

Biosystems) in triplicate. The PCR conditions were as follows: (1) stage 1, 50°C for 2 minutes; (2) stage 2, 95°C for 10 minutes; and (3) stage 3, 95°C for 15 seconds followed by amplification at 60°C for 1 minute. Stage 3 was repeated for 40 cycles. Specificity of the amplification products was confirmed by examination of dissociation reaction plots, and a distinct single peak indicated a single DNA sequence amplified by the real-time detection PCR. The PCR products were run on 2% agarose gels to confirm the molecular sizes as well as species-specific amplifications (Figure 6C). Data were analyzed by the 2<sup>-</sup>[Delta Delta C(t)] method using Sequence Detector version 1.7 software (Applied Biosystems),<sup>5</sup> and were normalized using human or mouse-specific glyceraldehyde-3-phosphate dehydrogenase. A standard curve was prepared by serial 10-fold dilutions of human or mouse cDNA. The curve was linear over 7 logs with a 0.998 correlation coefficient.

**Immunofluorescence** Immunofluorescence was performed as previously reported.<sup>1</sup> Briefly, fresh-frozen specimens were cut at 5–6 μm by cryostat, and fixed in acetone at room temperature for 10 minutes. Liver sections were blocked with Antibody Diluent (Dako, Glostrup, Denmark), incubated with rabbit anti-HBc antibody (Dako) at room temperature for 1 hour, and then

incubated with goat anti-rabbit IgG antibody conjugated with Cy3 (Chemicon) or goat anti-human albumin antibody labeled with FITC (Bethyl Laboratories Inc, Montgomery, TX). Sections were observed in a fluorescent microscopy (Eclipse E800M; Nikon, Tokyo, Japan).

#### *Statistical Analysis*

Group means were compared by an independent Student *t* test or 1-way analysis of variance.

#### **References**

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**Supplementary Table 1.** Sequence of Species-Specific Primers on Fibrosis-Related Genes

Primer	Sequence
hTIMP1/F1	5'-ATGGCCCCCTTTGAGCCC-3'
hTIMP1/R1	5'-GTCTGGTTGACTTCTGGTGC-3'
mTIMP1/F1	5'-ATGGCCCCCTTTGCATCT-3'
mTIMP1/R1	5'-GTCTCGTTGATTTCTGGGGAA-3'
hMMP2/F1	5'-CCTTCTTGTTCAATGGCAA-3'
hMMP2/R1	5'-GGACAGAAGCCGACTTGC-3'
mMMP2/F1	5'-CCTTCTGTTCAACGGTGC-3'
mMMP2/R1	5'-GGGCAGAAGCCATACTTGC-3'
hCOL1 $\alpha$ 2/F1	5'-AGGAAATGGCTACCCAATT-3'
hCOL1 $\alpha$ 2/R1	5'-TTAGAGCCCTGTAGAATG-3'
mCOL1 $\alpha$ 2/F1	5'-AGGAAATGGCAACTCAGCTC-3'
mCOL1 $\alpha$ 2/R1	5'-TTGGAACCCTGCAGAAGC-3'
hGAPDH/F2	5'-CACCAGGGCTGCTTTAACTC-3'
hGAPDH/R2	5'-AGATGGTGATGGGATTTCCA-3'
mGAPDH/F2	5'-CACCAGGGCTGCCATTTGCAG-3'
mGAPDH/R2	5'-AGATGGTGATGGGCTTCCCG-3'

COL1 $\alpha$ 2, collagen type 1 $\alpha$ 2; F, sense primer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h, human specific; m, mouse specific; MMP2, matrix metalloproteinase 2; R, antisense primer; TIMP1, tissue inhibitor of metalloproteinase 1.

## 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

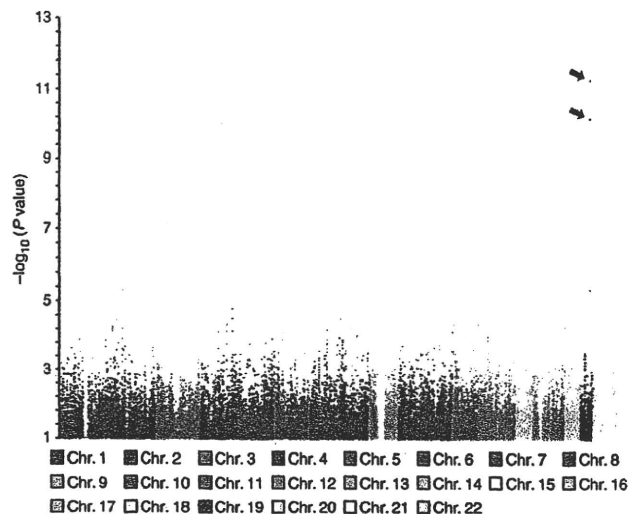
estimated that in 1999, there were 170 million HCV carriers worldwide, with 3–4 million new cases appearing each year. HCV infection affects more than 4 million people in the United States, where it represents the leading cause of cirrhosis and hepatocellular carcinoma as well as the leading cause of 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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**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

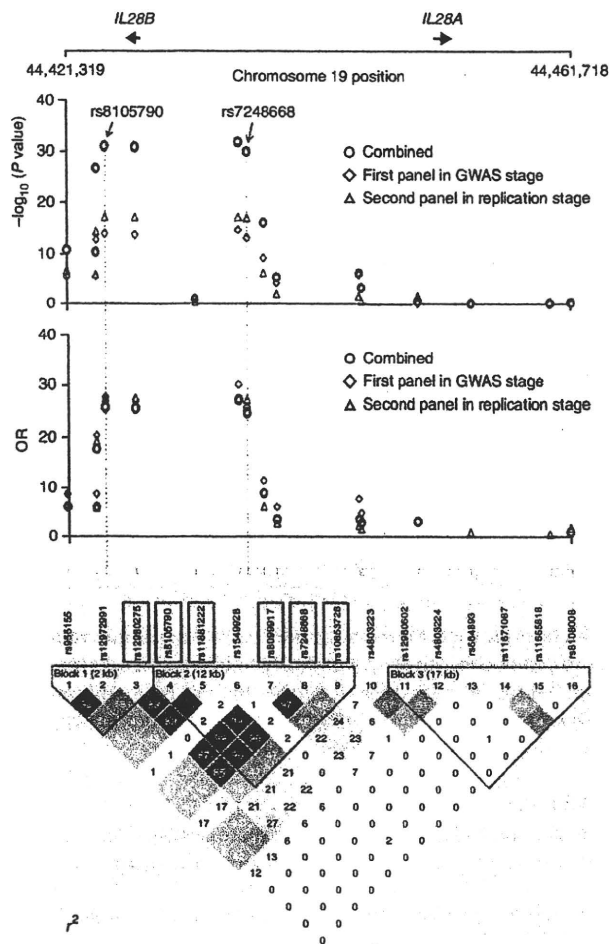
a few candidate genes, including those encoding type I interferon receptor-1 (*IFNAR1*) and mitogen-activated protein kinase-activated protein kinase 3 (*MAPKAPK3*), have been reported to be associated with treatment response<sup>13,14</sup>. We describe here a GWAS for response to PEG-IFN- $\alpha$ /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)	
					10	37	3	101	21	0	73	16	0	19.2	$5.46 \times 10^{-15}$	18.3	$8.37 \times 10^{-13}$
				Replication	(20.0)	(74.0)	(6.0)	(82.8)	(17.2)	(0.0)	(82.0)	(18.0)	(0.0)	(8.3–44.4)		(7.6–44.0)	
				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)	
					11	37	2	108	14	0	78	11	0	27.4	$9.47 \times 10^{-18}$	25.1	$1.00 \times 10^{-14}$
				Replication	(22.0)	(74.0)	(4.0)	(88.5)	(11.5)	(0.0)	(87.6)	(12.4)	(0.0)	(11.5–65.3)		(10.0–63.1)	
				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		$P$ value	OR (95% CI)
rs8105790	rs11881222	rs8103142	rs28416813	rs4803219	rs8099917	rs7248668	NVR group	VR group		
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).

