

## Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses

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**SUMMARY.** Reducing the dose of drug affects treatment efficacy in pegylated interferon (Peg-IFN) and ribavirin combination therapy for patients with hepatitis C virus (HCV) genotype 1. The aim of this study was to investigate the impact of drug exposure, as well as the baseline factors and the virological response on the treatment efficacy for genotype 2 patients. Two-hundred and fifty patients with genotype 2 HCV who were to undergo combination therapy for 24 weeks were included in the study, and 213 completed the treatment. Significantly more patients who achieved a rapid virological response (RVR), defined as HCV RNA negativity at week 4, achieved a sustained virological response (SVR) (92%, 122/133) compared with patients who failed to achieve RVR (48%, 38/80) ( $P < 0.0001$ ). Multivariate logistic-regression analysis showed that only platelet counts [odds ratio (OR), 1.68;

confidence interval (CI), 1.002–1.139] and RVR (OR, 11.251; CI, 5.184–24.419) were independently associated with SVR, with no correlation being found for the mean dose of Peg-IFN and ribavirin for RVR and SVR. Furthermore, in the stratification analysis of the timing of viral clearance, neither mean dose of Peg-IFN ( $P = 0.795$ ) nor ribavirin ( $P = 0.649$ ) affected SVR in each group. Among the patients with RVR, the lowest dose group of Peg-IFN ( $0.77 \pm 0.10 \mu\text{g}/\text{kg}/\text{week}$ ) and ribavirin ( $6.9 \pm 0.90 \text{ mg}/\text{kg}/\text{day}$ ) showed 100% and 94% of SVR. Hence, RVR served as an important treatment predictor, and drug exposure had no impact on both SVR and RVR in combination therapy for genotype 2 patients.

**Keywords:** chronic hepatitis C, drug exposure, genotype 2, peginterferon and ribavirin combination therapy.

### INTRODUCTION

The current standard of care for chronic hepatitis C (CHC) patients consists of combination therapy using pegylated

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CHC, chronic hepatitis C; c-EVR, complete early virological response; ETR, end of treatment response;  $\gamma$ -GTP,  $\gamma$ -glutamyl transpeptidase; HCV, hepatitis C virus; IFN, interferon; NPV, negative predictive value; Peg-IFN, pegylated interferon; RVR, rapid virological response; SVR, sustained virological response.

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interferon (Peg-IFN) and ribavirin [1–3]. Large, randomized clinical trials have demonstrated that 42–52% of hepatitis C virus (HCV) genotype 1 ‘difficult-to-treat’ patients achieved sustained virological response (SVR), whereas 76–84% of HCV genotype 2 or 3 infected patients treated with Peg-IFN and ribavirin achieved SVR [4–6]. It also has been shown that in HCV genotype 2 and 3 infected patients, 24-week treatment regimens are just as effective as 48-week regimens [6,7]. Therefore, current guidelines recommend a 24-week treatment for these patients in contrast to 48 weeks for genotype 1 patients [1–3]. However, as side effects are common and treatment is expensive for this therapy, it would be ideal to be able to further reduce the total amount of drug medication

without loss of treatment efficacy for genotype 2 and 3 patients.

In HCV genotype 1 patients, reducing drug doses affects treatment efficacy. In our investigation of HCV genotype 1 patients, the rate of complete early virological response (c-EVR), defined as HCV RNA negativity at week 12, was affected by the mean dose of Peg-IFN during the first 12 weeks dose-dependently ( $P < 0.0001$ ) [8]. Furthermore, we showed that only 4% relapse was found in patients given  $\geq 12$  mg/kg/day of ribavirin among those with c-EVR, and the relapse rate showed a decline in relation to the increase in the dose of ribavirin ( $P = 0.0002$ ) [9]. On the contrary, it remains to be determined whether treatment efficacy can be preserved by further reducing both drug doses in genotype 2 and 3 patients. Because lower doses are expected to cause fewer adverse effects, it is important to find whether reduced drug doses can be used while retaining efficacy.

In the present study, we retrospectively evaluated the efficacy of Peg-IFN alpha-2b and ribavirin combination therapy for 24 weeks in patients infected with HCV genotype 2 and analysed the factors that affected the treatment efficacy, with particular interests in the drug impact of Peg-IFN and ribavirin.

## PATIENTS AND METHODS

### *Patient selection and study design*

Patients considered to be eligible for this study were those infected with HCV genotype 2 who underwent Peg-IFN alpha-2b (Schering-Plough K.K., Tokyo, Japan) and ribavirin (Schering-Plough K.K.) combination therapy from December 2005 to July 2007 at 29 medical institutions taking part in the Osaka Liver Forum and had completed the 24-week observation after a clinical course of 24 weeks. Patients with the following criteria were excluded: hepatitis B virus or human immunodeficiency virus coinfection, decompensated liver disease, severe cardiac, renal, haematological or chronic pulmonary disease, poorly controlled psychiatric disease, poorly controlled diabetes and immunologically mediated disease. Liver biopsy had been performed within 24 months prior to the treatment, and histological results were classified according to the METAVIR scoring system [10].

Written informed consent was obtained from each patient, and the study protocol was reviewed and approved according to the ethical guidelines of the 1975 Declaration of Helsinki by institutional review boards at the respective sites.

Patients were treated with Peg-IFN alpha-2b plus ribavirin for the duration of the study of 24 weeks. Peg-IFN alpha-2b and ribavirin dosages were based on body weight according to the manufacturer's instructions: Peg-IFN alpha-2b was given subcutaneously weekly (45 kg or less, 60  $\mu$ g/dose; 46–60 kg, 80  $\mu$ g/dose; 61–75 kg, 100  $\mu$ g/dose; 76–90 kg,

120  $\mu$ g/dose; 91 kg or more, 150  $\mu$ g/dose), and ribavirin was given orally daily (60 kg or less, 600 mg/day; 61–80 kg, 800 mg/day; 81 kg or more, 1000 mg/day). The drug doses were also modified based on the manufacturer's instructions according to the intensity of the haematologic adverse effects.

### *Virological tests*

Serum HCV RNA level was quantified by PCR assay (COBAS Amplicor HCV Test v2.0, Chugai-Roche Diagnostics, Tokyo, Japan), with a sensitivity limit of 5000 IU/mL and a dynamic range from 5000 to 5 000 000 IU/mL [11].

Serum HCV RNA was assessed by qualitative PCR assay (COBAS Amplicor HCV Monitor Test v2.0, Chugai-Roche Diagnostics), with a detection limit of 50 IU/mL [12].

### *Assessment of efficacy*

Serum HCV RNA (qualitatively or quantitatively) was measured at weeks 4, 8, 12 and 24 during treatment and after 24 weeks of follow-up without treatment. Patients were classified as having a rapid virological response (RVR) if serum HCV RNA was undetectable ( $< 50$  IU/mL) at week 4 and at the end of treatment response (ETR) at week 24 of treatment. SVR was defined as undetectable HCV RNA at week 24 after treatment. Patients with an ETR who sero-reverted to HCV RNA during follow-up were classified as relapsers.

### *Drug exposure*

The amounts of Peg-IFN alpha-2b and ribavirin actually taken by each patient during the treatment period were evaluated by reviewing the medical records. The mean doses of both drugs were calculated individually as averages on the basis of body weight at baseline; Peg-IFN alpha-2b expressed as  $\mu$ g/kg/week and ribavirin as mg/kg/day.

### *Data collection*

The medical records were retrospectively reviewed and the factors necessary for this examination were extracted: age, sex, body weight, body mass index (BMI), basic laboratory assessments, liver histology, quantitative and qualitative HCV RNA, dose of Peg-IFN alpha-2b and ribavirin received at each administration, and the response to treatment.

### *Statistical analysis*

This study was a retrospective study and, for treatment results and the analysis of related factors, analysis was carried out only for cases in which the treatment had been completed (per-protocol analysis). Continuous variables are reported as the mean with standard deviation (SD) or

median level, while categorical variables are shown as the count and proportion. In univariate analysis, the Mann-Whitney *U*-test was used to analyse continuous variables, while chi-squared and Fisher's exact tests were used for analysis of categorical data. Variables with  $P < 0.05$  at univariate analysis were retained for the multivariate logistic-regression analysis. Stepwise and multivariate logistic-regression models were used to explore the independent factors that could be used to predict a virological response. The significance of trends in values was determined with the Mantel-Haenszel chi-square test. For all tests, two-sided *P*-values were calculated and the results were considered statistically significant if  $P < 0.05$ . Statistical analysis was performed using the SPSS program for Windows, version 15.0J (SPSS, Chicago, IL, USA).

## RESULTS

The baseline characteristics for the total cohort are shown in Table 1. Most of the patients were female (56%) with a mean age of 54 years. Seventy per cent of the patients were treatment naïve. Of the 250 patients, liver biopsies were performed for 174 patients, and 18 of them had advanced fibrosis (F 3–4).

Of the total of 250 patients, 37 (15%) were withdrawn from treatment because of adverse events: decreased haemoglobin ( $n = 10$ ), psychiatric problems including depression ( $n = 9$ ), fatigue ( $n = 3$ ), thrombocytopenia, neutropenia, pyrexia, rash, cerebral haemorrhage, bleeding of ocular fundus, dyspnea, dizziness, jaundice, transaminase rise, gastrointestinal symptoms ( $n = 1$ ) and other adverse

events ( $n = 4$ ). Eight of these patients who discontinued treatment prematurely had SVR (8/37; 22%).

### Drug adherence

Seventy-nine of the 213 patients (37%) required dose reduction of Peg-IFN alpha-2b, 99 (46%) of ribavirin because of adverse events (not including patients who later discontinued treatment because of adverse event). Neutropenia (24/79; 30%) and thrombocytopenia (24/79; 30%) were the most common adverse events for dose reduction of Peg-IFN alpha-2b, and decreased haemoglobin (82/99; 83%) for that of ribavirin.

### Virological response

Of the 213 patients who completed 24 weeks of treatment and 24 weeks of follow-up, 160 (75%) patients were clear of HCV RNA at week 4, 191 (90%) at week 8, 196 (92%) at week 12. ETR was observed for 195 (92%), and SVR for 160 (75%). The relapse rate was 18% (35/195).

