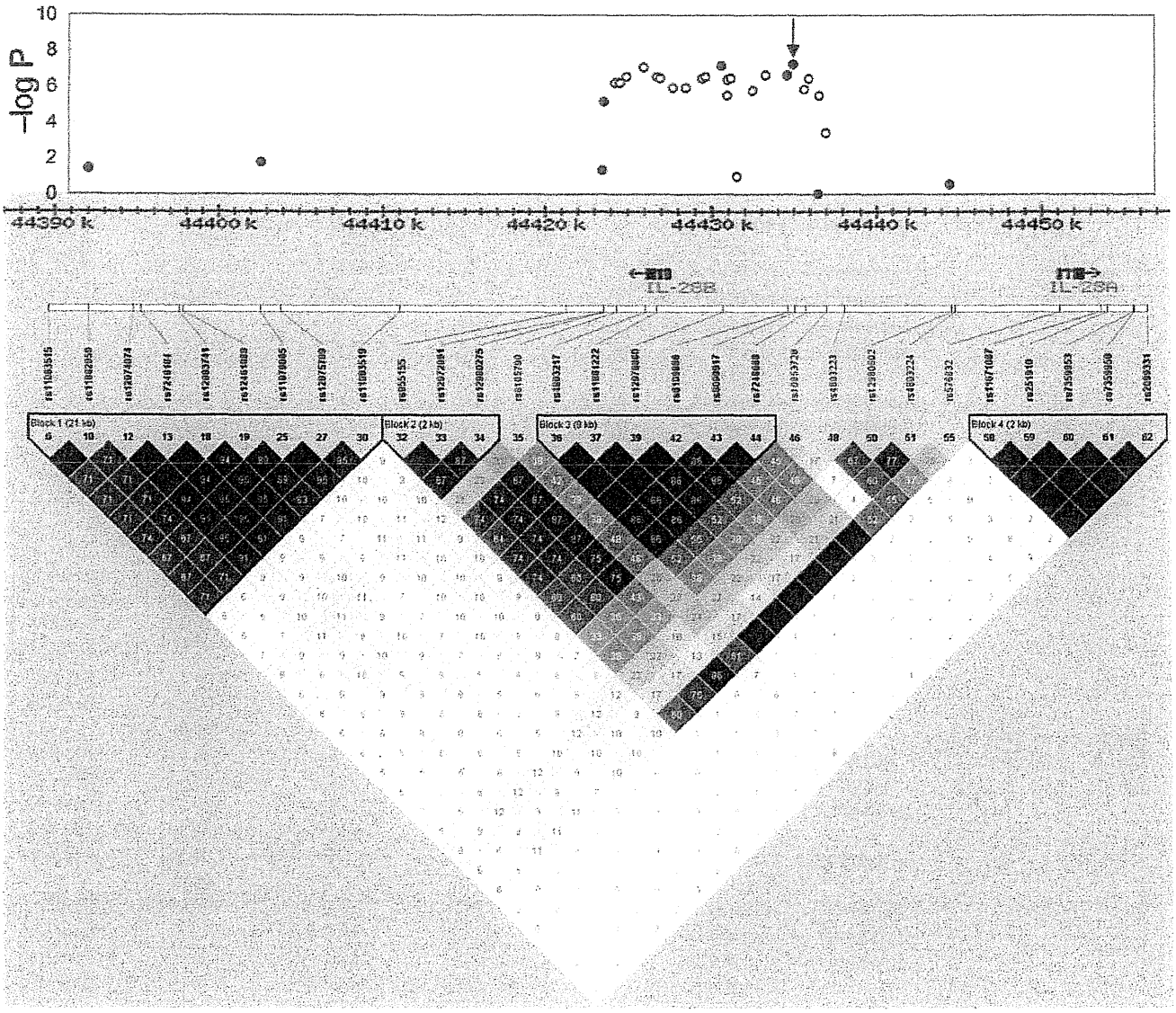

Fig. 1. LD mapping and probabilities for SNPs around the IL-28B locus. The lower panel depicts the haplotype structure around the IL-28B locus. Phase II HapMap JPT genotype data and the HAPLOVIEW program were used to define the LD block. The solid arrow represents the landmark SNP (rs8099917). The block structure is based on the criteria established by Gabriel *et al.* (2002) using pairwise estimates of the disequilibrium coefficient (r^2). Dark grey, regions with high r^2 values; light grey, regions with low r^2 values. The upper panel shows *P*-value plots of case–control association results. *P*-values by the Cochran–Armitage trend test are plotted on a $\log_{10}$ scale. Closed and open dots represent SNP *P*-value in the genome-wide association study (GWAS) and Set-1 fine-mapping stages, respectively.

