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Genome-wide association of *IL28B* with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C

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The recommended treatment for patients with chronic hepatitis C, pegylated interferon-α (PEG-IFN-α) 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, 3.11×10^{-15}). We replicated these associations in an independent cohort (combined *P* values, 2.84×10^{-27} (OR = 17.7; 95% CI = 10.0–31.3) and 2.68×10^{-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 ($P = 5.52 \times 10^{-28}$–2.68×10^{-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[1].The American Gastroenterological Association estimated that drugs are the largest direct costs of hepatitis C[1].

The most effective current standard of care in patients with chronic hepatitis C, a combination of PEG-IFN-α with ribavirin, does not produce SVR in all patients treated. Large-scale studies on 48-week-long PEG-IFN-α/RBV treatment in the United States and Europe showed that 42–52% of patients with HCV genotype 1 achieved SVR[2–4], 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[5,6]. 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[7]. 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-α/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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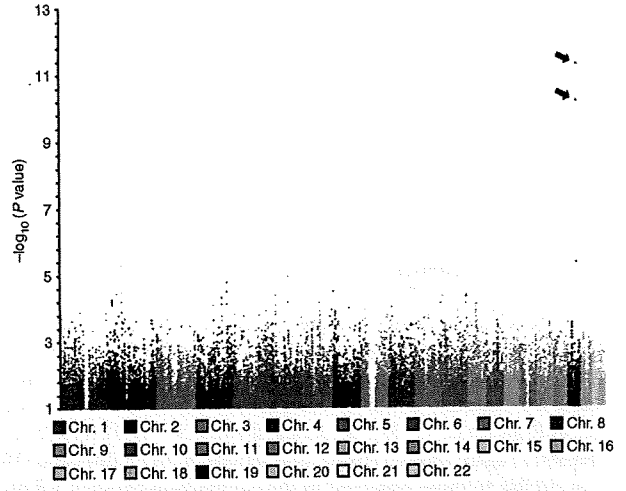

Figure 1 Genome-wide association results with PEG-IFN-α/RBV treatment in 142 Japanese patients with HCV (78 NVR and 64 VR samples). *P* values were calculated by using a χ^2 test for allele frequencies. The dots with arrows for chromosome 19 denote SNPs that showed significant genome-wide associations ($P < 8.05 \times 10^{-8}$) with response to PEG-IFN-α/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[8–10]. 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-α/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-α/RBV therapy outcome[11,12]. 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[13,14]. We describe here a GWAS for response to PEG-IFN-α/RBV treatment.

We conducted this GWAS to identify host genes associated with response to PEG-IFN-α/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 ≥95%, (ii) minor allele frequency (MAF) ≥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-α/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[15], 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 Cochrane-Armitage test on all the SNPs and found a genetic inflation factor, λ, 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-α/RBV treatment

					Null responder (NVR[a], $n = 128$)			Responder (VR[a], $n = 186$)			Responder (SVR[a], $n = 140$)			NVR vs. VR		NVR vs. SVR	
dbSNP rsID	Nearest gene	MAF[b] (allele)	Allele (1/2)	Stage	11	12	22	11	12	22	11	12	22	OR (95% CI)[c]	*P* value[d]	OR (95% CI)[c]	*P* value[d]
rs12980275	*IL28B*	0.15 (G)	A/G	GWAS	20 (25.6)	54 (69.2)	4 (5.1)	56 (87.5)	8 (12.5)	0 (0.0)	46 (90.2)	5 (9.8)	0 (0.0)	20.3 (8.3–49.9)	1.93×10^{-13}	26.7 (9.3–76.5)	7.41×10^{-13}
				Replication	10 (20.0)	37 (74.0)	3 (6.0)	101 (82.8)	21 (17.2)	0 (0.0)	73 (82.0)	16 (18.0)	0 (0.0)	19.2 (8.3–44.4)	5.46×10^{-15}	18.3 (7.6–44.0)	8.37×10^{-13}
				Combined	30 (23.4)	91 (71.1)	7 (5.5)	157 (84.4)	29 (15.6)	0 (0.0)	119 (85.0)	21 (15.0)	0 (0.0)	17.7 (10.0–31.3)	2.84×10^{-27}	18.5 (10.0–34.4)	3.99×10^{-24}
rs8099917	*IL28B*	0.12 (G)	T/G	GWAS	19 (24.4)	56 (71.8)	3 (3.8)	58 (90.6)	6 (9.4)	0 (0.0)	47 (92.2)	4 (7.8)	0 (0.0)	30.0 (11.2–80.5)	3.11×10^{-15}	36.5 (11.6–114.6)	5.00×10^{-14}
				Replication	11 (22.0)	37 (74.0)	2 (4.0)	108 (88.5)	14 (11.5)	0 (0.0)	78 (87.6)	11 (12.4)	0 (0.0)	27.4 (11.5–65.3)	9.47×10^{-18}	25.1 (10.0–63.1)	1.00×10^{-14}
				Combined	30 (23.4)	93 (72.7)	5 (3.9)	166 (89.2)	20 (10.8)	0 (0.0)	125 (89.3)	15 (10.7)	0 (0.0)	27.1 (14.6–50.3)	2.68×10^{-32}	27.2 (13.9–53.4)	1.11×10^{-27}

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

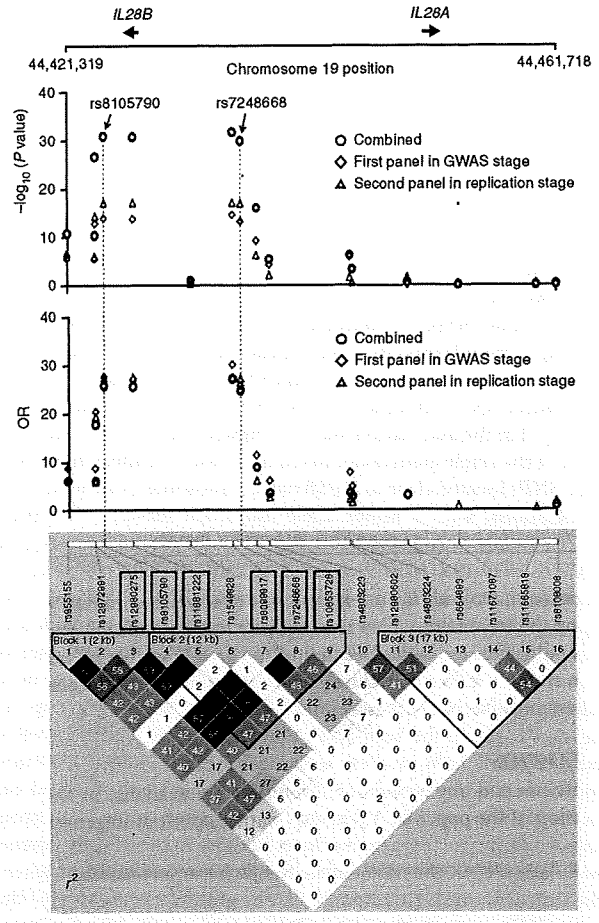

Figure 2 Genomic structure, *P* value and OR plots in association analysis and LD map around *IL28B* and *IL28A* (chr.19, nucleotide positions 44421319–44461718; build 35). *P* values by the χ^2 test for minor allele dominant effect model are shown for the first panel of 142 samples in the GWAS stage, 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-α/RBV. Boxes indicate the significantly associated SNPs with response to PEG-IFN-α/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.

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-α/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; $P = 9.47 \times 10^{-18}$, OR = 27.4 for rs8099917; **Table 1**). The combined *P* values for both stages reached 2.84×10^{-27} (OR = 17.7; 95% CI = 10.0–31.3) and 2.68×10^{-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 3.99×10^{-24} (OR = 18.5; 95% CI = 10.0–34.4) and 1.11×10^{-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-α/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 1.98×10^{-31} (OR = 25.7; 95% CI = 13.9–47.6), 2.84×10^{-31} (OR = 25.6; 95% CI = 13.8–47.3), 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3) and 1.84×10^{-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.

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	1.81×10^{-32}	0.1 (0.04–0.12)
C	**G**	**C**	**G**	**T**	**G**	**A**	0.387	0.054	1.35×10^{-25}	11.1 (6.6–18.6)

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

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[a]	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[20]	1.10	0.73–1.66	0.658
HCV-RNA level	1.01	0.99–1.02	0.139

[a]Re-treatment, non-response to previous treatment with interferon-α (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-α/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-α/RBV treatment. *IL28B* encodes a cytokine distantly related to type I (α and β) 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-λs) 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[16,17]. All three interact with a heterodimeric class II cytokine receptor that consists of IL-10 receptor beta (IL10Rβ) and IL-28 receptor alpha (IL28Rα, encoded by *IL28RA*)[16,17], 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*$^{-/-}$ and *IFNAR*$^{-/-}$ mice, indicating that IFN-λ is important in mediating antiviral protection by ligands for TLR3 and TRL9 (ref. 18). IFN-λ induced a steady increase in the expression of a subset of IFN-stimulated genes, whereas IFN-α induced the same genes with more rapid and transient kinetics[19]. Therefore, it is possible that IFN-λ 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-α/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-α/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-α/RBV 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.

