Discussion

A prospective, randomized, and treatment-controlled clinical trial was planned and conducted in Japan to compare the therapeutic efficacy and safety profiles between the triple therapy with T12PR24 and the SOC treatment with PR48. In this trial, 126 patients were assigned to receive T12PR24 (Group A) and the 63 to receive PR48 (Group B). They all were treatment-naive, and infected with HCV-1 in high viral loads ($\geqslant 5$ $\log_{10}$ IU/ml) and of genotype 1b in the great majority (98.9%). Randomization was not adopted due to ethnical concerns against giving intravenous placebo weekly for 24 weeks to patients in Group A.

Dynamics of circulating HCV RNA during treatment was quite different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, p <0.001) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Accordingly, SVR was achieved more frequently in patients with T12PR24 than PR48 (73.0% *vs.* 49.2%, $p = 0.0020$), while rates of relapse (16.7% *vs.* 22.2%) and breakthrough (3.2% and 1.6%) were not different between them. Due to the higher therapeutic efficacy and shorter treatment duration, T12PR24 would be more suitable for treatment of HCV-1 patients than the standard PR48, and lessen the total economic burden of patients and the nation.

Previous clinical trials with telaprevir were conducted in Europe or the United States and combined with PEG-IFN-α2a [8–11]. In the present study, Japanese patients have responded to a triple therapy with PEG-IFN-α2b, with an efficacy of 73% in comparison with 72–75% in phase 3 clinical trials [10,11]. In a recent report, PEG-IFN-α2a and -α2b were equally effective in triple therapies in combination with telaprevir and RBV [16]. Frequency of side effects demanding the discontinuation of all drugs is comparable between patients receiving the triple therapy with PEG-IFN-α2a in phase 3 trials [10,11] and -α2b in the present study (7–17% and 17%, respectively).

In our previous report [17], the IFN-responsive C/C genotype of *IL28B* at rs12979860 was detected in 42 out of the 72 (55%) patients infected with HCV-1 in Japan; the prevalence was not much different from that in 336 out of the 769 (44%) European-Americans [18]. The susceptibility to telaprevir depends on HCV genotypes, and is higher for genotypes 1 and 2 than genotypes 4 and 5 in *in vitro* experiments [19]. Further, it may differ between 1a and 1b, due to dissimilar evolution patterns of drug-resistant mutations [14]. Nevertheless, present patients infected with HCV-1b in the great majority (98.4%) were equally responsive to the triple therapy with telaprevir as those infected with HCV-1a [8,9,11].

High efficacy of T12PR24 was accompanied by increased adverse events, of which anemia and skin lesions were worrisome. Moderate and severe anemia (<9.5 g/dl) developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Since Japanese patients with chronic hepatitis C are older by >10 years than those in Western countries, with a higher proportion of women, they are prone to develop anemia during treatment with telaprevir. Stringent precaution had to be taken, therefore, by deducting the RBV dose in patients in whom hemoglobin levels decrease <12 g/dl, higher than the conventional threshold of <10 g/dl. The total RBV dose was lower in Group A than B (47.0% *vs.* 77.7% of the target, p <0.0001). However, decreased doses of RBV or PEG-IFN did not influence substantially the therapeutic efficacy of T12PR24.

Skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). It has to be noted that Grade 4 skin lesions, such as Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS), developed exclusively in patients in Group A. Since studied patients were monitored carefully and received immediate care by dermatologists, if and when skin lesions of Grades 2–4 developed, all patients eventually recovered. In the area of DAAs, potentially accompanying severe skin disorders, physicians would need close cooperation with dermatologists for the care of patients with hepatitis C.

In conclusion, this multicenter, randomized, and treatment-controlled study of T12PR24 in Japanese patients infected with HCV-1b has proven the efficacy and safety comparable to those in previous phase 3 studies [10,11]. Due to the excellence of T12PR24 over the standard PR48, we hope it will be used widely in patients with chronic hepatitis C over the world, who are expected to increase rapidly in the foreseeable future [20].

Conflict of interest

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

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Association of two polymorphisms of the *IL28B* gene with viral factors and treatment response in 1,518 patients infected with hepatitis C virus

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Abstract
Background Two nucleotide polymorphisms of the interleukin-28B (*IL28B*) gene, at rs8099917 and rs12979860, influence the response to interferon (IFN)-based therapies in patients infected with hepatitis C virus (HCV) of genotype 1. We aimed to investigate whether these polymorphisms showed complete linkage in Japanese patients.
Methods A total of 1,518 Japanese patients infected with HCV were genotyped for the two *IL28B* loci, and the two sets of genotypes were compared.
Results TT at rs8099917 and CC at rs12979860 were detected in 77.7 and 76.8%, respectively, of the 1,518 patients and TG/GG and CT/TT were detected in 22.3 and 23.2%. These two sets of *IL28B* genotype stood in strong linkage disequilibrium ($r^2 = 0.98$). Discordance between the two *IL28B* polymorphisms occurred in 16 (1.1%) patients, and 13 (0.9%) of them possessed IFN-sensitive TT at rs8099917 and IFN-resistant CT at rs12979860. Three of these 13 patients had HCV of genotype 1b and had received pegylated-interferon and ribavirin, and none of them gained a sustained virological response. At rs8099917, IFN-resistant TG/GG were more frequent in patients infected with HCV of genotype 1 than in those infected with HCV of genotype 2 [258/1,046 (24.7%) vs. 75/441 (17.0%), $p = 0.001$]. The response to pegylated-interferon/ribavirin in 279 patients who were infected with HCV-1 and the response to IFN monotherapy in 361 patients who were infected with HCV-1 , was higher in those with TT than in those with TG/GG at rs8099917, as well as being higher in those with CC than in those with CT/TT at rs12979860 ($p < 0.001$).
Conclusions Linkage disequilibrium between two *IL28B* polymorphisms at rs8099917 and rs12979860 is strong in Japanese HCV patients, but there are some discrepancies between the two sets of genotypes.

Keywords Hepatitis C virus · Chronic hepatitis C · Interleukin-*28B* gene · Interferon · Single nucleotide polymorphism

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Introduction

Worldwide, an estimated 170 million people are infected with hepatitis C virus (HCV) [1], and about 30% of those infected develop severe liver disease, such as decompensated cirrhosis and hepatocellular carcinoma [2, 3]. Currently, interferon (IFN) is the only antiviral drug that is capable of clearing HCV infection. The present standard-of-care therapy for patients infected with HCV of genotype 1 (HCV-1), the most prevalent genotype worldwide, is pegylated (PEG)-IFN combined with ribavirin (RBV) for

48 weeks. However, a sustained virological response (SVR), judged by the loss of HCV RNA from serum 24 weeks after the treatment completion, can be achieved in 50% of patients at most [4–6]. A number of host and viral factors are known to influence the response to IFN-based treatments [7–9]. Because IFN is expensive and can induce severe adverse effects, it is desirable to differentiate non-responders from responders before the treatment is started.

Single nucleotide polymorphisms (SNPs) at rs12979860 and rs8099917 have been reported, upstream of the interleukin-28B (*IL28B*) gene and separated by 4,378 bp, and they influence SVR to PEG-IFN/RBV in Caucasians, Hispanics, Africans, and Asians who are infected with HCV-1 [10–12]. However, it is not clear whether these two polymorphisms are completely linked in Japanese patients, and their clinical significance in large Japanese populations, including HCV-2 patients, has not been elucidated. At the Department of Hepatology at Toranomon Hospital in Metropolitan Tokyo, 1,518 patients with persistent HCV infection were genotyped by polymorphisms at rs1297980 and rs8099917, and the two sets of *IL28B* genotypes were compared to elucidate their association with host as well as viral factors, and the therapeutic response.

Patients and methods

Patients

From July 1996 through May 2010, 1,521 patients agreed to join the human genome study at the Department of Hepatology at Toranomon Hospital in Metropolitan Tokyo. All the patients possessed HCV RNA in the serum detectable by a commercial quantitative polymerase chain reaction (PCR) assay (COBAS Amplicor HCV Monitor Test, v2.0; Roche Diagnostics, Branchburg, NJ, USA). All the 1,521 patients were Japanese, and none were co-infected with hepatitis B virus or human immunodeficiency virus type-1, nor did they have apparent auto-immune hepatitis or alcoholic liver disease. They were tested for two sets of *IL28B* genotypes, specified by T or G at rs8099917 and C or T at rs12979860 [10–12].

Of the 1,521 patients, genotyping at rs8099917 was not possible in two, and that at rs12979860 was not feasible in another. Hence, the comparisons of *IL28B* genotypes between rs8099917 and rs12979860 were performed in the remaining 1,518 patients for whom genotypes at both loci were determined.

The two *IL28B* polymorphisms were evaluated for their association with therapeutic response in two cohorts of patients with chronic hepatitis C. In one cohort, combination therapy was given to 362 patients. Of them, 279 received PEG-IFN-α2b (PEG-Intron; MSD, Kenilworth, NJ, USA) subcutaneously at a median dose of 1.5 μg/kg (range: 1.3–2.0 μg/kg) once a week, and the remaining 83 received IFN-α2b (Intron; MSD) 6 million units daily for 2 weeks and then 3 times per week for 46–70 weeks. RBV was given to all 362 patients at a daily dose adjusted by body weight [Rebetol (600–1,000 mg); MSD]. The other cohort, of 633 patients, received monotherapy with IFN-α or IFN-β at a total dose ranging from 184 to 552 million units. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave written informed consent to participate in this study, with understanding of its purpose.