Despite the fact that the total dose of PEG-IFN or IFN that was administered to each patient varied according to HCV genotype and viral titre based on the current standard regimen, the effect of the therapy in all patient cohorts showed significant association with the SNP genotypes. For replication analyses, we also found significant associations in the case of IFN monotherapy (Sets 3 and 4 in Table 1). Although combination therapy with PEG-IFN and ribavirin is currently the recommended treatment for chronic HCV infection, such analysis is still important because patients cannot always receive PEG-IFN and/or ribavirin due to severe side effects or other reasons. Consistent with earlier findings, the heterozygous genotypes show an intermediate effect level of IFN resistance.

Recent studies have demonstrated that genotype frequency of IL-28B polymorphism varied considerably by ethnicity, which may explain much of the difference in response between different population groups and support the prediction that favourable alleles would be very much more

Table 3. Results of resequencing and fine-mapping of the IL-28B locus

Gene	Location	SNP Id	Position	Alleles		Minor allele frequency (%)	r^{2}*	P† (GWAS set)
			Chr 19	Major	Minor			
		rs11083519	44411103	T	A	46.9	0.04	
		rs60635720	44411511	A	G	47.9	0.05	
		rs57593994	44411591	G	C	46.9	0.04	
			44412803	C	G	6.3	0.02	
		rs12976234	44412840	T	A	49.0	0.06	
		rs8107090	44413755	A	T	49.0	0.07	
		rs35408086	44418650	A	G	47.9	0.04	
		rs11883239	44419320	A	G	46.9	0.04	
		rs11883201	44419330	G	A	47.9	0.02	
		rs12979140	44420380	C	T	49.0	0.00	
		rs12460005	44421017	G	C	33.3	0.10	
		rs955155	44421319	G	A	13.5	0.64	4.77e–02
		rs958039	44422141	T	A	17.7	0.66	4.79e–05
		rs35790907	44422595	A	T	17.7	0.66	1.24e–04
		rs12972991	44423587	A	C	17.4	0.69	4.53e–02
		rs12980275	44423623	A	G	18.8	0.73	6.97e–06
			44424552	A	G	18.8	0.87	6.15e–07
			44424592	G	A	19.8	0.81	6.95e–07
			44423744	T	C	19.8	0.81	8.78e–05
		rs8105790	44424341	T	C	16.7	1.00	6.33e–07
			44424963	C	T	19.1	0.87	3.21e–07
		rs4803217	44426060	C	A	18.8	0.87	9.18e–08
IL-28B	Exon 5		44426165	G	A	5.2	0.01	
IL-28B	Intron 2	rs11881222	44426763	A	G	18.8	0.87	3.12e–07
IL-28B	Exon 2	rs8103142	44426946	T	C	18.8	0.87	3.91e–07
		rs28416813	44427484	C	G	18.8	0.87	8.08e–07
			44427759	C	T	18.8	0.87	1.17e–06
		rs8107030	44428559	A	G	18.8	1.00	1.15e–06
			44428927	A	C	6.3	0.01	
		rs73930703	44429353	C	T	18.8	0.87	7.16e–07
		rs11882871	44429450	A	G	18.8	0.87	3.46e–07
		rs12971396	44429706	C	G	15.6	0.93	3.20e–07
			44430157	A	C	17.7	0.79	1.19e–05
		rs12979860	44430627	C	T	17.7	0.94	7.32e–08
		rs4803221	44430969	C	G	15.6	0.93	4.67e–07
			44430995	TT	G	18.8	0.87	3.12e–06
		rs4803222	44431193	G	C	18.8	0.87	4.03e–07
		rs1549928	44431549	T	C	6.3	0.01	1.20e–01
			44432515	C	A	14.1	0.74	1.68e–06
		rs12983038	44432964	G	A	12.8	0.41	
			44433305	C	T	13.8	0.64	2.33e–07
		rs8109886	44434602	C	A	19.8	0.81	2.41e–07
		rs8109889	44434610	C	T	16.7	1.00	9.67e–07
		rs8113007	44434943	A	T	18.8	0.87	8.20e–07
		rs8099917	44435005	T	G	16.7	–	6.62e–08
		rs7248668	44435661	G	A	16.0	1.00	1.61e–06
			44435942	–	GA	16.7	0.81	4.02e–07
		rs10612351	44436647	AC	–	19.8	0.74	3.32e–06
			44436767	C	T	5.2	0.01	
			44436898	C	T	14.6	0.71	3.73e–03
		rs10853728	44436986	C	G	29.2	0.39	4.38e–04
		rs57401101	44437536	–	C	37.5	0.25	
		rs61665163	44438053	–	AAT	13.5	0.00	
		rs4803223	44438059	A	G	8.3	0.19	1.23e–01