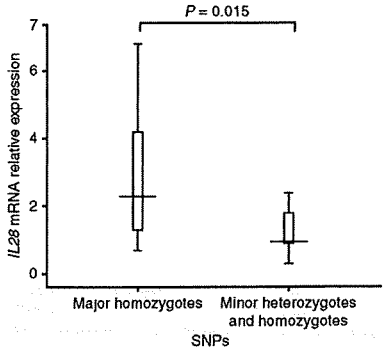

Figure 3 Quantification of *IL28* mRNA expression. The expression level of *IL28* genes was determined by real-time quantitative RT-PCR using RNA purified from peripheral blood mononuclear cells. Distribution of relative gene expression levels was compared between the individuals homozygous for major 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-α/RBV therapy.

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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AUTHOR CONTRIBUTIONS

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.I., 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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1. Ray Kim, W. Global epidemiology and burden of hepatitis C. *Microbes Infect.* 4, 1219–1225 (2002).
2. Manns, M.P *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C. a randomised trial. *Lancet* **358**, 958–965 (2001).

3. Fried, M.W. *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* **347**, 975–982 (2002).
4. Hadziyannis, S.J. *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann. Intern. Med.* **140**, 346–355 (2004).
5. Bruno, S. *et al.* Peginterferon alfa-2b plus ribavirin for naive patients with genotype 1 chronic hepatitis C: a randomized controlled trial. *J. Hepatol.* **41**, 474–481 (2004).
6. Sezaki, H. *et al.* Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. *Dig. Dis. Sci.* **54**, 1317–1324 (2009).
7 Fried, M.W. Side effects of therapy of hepatitis C and their management. *Hepatology* **36**, S237–S244 (2002).
8. Pascu, M. *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* **53**, 1345–1351 (2004).
9. Shirakawa, H. *et al.* Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* **48**, 1753–1760 (2008).
10. Akuta, N. *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J. Hepatol.* **46**, 403–410 (2007).
11. Walsh, M.J. *et al.* Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. *Gut* **55**, 529–535 (2006).
12. Gao, B., Hong, F. & Radaeva, S. Host factors and failure of interferon-alpha treatment in hepatitis C virus. *Hepatology* **39**, 880–890 (2004).
13. Matsuyama, N. *et al.* The dinucleotide microsatellite polymorphism of the IFNAR1 gene promoter correlates with responsiveness of hepatitis C patients to interferon. *Hepatol. Res.* **25**, 221–225 (2003).
14. Tsukada, H. *et al.* A polymorphism in MAPKAPK3 affects response to interferon therapy for chronic hepatitis C. *Gastroenterology* **136**, 1796–1805 (2009).
15. Nishida, N. *et al.* Evaluating the performance of Affymetrix SNP Array 6.0 platform with 400 Japanese individuals. *BMC Genomics* **9**, 431 (2008).
16. Kotenko, S.V. *et al.* IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol.* **4**, 69–77 (2003).
17 Sheppard, P *et al.* IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat. Immunol.* **4**, 63–68 (2003).
18. Ank, N. *et al.* An important role for type III interferon (IFN-lambda/IL-28) in TLR-induced antiviral activity. *J. Immunol.* **180**, 2474–2485 (2008).
19. Marcello, T. *et al.* Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology* **131**, 1887–1898 (2006).
20. Desmet, V.J., Gerber, M., Hoofnagle, J.H., Manns, M. & Scheuer, P.J. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* **19**, 1513–1520 (1994).

ONLINE METHODS

Study cohorts. From April 2007 to April 2009, samples were obtained from 314 patients with chronic HCV (genotype 1) infection who were treated at 15 multicenter hospitals (liver units with hepatologists) throughout Japan. Each patient was treated with PEG-IFN-α2b (1.5 μg per kg body weight (μg/kg) subcutaneously once a week) or PEG-IFN-α2a (180 μg/kg once a week) plus RBV (600–1,000 mg daily depending on body weight). As a reduction in the dose of PEG-IFN-α and RBV can contribute to a less sustained virological response[21], only patients with an adherence of >80% dose for both drugs during the first 12 weeks were included in this study. HBsAg-positive and/or anti-HIV–positive individuals were excluded from this study.

NVR (seen in ~20% of total treated patients) was defined as less than a 2-log-unit decline in the serum level of HCV RNA from the pre-treatment baseline value within the first 12 weeks and detectable viremia 24 weeks after treatment. VR was defined as the achievement of SVR or transient TVR in this study; SVR was defined as undetectable HCV RNA in serum 6 months after the end of treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after treatment was discontinued in a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. Of 878 patients with HCV genotype 1 treated by PEG-IFN-α/RBV at 14 hospitals, only 114 (13.0%) met the criteria for NVR in this study. For the GWAS stage of the study, a case-control study was conducted comparing individuals with NVR (82 individuals) and VR (72 individuals). For the replication stage, an independent cohort of samples from 172 Japanese patients with HCV genotype 1, including 50 with NVR and 122 with VR, was obtained from an independent cohort study at Tokyo Medical and Dental University Hospital (Ochanomizu Liver Conference Study Group) and Musashino Red Cross Hospital. Clinical data from the combined cohorts, with a total of 140 SVR, 46 TVR and 128 NVR patients, are shown in **Supplementary Table 4.**

Informed consent was obtained from each patient who participated in the study. The study protocol conforms to the relevant ethical guidelines as reflected in *a priori* approval by the ethics committees of all the participating universities and hospitals.

SNP genotyping and data cleaning. In the GWAS stage, we genotyped 154 Japanese patients with HCV receiving PEG-IFN-α/RBV treatment using the Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's instructions. After exclusion of 4 NVR samples and 8 SVR samples with QC call rates <95%, the remaining 142 samples were recalled using the Birdseed version 3 software (Affymetrix). The average overall call rate of 78 NVR and 64 VR samples reached 99.46% and 99.46%, respectively. We then applied the following thresholds for QC in data cleaning: SNP call rate ≥95% for all samples, MAF ≥1% for all samples and HWE P value ≥0.001 for VR group[22,23]. A total of 621,220 SNPs on autosomal chromosomes passed the QC filters and were used for association analysis. All cluster plots for the SNPs showing $P < 0.001$ in association analyses by comparing allele frequencies in NVR and VR groups were checked by visual inspection. SNPs with ambiguous genotype calls were excluded. **Supplementary Table 5** shows SNPs that might be weakly associated with NVR ($P < 10^{-4}$).

Although the 12 samples noted above were excluded from the GWAS stage by data cleaning, their quality was good enough for the SNP typing in the replication study, and thus they were included in the replication stage. In the subsequent replication stage with high-density association mapping, SNP genotyping in the independent set of 172 patients was completed using the DigiTag2 assay[24] and direct sequencing using the Applied Biosystems 3730 DNA Analyzer (Applied Biosystems). In addition, strongly associated SNPs identified in the GWAS stage were also genotyped for the GWAS samples using the DigiTag2 assay, and the results were 100% concordant to those from the GWAS platform.

Screening for new polymorphisms. To determine possible genomic variants in the region of *IL28B* and its promoter, we sequenced the 3.3-kb region in a total of 48 Japanese patients with HCV (28 NVR and 20 VR). We selected 7 samples from NVR patients who were minor allele homozygotes for 2 SNPs (rs12980275 and rs8099917), 11 samples from NVR and 10 samples from VR heterozygotes, and 10 samples from NVR and 10 samples from VR major allele homozygotes. The sequencing primers were designed using the Visual OMP Nucleic Acid software (**Supplementary Table 6**). PCR was carried using TaKaRa LA *Taq* polymerase (Takara Biochemicals) under the following thermal cycler conditions: stage 1, 94 °C for 1 min; stage 2, 98 °C for 10 s, 68 °C for 15 min, for a total of 30 cycles; stage 3, 72 °C for 10 min. A 50-μl PCR analysis was performed using 2.5 U TaKaRa LA *Taq* with 1× LA PCR buffer II, 0.4 mM dNTP, 10 pmol of each primer and 10 ng of genomic DNA. For sequencing, 7.0 μl of the PCR products were incubated with 3 μl of Exonuclease I/Shrimp Alkali Phosphatase (Takara Biochemicals) first for 90 min at 37 °C and then for another 10 min at 80 °C. Sequencing reactions were performed with the use of a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems). After purification with MultiScreen-HV (Millipore) and Sephadex G-50 Fine (GE Healthcare UK Ltd.), the reaction products were applied to the Applied Biosystems 3730 DNA Analyzer.

In the variation screening, three SNPs (rs8103142, rs28416813 and rs4803219) and a few infrequent variations were detected. We then typed these SNPs in all of the 314 patients.

Statistical analysis. The observed association between a SNP and response to PEG-IFN-α/RBV treatment was assessed by χ^2 test with a two-by-two contingency table in three genetic models: allele frequency model, dominant-effect model and recessive-effect model. SNPs on the X chromosome were removed because gender was not matched between the NVR group and the VR group. A total of 621,220 SNPs passed the QC filters in the GWAS stage; therefore, significance levels after the Bonferroni correction for multiple testing were $P = 8.05 \times 10^{-8}$ (0.05/621,220) in the GWAS stage and $P = 0.0031$(0.05/16) in the replication stage. None of the 16 markers genotyped in the replication stage showed deviations from Hardy-Weinberg equilibrium in the VR group ($P > 0.05$).