Determination of HCV RNA and genotypes

HCV RNA was determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan) with a linear dynamic range of 1.2–7.8 log IU/mL. Genotypes of HCV were determined by the PCR-Invader method [13].

Amino acid substitutions in the core protein

Substitutions of amino acid (aa) residues in the core protein of HCV-1b isolates were determined by direct sequencing. With the use of HCV-J (accession no. D90208) as a reference [14], the sequence of aa1–191 in the core protein was determined, and then it was compared with the consensus sequence constructed in 81 HCV-1b samples, for detecting substitutions of aa70 of arginine (Arg) in the wild-type for glutamine/histidine (Gln/His) in mutants [15].

Determination of *IL28B* genotypes

Some samples were genotyped in a genome-wide association study (GWAS), using the Illumina HumanHap610-Quad Genotyping BeadChip (Illumina, San Diego, CA, USA). Other samples were genotyped by the TaqMan Pre-Designed SNP Genotyping Assay or the Invader Assay [16, 17].

Statistical analysis

Differences in categorical variables between groups were evaluated by $\chi^2$and Fisher's exact tests. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA), and a p value of <0.05 was considered significant. Linkage disequilibrium (r^2 value) between the two loci was calculated with the Haploview program [18].

Results

IL28B genotypes at rs8099917 and rs12979860 in 1,518 Japanese patients with persistent HCV infection

The characteristics of the 1,518 Japanese patients infected with HCV are listed in Table 1. They had a median age of 50 years (range 13–81 years) when genotyping was performed, and included 887 (58.4%) men. Most of them had chronic hepatitis (89.5%), and cirrhosis or hepatocellular carcinoma had developed in the remaining patients. Genotype 1 predominated [68.9% (with HCV RNA at titers of >6.7 log IU/mL in the majority)], followed by genotypes 2 and 3 (29.1 and 0.5%, respectively). More than half of the patients (56.1%) had received IFN with or without RBV previously.

Of the *IL28B* genotypes at rs8099917, TT predominated at 77.7%, followed by TG at 20.6%; GG accounted for a far distant minority of 1.7% (Fig. 1a). Such a profile was closely mirrored in the distribution of the respective *IL28B* genotypes at rs12979860: CC (76.8%), CT (21.3%), and TT (1.9%) (Fig. 1b). The r^2 value between these two *IL28B* loci was calculated to be 0.98, thereby indicating strong linkage disequilibrium. Of the 1,518 patients, 16 (1.1%) possessed *IL28B* genotypes discordant between the rs8099917 and rs12979860 loci. All the 1,166 patients with the CC genotype at rs12979860 possessed the TT genotype at rs8099917. However, the 1,179 patients with the interferon-sensitive TT genotype at rs8099917 did not all possess the CC genotype, and this group included 13 patients who had the interferon-resistant CT genotype at rs12979860 (Fig. 1c). The other three patients possessed the TG genotype at rs8099917 and TT genotype at rs12979860; these are both IFN-resistant *IL28B* genotypes.

Both TT at rs8099917 and CC at rs12979860 are associated with increased chances for an SVR to PEG/RBV therapy [10–12]. Because the T and C alleles in the two loci, respectively, behave as recessive genes in terms of the response to PEG/RBV [10, 11], we combined the GG and TG genotypes into a non-TT category, and we combined the TT and CT genotypes into a non-CC category; these categories represent IFN-resistant phenotypes in clinical practice.

Table 1 Characteristics of the 1,518 patients with HCV infection for whom polymorphisms of the *IL28B* gene were determined

Demographic data[a]	
Male	887 (58.4%)
Age (years)	50 (13–81)
Family member(s) with liver disease	367 (24.2%)
History of transfusion	603 (39.7%)
Laboratory data	
Hemoglobin (g/dL)	14.7 (18.0–19.0)
Leukocytes (/mm^3)	4,700 (2,500–8,200)
Platelets ($\times 10^4$/mm^3)	18.2 (3.5–28.9)
Albumin (g/dL)	4.3 (2.4–6.0)
Aspartate aminotransferase (IU/L)	56 (4–1,520)
Alanine aminotransferase (IU/L)	83 (6–1,640)
Gamma-glutamyl transpeptidase (IU/L)	54 (4–810)
Alpha-fetoprotein (μg/L)	6 (1–406)
Cholinesterase (ΔpH)	1.2 (0.2–2.4)
Diagnosis of liver disease	
Chronic hepatitis	1,359 (89.5%)
Cirrhosis	118 (7.8%)
Hepatocellular carcinoma	41 (2.7%)
Virological data	
HCV genotypes: 1/2/3/untypeable	1,046 (68.9%)/441 (29.1%)/7 (0.5%)/ 24 (1.6%)
HCV RNA (log IU/mL)	>6.7 (<2.7–>6.7)
History of antiviral therapy	
IFN with or without ribavirin	852 (56.1%)

HCV hepatitis C virus, *IL28B* interleukin-28B, *IFN* interferon

[a] Data are numbers with percentages in parentheses or medians with ranges in parentheses

Characteristics of patients who had discordant *IL28B* genotypes in terms of the response to IFN-based treatments

Of the 1,518 patients, 13 (0.9%) patients possessed an IFN-sensitive (major) *IL28B* genotype that was different between rs8099917 and rs12979860. All these 13 patients possessed the IFN-sensitive TT genotype at rs8099917, in spite of having the IFN-resistant CT genotype at rs12979860 (Table 2). Ten of the 13 patients were infected with HCV-1, and six of the 13 had received antiviral treatments; three had been treated with PEG-IFN/RBV, one with IFN/RBV, and two with IFN monotherapy. Of the six patients with IFN-based therapies, four did not respond, and the remaining two relapsed after the treatment completion. Notably, all the three patients with HCV-1b who had received PEG-IFN/RBV failed to gain an SVR. All the three patients with mutant aa70 in the core protein were non-responders, whereas two of the three with the wild-type aa70 were relapsers.

Association of *IL28B* genotypes at rs8099917 and rs12979860 with HCV genotypes

IL28B genotypes were compared between the 1,046 patients infected with HCV-1 and the 441 patients infected with HCV-2. IFN-resistant TG/GG genotypes at rs8099917 were more frequent in the patients with HCV-1 than in

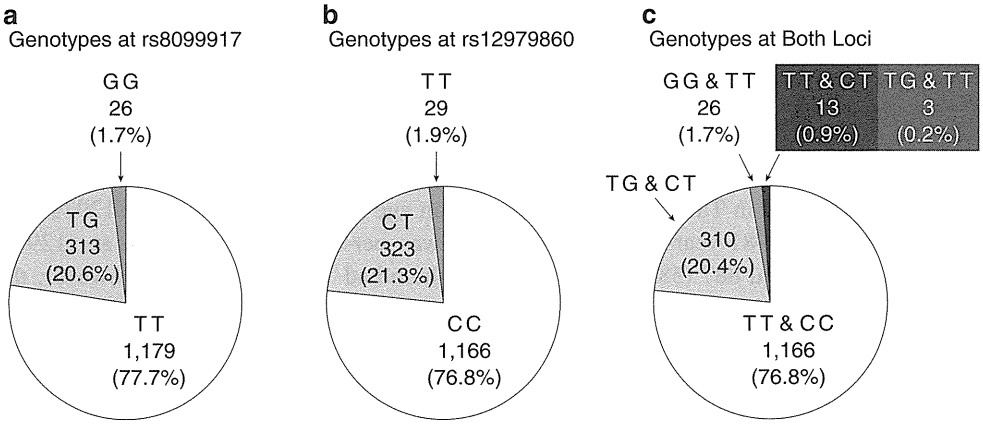

Fig. 1 Distribution of *IL28B* genotypes in the 1,521 Japanese patients with hepatitis C virus (HCV) infection. Distributions of *IL28* genotypes at rs8099917 (**a**) and rs12979860 (**b**) are shown. Distribution of concordant genotypes is shown (**c**), along with that of discordant genotypes in *black* and *gray shades*

Table 2 Virological characteristics and treatment responses in the 13 patients with discordant *IL28B* genotypes at rs8099917 and rs12979860

Patient no.	Age (years) and sex	HCV genotype	*IL28B* genotypes		aa70 in core protein[a]	HCV RNA (log IU/mL)	Antiviral treatment	
			rs8099917	rs12979860			Drugs[b]	Response
1	38M	1b	TT	CT	Wild-type	6.3	PEG/RBV	None
2	52M	1b	TT	CT	Mutant	4.8	PEG/RBV	None
3	53F	1b	TT	CT	Wild-type	6.8	PEG/RBV	Relapse
4	59F	1b	TT	CT	Wild-type	6.7	IFN/RBV	Relapse
5	52F	1a	TT	CT	Mutant	6.1	IFN	None
6	62M	1b	TT	CT	Mutant	6.0	IFN	None
7	49M	1b	TT	CT	Wild-type	5.4	None	
8	65M	1b	TT	CT	Mutant	8.7	None	
9	60F	1b	TT	CT	Mutant	5.8	None	
10	58F	1b	TT	CT	Wild-type	6.6	None	
11	44M	2a	TT	CT		5.4	IFN/RBV	SVR
12	45M	2a	TT	CT		5.1	PEG/RBV	SVR
13	45F	2b	TT	CT		5.6	IFN	Relapse

HCV hepatitis C virus, *IL28B* interleukin-28B, *IFN* interferon, *PEG* pegylated IFN, *RBV* ribavirin, *SVR* sustained virological response

[a] Wild-type and mutants possessed arginine and glutamine (or histidine), respectively, as aa70 in the core protein of HCV-1 isolates [19]. Substitutions were not determined in the three patients infected with HCV-2 in whom the response to IFN-based treatments was not influenced by substitutions of aa70 in the core protein

[b] PEG-IFN/RBV and IFN/RBV were continued for 46–70 weeks, and IFN alone for 12–24 weeks

those with HCV-2 [24.7 vs. 17.0%, $p = 0.001$ (Fig. 2a)]. Likewise, IFN-resistant CT/TT genotypes at rs12979860 were found more often in patients with HCV-1 than in those with HCV-2 [25.7 vs. 17.0%, $p = 0.001$ (Fig. 2b)].

