluated with respect to treatment outcome, after excluding patients with treatment discontinuation, drop out, and insufficient data collection.

SNP genotyping. We genotyped each subject for three SNPs on chromosome 19: rs8099917, rs12979860 and ss469415590 (refSNP no. rs368234815). The first two are IL-28B polymorphisms previously reported to be associated with therapy outcome (Ge *et al.*, 2009; Suppiah *et al.*, 2009; Tanaka *et al.*, 2009), and the last was recently found to cause a frame shift in the IFNL4 gene (Prokunina-Olsson *et al.*, 2013). Genotyping was performed using multiplex-PCR followed by the Invader assay (Third Wave Technology) as described previously (Ohnishi *et al.*, 2001). Because IFN-lambda family gene sequences are highly homologous, sequence-specific primers were designed to amplify the desired sequence.

HCV RNA levels. HCV RNA levels were measured using RT-PCR-based methods (the original Amplicor method, the high-range method, or the TaqMan real-time PCR test). The measurement ranges of these assays were 0.5–850 KIU ml^{-1}, 5–5000 KIU ml^{-1} and 1.2–7.8 log IU ml^{-1}, respectively. Saturated samples were diluted with PBS and reassayed. All values are reported as log IU ml^{-1}.

Statistical analysis. For general statistical analysis, we employed the R statistical package (http://www.r-project.org). Non-parametric tests (chi-squared test, Mann–Whitney U test) were used to detect significant associations. Deviation from HWE was evaluated by the chi-squared test. Discrimination ability between markers was compared using the IDI test under an additive-effect model in a logistic regression (Pencina *et al.*, 2008). All statistical analyses were two-sided, and $P<0.05$ was considered significant. The Haploview program (Barrett *et al.*, 2005) was used to estimate linkage disequilibrium between SNPs and haplotype construction.

Table 3. Demographic characteristics of subjects included in the study

Counts are listed for categorical values and the median and range are reported for continuous variables. P_{HWE}, P value for Hardy–Weinberg equilibrium test.

CHC patients	**(n=4630)**
Age	62 (7–95) years
Sex, M/F	2472/2158
Body mass index	22.6 (13.9–39.8)
Alanine transaminase concentration	54 (2–1500) IU l^{-1}
γ-GTP concentration	39 (6–1530) IU l^{-1}
Genotype, 1b/2a/2b/others + undetermined	2941/793/453/443
Fibrosis, F0/F1/F2/F3/F4/undetermined*	37/937/838/520/183/2115
Activity, A0/A1/A2/A3/undetermined*	26/859/1260/244/2241
Log viral titre†	5.9 (1.2–7.8)
rs12979860, CC/CT/TT (P_{HWE})	3526/1032/72 (0.72)
ss469415590, TTTT/TTΔG/ΔGΔG (P_{HWE})	3528/1030/72 (0.75)
rs8099917, TT/TG/GG (P_{HWE})	3570/996/64 (0.56)
Healthy controls	**(n=1122)**
Age	43 (14–93) years‡
Sex, M/F	440/682§
Body mass index	21.5 (14.1–39.2)‡
rs12979860 CC/CT/TT (P_{HWE})	905/206/11 (0.85)
ss469415590 TTTT/TTΔG/ΔGΔG (P_{HWE})	907/204/11 (0.90)
rs8099917 TT/TG/GG (P_{HWE})	912/200/10 (0.79)

*Based on the criteria for histological assessment by Desmet *et al.* (1994).
†Viral titre was measured in IU ml^{-1}.
‡$P<0.001$ compared with the chronic hepatitis C group by Mann–Whitney U test.
§$P<0.001$ compared with the chronic hepatitis C group by chi-squared test.

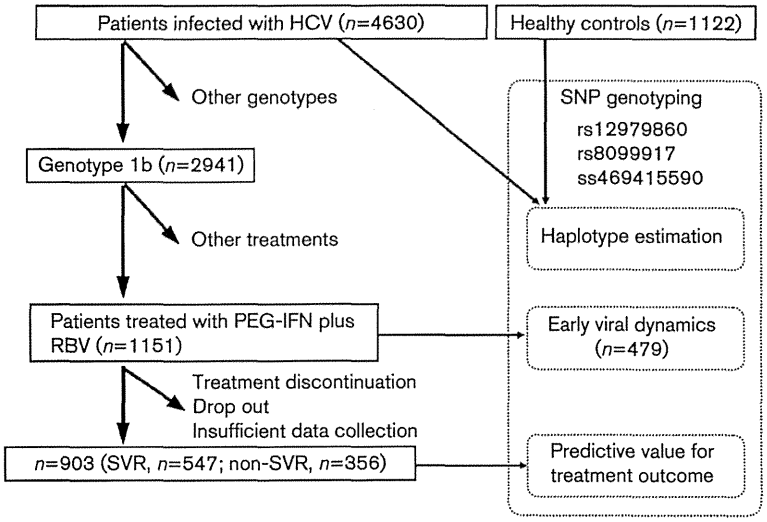


Fig. 5. Study design. Initially, 4630 patients infected with HCV and 1122 healthy controls were considered with respect to IFNL4/IL-28B SNPs (rs12979860, rs8099917 and ss469415590). Of these, 1151 patients had been treated with PEG-IFN plus RBV for chronic HCV-1b infection. Data were available for early viral dynamics and IFNL4/IL-28B SNP genotypes for 479 of these patients. After excluding patients with treatment discontinuation, drop out, and insufficient data collection, 903 patients were included in the analysis of treatment outcome and IFNL4/IL-28B SNPs.