The inflation factor λ was estimated based on the median χ^2 and revealed to be 1.029 (median) and 1.011 (mean), suggesting that the population substructure should not have any substantial effect on the statistical analysis (**Supplementary Fig. 1**). In addition, the principal component analysis on the 142 patients (78 NVR samples and 64 VR samples) analyzed in the GWAS stage together with the HapMap samples also revealed that the effect of population stratification was negligible (**Supplementary Fig. 2**).

For the replication study and the high-density association mapping, 16 SNPs were selected from the region of ~40 kb (chr. 9, nucleotide positions 44421319–44461718; build 35) containing the significantly associated SNPs (rs12980275 and rs8099917) in the GWAS stage by analyzing, using Haploview software, LD and haplotype structure based on the HapMap data for individuals of Japanese descent. These SNPs included tagging SNPs estimated on the basis of haplotype blocks, SNPs located within the *IL28B* and *IL28A* genes (rs11881222 and rs576832, respectively) and the significantly associated SNPs identified in the GWAS stage (**Supplementary Table 1**). On the basis of the genotype data from the total of 314 patients in the GWAS stage and replication stages, haplotype blocks were estimated using the four-gamete rule, and three blocks were observed (**Fig. 2**). Association of haplotype with response to PEG-IFN-α/RBV treatment was analyzed using Haploview software.

The logistic regression model was used to assess the factors associated with NVR. STATA 10 (Statacorp LP) was used for all analysis. Age, platelet count, and aminotransferase (ALT) and HCV-RNA levels were applied as continuous variables.

Real-time quantitative RT-PCR for *IL28* gene. A layer of mononuclear cells was collected via Ficoll from peripheral blood. Total RNA was isolated using the RNeasy Mini Kit and the RNase-Free DNase Set (Qiagen) according to the manufacturer's protocol. First-strand cDNA was synthesized using SuperScript II reverse transcriptase with Oligo $(dT)_{12-18}$ primer (Invitrogen). The relative quantification of the target gene was determined using Custom TaqMan Gene Expression Assays, and the expression of glyceraldehyde-3-phosphate dehydrogenase was used to normalize the gene expression level (Applied Biosystems) according to the manufacturer's protocol. The data were analyzed by the $2[-\Delta\Delta C_t]$ method using Sequence Detector version 1.7 software (Applied Biosystems). A standard curve was prepared by serial tenfold dilutions of

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human cDNA. The curve was linear over 7 logs with a correlation coefficient of 0.998. The specific detection of *IL28B* in real-time PCR is hard to establish, because the nucleotide differences between *IL28A* and *IL28B* consist of only 9 nucleotides scattered throughout the gene. Primers and probes are designed for the *IL28* gene (**Supplementary Table 6**).

URLs. The results of the present GWAS have been registered at a public database: https://gwas.lifesciencedb.jp/cgi-bin/gwasdb/gwas_top.cgi.

21. McHutchison, J.G. *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* **123**, 1061–1069 (2002).
22. Miyagawa, T. *et al.* Variant between *CPT1B* and *CHKB* associated with susceptibility to narcolepsy. *Nat. Genet.* **40**, 1324–1328 (2008).
23. Miyagawa, T. *et al.* Appropriate data cleaning methods for genome-wide association study. *J. Hum. Genet.* **53**, 886–893 (2008).
24. Nishida, N., Tanabe, T., Takasu, M., Suyama, A. & Tokunaga, K. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology* **131**, 1887–1898 (2006).

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ORIGINAL ARTICLE

A randomized trial of 24 versus 48 weeks of peginterferon α-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan

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Abstract In a country such as Japan with the average age of patients with chronic hepatitis C treated with antivirals sometimes well above 60 years, the standard combination therapy is not well tolerated. In this randomized, prospective, controlled trial, we investigated the efficacy of 24-week peginterferon α monotherapy for easy-to-treat patients. A total of 132 patients chronically infected with hepatitis C virus (HCV) genotype 2 ($n = 115$) or low viral load HCV genotype 1 (<100 kIU/ml, $n = 17$) were treated with peginterferon α-2a (180 μg/week). Patients with a rapid virological response (RVR, HCV RNA negative or <500 IU/ml at week 4) were randomized for a total treatment duration of 24 (group A) or 48 (group B) weeks. Patients who did not show RVR (group C) were treated for 48 weeks. Sustained virological response (SVR) was assessed by qualitative reverse-transcription polymerase chain reaction. One hundred eight of 132 (82%) patients with RVR were randomized. SVR rates were 60% (group A), 79% (group B), and 27% (group C), respectively. Similar SVR rates were achieved in patients infected with HCV genotype 2 with low pretreatment viral load (<1000 kIU/ml) in group A (81%) and group B (79%) ($P = 0.801$), whereas in those with higher viral load (≥1000 kIU/ml), a lower SVR rate was identified in group A (26%) than in group B (67%) ($P = 0.041$). In

This study is conducted on behalf of the Japanese Consortium for the Study of Liver Diseases.

Clinical Trial Registry: www.umin.ac.jp/ctr/index.htm; identifier: UMIN000001067.

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conclusion, in patients infected with HCV genotype 2 and pretreatment viral load below 1000 kIU/ml who achieve RVR, 24-week treatment with peginterferon α-2a alone is clinically sufficient. Those who show no RVR or have higher baseline viral load, require alternative therapies.

Keywords Randomized trial · Chronic hepatitis C · Peginterferon-α monotherapy · Rapid virological response · Genotype 2 · Pretreatment viral load

Introduction

Hepatitis C virus (HCV) infection may progress to chronic hepatitis, cirrhosis, and hepatocellular carcinoma [1–3]. Interferon (IFN)-based treatment of HCV-infected patients can achieve viral clearance and thereby improve histology and prognosis [4, 5]. Thus, the primary aim of antiviral therapy in patients with chronic hepatitis C is a sustained virological response (SVR), defined as undetectable serum HCV RNA by a sensitive molecular assay 24 weeks after the end of treatment.

A combination therapy of peginterferon and ribavirin is currently recognized as the standard treatment of chronic hepatitis C, resulting in 40–50% of SVR rate in patients infected with HCV genotype 1 and around 80% in those infected with HCV genotype 2 or 3 [6–8]. The combination therapy, however, tends to be associated with adverse events more frequently than those that occur with IFN monotherapy [9–14], resulting in dose reduction or discontinuation of therapy and thus impaired response rate particularly in elderly patients [15]. Furthermore, patients with renal failure, ischemic vascular disease, and congenital hemoglobin abnormalities never tolerate ribavirin treatment of their chronic hepatitis C.

In Japan, the Bureau of National Health Insurance provides reimbursement for 24-week interferon α-2b plus ribavirin combination therapy for patients with chronic hepatitis C with high viral load or re-treatment, irrespective of viral load, since December 2001 and for 48 weeks of peginterferon α-2a monotherapy for all patients with chronic hepatitis C since December 2003. The bureau started to provide reimbursement for 24-week peginterferon α-2b and ribavirin therapy for those infected with HCV genotype 2 and high viral load or re-treatment, irrespective of viral load, since December 2005. Thus, Japanese patients infected with HCV genotype 2 and high viral load or re-treatment irrespective of viral load have been able to receive either peginterferon α monotherapy or combination therapy with ribavirin since December 2003.

There are three major phase II/III or phase III clinical trials of peginterferon α monotherapy in patients with chronic hepatitis C [16–18]. All three studies indicate that the long-acting pegylated forms of IFN-α are more potent than standard IFN-α monotherapies. Factors independently associated with SVR to peginterferon α include viral genotype, low pretreatment viral load, age, no cirrhosis, and body surface area [18]. The reported SVR rate in patients with HCV genotype 2 infection and a baseline viral load of less than 2 million copies/ml is around 60% or more [16, 17]. A phase II study of 48-week peginterferon α-2a therapy conducted in Japan demonstrated an SVR rate as high as 71% in patients with HCV genotype 2 infection [19]. Furthermore, 85% of the patients, who had undetectable levels of HCV RNA after 4 weeks of therapy, had an SVR [19]. Thus, data on viral kinetics have led to the hypothesis that in these patients, 24 weeks of treatment may be as effective as the recommended course of 48 weeks. Therefore, 48-week therapy may lead to overtreatment in some patients who have a rapid virological response (RVR). Shorter treatment duration should also be associated with better tolerability and lower rate of premature discontinuation of therapy. This is particularly relevant to elderly patients with HCV genotype 2 infection who can less tolerate the combination therapy with ribavirin and/or a longer treatment period. However, whether the duration of treatment with peginterferon α alone can be reduced from 48 to 24 weeks in patients chronically infected with HCV genotype 2 or low viral load HCV genotype 1 without compromising antiviral efficacy is not clear at present.