Association of *IL28B* genotypes at rs8099917 and rs12979860 with amino acid substitutions in the HCV core protein

Substitutions of aa70 in the core protein of HCV-1b, from Arg in the wild-type to Gln or His in mutants, decrease the response to IFN-based treatments in patients with chronic hepatitis [15, 19]. There were 601 patients with HCV-1b for whom aa70 in the core protein was determined. Of them, 380 (63.2%) patients were infected with HCV-1b with the wild-type aa70, and 394 (65.6% of the 601) had received IFN with or without RBV. Most of these patients [555/601 (92.3%)] had chronic hepatitis.

HCV-1b mutants, with aa70 of Gln or His, were more frequent in patients with IFN-resistant than -sensitive genotypes at both rs8099917 and rs12979860. These mutants were found more often in patients with TG/GG than TT genotypes [62.4 vs. 28.0%, $p < 0.001$, (Fig. 3a)], as well as being more frequent in those with CT/TT than CC genotypes [62.0 vs. 27.6%, $p < 0.001$ (Fig. 3b)].

The influence of *IL28B* genotypes at rs8099917 on the substitution of aa70 may be subject to host factors such as sex and age. The frequency of aa70 mutants tended to

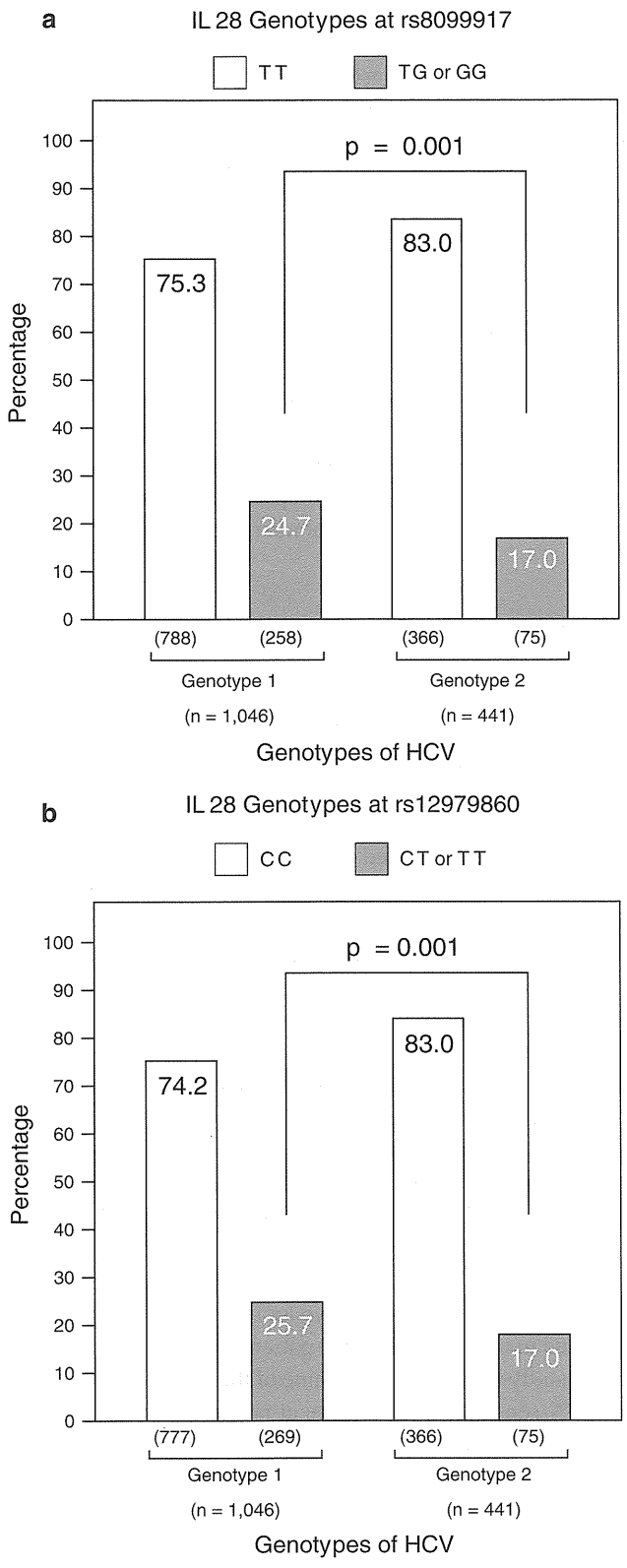

Fig. 2 Frequencies of interferon (IFN)-sensitive and -resistant *IL28B* genotypes in patients infected with HCV of genotype 1 or 2. Frequencies of TT and TG/GG genotypes at rs8099917 (**a**), as well as those of CC and CT/TT genotypes at rs12979860 (**b**), were compared in 1,046 patients with HCV-1 and 441 patients with HCV-2

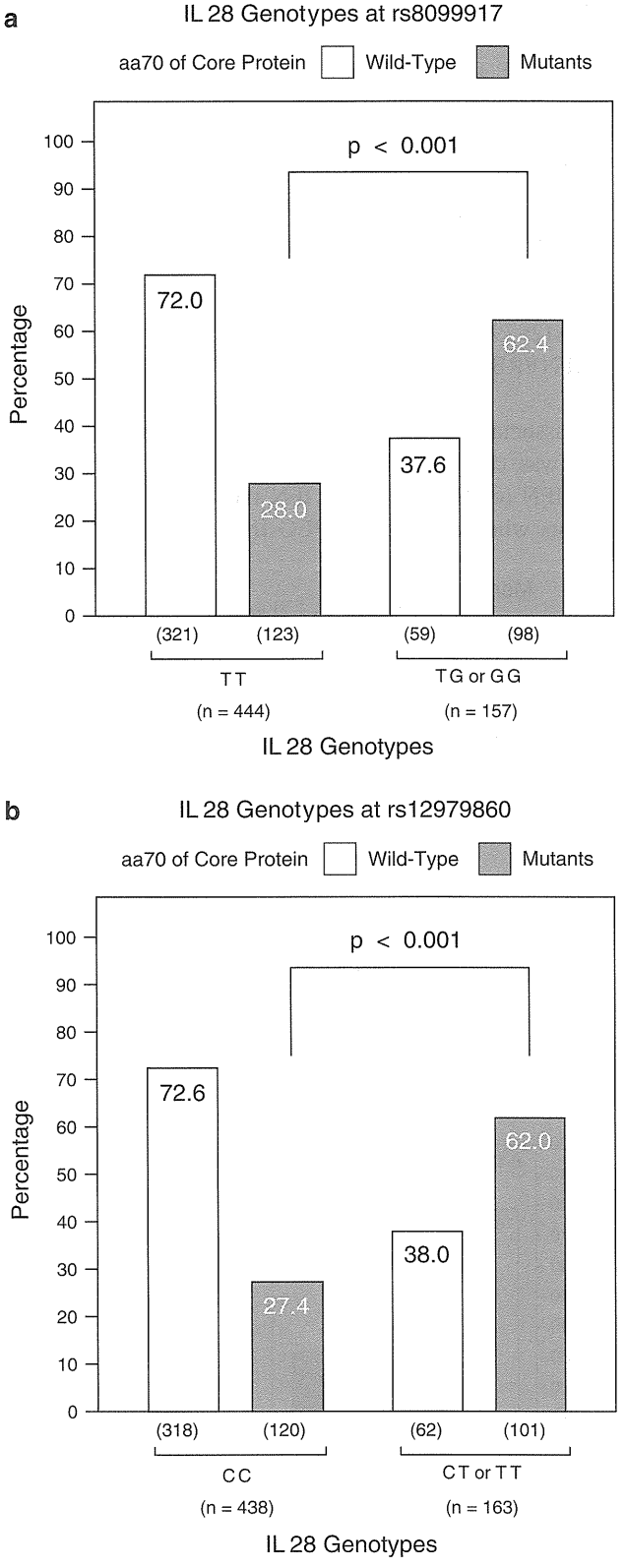

Fig. 3 Frequencies of aa70 substitution in the HCV-1b core protein in patients with IFN-sensitive and -resistant *IL28B* genotypes. Frequencies in *IL2B8* genotypes at rs8099917 (**a**) and rs12979860 (**b**) are shown for 380 patients infected with HCV-1b of the wild-type aa70 (arginine) and 221 patients infected with HCV-1b of mutant types (glutamine or histidine)

increase with age, both in men and women, and both in those with TT and those with TG/GG genotypes (Fig. 4a, b). Overall, aa70 mutants tended to be more frequent in women than men who had IFN-resistant *IL28B* genotypes. Thus, among the patients with TG/GG genotypes at rs8099917, aa70 mutants were the least frequent in men aged ≤50 years, and most common in women aged ≥51 years [11/18 (61.1%) vs. 35/50 (70.0%), $p = 0.561$].