Table 3. cont.

Gene	Location	SNP Id	Position	Alleles		Minor allele frequency (%)	r^{2}*	P† (GWAS set)
			Chr 19	Major	Minor			
			44438119	A	G	12.5	0.02	
		rs35811883	44438306	T	C	18.8	0.02	
		rs56116812	44438930	G	A	12.5	0.00	
		rs8101517	44439581	A	C	21.9	0.07	
			44440345	C	T	6.4	0.01	
			44441174	G	A	6.3	0.00	
		rs10424607	44441762	A	C	20.8	0.09	
		rs10407161	44443231	A	C	44.0	0.13	
		rs6508852	44444102	A	G	27.1	0.07	
			44445071	A	C	11.5	0.03	
		rs6508853	44445966	T	C	6.3	0.01	
			44448557	A	G	5.2	0.01	
		rs2596807	44449766	T	C	5.2	0.01	
			44450018	A	G	20.8	0.10	
IL-28A	Intron 3	rs62120533	44451758	T	G	6.3	0.00	
IL-28A	Intron 3		44451814	T	C	6.3	0.00	
IL-28A	Exon 5	rs59746524	44452275	T	C	12.5	0.32	

*Correlation coefficient (r^2) with the landmark SNP (rs8099917).
†Statistical comparisons were performed using the Cochran–Armitage trend test.