Therefore, the aim of this study was to compare the efficacy and safety of peginterferon α-2a administered alone for 24 or 48 weeks in patients with chronic HCV genotype 2 infection or low viral load HCV genotype 1 and had a virological response at week 4.

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Materials and methods

Patients

Adult patients with chronic HCV infection who had the following characteristics were eligible for the study: (1) a positive test for anti-HCV antibody, (2) HCV genotype 1 and an HCV RNA level less than 100,000 IU/ml or HCV genotype 2 irrespective of viral load, (3) entry neutrophil and platelet counts and hemoglobin level of at least 1500/μl, 90,000/μl, and 10 g/dL, respectively. Patients with the following criteria were excluded: other viral infections such as infection with hepatitis B virus or human immunodeficiency virus; any other cause of liver disease such as autoimmune hepatitis, primary biliary cirrhosis, drug-induced liver disease, and excessive daily intake of alcohol; relevant disorders including decompensated liver disease, hepatocellular carcinoma, and other malignant neoplastic disease; concomitant use of immunosuppressive or herbal medications such as Sho-saiko-to; current illicit drug use; neurological or psychiatric diseases; and allergic to peginterferon α-2a or other interferons and biological preparations including vaccines.

Study design

The current study was an investigator-initiated study. This multicenter, open-label, randomized, controlled trial was originally discussed and designed on 12 July 2003, by a committee composed of 36 staff members from 33 participating hospitals and universities (the Japanese Consortium for the Study of Liver Diseases). The diagnostic criteria for chronic hepatitis C, treatment regimens, and follow-up protocols were finalized by the committee on 9 November 2003. This study compared the efficacy and safety of 24 vs. 48 weeks of treatment with peginterferon α-2a in patients with chronic HCV genotype 2 infection or low viral load HCV genotype 1 and showed an RVR (serum HCV RNA negative [<50 IU/ml] or <500 IU/ml by HCV RNA test at week 4 of therapy).

Eligible patients were treated with peginterferon α-2a (PEGASYS; Chugai Pharmaceuticals Inc., Tokyo, Japan) at a dose of 180 μg once per week subcutaneously. Patients who showed RVR at 4 weeks of treatment were randomized into either total treatment duration of 24 (group A) or 48 (group B) weeks. Randomization was performed at Okayama University Graduate School, centrally accessed through fax. Patients were assigned upon a report of RVR to group A or B with a computer-based random allocation system by a researcher who was independent of the study, and the allocation system was not accessible by any of the investigators who enrolled patients for the study. Randomization was stratified according to genotype (genotype 1 or 2) and previous IFN treatment (naive or re-treated) and was not blocked. Patients who were still positive for HCV RNA (by qualitative or quantitative HCV RNA tests) at week 4 were treated for 48 weeks (group C, Fig. 1). After the end of treatment, all patients were followed for an additional 24-week period.

The study was approved by ethics committee of each center and carried out according to the Declaration of Helsinki and the guidelines of the International Conference

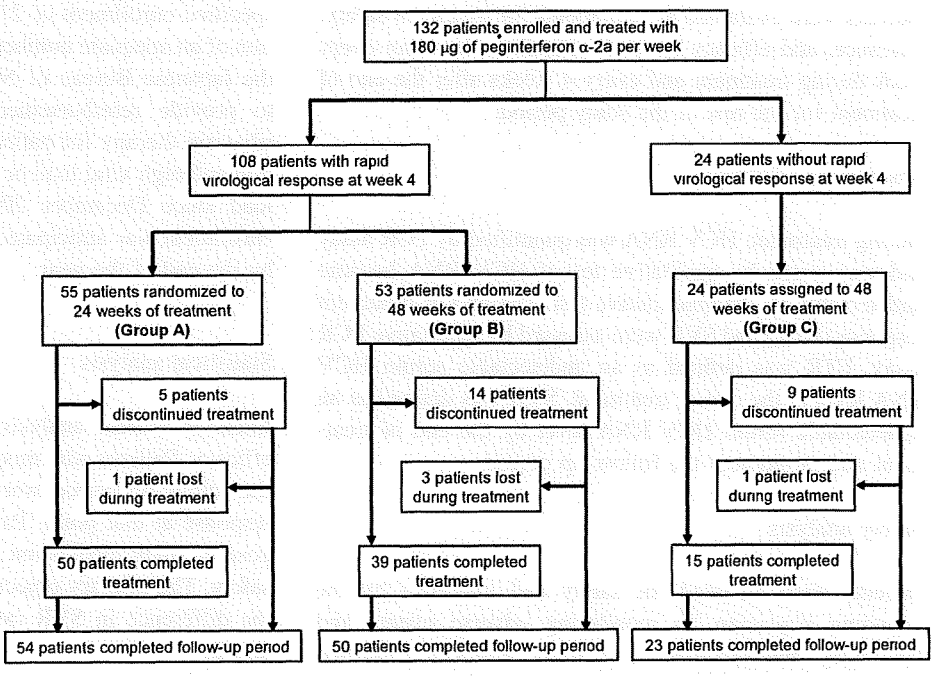

Fig. 1 Trial profile. Patients were randomized at week 8 for a total treatment duration of 24 (group A) or 48 (group B) weeks on the basis of the virological response at week 4. Patients who withdrew prematurely from treatment were encouraged to return for follow-up. For this reason, the number of patients who completed follow-up is higher than the number of patients who completed treatment

on Harmonization for Good Clinical Practice. All patients provided written informed consent before enrollment. Enrollment started in March 2004 and ended in December 2005.

Virological and histological evaluation

Serum HCV RNA was detected by qualitative reverse-transcription polymerase chain reaction (RT-PCR, Amplicor HCV, Roche Diagnostics Japan, Tokyo, Japan; low limit of detection 50 IU/ml). The serum HCV load was determined by quantitative RT-PCR (Amplicor HCV Monitor Test, Version 2.0, Roche Diagnostics Japan; low limit of detection 500 IU/ml). HCV RNA genotype was determined by RT-PCR with genotype-specific primers [20] or by serological grouping of serum antibodies determined by enzyme-linked immunosorbent assay (SRL Inc., Hachi-Oji, Tokyo) according to the method of Tanaka et al. [21] assuming that genotypes 1a and 1b corresponded to serological group 1 (genotype 1) and genotypes 2a and 2b corresponded to serological group 2 (genotype 2) [22].

Most patients underwent liver biopsy before therapy. In 40 patients, a liver biopsy was not available because the patients declined to have a biopsy specimen taken. Histopathological results were classified by local pathologists according to the METAVIR criteria reported previously [23, 24]. Treatment commenced within 12 months of liver biopsy.

Follow-up of patients

Patients were evaluated as outpatients for treatment safety, tolerance, and efficacy by each attending physician every week during treatment and every 4 weeks after the end of treatment for the rest of the study period.

Assessment of efficacy

During treatment, HCV RNA was quantified by PCR assay and was tested by qualitative test if HCV RNA became undetectable by the quantitative test. The end-of-treatment response (ETR) and SVR were assessed by qualitative PCR assay. ETR was defined as an undetectable serum HCV RNA level at the end of treatment. SVR was defined as an undetectable serum HCV RNA level by the end of treatment and throughout the follow-up period.

Safety analysis

Patients were assessed for safety and tolerance by the attending physician by monitoring adverse events and laboratory abnormalities. The study protocol permitted dose modification for patients who had clinically significant adverse events or important abnormalities in laboratory values. Adverse events were handled according to the instructions provided by the manufacturer for peginterferon α-2a, and therapy adjustments were applied. In general, dose reductions and discontinuation of therapy, if any, were made following the recommendations of the manufacturer. The dose was also reduced or the drug was discontinued at the discretion of the investigator at each of the participating clinical centers on the basis of the results of hematological, neuropsychiatric, and cutaneous or other adverse effects that were considered related to the medication. The dose of peginterferon could be restored to their original levels upon resolution of the event or abnormality.

Adherence to therapy was assessed as described previously [15], namely, by calculating the actual doses of IFN received as a percentage of the expected dose. Thus, patients who received 80% or more of their total IFN doses for 80% or more of the expected duration of therapy were considered to be 80% adherent. The dose of peginterferon received during the first 4 weeks was also assessed.

Sample size

The noninferiority margin was set at 10% between groups A and B. To obtain 80% statistical power with one-sided 5% significance level, a sample size of 81 patients per treatment group was necessary. With a dropout rate of 10% allowed, 90 patients per group were to be recruited. It was assumed that 70% of the patients would have undetectable HCV RNA at week 4. On the basis of this, the original plan specified enrollment of 270 patients to ensure randomization of an adequate number of patients at week 8. However, the Japanese Bureau of National Health Insurance started to provide reimbursement for peginterferon α-2b and ribavirin therapy for patients with HCV genotype 2 infection and high viral load or re-treatment irrespective of viral load since December 2005 and thus difficulty in new enrollment was anticipated; the enrollment was terminated by the end of the year.

Statistical analysis

Intention-to-treat analysis was used for all measures of efficacy. Patients who missed the examination at the end of the follow-up period were considered not to have had a response at that point. Patients who received at least one dose of study medication were included in the analysis of safety. The primary objective of the study was to establish the difference in SVR rates between treatment groups A and B.