**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 <sup>a</sup>	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

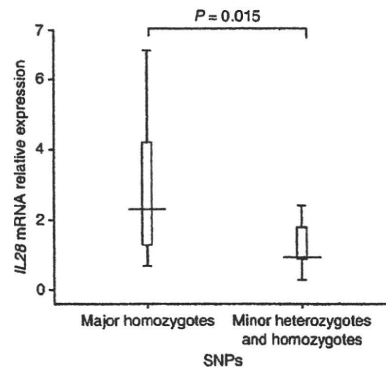
<sup>a</sup>Re-treatment, non-response to previous treatment with interferon- $\alpha$  (plus RBV).

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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## 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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## 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- $\alpha$ 2b (1.5  $\mu$ g per kg body weight ( $\mu$ g/kg) subcutaneously once a week) or PEG-IFN- $\alpha$ 2a (180  $\mu$ g/kg once a week) plus RBV (600–1,000 mg daily depending on body weight). As a reduction in the dose of PEG-IFN- $\alpha$  and RBV can contribute to a less sustained virological response<sup>21</sup>, 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- $\alpha$ /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- $\alpha$ /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  $\geq$ 95% for all samples, MAF  $\geq$ 1% for all samples and HWE  $P$  value  $\geq$ 0.001 for VR group<sup>22,23</sup>. 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<sup>24</sup> 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- $\mu$ l PCR analysis was performed using 2.5 U TaKaRa LA *Taq* with 1 $\times$  LA PCR buffer II, 0.4 mM dNTP, 10 pmol of each primer and 10 ng of genomic DNA. For sequencing, 7.0  $\mu$ l of the PCR products were incubated with 3  $\mu$ l 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 (rs18103142, 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- $\alpha$ /RBV treatment was assessed by  $\chi^2$  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  $\lambda$  was estimated based on the median  $\chi^2$  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- $\alpha$ /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 *IL28B* 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)<sub>12–18</sub> 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



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](https://gwas.lifesciencedb.jp/cgi-bin/gwasdb/gwas_top.cgi).

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&lt;特別寄稿&gt;

## 日本肝臓学会コンセンサス神戸 2009 : C 型肝炎の診断と治療

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索引用語 : C型慢性肝炎 診断 治療 ガイドライン

## はじめに

わが国の C 型肝炎の特徴は、欧米に比し高齢であり肝組織所見の進展例が多く、経過観察中に高率に肝癌が生じてくることである。このため、患者背景の異なる欧米のガイドライン<sup>1)</sup>はわが国では当てはまらない事項もあり、日本の患者の実態に即した独自のガイドラインの策定が必要である。このような指針を求めて、第 45 回日本肝臓学会総会 (工藤正俊会長) において、C 型肝炎 (病態・診断・予後・治療) をテーマとしたコンセンサス パネルディスカッションが開催された。すでに、第 5 回、第 7 回、第 10 回の日本肝臓学会大会においても、同一テーマで討議されているため、今回が 4 回目となる。エビデンスレベルが高く、発表者と座長のコンセンサスが得られた事項で有益な情報を Informative statement とし、推奨すべき指針を Recommendation として取り上げた。エビデンスレベルが低い欧米のガイドラインでは採用されていないか、発表者と座長の予備検討において全員の賛同が得られなかった事項については、アンサーパッドで学会参加者に意見を求めた。その際、回答者の 2/3 以上の承認が得られれば Consensus Statement として採用した。アンサーパッドの参加者は 200 人であり、内訳は内科医

が 88%、肝炎診療の経験年数が 10 年以上の医師が 83%、肝臓学会専門医も 83% を占めた。本稿では、紙面の都合で Informative statement や Recommendation は明記せず、パネルディスカッションにおいて活発な討議が行われ、結論が得られた Consensus Statement のみ全文を記載した。

## 1) 病態・診断・予後

## 1. C 型肝炎の発症機序

C 型肝炎ウイルス (HCV) の肝細胞への感染は HCV E2 タンパクが CD81 と結合することが必要であると報告されたが、その後 scavenger receptor class B type I (SR-B1) や claudin-1 (CLDN1) といった宿主タンパクも関与することが示された。さらに 2009 年になって occluding (OCLN) が HCV 感染に不可欠であることが明らかとなった。興味深いことに CLDN1 と OCLN はともに tight junction に存在する分子であり、HCV が肝細胞に接着した後の細胞内への取り込みに重要であると考えられている。さらに CD81 と OCLN は HCV 感染の種特異性に関与する分子であることも示されている<sup>2)</sup>。

HCV の持続感染が成立するためには、宿主の自然免疫からの回避が必要である。最近、HCV による自然免疫の抑制機構が明らかにされた。すなわち、複製中の HCV RNA の一部は PAMP として RIG-I や TLR に認識される。RIG-I に認識されたシグナルは IPS-1 を介して内因性のインターフェロン (IFN) シグナルを活性化する。産生された IFN は IFN レセプターに結合して Jak-STAT シグナルを活性化して IFN 応答遺伝子の発現を促す。しかし、HCV NS3/4A protease は IPS-1 を断裂することで IFN シグナルを阻害し IFN 産生を抑制する。また、HCV コアタンパクに誘導される SOCS-3 は Jak-