Influence of *IL28B* genotypes at rs8099917 and rs12979860 on the response to PEG-IFN (or IFN)/RBV, or IFN monotherapy

The association of the two *IL28B* polymorphisms with SVR was compared in patients who had received either PEG-IFN (or IFN)/RBV, or IFN monotherapy. In HCV-1 patients who had received PEG-IFN (or IFN)/RBV, SVR was more frequent in those with TT than TG/GG at rs8099917 (64.0 vs. 34.2%, $p < 0.001$), as well as being more frequent in those with CC than CT/TT at rs12979860 (64.8 vs. 33.7%, $p < 0.001$) (Fig. 5a). In HCV-2 patients, by contrast, SVR did not differ between those with TT (or CC) and TG/GG (or CT/TT) at rs8099917 (or rs12979860) (Fig. 5a).

The profiles of SVR in HCV-1 and HCV-2 patients with different genotypes at rs8099917 and rs12979860, who had received IFN monotherapy, resembled those in patients treated with PEG-IFN (or IFN)/RBV (Fig. 5b). SVR to IFN monotherapy, in HCV-1 patients with IFN-sensitive *IL28B* genotypes, was 53.8% for TT at rs8099917 and 54.2% for CC at rs12979860. Table 3 compares the response to IFN-based treatments in subpopulations of patients who were treatment-naive, who had relapsed after previous treatments, or who were non-responders.

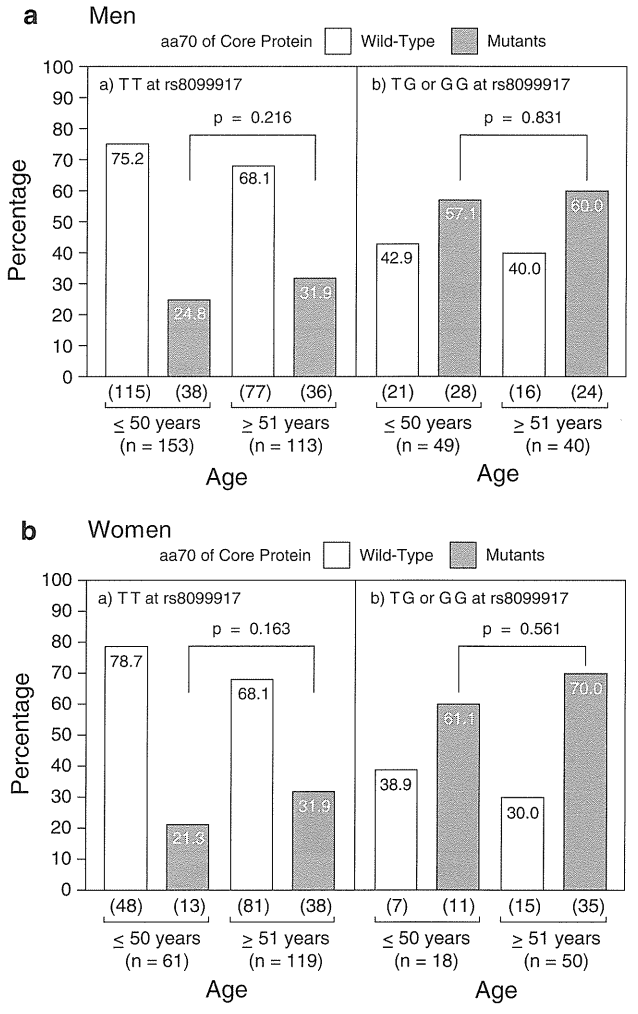

Fig. 4 Frequencies of aa70 substitution in the HCV-1b core protein in patients with IFN-sensitive and -resistant *IL28B* genotypes at rs8099917, stratified by sex and age. Frequencies of the wild-type aa70 (arginine) and mutant types (glutamine or histidine) are shown in male patients (**a**) and female patients (**b**) who were aged ≤50 or ≥51 years

Discussion

SNPs at rs12979860 and rs8099917, upstream of the *IL28B* gene in chromosome 19, are associated with SVR to PEG-IFN/RBV therapy in HCV-1 patients [10–12]. The SNP at rs8099917, defined by T or G, creates three *IL28B* genotypes, i.e., TT, TG, and GG. Likewise, the SNP at rs12979860 is specified by C or T, and creates three genotypes, CC, CT, and TT. Of the rs8099917 genotypes, TT was detected in 77.7%, TG in 20.6%, and GG in 1.7% of the 1,518 HCV patients in the present study, for whom *IL28*B polymorphisms at both loci had been determined. Such a distribution closely resembled that of genotypes at rs12979860, i.e., CC in 76.8%, CT in 21.3%, and TT in 1.9%. Reflecting such a close similarity in genotype distribution, rs8099917 and rs12979860 stood in strong linkage disequilibrium, with an r^2 value of 0.98. This is reasonably understood from their close positions, separated by merely 4,378 bp in a single haplotype block [20].

Despite their close proximity in the genome, the association between rs8099917 and rs12979860 is not perfect. Discordance between these two loci in *IL28B* genotypes was reported in nine of 708 (1.3%) patients with chronic hepatitis C in a study in Japan [21]; all nine of these patients possessed the IFN-sensitive TT genotype at rs8099917 and IFN-resistant non-CC genotypes (CT in all) at rs12979860. Also, among the 1,518 patients in the present study, 13 (0.9%) possessed TT at rs8099917 and CT at rs12979860 (Table 2). These 13 patients included six patients infected with HCV-1b who had received IFN-based treatment, three with PEG-IFN/RBV, one with IFN/RBV, and two with IFN monotherapy; none of them responded to the treatment. Our results would lend support to the view that *IL28B* genotypes at rs12979860, rather than those at 8099917, may reflect the

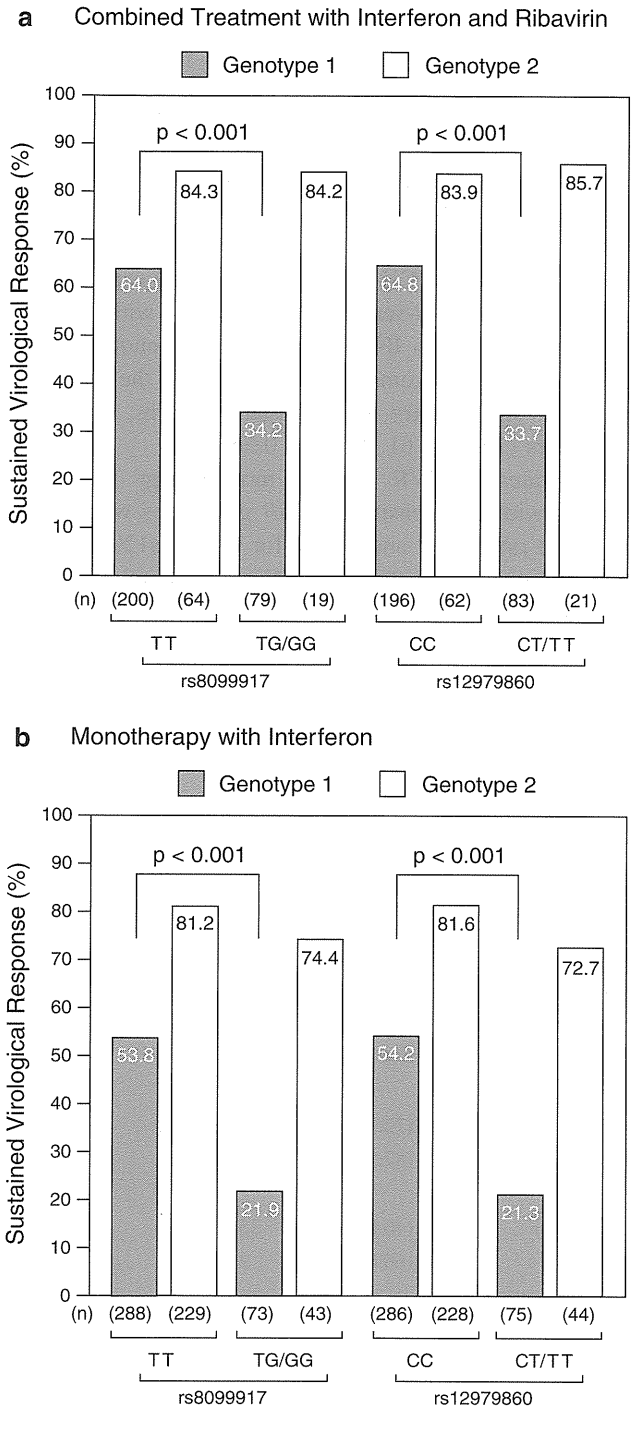

Fig. 5 Influence of *IL28B* polymorphisms on the response to IFN with or without ribavirin (RBV). SVR was compared in 279 HCV-1 and 83 HCV-2 patients treated with pegylated (PEG)-IFN (or IFN)/RBV (**a**); SVR was also compared in 361 HCV-1 and 272 HCV-2 patients treated with IFN monotherapy (**b**), who possessed IFN-sensitive (TT or CC) or IFN-resistant (TG/TT and CT/TT) genotypes at rs8099917 and rs12979860

response to IFN-based treatments in Japanese HCV-1 patients who possess genotypes discordant between the two loci, in terms of the response to IFN-based treatments.