Differences in baseline clinical characteristics, efficacy, and safety between the treatment groups were compared statistically by analysis of variance, χ^2 test, Fisher's exact test, Mann–Whitney *U* test, and Kruskal–Wallis test, where appropriate, using SAS, Version 9.1.3, software (SAS Institute, Inc., Cary, NC). Univariate and multivariate logistic regression analyses were used to establish those factors that contributed to the efficacy of peginterferon α-2a monotherapy. Variables with more than marginal statistical significance ($P < 0.10$) in univariate analysis were entered into multivariate analysis. A risk ratio with a 95% confidence interval was denoted for each analysis. Unless otherwise stated, *P* values below 0.05 were considered significant.

Results

Characteristics of patients

This study was performed between March 2004 and June 2007 at 33 centers in Japan. On the basis of the inclusion and exclusion criteria, 132 patients were enrolled (Fig. 1): 17 (13%) and 115 (87%) patients were infected with HCV genotypes 1 and 2, respectively. The baseline characteristics of the patients are summarized in Table 1.

Virological response

After 4 weeks of treatment with peginterferon α-2a, HCV RNA was below 500 IU/ml in 108 of 132 (82%) patients (Fig. 1). Among these 108 patients with RVR, HCV RNA was undetectable by qualitative test in 97 of 108 (90%) patients, whereas it was not tested by qualitative test in the rest of the patients. The RVR was achieved by 15 of 17 (88%) patients infected with HCV genotype 1 and low viral load and by 93 of 115 (81%) patients infected with HCV genotype 2 ($P = 0.737$). These patients were randomly assigned to group A ($n = 55$) and group B ($n = 53$). Patients with HCV RNA of 500 IU/ml or higher at week 4 were assigned to group C ($n = 24$) (Fig. 1). There were no significant differences in baseline parameters between groups A and B (Table 2).

An overall intention-to-treat virological response at the ETR was achieved in 122 of 132 (92%) patients and SVR in 81 of 132 (61%) patients. In groups A and B, 53 of 55 (96%) patients and 51 of 53 (96%) patients achieved ETR and 33 of 55 (60%) patients and 42 of 53 (79%) patients achieved SVR, respectively (Fig. 2). The SVR rate was

Table 1 Demographic, biochemical, molecular, and histological profiles of patients at baseline

	All patients	HCV RNA (kIU/ml) at week 4		*P*-value[†]
		<500	≥500	
Patients, *n*	132	108	24	
Gender, male/female (% male)	81/51 (61)	68/40 (63)	13/11 (54)	0.423[‡]
Age (years)[a]	56.4 ± 12.2	56.0 ± 12.2	57.8 ± 12.2	0.536[§]
Weight (kg)[a]	61.5 ± 12.6	62.0 ± 13.1	58.6 ± 9.5	0.257[§]
Naive/re-treatment	119/13	98/10	21/3	0.704[#]
Fibrosis staging, *n* (F1/F2/F3/F4)	57/26/8/1	48/22/6/1	9/4/2/0	0.881[‡]
Grading, *n* (A0-1/A2/A3)	52/38/2	44/31/2	8/7/0	0.868[‡]
Genotype, 1/2 (% genotype 1)	17/115 (13)	15/93 (14)	2/22 (8)	0.737[#]
HCV RNA (kIU/ml)[b]	285 (46–1620)	130 (37–1006)	1350 (360–3060)	<0.001[¶]
ALT (IU/l)[b] [7–42][c]	66 (35–117)	64 (36–119)	69 (31–109)	0.571[¶]
γ-GTP (IU/l)[b] [5–50][c]	45 (24–92)	54 (26–97)	33 (21–49)	0.014[¶]
Neutrophil count (/μl)[a] [1000–7500][c]	2844 ± 1036	2898 ± 1042	2615 ± 996	0.230[§]
Hemoglobin (g/dl)[a] [13.5–17.5][c]	14.0 ± 1.2	14.1 ± 1.2	14.0 ± 1.4	0.751[§]
Platelet count (10^3/μl)[a] [150–400][c]	175 ± 63	179 ± 66	155 ± 40	0.089[§]

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase

[†] Comparison between groups according to HCV RNA at week 4

[‡] Chi-square test

[#] Fisher's exact test

[§] Unpaired-t test

[¶] Mann–Whitney *U*-test

Data are [a] mean ± SD or [b] median (interquartile range), [c] normal range

Table 2 Comparison of baseline profiles between groups A (24 weeks) and B (48 weeks)

	Group A	Group B	*P*-value[†]
Patients, *n*	55	53	
Gender, male/female (% male)	35/20 (64)	33/20 (62)	0.883[‡]
Age (years)[a]	56.9 ± 11.3	55.2 ± 13.1	0.473[§]
Weight (kg)[a]	59.9 ± 11.6	64.3 ± 14.3	0.087[§]
Naive/re-treatment	48/7	49/4	0.374[#]
Fibrosis staging, *n* (F1/F2/F3/F4)	26/12/2/0	22/10/4/1	0.558[‡]
Grading, *n* (A0-1/A2/A3)	28/11/1	16/20/1	0.097[‡]
Genotype, 1/2 (% genotype 1)	9/46 (16)	6/47 (11)	0.580[#]
HCV RNA (kIU/ml)[b]	190 (35–1660)	120 (38–580)	0.282[¶]
ALT (IU/l)[b] [7–42][c]	70 (37–117)	59 (36–120)	0.813[¶]
γ-GTP (IU/l)[b] [5–50][c]	43 (26–78)	63 (28–116)	0.242[¶]
Neutrophil count (/μl)[a] [1000–7500][c]	2936 ± 1047	2859 ± 1088	0.711[§]
Hemoglobin (g/dl)[a] [13.5–17.5][c]	14.1 ± 1.0	14.1 ± 1.3	0.943[§]
Platelet count (10^3/μL)[a] [150–400][c]	177 ± 47	181 ± 81	0.793[§]

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase

[†] Comparison between groups A and B

[‡] Chi-square test

[#] Fisher's exact test

[§] Unpaired *t*-test

[¶] Mann–Whitney *U* test

Data are [a] mean ± SD or [b] median (interquartile range), [c] normal range

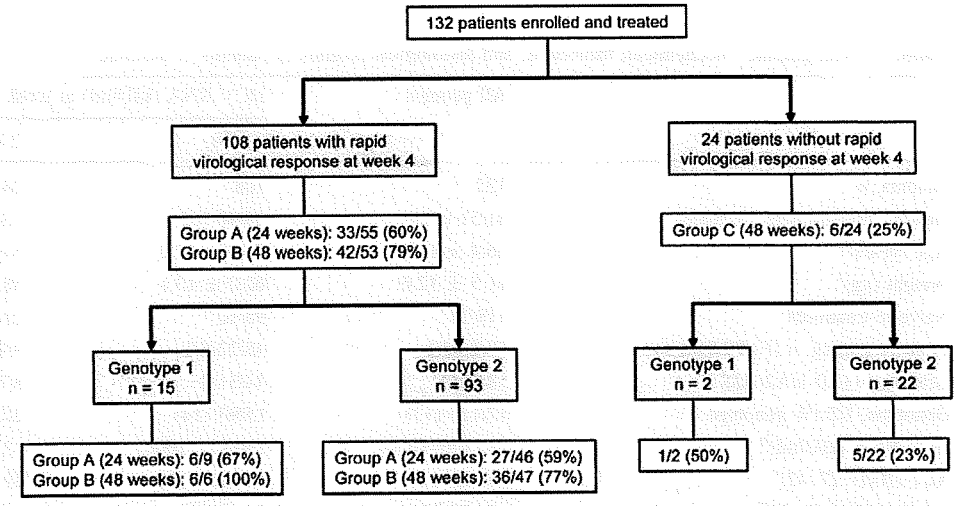

Fig. 2 Sustained virological response rate according to genotype in each treatment group

significantly higher in patients randomized to 48 weeks of therapy (group B) than in those randomized to 24 weeks of therapy (group A) ($P = 0.030$), namely, the relapse rate in group A was 40% (22/55), which was significantly higher than in group B (21%, 11/53, $P = 0.030$). Among patients with RVR confirmed by qualitative test (HCV RNA < 50 IU/ml), 29 of 48 (60%) patients achieved SVR in group A vs. 39 of 46 (85%) in group B ($P = 0.008$). The ETR and SVR rates in patients who did not show RVR and who were treated for 48 weeks (group C) were lower than in those who showed RVR (groups A and B) (75% vs. 96%, $P = 0.003$ for ETR and 25% vs. 69%, $P < 0.001$ for SVR, respectively) (Fig. 2).

Virological response according to HCV genotype and pretreatment viral load

The SVR rate in HCV genotype 1 and low viral load were not significantly different between treatment groups A and B (67% vs. 100%, respectively; $P = 0.229$) (Fig. 2),

although the number of patients of this subgroup was small for meaningful comparison.