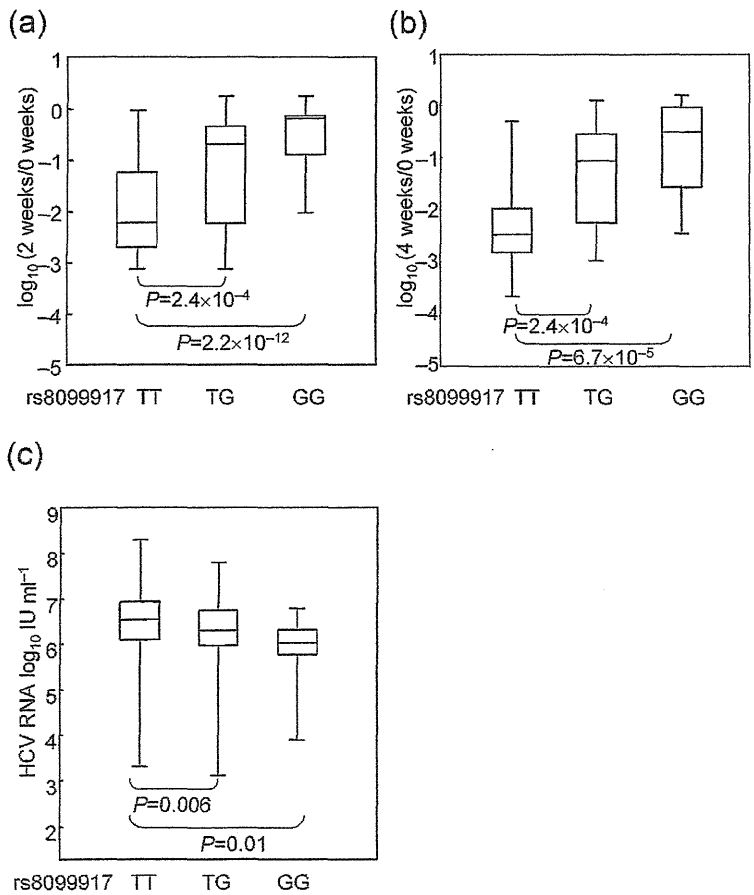

Fig. 2. Association between early response to combination therapy and the top SNP rs8099917 in the IL-28B locus. Viral reduction and basal viral load are shown according to rs8099917 genotype. (a) Viral response at 2 weeks; (b) viral response at 4 weeks; (c) baseline value of viral load. The box indicates the inter-quartile range (25 and 75 %) and the line within the box represents the median. The whiskers represent the range.

Table 4. Predictive factors for treatment outcome in patients with HCV genotype 1b determined by multivariate logistic regression analysis

Variable	*P*	OR	95 %CI
rs8099917	0.0022	0.24*	0.094–0.60
log viral load	0.0100	0.33†	0.140–0.77
Age	0.0180	0.67‡	0.480–0.93
Fibrosis	0.0200	0.40§	0.190–0.86

*Dominant model (GG+GT vs TT).
†Per 1 log increase.
‡Per 10 years increase.
§Severe versus mild.

common in Asian populations than in other ethnic groups (Ge *et al.*, 2009; Thomas *et al.*, 2009). We genotyped rs8099917 and rs12979860 in 282 healthy Japanese volunteers, and the allele frequencies of these SNPs were found to be comparatively high (91 % for both), consistent with the relatively high SVR rates of East Asian populations. Sarrazin *et al.* (2010) reported that genotype 1-infected patients had a lower frequency of the favourable genotype of rs12979860 compared with healthy controls and that genotypes 2/3 patients had an intermediate frequency. This finding was confirmed in this study (Table 2). On the other hand, they reported that the rs12979860 CC genotype, but not the rs8099917 and rs12980275 genotypes was significantly associated with SVR to (PEG-) IFN-α/ribavirin combination therapy in genotypes 2/3-infected patients. In this study, however, rs12979860 and rs8099917 were both strongly associated with SVR. This discrepancy may be due to differences among populations in the degree of linkage disequilibrium between these SNPs, and, in fact, these SNPs were in strong linkage disequilibrium in our healthy control subjects as well.

Of note in our study was that there was a significant difference in the initial viral reduction based on SNP genotype in patients treated with the PEG-IFN and ribavirin combination therapy. This finding holds important implications for the strategy of IFN-based anti-HCV therapy.

The mechanism by which polymorphisms within this locus influence the outcome of therapy has not been established. One of the polymorphisms in strong linkage disequilibrium with the two SNPs is a missense substitution within the IL-28B coding region. Recently, Urban *et al.* (2010) reported that there was no difference in antiviral potency between wild-type IL-28B and amino-acid substituted variant *in vitro* using an HCV replicon system. On the other hand, it has been reported that genetic variation in the IL-28B locus is associated with expression levels of IL-28B (plus IL-28A) IFN in peripheral blood mononuclear cells (Suppiah *et al.*, 2009; Tanaka *et al.*, 2009), although findings by Ge *et al.* (2009) are contradictory. Using a specific assay, Honda *et al.* (2010) and Urban *et al.* (2010) reported that hepatic IL-28B expression was not associated with IL-28B genotype in HCV-infected patients. Post-transcriptional regulation might be affected by the IL-28B variant, which could not be evaluated by the RT-PCR method, although further functional studies are needed to address this issue. IL-28A, IL-28B and IL-29 (IFN-λ II, III and I, respectively) are recently identified IFNs (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003) similar to type I IFNs in terms of biological activity and mechanism of action, but differ structurally and genetically (Maher *et al.*, 2008). The antiviral effects of IFN-λ against hepatitis B virus and HCV have already been reported (Robek *et al.*, 2005). Furthermore, IFNs α and λ act synergistically against HCV (Marcello *et al.*, 2006; Pagliaccetti *et al.*, 2008; Zhu *et al.*, 2005). Whether such synergy actually modulates the effect of IFN therapy against HCV in the liver requires further study. In this regard, recent studies have identified higher pre-treatment hepatic levels of IFN-stimulated genes in NRs than in responders (Chen *et al.*, 2005; Feld *et al.*, 2007; Sarasin-Filipowicz *et al.*, 2008). In addition, association between intrahepatic levels of ISG expression and IL-28B genotype have also been reported (Honda *et al.*, 2010; Urban *et al.*, 2010). On the other hand, the IL-28B variant has recently been reported to be associated with treatment response following liver transplantation in patients infected with HCV (Charlton *et al.*, 2011; Fukuhara *et al.*, 2010). These findings suggest that the IL-28B polymorphism may be associated with innate as well as adaptive immunity.