Our view stands at variance with that of Ito et al. [21], who have declared that the rs8099917 polymorphism is better than other *IL28B* polymorphisms, including those at rs12979860, for predicting the response to PEG-IFN/RBV in Japanese patients with hepatitis C. It needs to be pointed out that their conclusion is based on the concept of "viral response", which encompasses SVR and transient virological response (TVR); TVR was defined by either: (1) the reappearance of HCV RNA after treatment was discontinued, or (2) a decrease of HCV RNA levels by >2 log copies/mL within the first 12 weeks after treatment. Hence, SVR was confirmed in only two of the seven (29%) HCV-1b patients with discordant genotypes; they all possessed TT at rs8099917 and CT at rs12979860. Of the remaining five patients, one (14% of the seven) did not respond, one (14%) responded transiently, and the other three (43%) were under treatment or without known response, and therefore, SVR was not confirmed in these three.

Albeit rare, genotypes that are discrepant at two loci, in terms of the response to IFN-based treatment, do occur in Japan. It would be necessary to evaluate SVR to PEG-IFN/RBV in many more HCV-1b patients in Japan, who have genotypes discrepant between rs8099917 and rs12979860, to determine *IL28B* polymorphisms that are closely associated with SVR.

Based on HapMap entries of individuals of many ethnic backgrounds (http://www.hapmap.org), the linkage disequilibrium between rs8099917 and rs12979860 varies widely among individuals of various ethnicities. The r^2 value ranges from 0.50 in Swiss, as well as 0.52 in Caucasians, to 0.07 in African Americans [20]. Reflecting a strong linkage disequilibrium between the two SNPs in the Japanese ($r^2 = 0.98$), both rs8099917 and rs12979860 are highly predictive of the response to triple therapy with PEG-IFN/RBV and telaprevir in HCV-1b patients [22]. However, such parallelism may be subject to ethnicity. In particular, rs8099917 might not be as predictive of the response to IFN as rs12979860 in African populations who are known to be resistant to IFN. This would have to be confirmed, and taken into consideration in clinical studies, in order that ethnicity-specific predictors of IFN response should be adopted.

It remains to be seen whether IFN-sensitive and -resistant *IL28B* genotypes are applicable to patients who are infected with HCV of genotypes other than genotype 1. It appears that *IL28B* genotypes influence the response to PEG-IFN/RBV in patients infected with genotypes 1 and 4, but not in those infected with genotypes 2 and 3 [23, 24]. In the present study, IFN-resistant genotypes [TG/GG at rs8099917 (CT/TT at 12979860)] occurred more frequently in patients with HCV-1 than in those with HCV-2 [24.7 vs. 17.0%, $p = 0.001$ (25.7 vs. 17.0%, $p = 0.001$)] (Fig. 2). HCV-1 would be cleared faster in the individuals with

IFN-sensitive than -resistant IL28B genotypes, thereby producing such differences.

Changes of aa70 in the core protein, from the wild-type (Arg) to mutant types (Gln/His), decrease the response to IFN-based therapies [15, 19]. The effects of core aa substitutions on the response to PEG-IFN/RBV therapy would be inferior to the effects of *IL28B* genotypes, because these substitutions influence the response only in HCV-1 patients with IFN-resistant *IL28B* genotypes. HCV-1b patients with IFN-sensitive *IL28B* genotypes (TT at rs8099917 or CC at rs12979860) respond to the triple therapy with PEG-IFN/RBV and telaprevir, irrespective of the wild-type or mutant aa70 in the HCV core protein; aa70 substitutions are associated with a better response in the patients with IFN-resistant *IL28B* genotypes exclusively [22]. In the natural course of HCV infection, in which innate immunity operates, the wild-type aa70 of Arg may enhance the clearance of HCV-1b. It comes as a surprise that HCV-1b with the IFN-sensitive, wild-type aa70 was detected more frequently in patients with IFN-sensitive than -resistant *IL28B* genotypes [72.0 vs. 37.6%, $p < 0.001$ (for rs8099917 in Fig. 3a); and 72.6 vs. 38.0%, $p < 0.001$ (for rs12979860 in Fig. 3b)], in agreement with the results of a recent report [25].

In women after the menopause, the response to IFN-based treatments decreases remarkably, and becomes poorer than that in men of the same age [26]. In our study, we found that IFN-resistant *IL28B* genotypes (TG/GG at rs8099917) increased with age in both men and women, probably because HCV-1 would be cleared earlier in the individuals with IFN-sensitive than in those with -resistant genotypes. Although IFN-resistant *IL28B* genotypes were most prevalent in women aged ≥51 years, the frequency was not significantly higher than that in men aged ≤50 years (70.0 vs. 61.1%, $p = 0.561$).

The rates of SVR to IFN monotherapy in HCV-1 patients with IFN-sensitive *IL28B* genotypes were 53.8% for TT at rs8099917 and 54.2% for CC at rs12979860. Of the 633 patients who received IFN monotherapy, SVR was achieved in 389 (61%) patients (Table 3); SVR in this study was higher than 27% in a previous report [27]. Such a

Table 3 Genotypes of the *IL28B* gene at rs8099917 and rs12979860 in the 362 patients treated with IFN or PEG-IFN combined with ribavirin and the 633 patients treated with IFN alone

	n	Genotypes of the *IL28B* gene					
		rs8099917			rs12979860		
		TT	TG	GG	CC	CT	TT
(A) Combined IFN (or PEG-IFN) and ribavirin[a]							
Sustained virological responders							
Genotype 1	155	128 (82.6%)	25 (16.1%)	2 (1.3%)	127 (81.9%)	26 (16.8%)	2 (1.3%)
Genotype 2	70	54 (77.1%)	14 (20.0%)	2 (2.9%)	52 (74.3%)	16 (22.9%)	2 (2.9%)
Relapsed after treatment							
Genotype 1	59	44 (74.6%)	15 (25.4%)	0	43 (72.9%)	16 (27.1%)	0
Genotype 2	8	7 (87.5%)	1 (12.5%)	0	7 (87.5%)	1 (12.5%)	0
Null-responders							
Genotype 1	65	28 (43.1%)	33 (50.8%)	4 (6.1%)	26 (40.0%)	35 (53.8%)	4 (6.2%)
Genotype 2	5	3 (60.0%)	2 (40%)	0	3 (60.0%)	2 (40%)	0
(B) IFN monotherapy[b]							
Sustained virological responders							
Genotype 1	171	155 (90.6%)	15 (8.8%)	1 (0.6%)	155 (90.6%)	15 (8.8%)	1 (0.6%)
Genotype 2	218	186 (85.3%)	29 (13.3%)	3 (1.4%)	186 (85.3%)	27 (12.4%)	5 (2.3%)
Relapsed after treatment							
Genotype 1	49	44 (89.8%)	5 (10.2%)	0	44 (89.8%)	5 (10.2%)	0
Genotype 2	30	25 (83.3%)	5 (16.7%)	0	24 (80.0%)	6 (20.0%)	0
Null-responders							
Genotype 1	141	89 (63.1%)	47 (33.3%)	5 (3.5%)	87 (61.7%)	48 (34.0%)	6 (4.3%)
Genotype 2	24	18 (75.0%)	6 (25.0%)	0	18 (75.0%)	6 (25.0%)	0

IL28B interleukin-28B, *IFN* interferon, *PEG-IFN* pegylated IFN

[a] IFN or PEG-IFN combined with ribavirin was administered for 48–72 weeks. All the patients possessed HCV RNA at high titers (>5.0 log IU/mL)

[b] IFN was given not only to patients with high HCV RNA titers but also to those with low titers

good response as that seen in our study could be ascribed to the low HCV RNA titers (<5 log IU/mL) in the patients indicated for IFN monotherapy, as well as to the frequency of genotype 2 being higher in patients with IFN monotherapy than in those with PEG-IFN (or IFN)/RBV (43.0 vs. 22.9%, $p < 0.001$).

In conclusion, a very close interrelationship, with an r^2 value of 0.98, was found between two sets of *IL28*B genotypes at rs8099917 and rs12979860 in 1,518 patients with HCV at a single hepatology center in Japan. However, the linkage disequilibrium between these two loci was not perfect, and 13 of the 1,518 (0.9%) patients possessed the IFN-sensitive TT genotype at rs8099917 and IFN-resistant non-CC genotypes at rs12979860 (CT in all). It is to be hoped that the results of this study will help in promoting further epidemiological and clinical research on a larger scale, to extend the applicability of the remarkable establishment of the GWAS and HapMap projects in the hepatology arena. Studies along this line are expected to establish the role of host genetic polymorphisms in regulating the response to exogenous as well as endogenous IFN.

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Conflict of interest None.

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HEPATOLOGY

Interleukin-28B single nucleotide polymorphism of donors and recipients can predict viral response to pegylated interferon/ribavirin therapy in patients with recurrent hepatitis C after living donor liver transplantation

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Key words
core, hepatitis C virus, interferon sensitivity-determining region, interleukin-28B, liver transplantation.

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Abstract

Background and Aim: Interleukin-28B (*IL28B*) single nucleotide polymorphism (SNP) influences viral response (VR) to interferon (IFN) therapy in patients with hepatitis C. We studied the relationship between VR and the *IL28B* polymorphism (rs8099917) in patients on long-term pegylated IFN plus ribavirin (PEGIFN/RBV) therapy for recurrent hepatitis C after living-donor liver transplantation (LDLT).

Methods: Thirty-five patients with recurrent hepatitis C after LDLT were treated with PEGIFN/RBV. We evaluated the effect of *IL28B* SNP on the outcome in 20 patients infected with hepatitis C virus genotype 1 who completed IFN therapy.