The SVR rate in patients infected with HCV genotype 2 was higher in group B (77%) than in group A (59%, $P = 0.065$). There was an inverse correlation between SVR rate and baseline viral load (Fig. 3). This observation was significant in group A ($P < 0.001$) but not in group B ($P = 0.096$). On the basis of receiver operating characteristics analysis, 1,000,000 IU/ml was optimal for use as the cutoff point of baseline viral load to best discriminate patients who might achieve SVR in group A. The SVR rate of patients with HCV genotype 2 infection and a baseline viral load below 1,000,000 IU/ml was not compromised by 24-week treatment (group A) compared with 48-week treatment (group B) (81% and 79%, respectively), without significant difference between the two groups ($P = 0.801$). On the other hand, the SVR rate in those with a baseline viral load of 1,000,000 IU/ml or higher was significantly lower in group A than in group B (26% and 67%, respectively, $P = 0.041$) (Fig. 3).

Factors associated with RVR

Next, we analyzed the factors associated with RVR using data of all patients. The variables included were demographic features, baseline viral load, liver enzymes, and administered dose of peginterferon during the first 4 weeks (Table 3). Pretreatment HCV RNA level was lower and

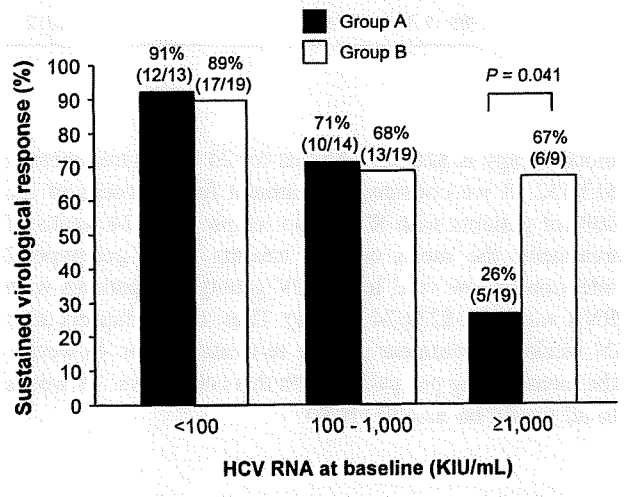

Fig. 3 Sustained virological response (SVR) rates stratified according to pretreatment HCV RNA level for patients of groups A (24 weeks) and B (48 weeks) infected with HCV genotype 2. The SVR rate was significantly lower in patients with higher baseline viral load in group A ($P < 0.001$) but not in group B ($P = 0.096$). The SVR rate in patients with a baseline viral load of less than 1,000,000 IU/ml was similar between group A (81%, 22/27) and group B (79%, 30/38) ($P = 0.801$). However, the SVR rate in those with a baseline viral load of 1,000,000 IU/ml or higher was lower in group A (26%, 5/19) than in group B (67%, 6/9) ($P = 0.041$)

γ-glutamyl transpeptidase (γ-GTP) level and platelet count were higher in patients with RVR (groups A and B) than in those without RVR (group C) (Table 1). On the basis of receiver operating characteristic analyses, 41 IU/l and 191 $\times$ 10^3/μl were optimal for use as the cutoff points of baseline γ-GTP level and platelet count, respectively. Multivariate analysis identified low baseline viral load (<1,000,000 IU/ml), high γ-GTP level (≥41 IU/l), and high platelet count (≥191 $\times$ 10^3/μl) were significant determinants of RVR (Table 3).

Factors associated with SVR

Next, the factors associated with SVR were analyzed using data of all patients. Univariate analysis indicated that grading score, pretreatment viral load, alanine aminotransferase (ALT) and γ-GTP levels, neutrophil count, RVR, and adherence to treatment were associated with SVR. On the basis of receiver operating characteristic analyses, 41 IU/l, 28 IU/l, and 3,155/μl were optimal for use as the cutoff points of baseline ALT level, γ-GTP level, and neutrophil count, respectively. Multivariate analysis was performed with the following variables: pretreatment viral load, ALT and γ-GTP levels, neutrophil count, RVR, and adherence to treatment but excluding grading score due to a significant association with ALT level and a substantial number of cases (40 cases) missing histological data. The analysis identified low viral load (<100,000 IU/ml), RVR, high ALT level (≥41 IU/l), and high γ-GTP level (≥28 IU/l) as independent determinants of SVR (Table 4).

Multivariate analysis for factors associated with SVR in patients with RVR identified low viral load (<100,000 IU/ml) and high ALT level (≥41 IU/l) as independent determinants of SVR. The SVR rates in patients with RVR and with high ALT levels (≥41 IU/l) were generally high except for group A patients with high viral load (≥1,000,000 IU/ml). On the other hand, the SVR rates for both groups A (black bars) and B (open bars) were entirely low in patients with low ALT levels (<41 IU/l) except those with low viral load (<100,000 IU/ml) (Fig. 4). In patients with high viral load (≥100,000 IU/ml) and low ALT levels (<41 IU/l), SVR was achieved only in group B patients, though at low rate.

Safety

Twenty-eight (21%) patients discontinued therapy and 14 of them discontinued because of adverse events, 4 because of laboratory abnormalities, 3 because of refusal of treatment, 2 because of insufficient response, and 5 because of failure to return (Table 5). Fatigue was the most common adverse event leading to discontinuation of therapy. The frequencies of discontinuation and discontinuation due to

Table 3 Logistic regression analysis of the factors associated with rapid virological response

Variable	RR (95% CI)	*P* value
Univariate analysis		
Pretreatment variables		
Gender (male vs. female)	1.438 (0.589–3.513)	0.425
Age (<55 vs. ≥55 years)	1.536 (0.583–4.049)	0.512
Weight (≥60 vs. <60 kg)	2.511 (0.938–6.723)	0.067
Treatment (naive vs. re-treatment)	1.400 (0.354–5.530)	0.631
Genotype (1 vs. 2)	1.773 (0.377–8.333	0.468
Fibrosis staging (F2-4 vs. F0-1)†	1.104 (0.356–3.425)	0.865
Grading (A2-3 vs. A0-1)†	1.167 (0.384–3.546)	0.786
HCV RNA (kIU/mL)		
<100	1	
100–1000	0.562 (0.140–2.251)	0.415
≥1000	0.159 (0.048–0.526)	0.003
ALT (≥60 vs. <60 IU/l)	1.437 (0.579–3.571)	0.435
γ-GTP (IU/l) (≥41 vs. <41 IU/l)	3.946 (1.504–10.352)	0.005
Neutrophil count (≥2500 vs. <2500/μL)	1.135 (0.465–2.771)	0.782
Hemoglobin (<14 vs. ≥14 g/dl)	1.427 (0.582–3.497)	0.437
Platelet count (≥191 vs. <191 × 10^3/μL)	6.567 (1.466–29.424)	0.014
Treatment-associated variables		
Adherence during 4 weeks of treatment (≥80% vs. <80%)	1.714 (0.419–7.011)	0.453
Stepwise multivariate analysis		
HCV RNA (kIU/ml)		
<100	1	
100–1000	0.399 (0.091–1.759)	0.225
≥1000	0.126 (0.034–0.464)	0.002
Platelet count (≥191 vs. <191 × 10^3/μl)	10.230 (2.056–50.902)	0.005
γ-GTP (IU/l) (≥41 vs. <41 IU/l)	3.989 (1.355–11.744)	0.012

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase

† A biopsy was not available from 40 patients

adverse events were significantly lower in the 24-week treatment group (group A) than in the 48-week treatment group (groups B and C) (9% vs. 30%, $P = 0.005$ and 4% vs. 16%, $P = 0.042$, respectively).

Adherence to scheduled therapy (median and interquartile range) was 100% (63–100%), 77% (54–100%), and 85% (55–100%), respectively, for groups A, B, and C ($P = 0.012$ by Kruskal–Wallis test). The rate of adherence in group A was higher than in groups B and C ($P = 0.003$ and $P = 0.082$, respectively, by Mann–Whitney U test). There was no difference in adherence to therapy between groups B and C ($P = 0.597$). Thus, adherence to therapy in the longer treatment course (48 weeks) was lower than in the shorter treatment course (24 weeks).

Costs

Based on the current prices in the United States, spending on medication for 48 weeks of peginterferon α-2a monotherapy is $26,305 and that for 24 weeks treatment is $13,152. If we consider re-treatment for 48 weeks of the 40% of patients with RVR who relapse after 24 weeks of treatment, the mean cost of treating HCV genotype 2 infection or low viral load HCV genotype 1 patients with RVR would be $23,674 (Fig. 5). Thus, if all relapsers after 24 weeks of treatment receive re-treatment for 48 weeks, the mean saving per patient with this concept vs. 48 weeks to all would be $2,630 (10%).