The OR for the effect of the PEG-IFN and ribavirin combination therapy associated with IL-28B polymorphisms in this study is much lower than the previous report that analysed a similar effect in Japanese patients (Tanaka *et al.*, 2009) and is closer to those of Caucasian, Hispanic and African patients (Ge *et al.*, 2009; Suppiah *et al.*, 2009). Tanaka *et al.*'s non-viral responder (NVR) criteria seem to be very constrained compared with those of other groups including ours. To evaluate possible effects of such limited criteria on ORs for treatment outcome, we divided our NRs into NVRs and transient viral responders (TVRs) by the use of Tanaka and colleagues criteria based on 2 log viral decline at 12 weeks. Among the Set-1 and Set-2 samples, 344 samples were available for this analysis (NVR, 58; TVR, 122; SVR, 164). The ORs in the analyses for week 12 NVR versus SVR and for NVR versus VR (TVR plus SVR) are much greater than those based on our criteria and nearly comparable to those of Tanaka *et al.* (2009) (OR: 20.2, 17.2, respectively). Accordingly, the extreme clinical phenotype of NRs, strongly dictated by early viral response, might account for the strongly associated OR values. Our viral kinetics study also supports this interpretation.

In conclusion, we have shown that polymorphisms located near the IL-28B locus (represented by rs8099917), reported to be associated with the outcome of PEG-IFN and

ribavirin combined therapy, are also associated with that of IFN monotherapy and significantly affect early viral decline. Our resequencing and fine-mapping study identified 15 common genetic variants on the IL-28B locus that are associated with the outcome of IFN therapy for HCV infection. These findings would contribute to better targeting of PEG-IFN plus ribavirin therapy and increase overall treatment efficacy. Genotyping of IL-28B polymorphisms may be useful for predicting treatment outcome as well as estimating the optimal duration of PEG-IFN plus ribavirin combination therapy for viral eradication in HCV patients. Further study is needed to clarify the mechanism and molecular function of the IL-28B polymorphism to establish a more optimal, tailor-made treatment regimen for each patient.

METHODS

Study populations. A total of 2112 patients with chronic HCV infection were included in the study who were treated either with PEG-IFN and ribavirin therapy or with IFN monotherapy at Toranomon Hospital Department of Hepatology, Hiroshima University Hospital, Hiroshima University-Affiliated Hospitals, and the National Taiwan University Hospital. All patients had abnormal levels of serum alanine transaminase for more than 6 months and were positive for both anti-HCV antibody and serum HCV RNA. All patients were negative for hepatitis B surface antigen, had no evidence of other liver diseases, and had not received immunosuppressive therapy before enrollment in the study. Table 5 lists the demographic features of the subjects. Patients were classified into the following two groups based on treatment outcome: SRs and NRs. SRs had no evidence of viraemia at 24 weeks after completion of IFN therapy, whereas NRs were still viraemic at this stage. For IFN monotherapy, patients were treated with 6 million units of IFN intramuscularly every day for 8 weeks, followed by the same dose twice a week for 16 weeks, with a total dose of 528 million units. In the PEG-IFN plus ribavirin combination therapy, subjects received weekly injections of PEG-IFN at 1.5 μg kg^{-1} body weight and oral administration of ribavirin for 48 weeks (for 24 weeks for Taiwanese patients). The amount of ribavirin was adjusted based on the subject's body weight (600 mg for <60 kg, 800 mg for 60–80 kg and 1000 mg for >80 kg). Only patients with greater than 75 % compliance with prescribed doses of PEG-IFN and ribavirin were included in this study. For patients who underwent IFN-based therapies more than once, only parameters related to the most recent therapy were analysed. In addition, 282 healthy Japanese volunteers were genotyped for rs8099917 and rs12979860. All subjects in the present study received a detailed explanation, and all signed a written informed consent. This study was approved by the Ethical Committee of each participating medical centre and by the Ethical Committee at the SNP Research Center, the Institute of Physical and Chemical Research (RIKEN), Yokohama.