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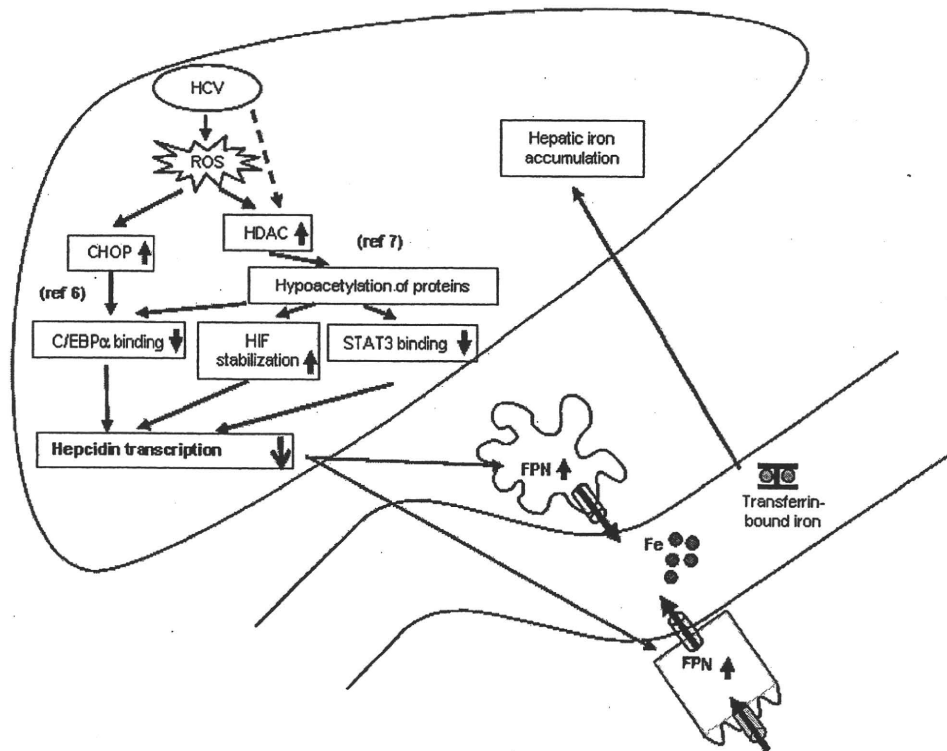
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**Fig. 1** Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation induced by HCV

HCV-induced ROS reduces hepcidin transcription through the inhibited binding of CHOP and/or STAT3 to the hepcidin promoter, and/or stabilization of HIF that is negative hepcidin regulator.

HCV, hepatitis C virus; ROS, reactive oxygen species; HDAC, histone deacetylase; CHOP, C/EBP homology protein; C/EBP, CCAAT/enhancer-binding protein; HIF, hypoxia inducible factor; STAT, signal transducer and activation of transcription; FPN, ferroportin

STAT シグナルを阻害して IFN 応答遺伝子の発現を抑制し、NS5A タンパクは IL-8 の産生を亢進し、おそらく IFN 応答遺伝子の発現を変化させることで IFN の抗ウイルス効果を減弱させる。更には、NS5A や E2 タンパクは PKR に結合して、PKR の酵素活性を抑制することで IFN のウイルスタンパク翻訳抑制効果を阻害する<sup>3)</sup>。HCV は以上に示したような様々な機構で宿主の自然免疫を回避すると考えられる。

HCV の持続感染成立後の肝細胞障害では、酸化ストレスが重要な役割を担っている。HCV コアタンパクはミトコンドリアを傷害し活性酸素を産生し肝臓に酸化ストレスを引き起こす<sup>4)5)</sup>。さらには TNF $\alpha$  や SOCS-3 を介した insulin receptor substrate (IRS) の抑制によるインスリン抵抗性の亢進、MTP 抑制や SREBP1 亢進による肝脂肪化、hepcidin の転写抑制を介した鉄蓄積などを引き起こし、C 型肝炎に特徴的な病態を引き起こす (Fig. 1)<sup>6)7)</sup>。これらの病態は肝発癌とも深く関連して

しており、さらにはペグインターフェロン (PEG-IFN) ・リバビリン (RBV) 併用療法の治療効果にも影響を与えることが報告されている。但し、肝内鉄過剰と抗ウイルス効果との関係については未だ一定の結論に至っていない。

#### Consensus Statement 1:

**インスリン抵抗性と肝脂肪化は PEG-IFN ・ RBV 併用療法の治療効果と関連する。(Level 2a, Grade C)**

このように C 型肝炎の発症機序は次第に明らかにされつつあるが、肝発癌予測と抗ウイルス療法の効果予測に不可欠なのが肝線維化の評価である。最近では elastography を用いた非侵襲的な肝線維化の評価もなされているが、中等度の線維化の評価は未だ困難である。「肝線維化の評価のために肝生検は必要か?」という質

**Table 1** Factors associated with sustained virological response to 48-week peginterferon/ribavirin combination therapy in patients infected with HCV genotype 1b, identified by multivariate analysis (n=114)<sup>11)</sup>

Factor	Category	Risk ratio (95% confidence interval)	P
Amino acid substitution in core region	1: double wild	1	0.004
	2: non-double wild	0.102 (0.022-0.474)	
LDL cholesterol (mg/dL)	1: < 86	1	0.005
	2: ≥ 86	12.87 (2.177-76.09)	
Gender	1: male	1	0.005
	2: female	0.091 (0.017-0.486)	
ICG R15 (%)	1: < 10	1	0.018
	2: ≥ 10	0.107 (0.017-0.678)	
γ-GTP	1: < 109	1	0.032
	2: ≥ 109	0.096 (0.0011-0.819)	
Ribavirin dose (mg/kg)	1: < 11.0	1	0.032
	2: ≥ 11.0	5.173 (1.152-23.22)	

問に対して、今回のアンサーパッドの集計では74%の賛同が得られた。

#### Consensus Statement 2:

肝発癌や抗ウイルス療法の治療効果と関連する宿主側因子として肝組織の線維化の程度 (staging) が重要であるが、stagingの評価には肝生検が推奨される。(Level 1, Grade C)