Discussion

The key finding of this study is that in patients infected with HCV genotype 2 and low viral load (<1,000,000 IU/ml) who achieve RVR, 24-week treatment with peginterferon α-2a alone may be sufficient in terms of efficacy. Patients treated for 24 weeks also discontinued treatment less frequently and showed higher adherence than those

Table 4 Logistic regression analysis of the factors associated with sustained virological response

Variable	RR (95% CI)	*P*-value
Univariate analysis		
Pretreatment variables		
Gender (male vs. female)	1.190 (0.581–2.438)	0.635
Age (≥55 vs. <55 years)	1.273 (0.618–2.625)	0.512
Weight (<60 vs. ≥60 kg)	1.064 (0.520–2.175)	0.865
Treatment (re-treatment vs. naive)	1.468 (0.428–5.051)	0.541
Genotype (1 vs. 2)	2.247 (0.690–7.299)	0.179
Fibrosis staging (F2-4 vs. F0-1)†	1.964 (0.812–4.749)	0.134
Grading (A2-3 vs. A0-1)†	4.343 (1.727–10.922)	0.002
HCV RNA (kIU/mL) <100	1	
100–1000	0.367 (0.138–0.975)	0.044
≥1000	0.115 (0.044–0.298)	<0.001
ALT (≥41 vs. <41 IU/l)	4.570 (2.104–9.927)	<0.001
γ-GTP (IU/l) (≥28 vs. <28 IU/l)	6.182 (2.657–14.384)	<0.001
Neutrophil count (<3155 vs. ≥3155/μl)	3.135 (1.479–6.623)	0.003
Hemoglobin (≥14 vs. <14 g/dl)	1.125 (0.555–2.283)	0.744
Platelet count (<150 vs. ≥150 × 10^3/μl)	1.091 (0.507–2.347)	0.824
Treatment-associated variables		
RVR (yes vs. no)	6.818 (2.482–18.733)	<0.001
Adherence (≥80% vs. <80%)	1.940 (0.949–3.966)	0.070
Stepwise Multivariate Analysis‡		
HCV RNA (kIU/ml)		
<100	1	
100–1000	0.165 (0.046–0.589)	0.006
≥1000	0.102 (0.029–0.352)	<0.001
RVR (yes vs. no)	6.223 (1.821–21.305)	0.003
ALT (≥41 vs. <41 IU/l)	4.775 (1.373–16.601)	0.014
γ-GTP (IU/l) (≥28 vs. <28 IU/l)	3.466 (1.092–11.000)	0.035

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase, *RVR* rapid virological response

† A biopsy was not available from 40 patients

‡ Grading was not included

treated for 48 weeks. Furthermore, the drug cost can be reduced by truncating treatment duration. Thus, by reducing the treatment period, these patients can avoid unnecessary treatment without compromising the chance for an SVR. In particular, the SVR rate in patients with HCV genotype 2 infection and low viral load (<1,000,000 IU/ml) who achieved RVR was as high as 81% by 24-week monotherapy. The SVR rate was comparable with that (84%, 81/96) reported previously in patients with HCV genotype 2 and 3 infection who received 24-week combination therapy of peginterferon α-2a plus ribavirin [7], although the latter included patients with HCV genotype 3 infection. On the other hand, the results of this study were not conclusive regarding patients with HCV genotype 1 infection and low viral load (<100,000 IU/ml). Further prospective controlled trial is warranted to confirm our findings in patients with HCV genotype 1 infection and low viral load or HCV genotype 2 infection and baseline viral loads of less than 1,000,000 IU/ml who achieve RVR.

In patients with HCV genotype 2 infection and high viral load (≥1,000,000 IU/ml), the SVR rate was lower for the 24-week treatment group than for the 48-week treatment group, even if the patients achieved RVR. Thus, a longer treatment (>24 weeks) with peginterferon is recommended for this group of patients. Furthermore, since the SVR rate was not more than 67% in this subgroup of patients, even if they were treated for 48 weeks, a combination with ribavirin or further extended treatment duration may be necessary as long as patients can tolerate the treatment.

The combination therapy of peginterferon and ribavirin is currently the therapeutic standard for chronic hepatitis C. However, the combination therapy tends to be associated with adverse events more frequently than those that occur with IFN monotherapy, resulting in dose reduction or discontinuation of therapy and thus impaired response rate [9–14]. This is true particularly in elderly patients. In a country such as Japan where the average age of patients with chronic hepatitis C to be treated by antivirals sometimes is well above 60, standard combination therapy is not well tolerated [15]. For example, in a phase III study of 48-week peginterferon α-2a plus ribavirin combination therapy

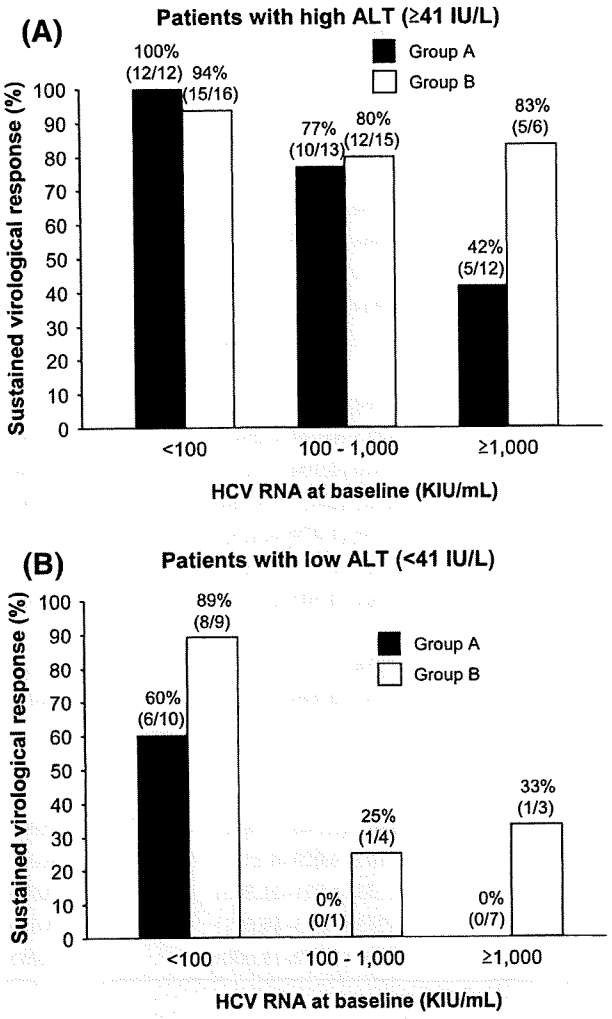

Fig. 4 Sustained virological response (SVR) rates stratified according to pretreatment HCV RNA and ALT levels in group A and B patients. The SVR rates in patients with high (≥41 IU/l) and low (<41 IU/l) ALT levels are shown in panels **a** and **b**, respectively. **a** The SVR rates in patients with high ALT levels (≥41 IU/l) were generally high except for group A patients with high viral load (≥1,000,000 IU/ml). **b** The SVR rates in both groups A and B were low in patients with low ALT levels (<41 IU/l) except those with low viral load (<100,000 IU/ml)

conducted in Japan [25], the SVR rate in the combination arm (78%, 18/23) was rather inferior to that of peginterferon α-2a monotherapy (placebo) arm (100%, 14/14) among patients with RVR ($P = 0.061$), although the difference did not reach statistical significance. In the same study, all of the patients who failed to achieve SVR in the combination arm discontinued treatment [25]. Thus, the combination therapy with ribavirin does not always lead to a better response than with monotherapy, at least in a subgroup of patients. It is noteworthy that most of the patients in the present trial were those who preferred peginterferon α monotherapy to combination therapy in spite

Table 5 Incidence and reason of discontinuation according to treatment group

Variable	All patients	Age (years)		
		A	B	C
n	132	55	53	24
Discontinuation	28 (21)	5 (9)	14 (26)	9 (38)
Adverse events	14 (11)	2 (4)	8 (15)	4 (17)
Fatigue	4	0	1	3
Depression	2	1	1	0
Arthralgia	2	0	2	0
Arrhythmia	2	0	1	1
Pyrexia	1	1	0	0
Headache	1	0	1	0
Hyperthyroidism	1	0	1	0
Colon cancer	1	0	1	0
Laboratory abnormality	4 (3)	1 (2)	1 (2)	2 (8)
High aminotransferase	2	1	0	1
Anemia	1	0	1	0
Neutropenia	1	0	0	1
Refusal of treatment	3 (2)	1 (2)	1 (2)	1 (4)
Insufficient response	2 (2)	0	1 (2)	1 (4)
Failure to return	5 (4)	1 (2)	3 (6)	1 (4)

Data are number of patients (percentage in each patient group)

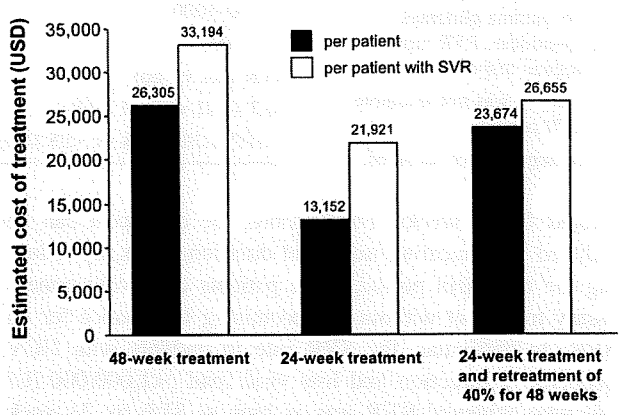

Fig. 5 The cost of treating patients infected with HCV genotype 2 or low viral load genotype 1 and RVR for 48, 28, or 24 weeks followed by 48 weeks of re-treatment of 40% of patients who relapse after the initial treatment

of the coverage for the latter therapy by the Bureau of National Health Insurance, as described previously.