### Virological response according to the timing of viral clearance

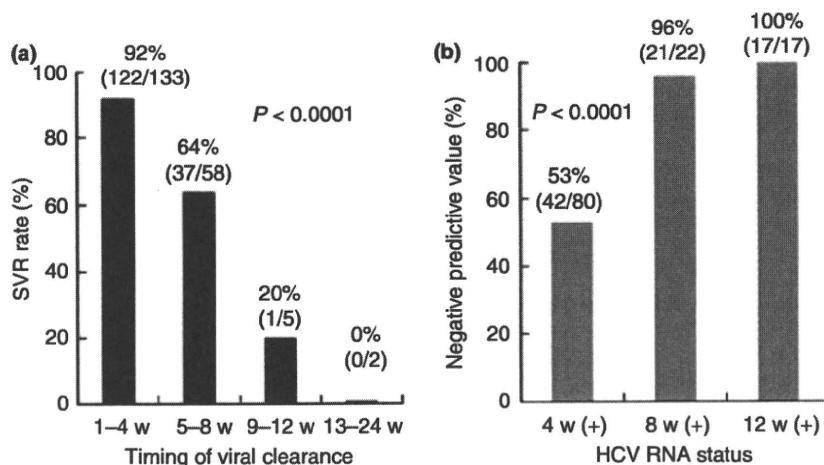
#### Positive and negative prediction of sustained virological response according to the timing of viral clearance

We examined SVR rates according to the timing of viral clearance for the case in which HCV RNA was cleared during the treatment (Fig. 1a). The SVR rate was 92% (122/133) for patients clear of HCV RNA until week 4, 64% (37/58) from week 5 until week 8, 20% (1/5) from week 9 until

**Table 1** Baseline demographic and viral characteristics of patients

Number of cases	250	
Age (years)*	54.0 ± 12.4	(22–76)
Sex (male/female)	110/140	
Body weight (kg)*	60.3 ± 11.7	(39–99)
Body mass index (kg/m <sup>2</sup> )*	23.1 ± 3.2	(16–35)
Past IFN therapy (naïve/experienced)†	175/70	
HCV RNA (KIU/mL)‡	1700	(4–5000 < )
Fibrosis (0/1/2/3/4)§	18/98/40/14/4	
Activity (0/1/2/3)§	15/81/70/8	
White blood cells (/mm <sup>3</sup> )*	5210 ± 1,750	(2100–13 870)
Neutrophils (/mm <sup>3</sup> )*	2700 ± 1,250	(590–9020)
Red blood cells (×10 <sup>4</sup> /mm <sup>3</sup> )*	436 ± 48	(307–554)
Haemoglobin (g/dL)*	13.9 ± 1.4	(10–18)
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )*	18.3 ± 6.4	(4–41)
ALT (IU/L)*	79 ± 77	(13–581)
γ-GTP (U/L)*	56 ± 65	(7–479)
Creatinine(mg/dL)*	0.7 ± 0.1	(0.4–1.1)

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase. \*Values expressed as mean ± SD (range), †interferon treatment history was not known for five patients, ‡values expressed as median (range), §data for 76 patients are missing.



**Fig. 1** (a) SVR rates according to timing of viral clearance. The number above each bar shows the percentage, and the numbers inside parentheses show the number of patients showing responses over the total number in the subgroup. The timing of viral clearance was time-dependently correlated with SVR ( $P < 0.0001$ ). (b) Negative predictive values according to time of HCV RNA positivity. The number above each bar shows the percentage, and the numbers inside parentheses show the number of patients showing responses over the total number in the subgroup. The time of HCV RNA positivity was time-dependently correlated with NPV ( $P < 0.0001$ ).

week 12 and 0% (0/2) from week 13 until week 24. The Mantel-Haenszel chi-square test showed that SVR rates were diminished with a delay in the timing of viral clearance becoming late ( $P < 0.0001$ ). Significantly, more patients who attained RVR achieved final SVR (92%, 122/133) than patients who failed to attain RVR (48%, 38/80;  $P < 0.0001$ ).

Next, we examined the negative predictive value (NPV) for the proportion of patients with treatment failure among those with HCV RNA persistence at week 4, 8 and 12 (Fig. 1b). NPV was 53% at week 4, 96% at week 8 and 100% at week 12. Only one of the 22 patients with positive HCV RNA at week 8 reached SVR.

#### Predictors of sustained virological response

Both pretreatment and treatment factors that could be associated with the response to Peg-IFN and ribavirin combination therapy were compared between patients with and without SVR in Table 2. This univariate analysis showed that age ( $P = 0.029$ ), baseline HCV RNA level ( $P = 0.033$ ), past IFN treatment history ( $P = 0.028$ ), platelets counts ( $P = 0.020$ ) and having RVR ( $P < 0.0001$ ) contributed to achievement of SVR. Factors that were significantly associated with SVR by univariate analysis were then analysed by multivariate logistic regression analysis. SVR was attained independent of high platelet counts [odds ratio (OR) 1.070, 95% confidence interval (CI) 1.003–1.140,  $P = 0.040$ ] and having RVR (OR 11.526, 95% CI 5.317–24.984,  $P < 0.0001$ ; Table 3). As for drug doses, the mean dose of Peg-IFN alpha-2b was  $1.32 \pm 0.27 \mu\text{g}/\text{kg}/\text{week}$  in patients with SVR and  $1.27 \pm 0.29 \mu\text{g}/\text{kg}/\text{week}$  in those without

SVR ( $P = 0.130$ ), while that of ribavirin was  $10.2 \pm 1.9$  and  $10.2 \pm 2.0 \text{ mg}/\text{kg}/\text{day}$  ( $P = 0.949$ ), respectively. Thus, neither Peg-IFN nor ribavirin drug exposure during the full treatment period affected attainment of SVR.

#### Predictors of rapid virological response

To delineate features that might help identify patients most likely to reach RVR, we also analysed these factors because having RVR turned out to be one of the most powerful predictors of SVR attainment. By univariate and multivariate logistic-regression analyses, RVR was attained independent of younger age (OR 0.648, 95% CI 0.494–0.850,  $P = 0.002$ ) and lower baseline HCV RNA level (OR 0.964, 95% CI 0.944–0.984,  $P < 0.0001$ ; Tables 4 & 5). The mean dose of Peg-IFN alpha-2b during the first 4 weeks was  $1.31 \pm 0.27 \mu\text{g}/\text{kg}/\text{week}$  in patients with RVR and  $1.31 \pm 0.29 \mu\text{g}/\text{kg}/\text{week}$  in those without RVR ( $P = 0.259$ ), that of ribavirin was  $10.1 \pm 1.8 \text{ mg}/\text{kg}/\text{day}$  and  $10.3 \pm 2.1 \text{ mg}/\text{kg}/\text{day}$  ( $P = 0.637$ ), respectively. Thus, neither Peg-IFN nor ribavirin drug exposure during the first 4 weeks had an impact on attainment of RVR.

#### Virological response according to drug exposure and the timing of viral clearance

##### Impact of drug exposure on sustained virological response

To more closely evaluate the impact of drug exposure on virological response, we classified the average doses of both drugs into four categories (Peg-IFN alpha-2b: up to  $0.9 \mu\text{g}/\text{kg}/\text{week}$ , from  $0.9$  to  $>1.2 \mu\text{g}/\text{kg}/\text{week}$ , from  $1.2$  to  $>1.5 \mu\text{g}/\text{kg}/\text{week}$ , from  $1.5 \mu\text{g}/\text{kg}/\text{week}$ ; ribavirin: up to

**Table 2** Factors associated with SVR among patients who completed the treatment – univariate analysis

Factor	SVR (n = 160)	Non-SVR (n = 53)	P-value
Age (years)*	52.4 ± 12.6	56.9 ± 10.2	0.029
Sex (male/female)	66 / 94	26 / 27	0.202
Body weight (kg)*	59.5 ± 11.5	59.9 ± 12.5	0.896
Body mass index (kg/m <sup>2</sup> )*	22.8 ± 3.1	22.8 ± 3.5	0.817
HCV RNA (KIU/mL) <sup>†</sup>	1170	1600	0.033
Past IFN therapy (naive/experienced) <sup>‡</sup>	116/41	31/22	0.028
Fibrosis (F 0–2/3–4) <sup>§</sup>	106/10	30/5	0.247
Activity (A 0–1/2–3) <sup>§</sup>	62/54	20/15	0.847
White blood cells (/mm <sup>3</sup> )*	5260 ± 1680	4720 ± 1500	0.078
Neutrophils (/mm <sup>3</sup> )*	2740 ± 1270	2420 ± 1020	0.186
Red blood cells (×10 <sup>4</sup> /mm <sup>3</sup> )*	435 ± 44	437 ± 55	0.820
Haemoglobin (g/dL)*	13.9 ± 1.3	14.0 ± 1.5	0.441
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )*	19.0 ± 6.0	16.5 ± 6.2	0.020
ALT (IU/L)*	86 ± 89	64 ± 45	0.514
γ-GTP (U/L)*	54 ± 67	58 ± 59	0.512
Creatinine (mg/dL)*	0.7 ± 0.1	0.7 ± 0.1	0.457
Mean Peg-IFN dose (µg/kg/week)*	1.32 ± 0.27	1.27 ± 0.29	0.130
Mean ribavirin dose (mg/kg/day)*	10.2 ± 1.9	10.2 ± 2.0	0.949
RVR (yes/no)	122/11	38/42	<0.0001

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; CI, confidence interval. \*Values expressed as mean ± sd, <sup>†</sup>values expressed as median, <sup>‡</sup>interferon treatment history was not known for three patients, <sup>§</sup>data for 62 patients are missing.

**Table 3** Factors associated with SVR among patients who completed the treatment – multivariate analysis

Factor	Category	Odds ratio	95% CI	P-value
Age (years)	By 10	–	–	NS
HCV RNA (KIU/mL)	By 100 KIU/mL	–	–	NS
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )	By 1 × 10 <sup>4</sup> /mm <sup>3</sup>	1.068	1.002–1.139	0.045
Past IFN therapy	Naïve/experienced	–	–	NS
RVR	Yes/no	11.251	5.184–24.419	<0.0001

IFN, interferon; HCV, hepatitis C virus; CI, confidence interval.