#### 2. ウイルス変異と病態

C型肝炎の診断にはHCV RNAの測定とともに、ウイルス量、型 (genotype) の測定が重要である。さらにHCV RNA遺伝子の変異について新たな知見が得られている。これらの因子はC型肝炎に対するIFN療法 (RBVの併用療法を含む) の治療効果の予測に非常に重要である。ウイルス量の測定法は、2000年以降アンプリコアHCVモニター法が用いられてきたが、2007年末から高感度かつ広範囲の測定レンジをもつreal-time PCR法を用いた測定が可能となっている。このようなウイルス量とウイルスの型 (genotype または serotype) の測定はIFN治療の効果予測や治療中の抗ウイルス効果をみるなど臨床的な有用性が高い<sup>8)</sup>。

ウイルスの遺伝子変異は、主としてgenotype 1b型のウイルスで多く検討されている。IFN単独投与におけるNS5A aa2209-2248 (interferon sensitivity determining region; ISDR) 領域のアミノ酸変異数が治療効果に関係することが明らかになった。HCV-Jのアミノ酸配

**Table 2** Effect of the IFN treatment on the annual incidence of hepatocellular carcinoma in each fibrosis staging

	Control	IFN-treated	
		All	SVR non-SVR
Patient's number	490	2400	789 1658
Staging			
F1	0.45%	0.08%	0.11% 0.07%
F2	1.99%	0.54%	0.10% 0.78%
F3	5.34%	1.95%	1.29% 2.20%
F4	7.88%	4.16%	0.49% 5.32%

Data were adopted from IHIT study<sup>16)</sup>

列と比較してISDRのアミノ酸変異数が多い場合、IFN単独療法でのSVR率が高いことが報告されている<sup>9)</sup>。さらに現在治療の主体である、PEG-IFNとRBV併用療法 (48週間) においてもISDRの変異数は効果予測に重要である<sup>10)</sup>。

#### Consensus Statement 3:

ISDRの変異は、IFN単独またはRBVとの併用療法におけるSVRに関係するので、治療前に測定すべきである。(Level 2a, Grade B)

さらに、HCV Core領域のアミノ酸置換の有無 (70番目と91番目の変異) がPEG-IFNとRBV併用療法の

治療効果に関係することが報告された (Table 1)<sup>11)</sup>. 米国の報告でも Core 領域の 70 番目のアミノ酸置換が抗ウイルス作用に関係することが示された<sup>12)</sup>.

#### Consensus Statement 4:

**Core 領域の 70 番目, 91 番目のアミノ酸置換は, IFN・RBV 併用療法における SVR, NVR に関係するため, 治療前に測定すべきである. (Level 2a, Grade B)**

また NS5A 領域の aa2334-2379 (IFN/ribavirin resistance determining region, IRRDR) のアミノ酸変異数が PEG-IFN・RBV 併用療法の治療効果に関係するという報告もある<sup>13)</sup>. さらに新規治療薬であるプロテアーゼ阻害剤では, NS3 領域の遺伝子変異が耐性に関係すると報告されている. 一方, 発癌との関係では, Core 領域のアミノ酸置換の有無や NS3 蛋白の二次構造が関係するという報告もなされているが, これらの点に関しては, さらなる検討が必要である.

### 3. 自然経過と IFN 治療適応 (高齢者, PNALT を含む)

C 型急性肝炎の 60~80% が慢性化するとされているが, 輸血後肝炎以外では感染時期が特定できないことが多く, また, 無症状で緩徐な経過をたどることが多いため C 型慢性肝炎の自然史には不明な点が多い. 比較的若く HCV に感染した者を追跡した欧米の報告では, HCV 感染が感染者全体の生命予後に与える影響は少なく, 20 年近く経過した症例でも多くは肝線維化の進展も軽度にとどまるとしている<sup>14)</sup>. この成績は, 輸血後肝炎患者においては平均 20 年~30 年の経過で肝硬変へ進展し, 平均 30 年~40 年の経過で肝癌を併発するというわが国の報告とは進展速度が大きく乖離する<sup>15)</sup>. 一方, C 型慢性肝炎の肝線維化の進展度と肝癌の発生との間の密接な関連性は多くの論文で示されており, わが国における肝硬変の年率発癌率は 5~8% に至る (Table 2)<sup>16)</sup>. このため, 以下のコンセンサスが得られた.

#### Consensus Statement 5:

**わが国の肝硬変患者の年率発癌率は欧米より高く, 5~8% であることを考慮して治療適応を選択すべきである. (Level 2b/3, Grade B)**

C 型慢性肝炎患者の線維化の進展速度は症例によりまちまちであるが, Poynard T ら<sup>17)</sup>は無治療の C 型慢性肝炎平均の年率肝線維化進展率が 0.133 (stage) であると報告し, Shiratori Y ら<sup>18)</sup>も同様に 0.10 (stage) であるとしている. ALT 持続正常の C 型慢性肝炎患者では線維化の進展はさらに緩徐で, 5 年後の肝組織の線維化

に著変なかったとする報告や, 年率肝線維化進展率が平均 0.05 (stage) であったとする報告がある<sup>19)</sup>. 最近では, アルコール多飲以外にも, 肝組織への鉄の過剰沈着, 肝脂肪化, インスリン抵抗性が C 型慢性肝炎の肝線維化を促進する因子であり, 生活習慣の改善が重要であるとされている.

以前より血清 ALT 値の高い肝硬変では発癌率が高かったが, ALT 値が 40 IU/l 以下の C 型慢性肝炎でも血清 ALT 値と発癌率が関連することが示された. 実際の臨床の場では, C 型慢性肝炎患者の血清 ALT 値は 30 IU/l 以下に治療の目標値を設定すべきである.

#### Consensus Statement 6:

**肝発癌予防のためには ALT 値を 30 IU 以下に保つべきである. (Level 2a, Grade A)**

また, わが国で C 型慢性肝炎患者に対する IFN 治療が始まって 20 年以上が経過し多くの患者が著効を得ているが, 著効後も肝癌が発症することが知られ, 治療前の肝組織の線維化進展例, 高齢者, 男性に肝癌併発のリスクが高いことが報告されている. Burno S ら<sup>20)</sup>は著効を示した肝硬変症例の年率発癌率は非著効例の 3 分の 1 ではあるが, 依然, 0.66% であることを示した.