Results: The sustained VR (SVR) rate was 54% (19/35) for all patients; 46% (13/28) for genotype 1. The SVR rate of donors' TT group (major genotype) was higher than that of donors' TG + GG group (minor genotype) (73% *vs* 20%), while that of recipients' TT group was similar to that of recipients' TG + GG group (64% *vs* 50%). With regard to the combined effect of donors' and recipients' *IL28B* SNP, the SVR rates of TT : TT (donors' : recipients'), TT : TG + GG, TG + GG : any group were 81%, 50%, and 20%, respectively. The VR rate of TT : TT, TT : TG + GG and TG + GG : any group at 12 weeks were 28%, 0%, and 0%; those at 48 weeks were 70%, 50%, 20%, and those at the end of treatment were 100%, 50%, 20%, respectively. The multivariate analysis identified *IL28B* of donors : recipients (TT : TT) as the only independent determinant of SVR (odds ratio 15.0, $P = 0.035$).

Conclusion: Measurement of donors' and recipients' *IL28B* SNP can predict the response to PEGIFN/RBV therapy, and the donors' *IL28B* SNP might be a more significant predictor than that of the recipients.

Introduction

Hepatitis C virus (HCV) has infected 170 million people worldwide, and such infection sometimes progresses to liver cirrhosis and/or hepatocellular carcinoma.[1] The current treatment for patients infected with HCV genotype 1 (HCV-1) is the combination of pegylated interferon-α and ribavirin (PEGIFN/RBV) for 48 weeks.[2] However, this treatment results in sustained viral response (SVR) in only approximately 50% of patients with HCV-1 infection.

In a recent genome-wide association study, a single nucleotide polymorphism (SNP) upstream of the interleukin (IL)-28B (*IL28B*) gene on chromosome 19, coding for IFN-λ-3, was found to be strongly associated with SVR rate in treatment-adherent HCV-1 patients.[3–8] The G nucleotide of rs8099917 was associated with a poor response to treatment (minor allele), whereas a T nucleotide was found to be associated with a fair response to treatment (major allele) in Japanese patients.

HCV-related end-stage liver disease is currently the leading indication for liver transplantation (LT). However, the outcome of LT for patients with HCV-related liver disease has been less satisfactory than those with HCV-negative liver disease.[9–15] HCV recurrence is universal after LT with accelerated progression of liver fibrosis. Approximately 20–25% of HCV-positive

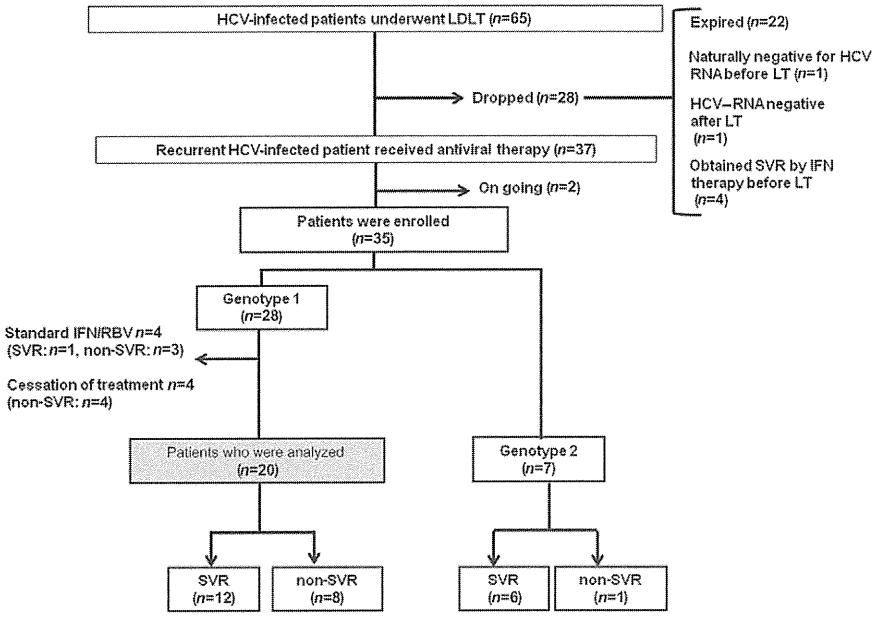

Figure 1 Flow diagram of patient recruitment. HCV, hepatitis C virus; IFN, interferon; LDLT, living-donor liver transplantation; LT, liver transplantation; RBV, ribavirin; SVR, sustained viral response.

patients develop cirrhosis within 5 years after LT, and approximately 50% within 10 years.[13,16,17] LT recipients with recurrent HCV are treated with a combination of PEGIFN/RBV for 48 weeks. However, eradication with IFN therapy after LT is hampered by the use of immunosuppressive agents, anemia, frequent side-effects, and the need to discontinue or reduce therapy. The outcome of PEGIFN/RBV antiviral therapy after LT is poor, with the SVR rate ranging from 10% to 30% for HCV-1-infected patients.[18–24]

However, Fukuhara *et al.*[8] reported that in patients with recurrent HCV infection after LT, combination analyses of SNP of *IL28B* in both the donor and recipient tissues and mutations in HCV–RNA allow the prediction of SVR to PEGIFN/RBV therapy.

We reported previously the effectiveness of the treatment of recipients with PEGIFN/RBV until HCV–RNA reaches undetectable levels, followed by continuation of treatment for at least 48 weeks (i.e. long-term IFN therapy).[25] Others also reported SVR rates of 34% and 50% under the same treatment, respectively.[26,27]

In the present study, we analyzed the viral response to long-term PEGIFN/RBV therapy in patients according to the major and minor genotypes of the polymorphic *IL28B* gene.

Methods

Patients. Sixty-five patients underwent living-donor LT (LDLT) for HCV-related end-stage liver disease between 2000 and January 2011. Among them, 22 patients died before the start of therapy, one was naturally negative for HCV–RNA before LT, one did not become positive for HCV–RNA after LDLT, and four obtained SVR by IFN therapy before LT, thus leaving 37 patients treated with IFN therapy at our institution. Of these, two patients are currently continuing antiviral therapy. A total of 35 patients were enrolled in this retrospective study.

There were 28 patients with HCV-1, and seven with HCV-2. The data of eight of the 28 patients with HCV-1 were excluded from the analysis due to the use of standard IFN/RBV in four patients, and cessation due to side-effects in four patients. Thus, the study included 20 patients with HCV-1 (Fig. 1).

Protocol of antiviral therapy. Patients received PEGIFN-α-2b subcutaneously once weekly combined with RBV (200 mg/day). The dose of the latter was increased to 800 mg/day in a stepwise manner, according to individual tolerance within the first 12 weeks of therapy. The combination PEGIFN/RBV therapy was continued for more than 48 weeks after the disappearance of serum HCV–RNA. At the end of the active treatment, patients were followed for another 24 weeks without treatment. In patients who remained positive for HCV–RNA in spite of treatment for more than 48 weeks, PEGIFN was switched to PEGIFN-α-2a, and treatment was continued as described earlier.

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the local ethics committees of all participating centers. Written, informed consent was obtained from all participating patients.

Assessment of therapy efficacy. HCV–RNA levels were measured using one of several reverse transcription–polymerase chain reaction (RT–PCR)-based methods (*Taq*Man RT–PCR test) at weeks 4, 8, and 12, and thereafter every 4 weeks of treatment, and at 24 weeks after the cessation of therapy.

SNP genotyping and quality control. Because the two reported significant *IL28B* SNP (rs8099917 and rs12979860) are in strong linkage disequilibrium, we examined only rs8099917 in this study. Some samples obtained from patients with HCV-1 were determined using the Illumina HumanHap610-Quad Genotyping BeadChip (San Diego, CA, USA), whereas the remaining samples were genotyped using the Invader assay (Third Wave Technologies, Madison, WI, USA), as described previously.[28,29]

Table 1 Characteristics of 20 patients with recurrent hepatitis C genotype 1 after living-donor liver transplantation

Age (years)†	58 (44–70)
Sex (male/female)	15/5
Body mass index (kg/m²)†	24.3 (18.8–42.2)
Viral load at therapy (LogIU/mL)†	6.6 (4.9–7.8)
Time from transplantation to therapy (months)†	4 (1–41)
No. mutations in the ISDR (0–1/2–5)	12/8
HCV core70 region (mutant/wild)	12/8
HCV core 91 region (mutant/wild)	10/10
Donors' *IL28B* genotype TT/TG + GG	15/5
Recipients' *IL28B* genotype TT/TG + GG	14/6
Combination of donors' and recipients' *IL28B* genotype (TT : TT/TT : TG + GG/TG + GG : TT/TG + GG : TG + GG)	11/4/3/2
Immunosuppression (tacrolimus/cyclosporine)	16/4
Adherence to PEGIFN ≥ 70/< 70 (%)†	11/9
Adherence to RBV ≥ 50/< 50 (%)†	8/12

†Values are median (range). HCV, hepatitis C virus; *IL28B*, interleukin-28B; ISDR, interferon sensitivity-determining region; PEGIFN, pegylated interferon; RBV, ribavirin.

Analysis of the nucleotide sequences of the core and non-structural 5A regions. The amino acid (aa) substitutions at aa 70 and aa 91 of the HCV core region and mutation at the IFN sensitivity-determining region were analyzed in the non-structural 5A region of HCV by the direct sequencing method, as described previously by our group.[25,30,31] Samples after LT were used.

Statistical analysis. Non-parametric tests (χ^2-test and Fisher's exact probability tests) were used to compare the characteristics of the groups. Univariate logistic regression analysis was used to determine those factors that significantly contributed to early viral dynamics. The odds ratios and 95% confidence intervals were also calculated. All P-values < 0.05 using two-tailed tests were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) in the univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Statistical analyses were performed using PASW 18 statistical software (SPSS, Chicago, IL, USA).