8 mg/kg/day, from 8 to >10 mg/kg/day, from 10 to >12 mg/kg/day, from 12 mg/kg/day). SVR rates relative to the mean drug doses during the full treatment period and the timing of HCV RNA clearance are shown in Table 6. As also shown in Fig. 1a, the respective rates for SVR according to the timing of viral clearance were 92% in patients clear of HCV RNA until week 4, 64% from week 5 until week 8 and 14% from week 9 until week 24. On the contrary, according to mean drug doses, the respective rates for SVR were 89% (24/27), 73% (11/15), 79% (85/107) and 82% (40/49) in patients who received Peg-IFN up to 0.9 µg/kg/week, from 0.9 to >1.2 µg/kg/week, from 1.2 to >1.5 µg/kg/week and from 1.5 µg/kg/week, respectively, and 80% (24/30), 80% (40/50), 82% (68/83) and 79% (27/34) in patients who received ribavirin up to 8 mg/kg/day, from 8 to >10 mg/kg/day, from 10 to >12 mg/kg/day and from 12 mg/kg/day,

respectively. If the category of the timing of viral clearance was the same, the respective rates for SVR attainment according to the mean doses of both Peg-IFN and ribavirin were similar. Furthermore, multivariate analysis by the Mantel–Haenszel chi-square test showed that neither the mean dose of Peg-IFN ( $P = 0.795$ ) nor ribavirin ( $P = 0.649$ ) affected SVR rates after stratification of the timing of viral clearance. Among the patients with RVR, SVR rates were as high as 88–100% regardless of Peg-IFN alpha-2b medication, and the least medicated group (<0.9 µg/kg/week, the mean dose with SD was  $0.77 \pm 0.10$  µg/kg/week, 0.50–0.89) showed 100% of SVR rate (19/19). Similarly, SVR rates were as high as 91–94% regardless of ribavirin medication among the patients with RVR, and 17 of 18 patients (94%) in the least medicated group (<8 mg/kg/day, the mean dose with SD was  $6.9 \pm 0.90$  mg/kg/day, 5.0–7.9)

Factor	RVR (n = 133)	Non-RVR (n = 80)	P-value
Age (years)*	51.9 ± 12.3	56.3 ± 11.3	0.010
Sex (male/female)	60/73	32/48	0.279
Body weight (kg)*	60.2 ± 11.6	58.6 ± 11.9	0.276
Body mass index (kg/m <sup>2</sup> )*	22.9 ± 3.2	22.6 ± 3.1	0.369
HCV RNA (KIU/mL) <sup>†</sup>	1050	1800	0.001
Past IFN therapy (naive/experienced) <sup>‡</sup>	97/34	50/29	0.068
Fibrosis (F 0–2/3–4) <sup>§</sup>	86/8	50/7	0.315
Activity (A 0–1/2–3) <sup>§</sup>	51/43	31/26	1.000
White blood cells (per mm <sup>3</sup> )*	5300 ± 1760	4850 ± 1400	0.205
Neutrophils (per mm <sup>3</sup> )*	2740 ± 1290	2530 ± 1090	0.340
Red blood cells (×10 <sup>4</sup> /mm <sup>3</sup> )*	440 ± 45	432 ± 49	0.628
Haemoglobin (g/dL)*	13.9 ± 1.4	13.9 ± 1.4	0.975
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )*	18.9 ± 6.1	17.5 ± 6.1	0.170
ALT (IU/L)*	87 ± 93	69 ± 52	0.630
γ-GTP (U/L)*	57 ± 71	53 ± 53	0.658
Creatinine (mg/dL)*	0.7 ± 0.1	0.7 ± 0.1	0.203
Mean Peg-IFN dose (µg/kg/week)*	1.31 ± 0.27	1.31 ± 0.29	0.259
Mean ribavirin dose (mg/kg/day)*	10.1 ± 1.8	10.3 ± 2.1	0.637

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; CI, confidence interval. \*Values expressed as mean ± SD, <sup>†</sup>values expressed as median, <sup>‡</sup>interferon treatment history was not known for three patients, <sup>§</sup>data for 62 patients are missing.

**Table 5** Factors associated with RVR among patients who completed the treatment – multivariate analysis

Factor	Category	Odds ratio	95% CI	P-value
Age (years)	By 10	0.648	0.494–0.850	0.002
HCV RNA (KIU/mL)	By 100 KIU/mL	0.964	0.944–0.984	<0.0001

HCV, hepatitis C virus; CI, confidence interval.

achieved SVR. In addition, we examined the drug impact on SVR in the patients with the least medication of both drugs (<0.9 µg/kg/week of Peg-IFN and <8 mg/kg/day of ribavirin). Nine patients were categorized into this group and six of these patients achieved SVR (67%); patients with RVR had a significantly higher SVR rate (100%, 5/5) than patients without RVR (25%, 1/4;  $P = 0.048$ ). Thus, SVR attainment was dependent on time, not on drug dose.

## DISCUSSION

In the present study, we found that having RVR and high platelet counts were statistically associated with reaching SVR according to multivariate analysis. The timing of viral clearance was closely related to the treatment effect in

**Table 4** Factors associated with RVR among patients who completed the treatment – univariate analysis

patients with genotype 2, similar to the case for those with genotype 1. Ninety-two per cent of SVR was observed for patients with RVR and, conversely, 96% of the patients with HCV RNA positivity at week 8 showed non-SVR. The predictability of SVR based on EVR, defined as a decline of at least 2-log from the baseline of the HCV RNA level at week 12, has been assessed, and genotype 1 patients who have failed to reach EVR are recommended to discontinue the treatment after 12 weeks, because the likelihood of SVR is 0–3% in the absence of EVR [5,13]. On the basis of our examination of patients with genotype 2, not EVR, but 8-week monitoring of the HCV RNA level can be used.

As a significant factor for SVR, not liver fibrosis, but the platelet count was selected. Everson *et al.* [14] reported that patients with low platelet counts ( $\leq 12.5 \times 10^4/\text{mm}^3$ ) achieved lower SVR rates than patients with normal platelet counts ( $> 12.5 \times 10^4/\text{mm}^3$ ) even in the case of patients with the same category of liver fibrosis treated by Peg-IFN plus ribavirin combination therapy. Thus, independent of liver fibrosis, thrombocytopenia itself seems to participate in treatment failure, although the mechanism remains unknown.

Our study also demonstrated that younger age (OR 0.648, 95% CI 0.494–0.850,  $P = 0.002$ ) and lower HCV RNA level (OR 0.964, 95% CI 0.944–0.984,  $P < 0.0001$ ) were statistically associated with reaching an RVR. Zeuzem *et al.* [7] previously reported that pretreatment viral load was not

Table 6 SVR rates according to Peg-IFN alpha-2b and ribavirin exposure and the timing of viral clearance among patients with virological response during the treatment

Timing of viral clearance (week)	Peg-IFN dose ( $\mu\text{g}/\text{kg}/\text{week}$ )				Ribavirin dose ( $\text{mg}/\text{kg}/\text{day}$ )				Total
	<0.9	0.9–1.2	1.2–1.5	1.5 $\leq$	<8	8–10	10–12	12 $\leq$	
1–4	100% (19/19)	91% (10/11)	92% (65/71)	88% (28/32)	94% (17/18)	92% (33/36)	91% (51/56)	91% (20/22)	92% (122/133)
5–8	63% (5/8)	33% (1/3)	64% (19/30)	71% (12/17)	58% (7/12)	54% (7/13)	74% (17/23)	60% (6/10)	64% (37/58)
9–24	–	0% (0/1)	17% (1/6)	–	–	0% (0/1)	0% (0/4)	50% (1/2)	14% (1/7)
Total	89% (24/27)	73% (11/15)	79% (85/107)	82% (40/49)	80% (24/30)	80% (40/50)	82% (68/83)	79% (27/34)	81% (160/198)

\* $P = 0.795$  for comparison of the four Peg-IFN groups after stratification of the timing of viral clearance. \*\* $P = 0.649$  for comparison of the four ribavirin groups after stratification of the timing of viral clearance.

associated with reaching RVR in genotype 2 patients. In contrast, Dalgard *et al.* [15] reported that independent predictors of RVR in genotype 2 or 3 patients were male gender, younger age ( $\leq 40$  years) and low viral load ( $\leq 400$  KIU/mL). The influence of viral load on reaching RVR remains controversial in the Peg-IFN and ribavirin combination therapy in genotype 2 patients, but patients with lower viral load seem favoured to reach HCV RNA levels below the detection limit, that is, to attain RVR, if the virological response is the same.

Recently, because of substantial adverse effects and costs associated with this therapy, studies have been carried out to determine the possibility of further reducing the total amount of drug medication without compromising antiviral efficacy in HCV genotype 2 and 3 patients. There seem to be two ways to achieve. One is by shortening the treatment duration, and the other is by decreasing the doses of the treatment drugs. With respect to the former, several studies on genotype 2 patients have been reported. At first, some studies of small numbers of subjects demonstrated that cumulatively analysed genotype 2 and 3 patients had high SVR rates up to 12 to 16 weeks of therapy (82–94%), similar to patients subjected to 24-week therapy (76–95%) [16–19]. However, further prospective investigation of large numbers of subjects revealed that shortening the treatment duration was associated with an increase in the rate of relapse and that significantly higher relapse rates led to lower SVR rates (71–81.1%), even among those with RVR [15,20,21]. The latest study by Mangia *et al.* [22] showed that shortened therapy after RVR was acceptable only for patients who had no signs of advanced liver fibrosis and low BMI. Considering the results of these trials, shortened therapy is regarded as optional treatment for selected patients displaying favourable baseline characteristics. Therefore, shortening treatment duration from 24 weeks should not be generally recommended for patients who are infected genotype 2 or 3 and can tolerate 24-week Peg-IFN and ribavirin combination therapy.

Another attempt to improve the treatment tolerability for genotype 2 or 3 patients has focused on dose reduction of treatment drugs. Weiland *et al.* [23] examined low-dose Peg-IFN alpha-2a (135  $\mu\text{g}$  weekly) with a weight-based standard-dose of ribavirin (11 mg/kg daily) for genotype 2 and 3 patients. They demonstrated that SVR rates of 86% were achieved, which is equal to those in previous representative randomized controlled studies of standard dose Peg-IFN therapy (76–84%) [4–6]. In contrast, Ferenci *et al.* [24] examined the efficacy of standard-dose Peg-IFN alpha-2a (180  $\mu\text{g}$  weekly) with low-dose ribavirin (400 mg daily) in comparison with standard-dose Peg-IFN alpha-2a (180  $\mu\text{g}$  weekly) and ribavirin (800 mg daily) for genotype 2 and 3 patients, and demonstrated that there was no difference between the two treatment groups with respect to SVR rates (64% with 400 mg/day compared with 69% with 800 mg/day) and relapse rates (20% with 400 mg/day compared

with 17% with 800 mg/day). These studies showed that either drug dose can be reduced for genotype 2 and 3 patients without compromising antiviral efficacy. In the present study, neither Peg-IFN nor ribavirin drug exposure participated in reaching RVR and SVR. In particular, more than 90% of patients having RVR achieved SVR regardless of the drug exposure level, as long as the mean Peg-IFN dose was over 0.5 µg/kg/week and ribavirin was over 5.0 mg/kg/day. The results of our study suggested that genotype 2 patients may receive reduced levels of both drug doses on the condition that they can complete the full 24-week course of combination therapy. Randomized, prospective trials that reduced both Peg-IFN and ribavirin should be conducted for CHC patients to clarify this.

In the present study, while the treatment outcome was independent of the individual ribavirin exposure in patients who had completed the 24-week treatment, the most common reason to withdraw the treatment was decreased haemoglobin because of ribavirin medication. Based on the results of randomized controlled trials [6], using a ribavirin dose of 800 mg/day is recommended for genotype 2/3 patients [1–3]. However, several studies have shown that some patients cannot tolerate even this suboptimal ribavirin dose. This is a serious problem for patients with the risk of anaemia, especially elderly patients. The ageing of patients is progressing around the world, requiring improvement in treatment tolerability. Recently, Andriulli *et al.* [25] examined the effect of ribavirin in a 12-week course of therapy on CHC genotype 2 patients with RVR in two groups, one continuing with ribavirin and the other receiving Peg-IFN alpha-2a alone after week 6. The relapse rates were higher (46% vs 17%;  $P < 0.001$ ) and overall SVR rates were lower (54 vs 82%;  $P < 0.001$ ) in patients who stopped receiving ribavirin at week 6. Thus, ribavirin medication throughout the treatment period is necessary to raise the SVR rate even in genotype 2 or 3 patients with RVR. In the present study, the ribavirin dose could be reduced without loss of efficacy for genotype 2 patients, as long as the patients were treated for 24 weeks. Therefore, in the patients with the risk of anaemia, it would be better to reduce the dose of ribavirin before anaemia arises rather than being forced to discontinue the combination therapy because of anaemia caused by ribavirin medication. We previously reported that in CHC patients treated by IFN or Peg-IFN in ribavirin combination therapy, a decline of haemoglobin concentration by 2 g/dL at the end of 2 weeks from the start of the treatment can be used to identify patients likely to develop severe anaemia [26,27]. This kind of predictive factor for the progression to severe anaemia can be of much help in reducing ribavirin with appropriate timing.