Genotyping. In the association study, we divided the Japanese patients who received PEG-IFN plus ribavirin into two independent groups based on the time of entry into the study (Set-1 and Set-2 in Table 5). For the GWAS stage, 594 Japanese patients with HCV genotype 1b treated with PEG-IFN plus ribavirin (315 SRs and 279 NRs) were initially genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip (Set-1 in Table 5). The other samples were genotyped using the Invader assay, the TaqMan assay, or by direct sequencing as described previously (Ohnishi *et al.*, 2001; Suzuki *et al.*, 2003). The other patients were organized into the following sets: Set-2, 185 genotype 1b Japanese patients treated with PEG-IFN plus ribavirin (67 SRs vs 118 NRs); Set-3, 750 genotype 1b Japanese patients treated with IFN monotherapy (217 SRs vs 533 NRs); Set-4, 513 genotype 2a Japanese patients treated with IFN monotherapy (342 SRs vs 171 NRs); Set-5, 44 genotype 1b Taiwanese patients treated with PEG-IFN plus ribavirin (25 SRs vs 19 NRs); and Set-6, 29 genotype 2a Taiwanese patients treated with PEG-IFN plus ribavirin (25 SRs vs 4 NRs) (Table 5).

Quality control criteria for the genome-wide study. For the genome-wide survey, we applied the following quality control criteria. Individual samples with genotype call rates less than 98 % and SNPs with call rates less than 99 % were removed, as were non-autosomal SNPs, minor alleles with a frequency less than 0.01, or SNPs deviating from the Hardy–Weinberg equilibrium ($P<1\times10^{-6}$). Related individuals were detected by identity-by-state analysis performed with the PLINK software. One subject from each of the cryptically related pairs of individuals (PI_HAT >0.4) was excluded. We assessed

Table 5. Clinical characteristics of cohorts

SR, Sustained responder; NR, non-responder. PEG+riba, pegylated IFN-α-2b plus ribavirin combination therapy; IFN, IFN-α monotherapy.

Group	Treatment outcome	No. samples	Male (%)	Mean age in years (±SD)	HCV genotype	Therapy	Ethnicity
Set-1	SR	304	197 (64.8)	54.4 (±13.2)	1b	PEG+riba	Japanese
	NR	279	139 (49.8)	59.9 (±10.7)			
Set-2	SR	67	33 (49.3)	57.1 (±13.3)	1b	PEG+riba	Japanese
	NR	118	49 (41.5)	62.7 (±8.5)			
Set-3	SR	217	147 (67.7)	57.2 (±13.8)	1b	IFN	Japanese
	NR	533	311 (58.3)	56.4 (±10.7)			
Set-4	SR	341	203 (59.5)	56.4 (±14.7)	2a	IFN	Japanese
	NR	169	86 (50.9)	54.9 (±12.3)			
Set-5	SR	25	12 (50.0)	50.5 (±9.8)	1b	PEG+riba	Taiwanese
	NR	19	8 (42.1)	51.7 (±7.0)			
Set-6	SR	25	14 (56.0)	49.4 (±12.4)	2a	PEG+riba	Taiwanese
	NR	4	4 (100.0)	48.2 (±13.0)			
Healthy control		282	151 (53.5)	44.1 (±15.0)			Japanese

population stratification using the smartpca program from the EIGENSOFT package (http://genepath.med.harvard.edu/~reich/Software.htm) using genotypes for about 70 000 SNPs informative for the Japanese population according to the method described previously (Yamaguchi-Kabata *et al.*, 2008). Analysis was performed using the Set-1 samples and all four of the HapMap population datasets (CEU/YRI/JPT/CHB). Outliers from JPT/CHB clusters were excluded from the association analysis. Genotype-based associations were tested with the Cochran–Armitage trend test. The genomic control method was applied to evaluate whether the inflation of false-positive rates, indicated by inflation factor λ, was within an acceptable level.