Results

Patient characteristics. Table 1 shows the baseline characteristics of the 20 patients with recurrent hepatitis C after LT who completed PEGIFN/RBV treatment. The median age of the patients (15 males and 5 females) was 58 years, and the median body mass index was 24.3. The median latency between transplantation and the initiation of antiviral therapy was 4 months. The median pretreatment serum HCV–RNA viral load was 6.6 LogIU/mL. The *IL28B* genotype (rs8099917) of the donors was TT in 15 patients, and TG + GG in five patients, whereas that of the recipients was TT in 14, and TG + GG in six. Immunosuppressive therapy included tacrolimus in 16, and cyclosporine in four.

Efficacy and tolerance of IFN therapy and side-effects. Figure 1 shows the effects of IFN therapy according to genotype. The SVR rate was 54.2% (19/35) for all patients. Among the patients infected with HCV-1, one of eight patients who were treated with mono-IFN/RBV or ceased treatment had SVR. Twelve of 20 patients with HCV-1 who completed IFN therapy achieved SVR. Thus, the SVR rate was 46.4% (13/28) for those with HCV-1, and 85.7% (6/7) with HCV-2. In patients with HCV-1, four ceased IFN therapy due to adverse effects. These included general fatigue in one, rejection in two, and cerebral hemorrhage in one patient.

Relationship between IL28B and viral response in patients infected with HCV genotype 1. Data or eight of 28 patients with HCV-1 were excluded from the analysis due to standard-IFN plus RBV in four patients, and the cessation of IFN therapy due to adverse effects in four patients. Thus, the data of 20 patients with HCV-1 were available for the analysis of *IL28B*.

In the donors, the SVR rate of the TT group (73.3% [$n = 11/15$]) was higher than that of the TG + GG group (20% [$n = 1/5$], $P = 0.053$, Fig. 2a). In the recipients, the SVR rate of the TT group (64.2% [$n = 9/14$]) was similar to that of the TG + GG group (50% [$n = 3/6$]) (Fig. 2b). The SVR rate of the TT : TT group (donors' *IL28B* : recipients' *IL28B*) was 81.8% ($n = 9/11$), which was higher than the SVR rate of the TT : TG + GG group (50% [$n = 2/4$], Fig. 2c). The SVR rate of the TG + GG : any group (donors' *IL28B* : recipients' *IL28B* of either TT or TG + GG) was 20% ($n = 1/5$), which was lowest among the three groups. There was significant difference between the SVR of the TT : TT group and TG + GG : any group ($P = 0.036$). We also analyzed the viral response (VR) rate according to the combination of donors' and recipients' *IL28B*. The VR rates of TT : TT, TT : TG + GG, TG + GG : any group at 12 weeks were 28%, 0%, and 0%; those at 48 weeks were 70%, 50%, and 20%; and those at the end of treatment were 100%, 50%, and 20%, respectively. The VR rate of the TT : TT group was 63.6% ($n = 7/11$), which was higher than the VR rate of the TG + GG : any group (0% [$n = 0/5$]) at 24 weeks. The VR rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the VR rate of the TG + GG : any group (20% [$n = 1/5$]) at the end of treatment. The SVR rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the SVR rate of the TG + GG : any group (20%, $n = 1/5$) at 24 weeks at the end of treatment (Fig. 3).

Analysis of factors associated with SVR in HCV-1 patients with recurrent hepatitis C. The univariate analysis identified three parameters that correlated with SVR either significantly or marginally: the combination of donors' and recipients' *IL28B* (TT : TT $P = 0.037$), donors' *IL28B* (TT genotype; $P = 0.053$), and adherence to RBV therapy (≥ 50; $P = 0.076$, Table 2). The combination of donors' and recipients' *IL28B* (TT : TT genotype) and adherence to RBV (> 50; $P = 0.076$) were entered into the multiple logistic regression analysis to identify significant independent predictive factors. The multivariate analysis identified the combination of donors' and recipients' *IL28B* (TT : TT) as the only significant and independent factor that influenced the SVR: (odds ratio: 15.0, 95% CI: 1.2–185.1, $P = 0.035$).

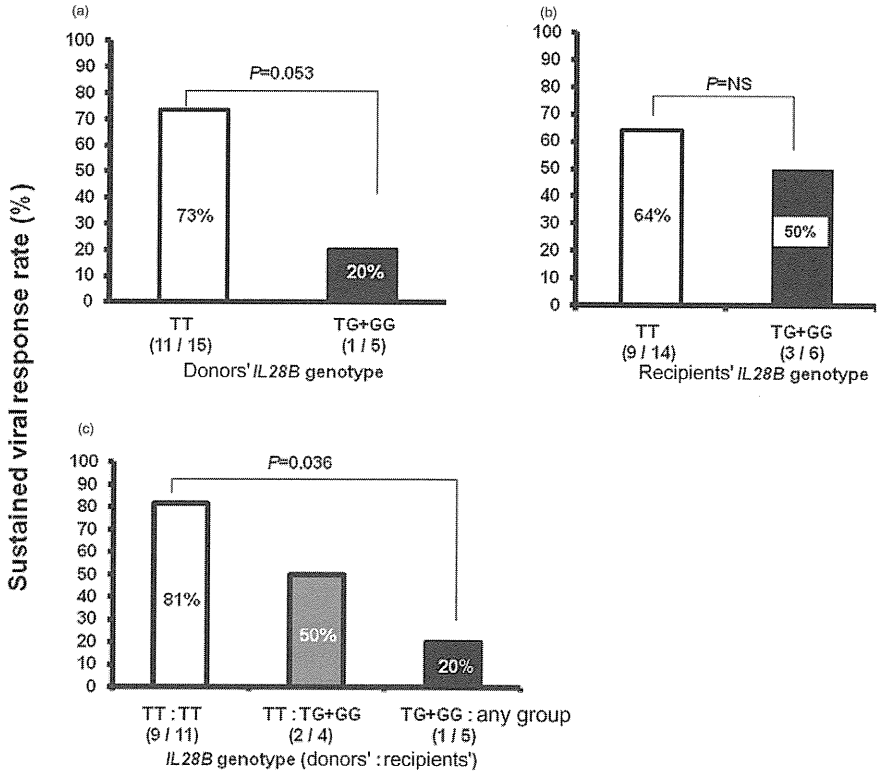

Figure 2 Sustained viral response rates according to (a) donors' interleukin-28B (*IL28B*), (b) recipients' *IL28B*, and (c) donors' and recipients' *IL28B* in patients infected with hepatitis C virus genotype 1. TT : TT group (donors' *IL28B* TT: recipients' *IL28B* TT), TT : TG + GG group (donors' *IL28B* TT: recipients' *IL28B* TG + GG), TG + GG : any group (donors' *IL28B* TG + GG: recipients' *IL28B* either TT or TG + GG). NS, not significant.

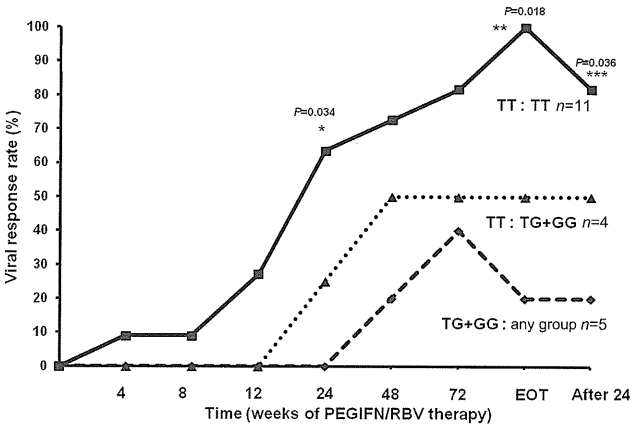

Figure 3 Viral response rates according to donors' and recipients' interleukin-28B (*IL28B*) genotyping. TT : TT group (donors' *IL28B* TT: recipients' *IL28B* TT), TT : TG + GG group (donors' *IL28B* TT: recipients' *IL28B* TG + GG), TG + GG : any group (donors' *IL28B* TG + GG: recipients' *IL28B* either TT or TG + GG). *Viral rate (VR) of the TT : TT group was 63.6% ($n = 7/11$), which was higher than the VR rate of the TG + GG : any group (0%, $n = 0/5$) at 24 weeks. **VR rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the VR rate of the TG + GG : any group (20%, $n = 1/5$) at the end of treatment (EOT). ***Sustained VR (SVR) rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the SVR rate of the TG + GG : any group (20%, $n = 1/5$) at 24 weeks at the EOT. PEGIFN, pegylated interferon; RBV, ribavirin.

Discussion

The SVR rate has improved since the introduction of PEGIFN/RBV for patients who undergo LT for HCV-related end-stage liver disease. The current estimated SVR rate for LT patients with a history of HCV-1 infection is 30–50%.[21–24,26,27] These results are much better than those reported in the 1990s and early 2000s; however, more than half of recipients still suffer from recurrent chronic hepatitis C.