Our study has some limitations. First, it is a retrospective study, and we could not obtain complete information for all patients. However, this is the first study of Peg-IFN and ribavirin combination therapy in which the drug dose of Peg-IFN and ribavirin taken by each patient was assessed

independently for HCV genotype 2 patients. Our results can be taken as an evidence offering suggestions for the treatment of CHC genotype 2 patients. Second, this cohort included patients with different histories of past IFN treatment. Patients who had failed to recover with previous IFN-based treatment were likely to experience treatment failure again [28]. Therefore, we examined the predictors of treatment response separately according to treatment history, and confirmed that in both naïve and treatment-experienced patients, the mean dose of Peg-IFN and ribavirin showed no correlation with SVR or RVR in both groups.

In conclusion, our study demonstrates that RVR is an important treatment predictor and more than 90% of patients having RVR achieve SVR with combination therapy of Peg-IFN and ribavirin for genotype 2 infected CHC patients regardless of the drug exposure. Further prospective, randomized studies are necessary to assess whether the standard or a reduced dose of each drug can produce equivalent outcomes.

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## Genome-wide association of *IL28B* with response to pegylated interferon- $\alpha$ and ribavirin therapy for chronic hepatitis C

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The recommended treatment for patients with chronic hepatitis C, pegylated interferon- $\alpha$  (PEG-IFN- $\alpha$ ) plus ribavirin (RBV), does not provide sustained virologic response (SVR) in all patients. We report a genome-wide association study (GWAS) to null virological response (NVR) in the treatment of patients with hepatitis C virus (HCV) genotype 1 within a Japanese population. We found two SNPs near the gene *IL28B* on chromosome 19 to be strongly associated with NVR (rs12980275,  $P = 1.93 \times 10^{-13}$ , and rs8099917,  $3.11 \times 10^{-15}$ ). We replicated these associations in an independent cohort (combined  $P$  values,  $2.84 \times 10^{-27}$  (OR = 17.7; 95% CI = 10.0–31.3) and  $2.68 \times 10^{-32}$  (OR = 27.1; 95% CI = 14.6–50.3), respectively). Compared to NVR, these SNPs were also associated with SVR (rs12980275,  $P = 3.99 \times 10^{-24}$ , and rs8099917,  $P = 1.11 \times 10^{-27}$ ). In further fine mapping of the region, seven SNPs (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668) located in the *IL28B* region showed the most significant associations ( $P = 5.52 \times 10^{-28}$ – $2.68 \times 10^{-32}$ ; OR = 22.3–27.1). Real-time quantitative PCR assays in peripheral blood mononuclear cells showed lower *IL28B* expression levels in individuals carrying the minor alleles ( $P = 0.015$ ).

Hepatitis C is a global health problem that affects a significant proportion of the world's population. The World Health Organization

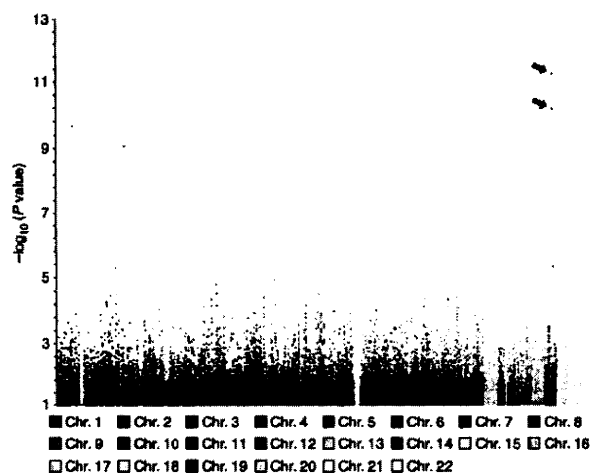
estimated that in 1999, there were 170 million HCV carriers worldwide, with 3–4 million new cases appearing each year. HCV infection affects more than 4 million people in the United States, where it represents the leading cause of cirrhosis and hepatocellular carcinoma as well as the leading cause of liver transplantation<sup>1</sup>. The American Gastroenterological Association estimated that drugs are the largest direct costs of hepatitis C<sup>1</sup>.

The most effective current standard of care in patients with chronic hepatitis C, a combination of PEG-IFN- $\alpha$  with ribavirin, does not produce SVR in all patients treated. Large-scale studies on 48-week-long PEG-IFN- $\alpha$ /RBV treatment in the United States and Europe showed that 42–52% of patients with HCV genotype 1 achieved SVR<sup>2–4</sup>, and similar results were found in Japan. However, older patients (greater than 50 years of age) had a significantly lower rate of SVR due to poor adherence resulting from adverse events and laboratory-detectable abnormalities such as neutropenia and thrombocytopenia<sup>5,6</sup>. Specifically, various well-described side effects (such as a flu-like syndrome, hematologic abnormalities and adverse neuropsychiatric events) often necessitate dose reduction, and 10–14% of patients require premature withdrawal from interferon-based therapy<sup>7</sup>. To avoid these side effects in patients who will not be helped by the treatment, as well as to reduce the substantial cost of PEG-IFN- $\alpha$ /RBV treatment, it would be useful to be able to predict an individual's response before or early in treatment. Several viral factors, such as genotype 1, high baseline viral load, viral

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# LETTERS



**Figure 1** Genome-wide association results with PEG-IFN- $\alpha$ /RBV treatment in 142 Japanese patients with HCV (78 NVR and 64 VR samples). P values were calculated by using a  $\chi^2$  test for allele frequencies. The dots with arrows for chromosome 19 denote SNPs that showed significant genome-wide associations ( $P < 8.05 \times 10^{-8}$ ) with response to PEG-IFN- $\alpha$ /RBV treatment.

kinetics during treatment, and amino acid pattern in the interferon sensitivity-determining region, have been reported to be significantly associated with the treatment outcome in a number of independent studies<sup>8–10</sup>. Studies have also provided strong evidence that ~20% of patients with HCV genotype 1 and 5% of patients with genotype 2 or 3 have a null response to PEG-IFN- $\alpha$ /RBV. No definite predictor of this resistance is currently available that make it possible to bypass the initial 12–24 weeks' treatment before deciding whether treatment should be continued. If a reliable predictor of non-response were identified for use in patients before treatment initiation, then an estimated 20%, including those who have little or no chance to achieve SVR, could be spared the side effects and cost of treatment.

Host factors, including age, sex, race, liver fibrosis and obesity, have also been reported to be associated with PEG-IFN- $\alpha$ /RBV therapy outcome<sup>11,12</sup>. However, little is known about the host genetic factors that might be associated with the response to therapy: thus far only

a few candidate genes, including those encoding type I interferon receptor-1 (*IFNAR1*) and mitogen-activated protein kinase-activated protein kinase 3 (*MAPKAPK3*), have been reported to be associated with treatment response<sup>13,14</sup>. We describe here a GWAS for response to PEG-IFN- $\alpha$ /RBV treatment.

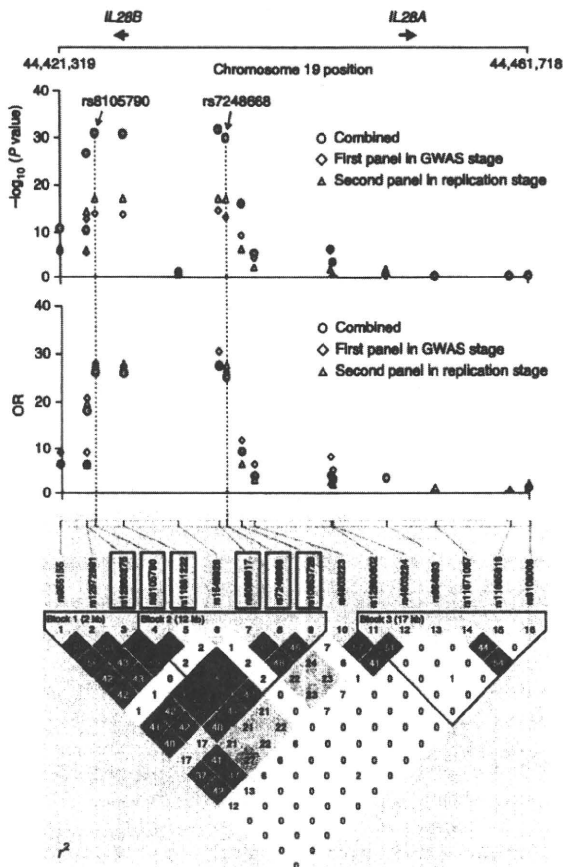
We conducted this GWAS to identify host genes associated with response to PEG-IFN- $\alpha$ /RBV treatment in 154 Japanese patients with HCV genotype 1 (82 with NVR and 72 with virologic response (VR), based on the selection criteria as described in Online Methods). We used the Affymetrix SNP 6.0 genome-wide SNP typing array for 900,000 SNPs. A total of 621,220 SNPs met the following criteria: (i) SNP call rate  $\geq 95\%$ , (ii) minor allele frequency (MAF)  $\geq 1\%$  and (iii) deviation from Hardy-Weinberg equilibrium (HWE)  $P \geq 0.001$  in VR samples. After excluding 4 NVR and 8 VR samples that showed quality control (QC) call rates of  $< 95\%$ , 78 NVR and 64 VR samples were included in the association analysis. Figure 1 shows a genome-wide view of the single-point association data based on allele frequencies. Two SNPs located close to *IL28B* on chromosome 19 showed strong associations, with a minor allele dominant model (rs12980275,  $P = 1.93 \times 10^{-13}$ , and rs8099917,  $P = 3.11 \times 10^{-15}$ , respectively), with NVR to PEG-IFN- $\alpha$ /RBV treatment (Table 1). The rs8099917 lies between *IL28B* and *IL28A*, ~8 kb downstream from *IL28B* and ~16 kb upstream from *IL28A*. These associations reached genome-wide levels of significance for both SNPs in this initial GWAS cohort (Bonferroni criterion  $P < 8.05 \times 10^{-8}$  (0.05/621,220)). The frequencies of minor allele-positive patients were much higher in the NVR group than in the VR group for both SNPs (74.3% in NVR, 12.5% in VR for rs12980275; 75.6% in NVR, 9.4% in VR for rs8099917). Notably, individuals homozygous for the minor allele were observed only in the NVR group. The VR group, as compared to the NVR group, showed genotype frequencies closer to those in the healthy Japanese population<sup>15</sup>, yet the minor allele frequencies were slightly higher in the transient virologic response (TVR) group (23.1%, 15.4%) than in the SVR group (9.8%, 7.8%) (Table 1). We applied the Cochran-Armitage test on all the SNPs and found a genetic inflation factor,  $\lambda$ , of 1.029 for the GWAS stage (Supplementary Fig. 1). We also carried out principal component analysis in 142 samples for the GWAS stage together with the HapMap samples (CEU, YRI, CHB and JPT) (Supplementary Fig. 2); this suggested that the effect of population stratification was negligible.