Resequencing. Resequencing around the IL-28B locus was performed by direct sequencing of DNA from 48 unrelated Japanese HCV patients from the enrolled subjects. We used Phase II HapMap JPT genotype data and the HAPLOVIEW program to define an LD block.

HCV RNA level. The HCV RNA level was analysed at three time points: before IFN therapy, at the end of the second week and at the end of the fourth week, using an RT-PCR-based method (the original method, the high range method or the TaqMan RT-PCR test). The measurement ranges of these assays were 0.5–850 kilo (K) IU ml^{-1}, 5–5000 KIU ml^{-1} and 1.2–7.8 log IU, respectively. Set-1 and Set-2 patients with sufficient viral load data falling within the measurable range were included in the viral kinetics analysis.

Liver biopsy. Liver biopsy specimens were evaluated by a pathologist at each institution and were scored for the stage of liver fibrosis according to the classification of Desmet *et al.* (1994). The patients were divided into two categories: mild fibrosis (F0–1) and severe fibrosis (F2–4).

Statistical analysis. Genotype-based associations were tested using the allele model χ^2-test. SNP effects were also evaluated using the dominant, recessive and co-dominant models (using the Cochran–Armitage trend test). Combined analysis was performed following the Mantel–Haenszel method. Viral kinetics were assessed using the non-parametric Kruskal–Wallis test followed by the Steel–Dwass test. Multivariate logistic regression analysis with stepwise forward selection was performed with criteria of $P<0.05$ for inclusion and removal of variables using the StatFlex 5.0 software package (Artec Inc.).

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Amino Acid Substitution in HCV Core/NS5A Region and Genetic Variation Near *IL28B* Gene Affect Treatment Efficacy to Interferon plus Ribavirin Combination Therapy

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Core region · NS5A region · ISDR · IRRDR · *IL28B*

Abstract
Objective: To evaluate predictive factors of treatment efficacy to interferon (IFN)/ribavirin in patients infected with HCV genotype 1b (HCV-1b). ***Methods:*** This study investigated pretreatment predictors, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in 490 Japanese adults infected with HCV-1b. ***Results:*** The proportion of patients who showed end-of-treatment response (ETR), sustained virological response (SVR), and SVR after ETR was 76, 54, and 76%, respectively. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR. Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. Furthermore, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR. By multivariate analysis, rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core aa 70/91 was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. ***Conclusion:*** aa substitution in core/NS5A region and genetic variation near *IL28B* were important predictors of treatment efficacy to IFN/ribavirin.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) combined with ribavirin carries potential serious side effects and is costly, especially when used long enough to achieve a high sustained virological response (SVR) in patients infected with HCV genotype 1b (HCV-1b) and high viral loads. For these rea-

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sons, those patients who do not achieve SVR need to be identified, so as to free them of unnecessary side effects and reduce costs, preferably before the start of the combination therapy.

Viral- and host-related factors are useful as predictors of treatment efficacy to 48-week IFN/ribavirin combination therapy. With regard to viral factors, amino acid (aa) substitutions at position 70 and/or 91 in the core region of HCV-1b are pretreatment predictors of virological response to combination therapy [1–4], and also affect clinical outcome, including hepatocarcinogenesis [5, 6]. Furthermore, the NS5A region of HCV-1b, including IFN-sensitivity-determining region (ISDR) [7, 8] and IFN/ribavirin resistance-determining region (IRRDR) [9, 10], are also useful as pretreatment predictors of virological response to combination therapy [11, 12]. With regard to host factors, genetic variations near *IL28B* gene (rs8099917, rs12979860) on chromosome 19, which encodes IFN-λ-3, are pretreatment predictors of virological response to combination therapy in individuals infected with HCV-1 [13–16], and also affect clinical outcome, including spontaneous clearance of HCV [17]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of SVR to triple therapy of telaprevir/pegylated (PEG)-IFN/ribavirin in Japanese patients infected with HCV-1b [18]. However, to our knowledge, there are no previous reports of IFN/ribavirin combination therapy based on multivariate analysis to investigate pretreatment predictors, including all of aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR, and genetic variation near *IL28B* gene.