#### Consensus Statement 7:

**C 型慢性肝炎や肝硬変患者では定期的な肝癌のスクリーニング検査を行うべきである. IFN 治療で著効が得られても, 特に肝線維化進展例, 高齢, 男性患者では肝発癌のリスクが高く, 定期的な画像診断・腫瘍マーカーによる検査が引き続き必要である. (Level 2b, Grade A)**

C 型慢性肝炎に対する抗ウイルス療法では Peg-IFN・RBV 併用療法が第一選択の治療法であるが, 両薬剤には多くの副作用がある. 特に高齢者ではグレード 3 以上の副作用の発生率が高く, 両薬剤の減量を余儀なくされることも少なくない. しかし, IFN 治療の年齢制限については, 上限なし 35%, 75 歳まで 64% という意見であり, わが国では高齢者にも積極的に IFN 治療を導入していることが明らかとなった. AASLD のガイドラインでは, 治療適応は病態の重症度, 副作用のリスク, 完治の可能性, 生命予後への影響, 患者の治療への意欲などを総合的に捉え, 個別化して判断すべきであるとしている<sup>1)</sup>. さらに, Zeuzem S ら<sup>21)</sup>が遺伝子型 1 型の ALT 持続正常の C 型慢性肝炎患者に対する Peg-IFN・RBV 併用療法の著効率が 40% であることを報告

して以来、わが国でも ALT 正常の C 型慢性肝炎患者に対する治療が広く行われるようになり、「肝癌抑制を目指した ALT 正常の C 型慢性肝炎患者に対する治療ガイドライン」が示されている（厚生労働省肝炎等克服緊急対策研究事業熊田班）。同班の共同研究において、ALT 正常例であっても血小板数が 15 万以下では、組織学的に繊維化が進展している症例が多いことが明らかにされた (Fig. 2)<sup>22)</sup>。今回のパネルディスカッションにおいても、IFN の投与対象として下記のコンセンサスが得られた。

#### Consensus Statement 8:

IFN 治療は肝組織の Grade/Stage をふまえ、心身の状態、完全著効や生命予後改善の可能性、重篤な副作用を惹起する可能性を個別に評価して考慮する。特に高齢者においても、肝疾患が生命予後を規定する場合には、安全性に十分配慮し IFN 治療を考慮すべきである。(Level 6/3, Grade A)

#### Consensus Statement 9:

HCV RNA 陽性で治療禁忌に該当しない成人は、原則として IFN 治療適応がある。肝発癌抑制を目指した場合、ALT 30 IU/l 以下かつ血小板数 15 万/ $\mu$ l 未満であれば IFN 治療の適応であり、経過観察中に ALT 31 IU/l 以上となった症例も治療を考慮すべきである。ALT 30 IU/l 以下かつ血小板数 15 万/ $\mu$ l 以上では原則経過観察であるが、治療の希望が強い場合、年齢、ウイルス量と遺伝子型、肝線維化の進展度、合併症の有無、副作用の素因を総合的に評価し、治療の可能性と治療のリスクをふまえて治療を考慮すべきである。(Level 3, Grade B)

## 2) 治療

### 1. PEG-IFN・RBV 併用療法

わが国では、PEG-IFN・RBV 併用療法に対して 2 つの全国臨床試験が行われた<sup>23)24)</sup>。その結果、本療法は低ウイルス量の初回治療症例を除く C 型慢性肝炎の標準治療となっている。これらの試験では、genotype 1b かつ高ウイルス量症例におけるウイルス学的著効 (SVR) を期待しがたい因子として、高齢者、女性、線維化進行例、前インターフェロン治療無効例、投与期間 80% 以下が、挙げられている。これらの解析結果で欧米と特に異なる点は、女性が男性より SVR 率が低いことである。しかし、わが国における市販後の複数の臨床研究ではこれを支持するデータが多く認められ<sup>25)26)</sup>、次の

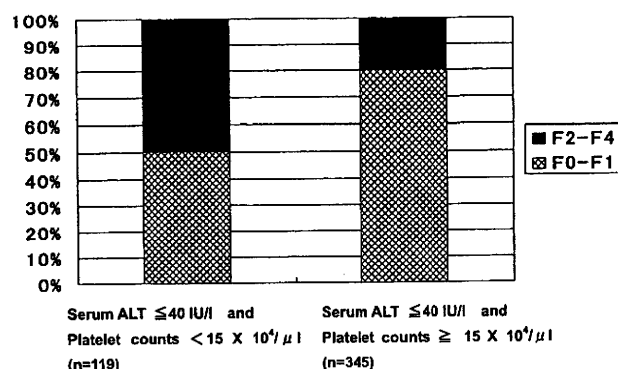


Fig. 2 The relation between platelet counts and the histological findings in the patients with normal ALT.

Forty hundred and sixty four HCV carriers with normal serum ALT ( $\leq$  40 IU/l) were classified according to the platelet counts. Around 20% of patients with ALT  $\leq$  40 IU/l and platelet counts  $\geq$   $15 \times 10^4/\mu$ l (n=345) were in stage F2-4, whereas approximately 50% of patients with ALT  $\leq$  40 IU/l and platelet count  $<$   $15 \times 10^4/\mu$ l (n=119) were in stage F2-4<sup>22)</sup>.

consensus statement が採用された。

#### Consensus Statement 10:

PEG-IFN・RBV 併用療法において、ウイルス学的非著効に至りやすい症例の特徴は、60 歳以上の高齢者、とくに高齢女性、線維化進行例、過去の IFN 単独治療無効例、投与期間 80% 以下の症例、などが挙げられる。(Level 2a, Grade B)

治療中の HCV RNA の消失時期と SVR との間には密接な関連がある。全国臨床試験の成績では<sup>23)24)</sup>、アンプリコア法で測定した HCV RNA が投与開始後 4 週で消失した症例の SVR 率は 100%~76%、5 週以降 12 週までに消失した症例でも 73%~71% と高率であった。しかし、13 週以降 24 週までに消失した症例では 36%~29% で 48 週治療では再燃が増え、24 週以降に陰性化した症例からは 1 例も SVR が得られなかった。従って、治療中の HCV RNA 陰性化時期および減少率は治療効果の予測に有用であり、HCV RNA 測定時期は、4 週、12 週、24 週が推奨される。

HCV RNA (アンプリコア法) が 12 週で 2 log 以上の低下または 24 週で陰性化が得られなければ、SVR は得られない。従って、欧米の practice guideline では、このような症例に対して治療中止が推奨されている。しかし、わが国における 52 例の後ろ向き検討では、再燃

例, 無効例における治療終了後 6 カ月の ALT 正常化率はそれぞれ 56% (5/9), 62% (8/13) で, 1 例を除いて全例で治療終了 2 年後までの長期の biochemical response が得られた<sup>27)</sup>. 従って, 欧米と異なりわが国においては以下のコンセンサスが得られた.