**Table 1** Significant association of two SNPs (rs12980275 and rs8099917) with response to PEG-IFN- $\alpha$ /RBV treatment

dbSNP rsID	Nearest gene	MAF <sup>b</sup> (allele)	Allele (1/2)	Stage	Null responder (NVR <sup>a</sup> , n = 128)			Responder (VR <sup>a</sup> , n = 186)			Responder (SVR <sup>a</sup> , n = 140)			NVR vs. VR		NVR vs. SVR	
					11	12	22	11	12	22	11	12	22	OR (95% CI) <sup>c</sup>	P value <sup>d</sup>	OR (95% CI) <sup>c</sup>	P value <sup>d</sup>
rs12980275	<i>IL28B</i>	0.15 (G)	A/G	GWAS	20	54	4	56	8	0	46	5	0	20.3	$1.93 \times 10^{-13}$	26.7	$7.41 \times 10^{-13}$
					(25.6)	(69.2)	(5.1)	(87.5)	(12.5)	(0.0)	(90.2)	(9.8)	(0.0)	(8.3–49.9)		(9.3–76.5)	
					Replication	10	37	3	101	21	0	73	16	0	19.2	$5.46 \times 10^{-15}$	18.3
Combined	30	91	7	157	29	0	119	21	0	17.7	$2.84 \times 10^{-27}$	18.5	$3.99 \times 10^{-24}$				
					(23.4)	(71.1)	(5.5)	(84.4)	(15.6)	(0.0)	(85.0)	(15.0)	(0.0)	(10.0–31.3)		(10.0–34.4)	
rs8099917	<i>IL28B</i>	0.12 (G)	T/G	GWAS	19	56	3	58	6	0	47	4	0	30.0	$3.11 \times 10^{-15}$	36.5	$5.00 \times 10^{-14}$
					(24.4)	(71.8)	(3.8)	(90.6)	(9.4)	(0.0)	(92.2)	(7.8)	(0.0)	(11.2–80.5)		(11.6–114.6)	
					Replication	11	37	2	108	14	0	78	11	0	27.4	$9.47 \times 10^{-18}$	25.1
Combined	30	93	5	166	20	0	125	15	0	27.1	$2.68 \times 10^{-32}$	27.2	$1.11 \times 10^{-27}$				
					(23.4)	(72.7)	(3.9)	(89.2)	(10.8)	(0.0)	(89.3)	(10.7)	(0.0)	(14.6–50.3)		(13.9–53.4)	

<sup>a</sup>NVR, null virologic response; VR, virologic response; SVR, sustained virologic response. The 186 VRs consisted of 46 transient virologic response (TVRs) and 140 SVRs. <sup>b</sup>Minor allele frequency and minor allele in 184 healthy Japanese individuals<sup>15</sup>. The MAF of the SNPs in SVR is similar to that of TVR group, whereas that of NVR is much higher (76.6%). <sup>c</sup>Odds ratio for the minor allele in a dominant model. <sup>d</sup>P value by  $\chi^2$  test for the minor allele dominant model.





**Figure 2** Genomic structure,  $P$  value and OR plots in association analysis and LD map around *IL28B* and *IL28A* (chr.19, nucleotide positions 44421319–44461718; build 35).  $P$  values by the  $\chi^2$  test for minor allele dominant effect model are shown for the first panel of 142 samples in the GWAS stage, the second panel of 172 samples in the replication stage, and the combined analysis. Below are estimates of pairwise  $r^2$  for 16 SNPs selected in the replication study using a total of 314 Japanese patients with HCV treated with PEG-IFN- $\alpha$ /RBV. Boxes indicate the significantly associated SNPs with response to PEG-IFN- $\alpha$ /RBV treatment both in the GWAS stage and in the replication stage. Dotted lines indicate the region with the strongest associations from the positions of rs8105790 to rs7248668.

OR = 27.4 for rs8099917; **Table 1**). The combined  $P$  values for both stages reached  $2.84 \times 10^{-27}$  (OR = 17.7; 95% CI = 10.0–31.3) and  $2.68 \times 10^{-32}$  (OR = 27.1; 95% CI = 14.6–50.3), respectively (**Table 1**). Notably, when we compared the SVR ( $n = 140$ ) with the NVR group ( $n = 128$ ), the original two SNPs (rs12980275 and rs8099917) again showed strong associations: both  $P$  values and ORs were similar to those observed in the comparison between VR and NVR, and the combined  $P$  values for both stages reached  $3.99 \times 10^{-24}$  (OR = 18.5; 95% CI = 10.0–34.4) and  $1.11 \times 10^{-27}$  (OR = 27.2; 95% CI = 13.9–53.4), respectively (**Table 1**). Comparing SVR ( $n = 140$ ) versus NVR plus TVR ( $n = 174$ ), we again found that these SNPs were significantly associated ( $P = 1.71 \times 10^{-16}$ , OR = 8.8; 95% CI 5.1–15.4 for rs12980275;  $P = 1.18 \times 10^{-18}$ , OR = 12.1; 95% CI 6.5–22.4 for rs8099917, **Supplementary Table 2**), suggesting that these SNPs would predict NVR as well as SVR before PEG-IFN- $\alpha$ /RBV therapy.

Among the newly analyzed SNPs in the replication study, six (rs12980275, rs8105790, rs11881222, rs8099917, rs7248668 and rs10853728) showed significant associations both in the GWAS stage ( $P < 8.05 \times 10^{-8}$ ) and in the replication stage ( $P < 0.0031$  (0.05/16)) after Bonferroni correction. These SNPs are located within a 15.7-kb region that includes *IL28B* (**Fig. 2** and **Supplementary Table 1**). In particular, the strongest associations with NVR were observed for four SNPs, rs8105790, rs11881222, rs8099917 and rs7248668, that are located in the downstream flanking region, the third intron and the upstream flanking region of *IL28B*. The combined  $P$  values for these polymorphisms were  $1.98 \times 10^{-31}$  (OR = 25.7; 95% CI = 13.9–47.6),  $2.84 \times 10^{-31}$  (OR = 25.6; 95% CI = 13.8–47.3),  $2.68 \times 10^{-32}$  (OR = 27.1; 95% CI = 14.6–50.3) and  $1.84 \times 10^{-30}$  (OR = 24.7; 95% CI = 13.3–45.8), respectively (**Supplementary Table 1**). We then sequenced this region to identify further variants and found three SNPs (rs8103142, rs28416813 and rs4803219) located in the third exon, the first intron and the upstream flanking region of *IL28B*, and a few infrequent variations. These SNPs also showed strong associations in the combined dataset of 128 NVR and 186 VR samples ( $P = 1.40 \times 10^{-29}$ , OR = 26.6 for rs8103142;  $P = 5.52 \times 10^{-28}$ , OR = 22.3 for rs28416813;  $P = 2.45 \times 10^{-29}$ , OR = 23.3 for rs4803219; **Supplementary Table 3**). We also performed LD and haplotype analyses with seven SNPs. These SNPs were in strong LD, and the risk haplotype showed a level of association similar to those of individual SNPs ( $P = 1.35 \times 10^{-25}$ , OR = 11.1; 95% CI = 6.6–18.6) (**Table 2**). These results suggest that the association with NVR was primarily driven by one of these SNPs.

We analyzed the region of ~40 kb (chr. 19, nucleotide positions 44421319–44461718; build 35) containing the significantly associated SNPs (rs12980275 and rs8099917) using Haploview software for linkage disequilibrium (LD) and haplotype structure based on the HapMap data for individuals of Japanese ancestry. The LD blocks were analyzed using the four-gamete rule, and four blocks were observed (**Supplementary Fig. 3**). We selected 16 SNPs for both replication study and high-density association mapping, including tagging SNPs estimated on the basis of the haplotype blocks, one SNP located within *IL28B* (rs11881222) and the significantly associated SNPs from the GWAS stage (rs12980275 and rs8099917) (**Supplementary Table 1**).

To validate the results of the GWAS stage, 16 SNPs selected for the replication stage, including the original SNPs, were genotyped using the DigiTag2 assay in an independent set of 172 Japanese patients with HCV treated with PEG-IFN- $\alpha$ /RBV treatment (50 NVR and 122 VR samples), together with the first panel of 142 samples analyzed in the GWAS stage (**Supplementary Table 1**). The associations of the original SNPs were replicated in the replication cohort of 172 patients ( $P = 5.46 \times 10^{-15}$ , OR = 19.2 for rs12980275;  $P = 9.47 \times 10^{-18}$ ,

**Table 2** Association analysis of response to treatment by *IL28B* haplotype

SNP							Frequencies			
rs8105790	rs11881222	rs8103142	rs28416813	rs4803219	rs8099917	rs7248668	NVR group	VR group	$P$ value	OR (95% CI)
T	A	T	C	C	T	G	0.543	0.942	$1.81 \times 10^{-32}$	0.1 (0.04–0.12)
C	G	C	G	T	G	A	0.387	0.054	$1.35 \times 10^{-25}$	11.1 (6.6–18.6)

Association analysis of haplotypes consisting of seven SNPs with response to PEG-IFN- $\alpha$ /RBV treatment in 314 Japanese patients with HCV. Boldface letters: rs11881222 (third intron); rs8103142 (third exon).

## LETTERS

**Table 3 Factors associated with NVR by logistic regression model**

Factors	Odds ratio	95% CI	P value
rs8099917 (G allele)	37.68	16.71–83.85	<0.0001
Age	1.02	0.98–1.07	0.292
Gender (Female)	3.32	1.49–7.39	0.003
Re-treatment*	1.12	0.55–2.33	0.750
Platelet count	0.93	0.87–1.01	0.080
Aminotransferase level	1.00	0.99–1.00	0.735
Fibrosis stage <sup>20</sup>	1.10	0.73–1.66	0.658
HCV-RNA level	1.01	0.99–1.02	0.139

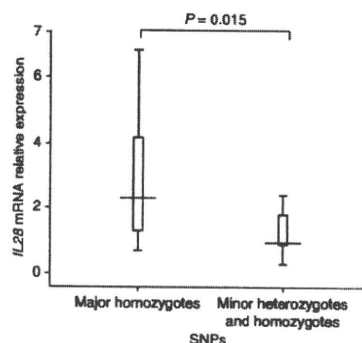
\*Re-treatment, non-response to previous treatment with interferon- $\alpha$  (plus RBV).