In addition to elderly patients, those with renal failure, ischemic vascular diseases, and congenital hemoglobin abnormalities never tolerate ribavirin for chronic hepatitis C treatment [26]. Therefore, data on peginterferon α monotherapy are particularly relevant to these patients. The possibility of shorter combination therapy with peginterferon α and ribavirin in easy-to-treat patients such as those

chronically infected with HCV genotype 2 or 3 has been investigated in several trials [27–31]. However, to the best of our knowledge, there has been no randomized, controlled trial to identify optimal treatment duration of peginterferon α monotherapy. A further prospective randomized, controlled trial aiming at patients who cannot receive combination therapy with ribavirin is warranted.

A high ALT level has been identified as a significant factor for SVR [18]. The reason why patients with low or normal ALT levels do not respond well to peginterferon α monotherapy is currently unknown. The SVR rates were low in our patients with low ALT levels and HCV RNA levels of 100,000 IU/ml or higher in the two randomized groups (Fig. 4). Thus, these patients may not benefit by simply extending therapy from 24 to 48 weeks. Since a similar efficacy has been demonstrated in patients with persistently normal ALT levels compared with those with elevated ALT levels by combination therapy of peginterferon α-2a plus ribavirin [32], combination therapy should be considered for these patients.

A high γ-GTP level was unexpectedly identified as a factor for both RVR and SVR, independent of ALT levels. Again, the reason for this finding is unknown at present. It is well known that a low γ-GTP level is associated with SVR to combination therapy comprising peginterferon and ribavirin; the reason also being unexplained so far [33]. Thus, the present finding at least suggests that entirely different mechanisms may underlie these observations.

In this trial, RVR was defined as serum HCV RNA level below 500 IU/ml at week 4, although most of the patients who achieved RVR had HCV RNA levels below 50 IU/ml. The criterion of RVR used in this study was less strict than those reported recently, in which serum HCV RNA level below 50 IU/ml at week 4 has been utilized [30, 31]. This may result in a higher rate of achieving RVR and lower SVR rates, resulting in more relapsers, particularly in patients treated for a shorter duration of 24 weeks than a standard duration of 48 weeks. By using more strict criteria of serum HCV RNA level below the detection limit of qualitative PCR (≤50 IU/ml) at week 4 or negativity of HCV RNA at earlier time points during therapy, such as at week 2 [34], a subgroup of patients, who can be sufficiently treated with a shorter duration of therapy (such as 24 weeks) without compromising the chance for SVR, could be more specifically identified.

In conclusion, patients infected with HCV genotype 2 and have low baseline viral load (<1,000,000 kIU/ml), who can achieve RVR, can satisfactorily be treated for 24 weeks with peginterferon α-2a alone without compromising the SVR. We propose that these patients should first be treated with peginterferon α monotherapy for 24 weeks, as long as RVR is achieved, otherwise they should be switched to combination therapy with ribavirin at the time for another 24–48 weeks, depending on the response thereafter. However, the data of this study are less conclusive for patients with low viral load genotype 1 or 2 and viral load of more than 1,000,000 IU/ml. Additional trials are required to optimize treatment schedule in these patients.

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Conflict of interest statement None declared.

References

1. El Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–823.
2. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52. doi:10.1056/NEJM200107053450107.
3. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002;36:S35–46. doi:10.1002/hep.1840360706.
4. Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, et al. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. Ann Intern Med 1997;127:875–881.

5. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–1695. doi: 10.1002/hep.510280632.
6. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982. doi:10.1056/NEJMoa020047.
7. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355.
8. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965. doi:10.1016/S0140-6736(01)06102-5.
9. Cummings KJ, Lee SM, West ES, Cid-Ruzafa J, Fein SG, Aoki Y, et al. Interferon and ribavirin vs interferon alone in the re-treatment of chronic hepatitis C previously nonresponsive to interferon: a meta-analysis of randomized trials. JAMA 2001; 285:193–199. doi:10.1001/jama.285.2.193.
10. Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1493–1499. doi:10.1056/NEJM199811193392102.
11. Kjaergard LL, Krogsgaard K, Gluud C. Interferon alfa with or without ribavirin for chronic hepatitis C: systematic review of randomised trials. Br Med J 2001;323:1151–1155. doi:10.1136/bmj.323.7322.1151.
12. Maddrey WC. Safety of combination interferon alfa-2b/ribavirin therapy in chronic hepatitis C-relapsed and treatment-naive patients. Semin Liver Dis 1999;19(Suppl 1):67–75.
13. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339: 1485–1492. doi:10.1056/NEJM199811193392101.
14. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352:1426–1432. doi:10.1016/S0140-6736(98)07124-4.
15. Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63. doi:10.1002/hep.20984.
16. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673–1680. doi:10.1056/NEJM200012073432302.
17. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34:395–403. doi: 10.1053/jhep.2001.26371.
18. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343:1666–1672. doi:10.1056/NEJM200012073432301.
19. Data on file. PEGASYS. Tokyo (Japan): Chugai Pharmaceuticals Inc; 2002.
20. Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, et al. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 1992;73:673–679. doi: 10.1099/0022-1317-73-3-673.
21. Tanaka T, Tsukiyama-Kohara K, Yamaguchi K, Yagi S, Tanaka S, Hasegawa A, et al. Significance of specific antibody assay for genotyping of hepatitis C virus. Hepatology 1994;19:1347–1353. doi:10.1002/hep.1840190605.
22. Shiratori Y, Kato N, Yokosuka O, Imazeki F, Hashimoto E, Hayashi N, et al. Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. Gastroenterology 1997;113:558–566. doi: 10.1053/gast.1997.v113.pm9247476.
23. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289–293. doi:10.1002/hep.510240201.
24. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–1520. doi:10.1002/hep.1840190629.
25. Sakai T, Iino S, Okuno T, Omata M, Kiyosawa K, Kumada H, et al. High response rates with peginterferon alfa-2a (40 KD) (PEGASYS) plus ribavirin (COPEGUS) in treatment-naive Japanese chronic hepatitis C patients: a randomized, double-blind, multicenter phase III trial. Gastroenterology 2006;130(Suppl):A839. abstract.
26. Dienstag JL, McHutchison JG. American Gastroenterological Association technical review on the management of hepatitis C. Gastroenterology 2006;130:231–264. doi:10.1053/j.gastro.2005.11.010.
27. Lagging M, Langeland N, Pedersen C, Farkkila M, Buhl MR, Morch K, et al. Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. Hepatology 2008;47:1837–1845. doi: 10.1002/hep.22253.
28. Shiffman ML, Suter F, Bacon B, Nelson D, Harley H, Sola R, et al. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med 2007;357:124–134. doi: 10.1056/NEJMoa066403.
29. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40 kD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129:522–527.
30. Yu ML, Dai CY, Huang JF, Hou NJ, Lee LP, Hsieh MY, et al. A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. Gut 2007;56:553–559. doi:10.1136/gut.2006.102558.
31. Dalgard O, Bjøro K, Ring-Larsen H, Bjornsson E, Holberg-Petersen M, Skovlund E, et al. Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. Hepatology 2008;47:35–42. doi:10.1002/hep.21975.
32. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, et al. Peginterferon alfa-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology 2004;127:1724–1732. doi:10.1053/j.gastro.2004.09.050.
33. Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, et al. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. Hepatology 2003;37:600–609. doi:10.1053/jhep.2003.50106.
34. Jeong S, Kawakami Y, Kitamoto M, Ishihara H, Tsuji K, Aimitsu S, et al. Prospective study of short-term peginterferon-alpha-2a monotherapy in patients who had a virological response at 2 weeks after initiation of interferon therapy. J Gastroenterol Hepatol 2008;23:541–545. doi:10.1111/j.1440-1746.2008.05356.x.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study

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Abstract

Background Chronic hepatitis C (CHC) genotype 1b patients with high viral load are resistant to peginterferon (PEG-IFN) and ribavirin (RBV) combination therapy, especially older and female patients.

Methods To elucidate the factors affecting early and sustained viral responses (EVR and SVR), 409 genotype 1b patients CHC with high viral loads who had received 48 weeks of PEG-IFN/RBV therapy were enrolled. The amino acid (aa) sequences of the HCV core at positions 70 and 91 and of the interferon sensitivity determining region (ISDR) were analyzed. Host factors, viral factors, and treatment-related factors were subjected to multivariate analysis.

Results Male gender, low HCV RNA load, high platelet count, two or more aa mutations of ISDR, and wild type of core aa 70 were independent predictive factors for SVR. In patients with over 80% adherences to both PEG-IFN and RBV, male gender, mild fibrosis stage, and wild type of core aa 70 were independent predictors for SVR.

Conclusions Independent predictive factors for SVR were: no aa substitution at core aa 70, two or more aa mutations in the ISDR, low viral load, high values of platelet count, mild liver fibrosis and male gender.

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