#### Consensus Statement 11:

**Genotype 1 型において, HCV RNA が 12 週で 2 log 以上の低下または 24 週で陰性化が得られなければ, 48 週間の標準治療ではウイルス学的著効は得られない (Level 1, Grade A).** しかし, 24 週時 HCV RNA が陰性化しなくても長期の biochemical response が得られることがあり, ALT が正常化していれば治療を継続する意義がある. (Level 4, Grade C)

Genotype 1 に対する 72 週投与の有用性については, これまで 5 つランダム化比較試験が報告されている<sup>28)~32)</sup>. いずれも IFN の治療法や無作為化する対象症例が異なるため, これらの結果を画一的に評価することはできない. しかし, サブ解析をすると HCV RNA が 13~24 週に陰性化する, いわゆる late virological responder では 72 週投与の有用性が示されている.

わが国においても, genotype 1b 高ウイルス量 113 例において, 48 週投与群と HCV RNA が陰性化してから 44 週間延長投与する群で無作為比較試験が行われており, SVR 率は通常投与群で 36%, 延長投与で 53% であり, 特に HCV RNA が 16~24 週に陰性化した症例で延長投与の SVR 率が有意に高かった (9% vs. 78%,  $p=0.005$ )<sup>33)</sup>. また, Akuta らは, 年齢, 性別, HCV RNA 陰性化時期を合わせた case-control study を行い, 48 週投与 ( $n=130$ ) のウイルス学的著効率が 33% であったのに対し, 72 週投与 ( $n=65$ ) では 62% と高率であり, 特に 70 番 91 番コア変異例と ISDR 野生例で 72 週投与の有用性があったと報告している<sup>34)</sup>. 従って, Genotype 1 型において, HCV RNA (アンプリコア法) が 12 以降 24 週までに陰性化する症例では 72 週延長投与を推奨する.

最近では, HCV RNA の陰性化の判定には, 従来法より感度の高いリアルタイム PCR 法を用いている. リアルタイム PCR を用いた 72 週投与の有用性については十分なエビデンスはないが, 36 週までに HCV RNA が陰性化した症例からでも 72 週投与で SVR が得られている. 従って, 次の consensus statement が採用された.

#### Consensus Statement 12:

**リアルタイム PCR 法を用いた場合, 36 週までに陰性化すれば 72 週投与でウイルス学的著効率の向上が得られる. (Level 2b, Grade C)**

一方, Genotype 2/3 型における短期投与の有用性については, これまで 6 つのランダム化比較試験が報告されている<sup>35)~40)</sup>. しかし, その有用性については一致した見解が得られていない. 最近 Mangia らは, 12 週短期投与における再燃に因する因子を解析し, 年齢 45 歳以上, 血小板数 14 万/ $\mu$ L 未満, BMI 30  $\text{kg}/\text{m}^2$  以上が関連すると報告した<sup>41)</sup>. すなわち, これらの再燃因子を有する症例では短期投与は行うべきではなく, 高齢者や線維化進展例の多いわが国では, 一般的に短期投与を推奨されない.

欧米では, 個々の治療効果の規定因子は HCV 陰性化時期などの治療に対するウイルスの反応性に帰納するとの考え方が支配的である. 従って, 欧米の practice guideline では治療中のウイルスの反応性のみによる画一的な推奨が行われている. しかしわが国では, 再燃因子の有無によりたとえ同様の治療中の反応性が得られたとしても最終治療効果は異なることが示唆されている. 例えば, Akuta らは viral kinetics に因する因子を検討し, 同じ EVR が得られても女性と高度線維化例では SVR 率が低いことを報告している<sup>42)</sup>. 従って, PEG-IFN・RBV 併用療法では, 治療中のウイルス反応性を考慮して治療期間を設定すべきであるが, 再燃リスクを有する症例の多いわが国では, 治療期間の最適化には年齢や性別などの再燃因子を考慮すべきである.

Helibling は代償性肝硬変 124 例を RBV 1000/1200 mg (標準投与量) 群と 600/800 mg (低用量) 群に無作為に割付し PEG-IFN と併用療法を行い, 有用性を検討した<sup>43)</sup>. その結果 SVR 率は標準投与量群で 52%, 低用量群で 38% と前者で良好であった. 重篤な副作用はそれぞれ 14%, 18% で, 薬剤減量を必要とした症例は 78%, 57% であった. SVR に寄与する因子は genotype 2/3 と血小板  $150 \times 10^9/\text{L}$  以上であった. 従って, わが国においても代償性肝硬変に対して, PEG-IFN/RBV 併用療法は可能であるが, 副作用出現に対して注意が必要である. (Level 1, Grade A)

過去の通常型の IFN (RBV 併用例を含む) に対して無効または再燃した症例に対する, PEG-IFN・RBV 併用療法の有用性を検討したランダム化比較試験はこれまで 7 つある<sup>44)~50)</sup>. これらの SVR 率は 6% から 45%

と様々であるが、とくに過去の治療における再燃例では、再治療効果が高い。一方、AASLDのpractice guideline<sup>1)</sup>では、過去に十分なPEG-IFN・RBV併用療法が行われた再燃・無効例に対しては、PEG-IFN・RBV併用療法の再投与は推奨されていない。しかし最近、過去のPEG-IFN・RBV併用療法の再燃・無効例であっても、PEG-IFN・RBVの再投与により12週でHCV RNAの陰性化が達成されれば、48~72週投与により48%~68%のSVR率が得られたとの報告がある<sup>45)~52)</sup>。再治療により12週陰性化が得られる症例を治療前に同定することは現時点では困難であることや、プロテアーゼ阻害薬の開発状況を考慮すると依然議論の余地はあるが、以下のconsensus statementが採用された。

#### Consensus Statement 13:

**過去のIFN療法の再燃・無効例であっても、PEG-IFN・RBV併用療法の再治療で、HCV RNAの12週陰性化が達成されれば、ウイルス学的著効が期待できる。(Level 2b, Grade B)**

#### 2. 従来型IFNあるいはPEG-IFN単独療法の位置づけ

我が国では、1992年にC型慢性肝炎に対するIFN単独治療が開始され、その臨床的効果が多数報告されている。しかし現在では、1b・高ウイルス量例に対する標準治療はPEG-IFN・RBV併用療法となり、HCV排除を目的とする単独療法の適応は、一部の患者に限られている。IFNあるいはPEG-IFN単独治療の対象として、1. 低ウイルス症例、2. リバビリン併用困難例(慢性腎不全など)、3. 急性肝炎例、4. 肝癌根治例、5. 維持療法(線維化進展例や抗ウイルス療法抵抗例)が挙げられている<sup>53)</sup>。