To examine the relative contribution of factors associated with NVR, we used a logistic regression model. One tagging SNP located within *IL28B* (minor allele of rs8099917) was the most significant factor for predicting NVR, followed by gender (Table 3). Clinically, viral factors such as HCV genotype and HCV RNA level are important for the outcome of PEG-IFN- $\alpha$ /RBV therapy. Indeed, mean HCV-RNA level was significantly lower in SVR (SVR versus TVR,  $P = 0.002$ ; SVR versus NVR,  $P = 0.016$ ; Supplementary Table 4). Mean platelet count and the proportion of mild fibrosis (F1–F2) were significantly higher in SVR than in NVR.

Real-time quantitative PCR assays in peripheral blood mononuclear cells revealed a significantly lower level of *IL28* mRNA expression in individuals with the minor alleles (Fig. 3), suggesting that variant(s) regulating *IL28* expression is associated with a response to PEG-IFN- $\alpha$ /RBV treatment. *IL28B* encodes a cytokine distantly related to type I ( $\alpha$  and  $\beta$ ) interferons and the interleukin (IL)-10 family. This gene and *IL28A* and *IL29* (encoding IL-28A and IL-29, respectively) are three closely related cytokine genes that encode proteins known as type III IFNs (IFN- $\lambda$ s) and that form a cytokine gene cluster at chromosomal region 19q13 (ref. 16). The three cytokines are induced by viral infection and have antiviral activity<sup>16,17</sup>. All three interact with a heterodimeric class II cytokine receptor that consists of IL-10 receptor beta (IL10R $\beta$ ) and IL-28 receptor alpha (IL28R $\alpha$ , encoded by *IL28RA*)<sup>16,17</sup>, and they may serve as an alternative to type I IFNs in providing immunity to viral infection.

Notably, a recent report showed that the strong antiviral activity evoked by treating mice with TLR3 or TLR9 agonists was significantly reduced in both *IL28RA*<sup>-/-</sup> and *IFNAR*<sup>-/-</sup> mice, indicating that IFN- $\lambda$  is important in mediating antiviral protection by ligands for TLR3 and TLR9 (ref. 18). IFN- $\lambda$  induced a steady increase in the expression of a subset of IFN-stimulated genes, whereas IFN- $\alpha$  induced the same genes with more rapid and transient kinetics<sup>19</sup>. Therefore, it is possible that IFN- $\lambda$  induces a slower but more sustained response that is important for TLR-mediated antiviral protection. This might be one of the ways that a genetic variant regulating *IL28* expression influences the response to PEG-IFN- $\alpha$ /RBV treatment. Further research will be required to fully understand the specific mechanism by which a genotype might affect the response to treatment.

In conclusion, the strongest associations with NVR were observed for seven SNPs, rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668, that are located in the downstream flanking region, the third intron, the third exon, the first intron and the upstream flanking region of *IL28B*. Further studies following our report of this robust genetic association to NVR may make it possible to develop a pre-treatment predictor of which individuals are likely to respond to PEG-IFN- $\alpha$ /RBV treatment. This would remove the need for the initial 12–24 weeks of treatment that is currently used as a basis for a clinical decision about whether treatment should be continued. That would allow better targeting of PEG-IFN- $\alpha$ /RBV



**Figure 3** Quantification of *IL28* mRNA expression. The expression level of *IL28* genes was determined by real-time quantitative RT-PCR using RNA purified from peripheral blood mononuclear cells. Distribution of relative gene expression levels was compared between the individuals homozygous for major alleles ( $n = 10$ ) and the heterozygous or homozygous individuals carrying minor alleles ( $n = 10$ ) of rs8099917 by using the Mann-Whitney  $U$ -test. The bars indicate the median. All samples were obtained from HCV-infected patients before PEG-IFN- $\alpha$ /RBV therapy.

treatment, avoiding the unpleasant side effects that commonly accompany the treatment where it is unlikely to be beneficial, and reduce overall treatment costs. Because of the small number of samples in this study, we plan to conduct a further prospective multicenter study to establish these SNPs as a clinically useful marker.

### METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

Note: Supplementary information is available on the Nature Genetics website.

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Study design and discussion: Y.T., N.N., N.M., K.T., M.M.; sample collection: Y.T., M.K., K.M., N.S., M.N., M.K., K.H., S.H., Y.I., E.M., E.T., S.M., Y.M., M.H., A.S., Y.H., S.N., I.S., M.I., K.L., K.Y., F.S., N.I.; genotyping: N.N.; statistical analysis: N.N., A.K., K.I.; quantitative RT-PCR: M.S.; manuscript writing: Y.T., N.N., K.T., M.M.

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## Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin

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**SUMMARY.** The impact of ribavirin exposure on virologic relapse remains controversial in combination therapy with pegylated interferon (Peg-IFN) and ribavirin for patients with chronic hepatitis C (CH-C) genotype 1. The present study was conducted to investigate this. Nine hundred and eighty-four patients with CH-C genotype 1 were enrolled. The drug exposure of each medication was calculated by averaging the dose actually taken. For the 472 patients who were HCV RNA negative at week 24 and week 48, multivariate logistic regression analysis showed that the degree of fibrosis ( $P = 0.002$ ), the timing of HCV RNA negativation ( $P < 0.001$ ) and the mean doses of ribavirin ( $P < 0.001$ ) were significantly associated with relapse, but those of Peg-IFN were not. Stepwise reduction of the ribavirin dose was associated with a stepwise increase in relapse rate from 11%

to 60%. For patients with complete early virologic response (c-EVR) defined as HCV RNA negativity at week 12, only 4% relapse was found in patients given  $\geq 12$  mg/kg/day of ribavirin and ribavirin exposure affected the relapse even after treatment week 12, while Peg-IFN could be reduced to 0.6  $\mu$ g/kg/week after week 12 without the increase of relapse rate. Ribavirin showed dose-dependent correlation with the relapse. Maintaining as high a ribavirin dose as possible ( $\geq 12$  mg/kg/day) during the full treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to Peg-IFN alpha-2b plus ribavirin, especially in c-EVR patients.

**Keywords:** chronic hepatitis C, drug exposure, pegylated interferon plus ribavirin, virologic relapse.

### INTRODUCTION

Combination therapy of pegylated interferon (Peg-IFN) plus ribavirin is very effective for patients with chronic hepatitis C

Abbreviations: CH-C, chronic hepatitis C; c-EVR, complete early virologic response; ETR, end-of-treatment virologic response; Hb, haemoglobin; HCV, hepatitis C virus; IFN, interferon; LVR, late virologic response; Peg-IFN, pegylated interferon; PP, per protocol; Plt, platelet; RVR, rapid virologic response; SVR, sustained virologic response; VR, virologic response; WBC, white blood cell.

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(CH-C). However, sustained virologic response (SVR) in current therapy occurs in only 40–50% of patients with hepatitis C virus (HCV) genotype 1 [1–4]. Also, SVR is reduced in patients with genotype 1 who require reduction of either Peg-IFN or ribavirin, although dose reduction has little influence on SVR in those with genotype 2 or 3 [1–3,5,6]. Therefore, it is important to clarify the degree to which these medications can be reduced without adversely affecting SVR in patients with CH-C genotype 1.

In an early report on the relationship between drug exposure and antiviral effect in patients with CH-C genotype 1, patients who received  $\geq 80\%$  of their total planned cumulative doses of Peg-IFN and ribavirin for  $\geq 80\%$  of the scheduled duration of therapy had an SVR of 51% compared with only 34% for patients who received lesser amounts of one or both

medications [7]. On the other hand, Shiffman *et al.* [8] recently reported that reducing ribavirin did not affect SVR as long as the dose of Peg-IFN was maintained, while reducing the Peg-IFN dose significantly reduced SVR. The results of these observations are consistent with respect to the effect of Peg-IFN on SVR. However, what is controversial is whether or not reducing the ribavirin dose affects the antiviral effect.

Adding ribavirin to either interferon (IFN) or Peg-IFN monotherapy for patients with CH-C genotype 1 has been shown to reduce the relapse rate in large randomized trials [1,2,9–11]. In detail, adding ribavirin to the usual IFN monotherapy (3MIU, three-times-weekly) in 48-week treatment raised the end-of-treatment virologic response (ETR) rate from approximately 30% to 50% and also lowered the relapse rate from mid-40% to approximately 20% [9–11]. Lindsay *et al.* [12] reported that Peg-IFN alpha-2b (Peg-IFN  $\alpha$ -2b) monotherapy (1.5  $\mu$ g/kg, once-weekly), as compared with IFN alpha-2b (IFN $\alpha$ -2b) monotherapy (3MIU, three-times-weekly), improved ETR (49% vs. 24%), but not the relapse rate (53% vs. 50%). In the trial of Peg-IFN alpha-2a (Peg-IFN  $\alpha$ -2a) plus ribavirin vs IFN  $\alpha$ -2b plus ribavirin or Peg-IFN  $\alpha$ -2a alone, the ETR rates were 69%, 52% and 59%, and the relapse rates were 19%, 15% and 52%, respectively [2]. These findings from large-scale trials indicate that the main role of ribavirin is to reduce relapse in the combination therapy with Peg-IFN, although ribavirin affects both ETR and relapse in combination therapy with the usual IFN.

In the present study, we tried to determine whether or not dose reduction of ribavirin (or Peg-IFN) has an effect on virologic relapse in Peg-IFN plus ribavirin treatment for patients with CH-C genotype 1.

## PATIENTS AND METHODS

### Patients

This study was a multicentre trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 984 patients with CH-C were enrolled in this study between December 2004 and September 2006, and treated with a combination of Peg-IFN  $\alpha$ -2b plus ribavirin. The baseline characteristics of the patients are shown in Table 1. All patients were Japanese infected with HCV genotype 1 and a viral load of more than  $10^5$  IU/mL. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), coinfection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

### Treatment

All patients received Peg-IFN  $\alpha$ -2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL;

**Table 1** Baseline characteristics of patients and drug doses at start of treatment

Factor	Mean $\pm$ SD or n
n	984
Age (years)	56.3 $\pm$ 10.1
Sex (male/female)	555/429
Body weight (kg)	61.8 $\pm$ 11.5
History of IFN treatment	575/409 (160/182)
Naïve/experienced	
(relapser/nonresponder)*	
White blood cells (/mm <sup>3</sup> )	5052 $\pm$ 1550
Neutrophils (/mm <sup>3</sup> )	2577 $\pm$ 1092
Red blood cells ( $\times 10^4$ /mm <sup>3</sup> )	442 $\pm$ 47
Haemoglobin (g/dL)	14.1 $\pm$ 1.4
Platelets ( $\times 10^4$ /mm <sup>3</sup> )	15.9 $\pm$ 5.5
AST (IU/L)	66 $\pm$ 45
ALT (IU/L)	79 $\pm$ 61
Serum HCV RNA (kIU/mL) <sup>†</sup>	1600
Histology (METAVIR) <sup>‡</sup>	
Fibrosis; 0/1/2/3/4	49/314/197/105/18
Activity; 0/1/2/3	23/329/304/27
Peg-IFN dose ( $\mu$ g/kg/week)	1.45 $\pm$ 0.17
Ribavirin dose (mg/kg/day)	11.4 $\pm$ 1.6

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus. \*Viral response to previous treatment was unknown in 57 patients, and 10 patients had discontinued treatment. <sup>†</sup>Data shown are median values. <sup>‡</sup>301 missing.