ACKNOWLEDGEMENTS

The authors thank the subjects who agreed to participate in this study. We also thank the following team members at Hiroshima University Hospital and Hiroshima Liver Study Group for clinical sample collection: Kazuaki Chayama, Hidenori Ochi, Hiroshi Aikata, Yoshiiku Kawakami, Hideyuki Hyogo, Michio Imamura, Akira Hiramatsu, Masataka Tsuge, Nobuhiko Hiraga, Tomokazu Kawaoka, Daiki Miki, Yoshio Katamura, Satoe Yokoyama, Daisuke Miyaki, Eisuke Murakami, Hiromi Abe, C. Nelson Hayes and Niu Shi (Hiroshima University); Yasuyuki Aisaka, Nami Mori and Shintaro Takaki (Hiroshima Red Cross Hospital); Makoto Ohbayashi and Hajime Amano (Onomichi General Hospital); Keiji Tsuji and Koji Waki (Hiroshima City Asa Hospital); Toshio Nakanishi and Takahiro Azakami (Miyoshi Central Hospital); Hiroshi Kohno and Hirotaka Kohno (Kure Medical Center); Shiomi Aimitsu and Keitaro Yamashina (Hiroshima General Hospital of West Japan Railway Company); Tomokazu Ishitobi and Yutaka Nabeshima (Chuden Hospital); Kunio Ishida and Michihiro Nonaka (Hiroshima General Hospital); Mikiya Kitamoto (Hiroshima Prefectural Hospital); Toru Tamura (Mazda Hospital); Shinsuke Kira (Saiseikai Hiroshima Hospital); Hideaki Kodama (Hiroshima Kinen Hospital); Keiko Arataki (Tsuchiya General Hospital); Hiroyuki Ito (Saiseikai Kure Hospital); Takashi Moriya (Chugoku Rousai Hospital); Eichi Takesaki and Yuko Nagaoki (Higashihiroshima Medical Center); Hiroiku Kawakami (Kawakami Clinic); Syuji Yamaguchi (Kure Kyosai Hospital); Hiroto Ishihara (Hiroshima Nishi Medical Center); Toshio Miura (Akitsu Prefectual Hospital); Koji Kamada (Shobara Red Cross Hospital); Ryo Nakashio (Nakashio Clinic); Kazunari Masuda (Masuda Clinic); Masaya Kikkawa (Kikkawa Clinic); Shoichi Takahashi (Koyo New Town Hospital). The authors thank Kana Izumoto and Tomoko Imai for technical assistance and Junko Sakamiya for technical and clerical assistance. This study was supported, in part, by a Grant-in-Aid for Scientific Research from the Ministry of Labour, Health, and Welfare and Ministry of Education Culture Sports Science and Technology, Government of Japan.

REFERENCES

Abe, H., Ochi, H., Maekawa, T., Hayes, C. N., Tsuge, M., Miki, D., Mitsui, F., Hiraga, N., Imamura, M. & other authors (2010). Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* **53**, 439–443.

Abe, H., Hayes, C. N., Ochi, H., Maekawa, T., Tsuge, M., Miki, D., Mitsui, F., Hiraga, N., Imamura, M. & other authors (2011). IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. *J Hepatol* **54**, 1094–1101.

Akuta, N., Suzuki, F., Sezaki, H., Suzuki, Y., Hosaka, T., Someya, T., Kobayashi, M., Saitoh, S., Watahiki, S. & other authors (2006). Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* **78**, 83–90.

Akuta, N., Suzuki, F., Hirakawa, M., Kawamura, Y., Yatsuji, H., Sezaki, H., Suzuki, Y., Hosaka, T., Kobayashi, M. & other authors (2009). A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* **81**, 452–458.

Akuta, N., Suzuki, F., Hirakawa, M., Kawamura, Y., Yatsuji, H., Sezaki, H., Suzuki, Y., Hosaka, T., Kobayashi, M. & other authors (2010). Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* **52**, 421–429.

Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265.

Bergmann, J. F., Vrolijk, J. M., van der Schaar, P., Vroom, B., van Hoek, B., van der Sluys Veer, A., de Vries, R. A., Verhey, E., Hansen, B. E. & other authors (2007). Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* **27**, 1217–1225.

Bibert, S., Roger, T., Calandra, T., Bochud, M., Cerny, A., Semmo, N., Duong, F. H., Gerlach, T., Malinverni, R. & other authors (2013). IL28B expression depends on a novel TT/-G polymorphism which improves HCV clearance prediction. *J Exp Med* **210**, 1109–1116.

Bucci, C., von Delft, A., Christian, A., Flemming, V. M., Harrison, A., Halliday, J., Collier, J., Manganis, C., Klenerman, P. & other authors (2013). 'Favourable' IL28B polymorphisms are associated with a marked increase in baseline viral load in hepatitis C virus subtype 3a