アンサーパッドにおいて、「初回治療例において、低ウイルス量の患者にふさわしい治療は？」と質問したところ、PEG-IFNまたはIFN単独療法は60%に支持されたが、最初からIFN・RBVの併用療法を行うとする意見が37%に見られた。欧米のガイドラインではPEG-IFN・RBV併用療法のみを推奨しているが、我が国では初回の低ウイルス量例にはPEG-IFNあるいはIFN単独療法も一定の評価を受けていることが示された。

急性HCV感染は、70%程度が慢性感染に移行するため、治療介入が必要である。急性C型肝炎患者で持続感染への移行が疑われる症例では、発症後12週から24週以内にIFN6MIU週3回あるいはPEG-IFN週1回12~24週間の単独治療が推奨される。しかし、リバビリンの併用が治療効果を向上した成績がなく、単独療法で

十分な有効性が期待できる<sup>54)</sup>。

わが国ではIFNの発がん抑制効果について多くの臨床的検討が行われてきた<sup>55)~57)</sup>。肝硬変例を対象とするメタアナリシス解析ではIFN投与により発癌率が低下することが確認されている。また線維化進展例(F3-F4)においてもIFN投与にてHCVが排除されると発癌率が低下し予後改善効果があることも報告されている<sup>16)</sup>。我が国の成績では、60歳以上の患者への少量長期IFN単独治療は、ALTおよびAFPの低下を誘導し非治療群と比較して肝発癌を抑制することが示されている<sup>56)</sup>。さらに、我が国を含めて複数の施設から肝癌根治治療例に対するIFNの発癌抑制効果が報告されている<sup>57)</sup>。特に、IFN投与群では二次再発、三次再発が低下することも注目すべき効果である<sup>58)</sup>。一方、欧米で実施された前向きランダム化試験(HALT-C)ではPEG-IFN・RBV無反応例に対するPEG-IFN少量長期投与が肝疾患の進展を阻止しなかったことが示された<sup>59)</sup>。すなわちIFN維持療法に関する欧米と我が国の臨床成績が相反する結果が示された。この理由として、我が国の治療対象が高齢であり、かつ肝発癌率が高いことが治療介入による効果の差になっていることが推測され、今後明らかにすべき課題である。

今回、SVRが期待できない場合でも、「IFN長期投与はALT値の低下が見られれば、肝発癌抑制や生命予後の改善効果を期待できるか？」という質問に対し、89%の同意が得られた。

#### Consensus Statement 14:

**肝癌根治例では生命予後延長効果を期待したIFN投与を推奨する。(Level 1, Grade A)**

#### Consensus Statement 15:

**SVRが期待できない場合でも、IFN長期投与はALT値の低下が見られれば、肝発癌抑制や生命予後の改善効果が期待できる。(Level 2a, Grade B)**

#### 3. 治療方針のコンセンサス

C型肝炎に対する治療方針として、C型肝炎の治療目標、抗ウイルス療法の治療適応、Peg-IFN・RBV併用療法(薬剤投与量との関係)、ウイルス排除不能例・肝硬変例の治療、新規抗ウイルス剤の各項目について、下記のようにまとめた。

##### 治療目標

治療目標の第一は、HCV RNA排除による肝炎治癒であり、IFN治療によってウイルス排除が得られた場合



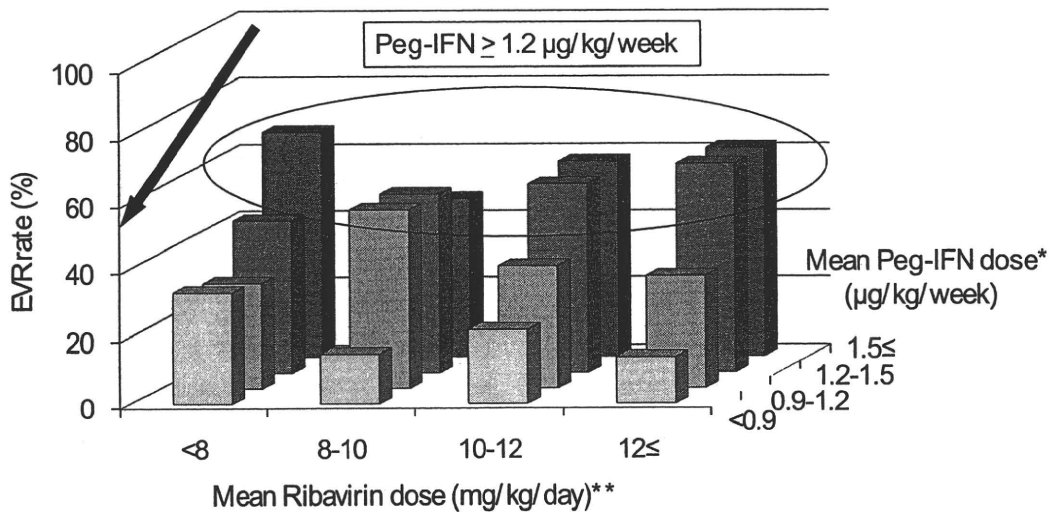


Fig. 3 C-EVR rate according to PEG-IFN alfa-2b and ribavirin doses during 12 weeks after start of therapy. \*, p<0.0001; Peg-IFN \*\*, p=0.34; Ribavirin (Mantel-Haentzel chi-square test). The c-EVR rates were 54% and 56% for patients who received more than 1.5 µg/kg/week and 1.2-1.5 µg/kg/week of Peg-IFN alfa-2b and declined to an average rate of 38% in patients given 0.9-1.2 µg/kg/week of Peg-IFN alfa-2b, to an average rate of 22% in patients given less than 0.9 µg/kg/week of Peg-IFN alfa-2b. <sup>61)</sup>