Schering-Plough) for the duration of the study of 48 weeks. As a starting dose, Peg-IFN  $\alpha$ -2b was given subcutaneously once weekly at a dosage of 60–150  $\mu$ g/kg based on body weight (body weight 35–45 kg, 60  $\mu$ g; 46–60 kg, 80  $\mu$ g; 61–75 kg, 100  $\mu$ g; 76–90 kg, 120  $\mu$ g; 91–120 kg, 150  $\mu$ g) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight (body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg) according to the manufacturer's drug information available in Japan.

### Dose reduction and discontinuance

Dose modification also followed, as a rule, the manufacturer's drug information according to the intensity of the haematologic adverse effects. The dose of Peg-IFN  $\alpha$ -2b was reduced to 50% of the assigned dose when the white blood cell (WBC) count was below 1500/mm<sup>3</sup>, the neutrophil count below 750/mm<sup>3</sup> or the platelet (Plt) count below  $8 \times 10^4$ /mm<sup>3</sup>, and was discontinued when the WBC count was below 1000/mm<sup>3</sup>, the neutrophil count below 500/mm<sup>3</sup> or the Plt count below  $5 \times 10^4$ /mm<sup>3</sup>. Ribavirin was also reduced from 1000 mg to 600 mg, 800 mg to 600 mg, or 600 mg to 400 mg when the haemoglobin (Hb)



concentration decreased to less than 10 g/dL, and was discontinued when the Hb concentration decreased to less than 8.5 g/dL. Both Peg-IFN  $\alpha$ -2b and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. No ferric medicine or haematopoietic growth factors, such as epoetin alpha, or granulocyte-macrophage colony stimulating factor, were administered.

#### *Virologic assessment and definition of virologic response*

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 kIU/mL; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analysed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/mL; Roche Diagnostics). Complete early virologic response (c-EVR) was defined as the absence of detectable serum HCV RNA at treatment week 12, the late virologic response (LVR) was defined as undetectable serum HCV RNA for the first time at 13–24 weeks of treatment, and the virologic response (VR) was defined as HCV RNA negativity at week 24 and week 48. SVR was defined as the absence of detectable serum HCV RNA at week 72. Patients with less than a 2-log decrease in HCV RNA level at treatment week 12 compared with the baseline had to stop treatment according to the protocol and were regarded as nonresponders. All patients with detectable serum HCV RNA at treatment week 24 were also considered to be nonresponders and were excluded from further treatment.

#### *Assessment of drug exposure*

The amounts of Peg-IFN  $\alpha$ -2b and ribavirin actually taken by each patient during the full treatment period were evaluated by reviewing the medical records. The mean doses of Peg-IFN  $\alpha$ -2b and ribavirin were calculated individually as averages on the basis of body weight at baseline: Peg-IFN  $\alpha$ -2b expressed as  $\mu$ g/kg/week, ribavirin expressed as mg/kg/day.

#### *Evaluation of impact of drug exposure on virologic relapse*

We evaluated the relationship between the drug exposure of both drugs and relapse by two different methods, univariate and multivariate analysis for relapse and independent evaluation of both drugs for relapse according to the degree of drug exposure. The former was performed with the factors of mean administration doses of both drugs, including the factors at baseline and the timing of HCV RNA negativation. The latter was examined by classifying Peg-IFN  $\alpha$ -2b exposure into five categories (up to 0.6  $\mu$ g/kg; from 0.6 to less than 0.9  $\mu$ g/kg; from 0.9 to less than 1.2  $\mu$ g/kg; from 1.2 to less than 1.5  $\mu$ g/kg; from 1.5  $\mu$ g/kg) and ribavirin exposure into five categories (up to 6 mg/kg; from 6 to less than 8 mg/kg; from 8 to less than 10 mg/kg; from 10 to less than 12 mg/kg; from 12 mg/kg).

#### *Statistical analysis*

Baseline data are expressed as means  $\pm$  SD or median values. Virologic response was evaluated using per protocol (PP) analysis. To analyse the difference between baseline data including drug exposure and virologic response, univariate analysis using the Mann-Whitney *U*-test or chi-square test and multivariate analysis using logistic regression analysis were performed. The significance of trends in values was determined with the Mantel-Haenszel chi-square test. A two-tailed *P* value  $<0.05$  was considered significant. The analysis was conducted with SPSS version 15.0J (SPSS Inc., Chicago, IL, USA).

## RESULTS

#### *Progress of patients and dose reduction of Peg-IFN $\alpha$ -2b and ribavirin*

The progress of patients in this study is shown in Fig. 1. Of the 984 patients, 903 completed 12 weeks of treatment and the c-EVR rate was 49% (445/903), based on PP study. To analyse for relapse, 472 patients with VR were assessed, with 178 (38%) showing Peg-IFN dose reduction without discontinuation and 246 (52%) with ribavirin dose reduction without discontinuation during the full (48 weeks) treatment period. The relapse rate was 26% (125/472) in the patients with undetectable HCV RNA level at the end of treatment. No difference was found in relapse rates between the IFN naïve patients and IFN experienced patients (IFN naïve; 25%, 72/287 vs IFN experienced; 29%, 53/185, *P* = 0.40). The SVR rate was 43% (347/812) in the PP study.

#### *Impact of drug exposure during 0–48 weeks on relapse among patients with VR*

The mean dose of Peg-IFN  $\alpha$ -2b actually taken during the full treatment period by each patient was 1.32  $\mu$ g/kg/week (range, 0.49–2.16  $\mu$ g/kg/week; median, 1.38  $\mu$ g/kg/week) and that of ribavirin was 9.8 mg/kg/day (range, 3.3–16.2 mg/kg/day; median, 10.1 mg/kg/day) in patients with VR.

The result of univariate analysis for relapse among the patients with VR is shown in Table 2a. The degree of fibrosis, the timing of HCV RNA negativation, Plt value and the mean doses of ribavirin were factors significantly associated with relapse, but those of Peg-IFN  $\alpha$ -2b were not. The mean dose of ribavirin as well as the degree of fibrosis and the timing of HCV RNA negativation was selected as a significant independent factor by multivariate logistic regression analysis (Table 2b).

Next, we analysed the relationship of the relapse rate and the mean ribavirin dose. The overall relapse rate among patients with VR was 26% (125/472). The

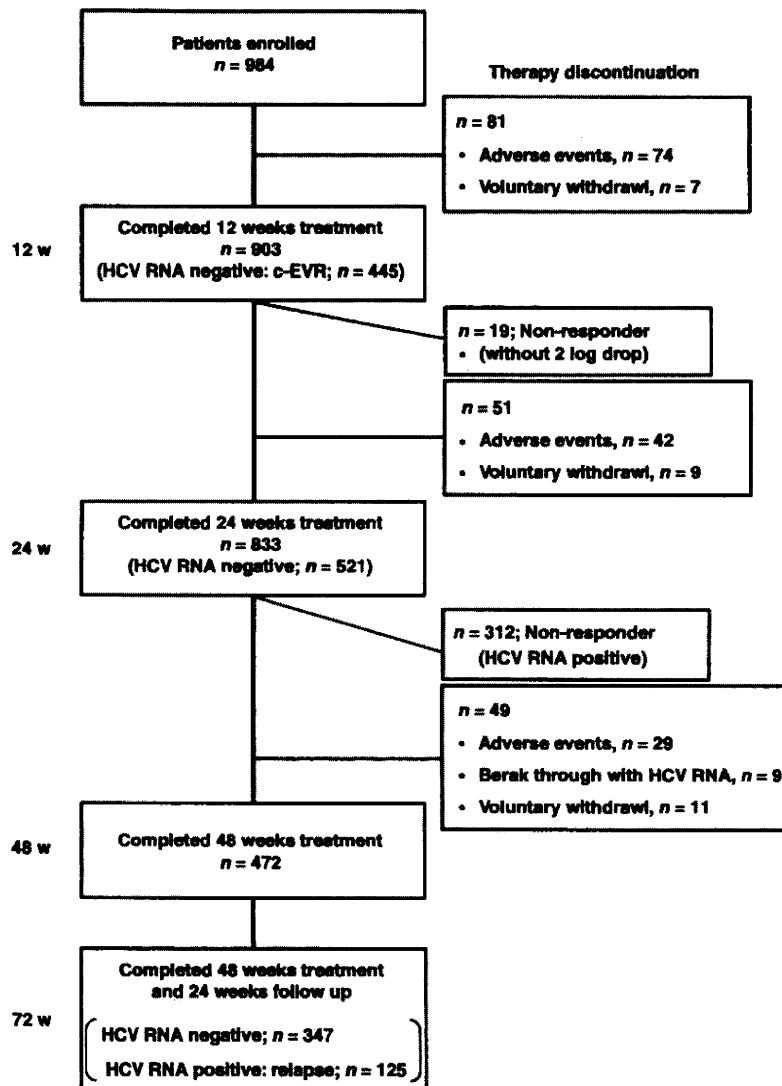


Fig. 1 Flow of patients throughout the study.

relapse rate was 60% (9/15) in patients receiving less than 6 mg/kg/day of ribavirin, and declined to 41% (32/79) at 6–8 mg/kg/day, 27% (34/124) at 8–10 mg/kg/day, 22% (43/193) at 10–12 mg/kg/day and 11% (7/61) in patients given  $\geq 12$  mg/kg/day ( $P < 0.0001$ ). Figure 2 shows the relationship of the relapse rate and the mean ribavirin dose for two dosage groups of Peg-IFN  $\alpha$ -2b: the group given  $\geq 1.4$   $\mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN and that given  $< 1.4$   $\mu\text{g}/\text{kg}/\text{week}$  (1.4  $\mu\text{g}/\text{kg}/\text{week}$  was the median value). In both groups, ribavirin was dose-dependently correlated with relapse. More than 12 mg/kg/day of the mean ribavirin exposure could suppress the relapse rate to 20% (4/20) in the group given  $< 1.4$   $\mu\text{g}/\text{kg}/\text{week}$  and strongly suppress it to 7% (3/41) in the group given  $\geq 1.4$   $\mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN.