Although many studies have determined the predictive factors of the viral response for PEGIFN/RBV among patients with chronic hepatitis C, recent molecular biological analyses and genome-wide analyses of the human genome have identified genetic variations of *IL28B* and amino-acid substitution of HCV core 70 as the most significant predictive factors for IFN response.[3–5,32,33] *IL28B* encodes a cytokine distantly related to type I IFN and the IL-10 family. It has been reported that the expression level of the *IL28* gene in peripheral blood mononuclear cells is significantly lower in individuals with minor alleles than in individuals with major alleles.[5]

Several studies have determined the predictive factors for the viral response to PEGIFN/RBV in patients with recurrent post-LT hepatitis C viral infection, and recent molecular and genome wide analyses of the human genome have demonstrated that genetic variation of *IL28B* is the most significant predictive factor of the response to IFN.[8,34–37] In the present study, we examined whether the same factors can also predict the response to PEGIFN/RBV in LT recipients. Several groups have reported that recipients' and donors' *IL28B* influenced the SVR to PEGIFN/RBV in patients with recurrent hepatitis C after LT.[8,36,37] Furthermore, others

Table 2 Univariate analysis of factors associated with sustained viralresponse (SVR) during interferon therapy in genotype 1 patients with recurrent hepatitis C

	SVR ($n = 12$)	Non-SVR ($n = 8$)	*P*-value
Age (years)†	60 (44–69)	57 (47–65)	0.48
Sex (male/female)	10/2	5/3	0.3
Body mass index (kg/m^2)†	24.1 (21.4–26.5)	24.2 (18.9–42.2)	0.4
Viral load at therapy (LogIU/mL)†	6.3 (5.8–6.6)	6.6 (5.9–7.2)	0.52
Time from transplantation to therapy (months)†	4 (1–41)	3 (1–6)	1.7
No. mutations in the ISDR (0–1/2–5)	7/5	5/3	1.0
HCV core70 region (mutant/wild)	7/5	5/3	1.0
HCV core 91 region (mutant/wild)	7/5	3/5	0.6
Donors' *IL28B* genotype TT/TG + GG	11/1	4/4	0.053
Recipients' *IL28B* genotype TT/TG + GG	9/3	5/3	0.6
Donors' and recipients' *IL28B* genotype TT : TT/others	9/3	2/6	0.037
Immunosuppression (tacrolimus/cyclosporine)	9/3	7/1	1.0
Adherence to PEGIFN ≥ 70/< 70 (%)†	8/4	3/5	0.3
Adherence to RBV ≥ 50/< 50 (%)†	7/5	1/7	0.076

†Values are median (range). HCV, hepatitis C virus; *IL28B*, interleukin-28B; ISDR, interferon sensitivity-determining region; PEGIFN, pegylated interferon; RBV, ribavirin.

reported that donors' *IL28B* influenced the SVR in patients treated with PEGIFN/RBV for recurrent hepatitis C after LT,[34] and that recipients' *IL28B* influenced the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C.[35,36]

The results of the present study indicate that both donors' and recipients' *IL28B* influence the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C. Both recipients' and donors' *IL28B* influenced the SVR to PEGIFN/RBV in recurrent hepatitis C after LT; however it is not clear whether the recipients' or donors' *IL28B* influenced the SVR to PEGIFN/RBV.

However, the donors' *IL28B* might have influenced the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C more than the recipients' *IL28B*. This conclusion is based on the following results: although the SVR rate of the TT group (64.2%) was similar to that of the TG + GG group (50%), according to the recipients' *IL28B*, the SVR rate of the TT group (73.3%) was higher than that of the TG + GG group (20%), according to the donors' *IL28B*. Furthermore, the VR rates of TT : TT, TT : TG + GG, TG + GG : any group at 12 weeks were 28%, 0%, and 0%; those at 48 weeks were 70%, 50%, and 20%; and those at the end of treatment were 100%, 50%, and 20%, respectively. That is, the time to VR of the TG + GG : any group was the latest among the three groups. Lange *et al.* reported that donors' *IL28B* influenced the SVR in patients treated with PEGIFN/RBV for recurrent hepatitis C after LT.[34] In this regard, Hiraga *et al.*[38] reported that IFN-stimulated gene expression levels in mice livers measured at 2 weeks after IFN treatment were significantly higher in mice transplanted with donor human hepatocytes (*IL28B*; TT) than from donor (*IL28B*; TG + GG) mice. Furthermore, previous studies reported that the expression level of IFN- λ-3, coded for the *IL28B* gene, was higher in hepatocytes than hematopoietic cells.[39]

However, we demonstrated the feasibility of treatment of LT recipients with PEGIFN/RBV until HCV–RNA reached undetectable levels, followed by the continuation of treatment for at least 48 weeks (i.e. long-term IFN therapy). In fact, the SVR rate (50%) of the recipients' *IL28B* TG + GG group was higher than that reported by others[8] (SVR rate: 11%). Furthermore, the SVR rate (81%) of the combination of donors' and recipients' *IL28B* (TT : TT) group was higher than that reported by Fukuhara *et al.*[8] (SVR rate: 56%). However, the SVR rate of the donors' *IL28B* TG + GG group (SVR rate: 20%) was similar to that reported by Fukuhara *et al.*[8] (SVR rate: 9%). We believe that the treatment of LT recipients with PEGIFN/RBV until HCV–RNA reaches undetectable levels, followed by the continuation of treatment for at least 48 weeks, is not useful for donors with *IL28B* TG + GG.

In Japan, LDLT is more common than orthotopic LT. In finding a suitable donor, it is better to select a donor with TT of the *IL28B* gene than a TG or GG donor. In conclusion, our results demonstrated the suitability of donors with the TT *IL28B* genotype, and that long-term PEGIFN/RBV therapy seems useful for recipients of LDLT who develop recurrent hepatitis C after transplantation.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study

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Abstract
Background We investigated whether the administration of maintenance doses of interferon prevented hepatocellular carcinoma (HCC) in patients with chronic hepatitis C.
Methods Study 1: A multicenter, retrospective, cooperative study was carried out to determine whether long-term administration of low-dose peginterferon alpha-2a (PegIFNα-2a) prevented HCC development in patients with chronic hepatitis C. In total, 594 chronic hepatitis C patients without a history of HCC were enrolled and treated with 90 μg PegIFNα-2a administered weekly or bi-weekly for at least 1 year. Study 2: HCC developed in 16 of 99 additional patients without PegIFNα-2a treatment during 3.8 years of observation. A propensity-matched control study was then carried out to compare the incidence of

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HCC between the 59 patients who received low-dose PegIFNα-2a (PegIFNα-2a group) and 59 patients who did not receive PegIFNα-2a treatment (control group), matched for sex, age, platelet count, and total bilirubin levels.
Results Study 1: HCC developed in 49 patients. The risk of HCC was lower in patients with undetectable hepatitis C virus RNA, ≤40 IU/L alanine aminotransferase (ALT), or ≤10 ng/L alpha-fetoprotein (AFP) 24 weeks after the start of therapy. Study 2: The incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group.
Conclusions Low-dose and long-term maintenance administration of PegIFNα-2a decreased the incidence of HCC in patients with normalized ALT and AFP levels at 24 weeks compared with patients without normal ALT and AFP levels.

Keywords Chronic hepatitis C · Hepatocellular carcinoma · Peginterferon

Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, often develops because of long-term hepatitis B or C virus infection [1, 2]. In particular, chronic hepatitis C and hepatic cirrhosis increase the risk of HCC; the annual incidence of tumor development in such patients may be as high as 2–4 % [3–5]. The incidence of HCC decreases in patients who achieve a sustained virological response (SVR) to interferon (IFN) treatment, although the incidence remains high in non-SVR patients [6–9]. A detailed analysis of HCC development revealed that chronic hepatitis C patients aged 65 years or more, especially those with advanced fibrosis of the liver, were at an increased risk of developing HCC [10]. For patients 65 years or older with advanced liver fibrosis, the dose of ribavirin is often reduced or the agent is discontinued, resulting in lower SVR rates in those with discontinuation of ribavirin. Establishing an effective treatment strategy for preventing the development of HCC is important for these high-risk patients.

Factors related to the development of HCC have been analyzed in patients who did not achieve an SVR even after IFN treatment; advanced fibrosis of the liver and high levels of serum alanine aminotransferase (ALT), and alpha-fetoprotein (AFP) are risk factors for HCC development [11, 12]. A randomized controlled trial was conducted in Western countries to determine whether combined peginterferon and ribavirin treatment with weekly administration of 90 μg peginterferon alpha-2a (PegIFNα-2a) could prevent HCC in non-responders. A 3.5-year follow up showed that administration of a maintenance dose of PegIFNα-2a did not reduce tumor incidence in these patients [13]. However, after 8.5 years of observation, the incidence of HCC was decreased among those in the PegIFNα-2a group with cirrhosis [14]. Meanwhile, Bruix et al. [15] reported that maintenance therapy with PegIFNα-2b did not prevent HCC in chronic hepatitis C patients with cirrhosis. In Japan, long-term low-dose administration of natural IFN has been reported to decrease the incidence of HCC [16]. In light of these conflicting results, investigations should be carried out in a large number of patients with chronic hepatitis C to resolve the question of whether IFN treatment prevents the development of HCC.

We carried out a multicenter retrospective cooperative study of patients with chronic hepatitis C to determine whether those treated with 90 μg PegIFNα-2a without ribavirin had a reduced incidence of HCC compared with those not treated with IFN.

Patients and methods

Study 1: analysis of risk factors for HCC in patients treated with long-term low-dose-PegIFNα-2a

In total, at 21 hepatitis centers throughout Japan, 743 patients with hepatitis C who had received 90 μg of PegIFNα-2a therapy weekly or bi-weekly for 1 year or more without having received the full dose (180 μg) since December 2003 were examined retrospectively for the development of HCC. The end of enrollment in this study was the end of December 2008 and the end of follow up was the end of December 2010. Patients with a history of HCC before the start of therapy and those with a therapy period of less than 48 weeks were excluded, leaving 594 patients who had undergone long-term administration of PegIFNα-2a for analysis. At the 21 centers involved in this

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