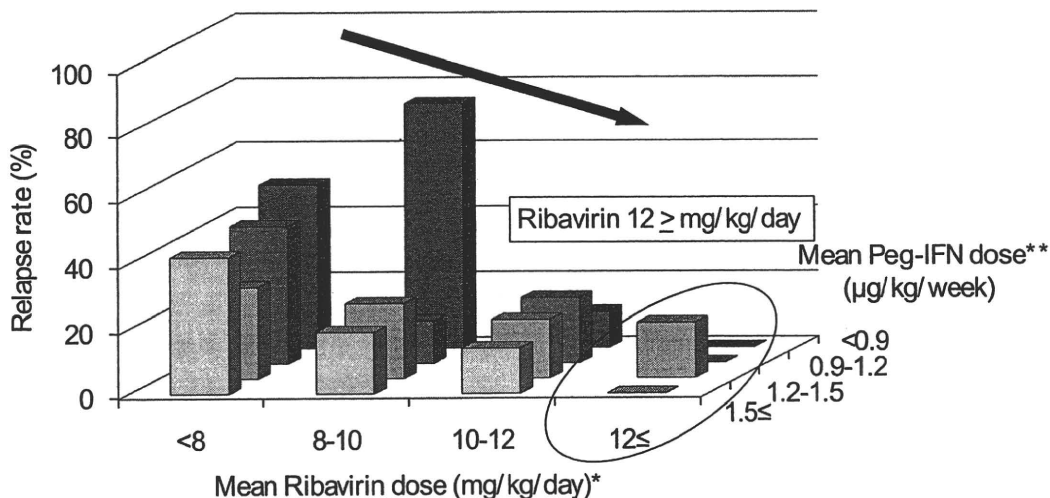


Fig. 4 Relapse rate according to Peg-IFN alfa-2b and ribavirin doses during treatment of patients who completed treatment. \*, p=0.0001; Ribavirin \*\*, p=0.15; Peg-IFN (Mantel-Haentzel chi-square test). The relapse rate was 60% in patients receiving less than 6 mg/kg/day of ribavirin, and declined to 41% at 6-8 mg/kg/day, 27% at 8-10 mg/kg/day, 22% at 10-12 mg/kg/day and 11% in patients given ≥ 12 mg/kg/day. <sup>62)</sup>

には、肝発癌抑止効果や生命予後改善が得られる。現時点で最も治療効果の高い抗ウイルス療法は Peg-IFN・RBV 併用療法である。第二の目標として、ウイルス排除ができない場合には肝病変進展予防あるいは肝発癌予防を目指すことが重要である。

治療適応

治療適応については、C 型肝炎患者の予後を規定し、薬剤の副作用への対処が可能と考えられる症例を抗ウイルス療法の対象とする。このうち、良好な治療効果が予測される症例が、“良い適応”症例である。さらに、SVR の可能性が低い高齢者や線維化進展例でも、合併疾患がなければ抗ウイルス療法を提示す

べきであるということに88%の同意が得られた。

#### Consensus Statement 16:

**C型肝炎患者の予後を規定し、薬剤の副作用への対処が可能であると考えられる症例は抗ウイルス療法の対象とする。(Level 6, Grade B/C)**

たとえ、SVRの可能性が低い症例でも合併疾患がなければ抗ウイルス療法を提示すべきである。

#### Peg-IFN・RBV併用療法(薬剤投与量との関係)

Genotype1型におけるEVR(治療開始12週のHCV RNA陰性化)の達成には、Peg-IFN投与量が用量依存性に関与する(Fig. 3)。Peg-IFN $\alpha$ 2a投与量80%以上<sup>60)</sup>あるいはPeg-IFN $\alpha$ 2b平均投与量1.2 $\mu$ g/kg/週以上<sup>61)</sup>を目標とし、極力、減量投与開始は避ける(Level 2b/3, Grade B)。また、Genotype1型のウイルス陰性化例における治療後再燃には、RBVが用量依存性に関与し、予定投与量の80%以上あるいは平均投与量10mg/kg/日(可能であれば12mg/kg/日)以上を目標とする<sup>62)</sup>(Level 2b/3, Grade B)(Fig. 4)。一方、Genotype2/3型における減量(PegIFN $\alpha$ 2a 135 $\mu$ g/週あるいはPegIFN $\alpha$ 2b 1.0 $\mu$ g/kg/週, RBV 400mg/day)については、治療効果に有意な影響を及ぼさないものと考えられる(Level 2a, Grade B)<sup>63)~65)</sup>。

#### ウイルス排除不能例・肝硬変例の治療

IFN療法を積極的に行うことが治療の第一選択であるが、IFN非適応例や無効例に対しては肝庇護療法や瀉血療法の重要性は認識されており、77%の同意が得られた。

#### Consensus Statement 17:

**IFN非適応例やIFNでALT値やAFPの改善が得られない症例には、肝庇護剤による治療を行い、効果不十分な場合は、瀉血療法を併用する。(Level 3/6, Grade B/C)**

次に、代償性肝硬変では、IFNを主体とした治療でHCV RNA排除を目指し、非代償性肝硬変では、肝予備能の改善や発癌予防を目標とした治療を行う。代償性肝硬変で発癌予防を目指す場合には、AST・ALT値、AFP値の改善を目標とし、IFNのみでなく肝庇護剤、瀉血療法、分岐鎖アミノ酸製剤を単独あるいは組み合わせて治療することが望ましい。AASLD guideline<sup>1)</sup>では、“C型慢性肝疾患に対して抗ウイルス療法が奏功しない場合、肝移植を考慮する”という方針のみにとどまるのに対し、肝不全への進展、発癌予防に向けた

わが国独特の肝庇護療法に対して、91%の同意が得られた。

#### Consensus Statement 18:

**代償性肝硬変で発癌予防を目指す場合には、AST・ALT値、AFP値の改善を目標とし、IFNのみでなく肝庇護剤、瀉血療法、分岐鎖アミノ酸製剤を単独あるいは組み合わせて治療する。**

#### 新規抗ウイルス剤

新規抗ウイルス剤として、プロテアーゼ阻害剤、ポリメラーゼ阻害剤などが有効であり、PEG-IFN/RBVとの併用で著効率が向上する(Level 1b, B)。特に、プロテアーゼ阻害剤であるTelaprevir(VX950)にPeg-IFN・RBVを加えた3者併用療法では、新規症例で6~7割<sup>66)</sup>、PEG-IFN・RBV併用療法の再燃例で約7割、無効例でも約4割にSVRを認めている。

今後の治療方針として、より早期のウイルス排除が期待される線維化進展例や高齢者ではPEG-IFN・RBV併用療法を行うが、それ以外の症例では新規治療を考慮に入れた治療選択が必要となる。

#### おわりに

わが国のC型肝炎は、欧米に比し高齢で組織進展例が多い。このため、年率発がん率が高く、IFN治療に対しても有効率が低く副作用の発現が多い。このような患者背景に即したわが国独自のエビデンスの確立が求められるが、現状では信頼度の高い情報が集積されていないものも多い。今回、アンサーパッドを用いて聴取したわが国の肝臓専門医の意見を基に、これらの多くをコンセンサスステートメントとしてまとめた。肝臓専門医の共感が得られた提言ではあるが、国際的に承認されるためには全国的な多施設研究による今後の検証が必要である。その一方で、わが国の患者の現状は欧米の患者の未来像を示している可能性が高いことから、日本の実情に即したガイドラインを海外にむけて発信し批判の俎上に載せることは、日本肝臓学会が果たすべき重要な役割と考える。このため、本論文にInformative statementやRecommendationを追記してHepatology Res.にも掲載する。

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