#### Impact of drug exposure during 0–48 weeks on relapse according to the timing of HCV RNA negativiation

##### Relapse rates among patients with c-EVR

The overall relapse rate among patients with c-EVR was 19% (75/391). We separately analysed the relapse rate among the patients with c-EVR according to the degree of exposure to both drugs. Table 3a shows the relapse rates among the patients with c-EVR according to the categories of Peg-IFN  $\alpha$ -2b and ribavirin doses during the full treatment period. The relapse rate showed a decline according to the increase in the dose of ribavirin ( $P = 0.0002$ ). The relapse rate was suppressed at an average of 15% (13–16%) in the patients who received 10–12 mg/kg/day of ribavirin, and the average was only 4% for those who received more than 12 mg/kg/day

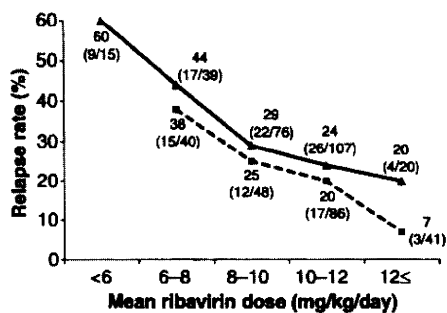
**Table 2** Factors associated with relapse among the patients with virologic response

(a) Univariate analysis				
Factor	Nonrelapser	Relapser	P value	
<i>n</i>	347	125		
Age (years)	53.9 ± 10.7	56.2 ± 9.2	0.07	
Sex (male/female)	213/134	66/59	0.09	
Serum HCV RNA (kIU/mL)*	1600	1800	0.34	
White blood cells (/mm <sup>3</sup> )	5335 ± 1517	5075 ± 1428	0.08	
Neutrophils (/mm <sup>3</sup> )	2797 ± 1143	2625 ± 1021	0.17	
Red blood cells (×10 <sup>4</sup> /mm <sup>3</sup> )	450 ± 45	446 ± 50	0.25	
Haemoglobin (g/dL)	14.3 ± 1.4	14.2 ± 1.5	0.45	
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )	17.6 ± 5.3	16.4 ± 5.1	0.03	
AST (IU/L)	60 ± 42	58 ± 33	0.75	
ALT (IU/L)	75 ± 60	71 ± 50	0.98	
Histology (METAVIR) <sup>†</sup>				
Fibrosis: 0–2/3–4	222/20	74/19	0.002	
Activity: 0–1/2–3	140/102	52/41	0.75	
Peg-IFN dose (µg/kg/week) <sup>‡</sup>	1.33 ± 0.26	1.27 ± 0.29	0.07	
Ribavirin dose (mg/kg/day) <sup>‡</sup>	10.1 ± 1.9	9.1 ± 2.1	<0.001	
Virologic response <sup>§</sup> : c-EVR/LVR	316/31	75/50	<0.001	
(b) Multivariate analysis				
Factor	Category	Odds ratio	95% CI	P value
Platelets	By 1 × 10 <sup>4</sup> /mm <sup>3</sup>	–	–	NS
Fibrosis <sup>†</sup>	0–2/3–4	1/3.192	1.515–6.725	0.002
Ribavirin dose <sup>‡</sup>	By 1 mg/kg/day	0.790	0.696–0.896	<0.001
Virologic response <sup>§</sup>	c-EVR/LVR	1/6.290	3.385–11.690	< 0.001

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus; c-EVR, complete early virologic response; LVR, late virologic response; NS, not significant difference Peg-IFN, pegylated interferon.

\*Data shown are median values. <sup>†</sup>137 missing. <sup>‡</sup>Mean doses during 0–48 weeks. <sup>§</sup>The timing of HCV RNA negativation.

<sup>†</sup>METAVIR fibrosis score.



**Fig. 2** Relapse rate according to Peg-IFN  $\alpha$ -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses. (— $\blacktriangle$ ) Group with the mean Peg-IFN dose <1.4  $\mu$ g/kg/week; (--- $\blacksquare$ ) Group with the mean Peg-IFN dose  $\geq$ 1.4  $\mu$ g/kg/week. The ribavirin dose was dose-dependently correlated with the virologic relapse in both groups ( $P < 0.0001$ ). There was no significant difference between the two Peg-IFN  $\alpha$ -2b-dose groups ( $P = 0.17$ ).

of ribavirin. In contrast, the relapse rate was not affected by the dose of Peg-IFN  $\alpha$ -2b when the patients were given more than 0.9  $\mu$ g/kg/week of Peg-IFN  $\alpha$ -2b. On the other hand, with respect to patients with rapid virologic response (RVR) defined as the absence of detectable serum HCV RNA at treatment week 4 ( $n = 41$ ), none showed relapse and all attained SVR irrespective of the dose of Peg-IFN  $\alpha$ -2b or ribavirin (prevalence of patients: the mean dose of Peg-IFN  $\alpha$ -2b; <0.9: 0.9–1.2: 1.2–1.5: 1.5  $\mu$ g/kg/week  $\leq$  7: 17: 34: 42%, the mean dose of ribavirin; <8: 8–10: 10–12: 12 mg/kg/day  $\leq$  15: 24: 41: 20%).

#### Relapse rates among patients with LVR

Among the patients with LVR, the ribavirin exposure during treatment was also the factor correlated adversely with the relapse rate ( $P = 0.03$ ). However, the overall relapse rate was 62% (50/81), which was much higher than that of the c-EVR patients ( $P < 0.0001$ ) and 45% (5/11) of patients with LVR relapsed even in the group given more than 12 mg/kg/day of the average ribavirin dose (Table 3b).

**Table 3** Relapse rate according to Peg-IFN and ribavirin doses during week 0–48 for patients with c-EVR and LVR who completed 48 weeks of treatment

(a) C-EVR										
Peg-IFN dose ( $\mu\text{g}/\text{kg}/\text{week}$ ) <sup>†</sup>	Ribavirin dose (mg/kg/day)*								Total	
	12 $\leq$	10–12		8–10		<8				
$\geq 1.5$	0%	(0/28)	13%	(4/31)	14%	(3/21)	29%	(5/17)	12%	(12/97)
1.2–1.5	20%	(2/10)	16%	(16/100)	25%	(16/65)	23%	(7/30)	20%	(41/205)
0.9–1.2	0%	(0/7)	13%	(2/15)	15%	(2/13)	38%	(6/16)	20%	(10/51)
<0.9	0%	(0/5)	15%	(2/13)	55%	(6/11)	44%	(4/9)	32%	(12/38)
Total	4%	(2/50)	15%	(24/159)	25%	(27/110)	31%	(22/72)	19%	(75/391)

(b) LVR										
Peg-IFN dose ( $\mu\text{g}/\text{kg}/\text{week}$ ) <sup>§</sup>	Ribavirin dose (mg/kg/day) <sup>‡</sup>								Total	
	12 $\leq$	10–12		8–10		<8				
$\geq 1.5$	43%	(3/7)	50%	(1/2)	100%	(2/2)	100%	(4/4)	67%	(10/15)
1.2–1.5		(1/1)	60%	(12/20)	29%	(2/7)	82%	(9/11)	62%	(24/39)
<1.2	33%	(1/3)	50%	(6/12)	60%	(3/5)	86%	(6/7)	59%	(16/27)
Total	45%	(5/11)	56%	(19/34)	50%	(7/14)	86%	(19/22)	62%	(50/81)

Peg-IFN, pegylated interferon; c-EVR, complete early virologic response; LVR, late virologic response.

\* $P = 0.0002$  for comparison of the four ribavirin groups. <sup>†</sup> $P = 0.08$  for comparison of the four Peg-IFN groups. <sup>‡</sup> $P = 0.03$  for comparison of the four ribavirin groups. <sup>§</sup> $P = 0.57$  for comparison of the three Peg-IFN groups.

#### Impact of dose reduction after week 12 on relapse among patients with c-EVR

Among c-EVR patients with no or little reduction of Peg-IFN  $\alpha$ -2b (the average dose  $\geq 1.2 \mu\text{g}/\text{kg}/\text{week}$ ) during the first 12 weeks, no significant difference was found in the relapse rate between those whose average dose of Peg-IFN  $\alpha$ -2b was reduced to 0.6–1.2  $\mu\text{g}/\text{kg}/\text{week}$  during 12–48 weeks (17%, 7/41) and those without reduction of Peg-IFN  $\alpha$ -2b (average dose  $\geq 1.2 \mu\text{g}/\text{kg}/\text{week}$ ) (18%, 53/295) ( $P = 0.86$ ) (Table 4a). Reducing the dose of Peg-IFN  $\alpha$ -2b after week 12 in patients in whom HCV RNA had already become undetectable before week 12 did not appear to adversely influence virologic relapse when the average dose of Peg-IFN  $\alpha$ -2b was more than 0.6  $\mu\text{g}/\text{kg}/\text{week}$  during 12–48 weeks, irrespective of the mean dose of Peg-IFN  $\alpha$ -2b during the first 12 weeks. On the other hand, the ribavirin dose reduction after week 12 tended to affect the relapse rate in patients given  $\geq 10 \text{ mg}/\text{kg}/\text{day}$  of the ribavirin dose during the first 12 weeks (Table 4b).

#### Impact of drug exposure during 0–48 weeks on relapse among VR patients with advanced fibrosis

In the evaluation of the 39 patients with VR with progression of fibrosis or cirrhosis (METAVIR fibrosis score 3 or 4) enrolled in this study, ribavirin exposure during treatment significantly correlated with relapse (nonrelapser,  $10.5 \pm 2.1 \text{ mg}/\text{kg}/\text{day}$  vs relapser,  $8.8 \pm 2.3 \text{ mg}/\text{kg}/\text{day}$ ;  $P = 0.007$ ). Among patients with advanced fibrosis (score 3–4),

the relapse rate in patients given  $\geq 10 \text{ mg}/\text{kg}/\text{day}$  of the average ribavirin dose was significantly low (36%, 9/25) in comparison with that in patients given  $< 10 \text{ mg}/\text{kg}/\text{day}$  of ribavirin (71%, 10/14) ( $P = 0.048$ ).

#### DISCUSSION

Previous studies have suggested that reducing the ribavirin dose within the first 12–20 weeks of treatment in patients with HCV genotype 1 was associated with a decline of SVR [7,13,14]. However, Shiffman *et al.* [8] recently reported that reducing the mean dose of ribavirin during the first 20 weeks of treatment had little impact on relapse for patients with CH-C genotype 1 and that SVR may not be adversely affected as long as the total cumulative ribavirin dose remains above 60%. As the reason for the inconsistency in the impact of reducing ribavirin on the antiviral effect, it was suggested that sample sizes of the previous studies were insufficient to assess the impact of reducing the dose of ribavirin independent of Peg-IFN. However, in Shiffman's study, while the impact of reducing the dose of Peg-IFN or ribavirin on SVR was indeed closely examined independently of each other with a large sample size, the subjects were limited to patients with advanced fibrosis or cirrhosis and prior nonresponse to Peg-IFN  $\pm$  ribavirin who were enrolled in the Hepatitis Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial. Reddy *et al.* [15] analysed the drug exposure retrospectively for 569 CH-C patients with genotype 1 enrolled in clinical trials of Peg-IFN  $\alpha$ -2a plus