The aim of the present study was to investigate predictive factors of treatment efficacy, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in Japanese adults infected with HCV-1b.

Patients and Methods

Study Population

A total of 1,249 HCV-1b-infected Japanese adult patients were consecutively recruited into the study protocol of combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) plus ribavirin between December 2001 and January 2009 at Toranomon Hospital, Tokyo, Japan. Among these, 490 patients, who could complete a total of 48 weeks of combination therapy, were enrolled in this retrospective study, and fulfilled the following criteria: (1) negativity for hepatitis B surface antigen (HBsAg) in serum; (2) HCV-1b only confirmed by sequence analysis; (3) HCV-RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan) within the preceding 2 months of enrolment; (4) no hepatocellular carcinoma; (5) body weight >40 kg; (6) lack of coinfection with human immunodeficiency virus; (7) no previous treatment with antiviral or immunosuppressive agents within the preceding 3 months of enrolment; (8) none was an alcoholic; lifetime cumulative alcohol intake was <500 kg; (9) none had other forms of liver diseases, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, or autoimmune liver disease, and (10) none of the females was pregnant or breastfeeding.

The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave their informed consent before participating in this trial.

The treatment efficacy was evaluated in terms of HCV-RNA negativity at the end of treatment (end-of-treatment response (ETR)) and 24 weeks after the completion of therapy (SVR), based on the COBAS TaqMan HCV test (Roche Diagnostics). SVR in patients who achieved ETR was defined as SVR after ETR. ETR, SVR, and SVR after ETR could be evaluated in 487 (99%), 448 (91%), and 321 (66%) of 490 patients, respectively.

422 (86%) patients received PEG-IFNα-2b at a median dose of 1.4 μg/kg (range 0.7–1.9) subcutaneously each week plus oral ribavirin at a median dose of 11.1 mg/kg (range 3.7–15.1) daily for 48 weeks. The remaining 68 (14%) patients received 6 million units of IFNα-2b intramuscularly each day for 48 weeks (daily for the initial 2 weeks, followed by three times per week for 46 weeks), and oral ribavirin at a median dose of 11.3 mg/kg (range 6.8–13.4) daily for 48 weeks.

Table 1 summarizes the profiles and laboratory data of the 490 patients at the commencement of treatment. They included 310 males and 180 females aged 20–75 years (median 54).

Measurement of HCV RNA

The antiviral effects of treatment on HCV were assessed by measuring plasma HCV-RNA levels. In this study, HCV-RNA levels were evaluated at least once every month before, during, and after therapy. HCV-RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative.

Detection of aa Substitutions in Core, and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [19], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on the previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [1]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [7, 8] was determined, and the number of aa substitutions in ISDR was defined as wild-type (WT) (0, 1) or non-wild-type (non-WT) (≥2) in comparison with HCV-J. Furthermore, the sequence of 2,334–2,379 aa in the NS5A of HCV-1b (IRRDR) reported by El-Shamy et al. [9, 10] was determined and then compared with the consensus sequence constructed on the previous study. In the present study, aa substitutions of the core region and NS5A-ISDR/IRRDR of HCV-1b were analyzed by direct sequencing [10, 18].

Genetic Variation near IL28B Gene

Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of Invader assay, TaqMan assay, or direct sequencing as described previously [20, 21].

In this study, genetic variations near *IL28B* gene (rs8099917), reported as the pretreatment predictors of treatment efficacy in Japanese patients [14, 18], were investigated.

Statistical Analysis

Non-parametric tests (Mann-Whitney U test, χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Correlation analysis was evaluated by the Spearman rank correlation test. Uni- and multivariate logistic regression analyses were used to determine those factors that significantly contributed to ETR, SVR, and SVR after ETR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All p values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for uni- and multivariate analyses. Potential predictive factors associated with ETR, SVR, and SVR after ETR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, γ-glutamyl transpeptidase (GGT), leukocyte count, hemoglobin, platelet count, level of viremia, α-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, ribavirin dose/body weight, genetic variation near *IL28B* gene, and aa substitution in the core region, and NS5A-ISDR/IRRDR. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, Ill., USA).

Table 1. Patient profile and laboratory data at commencement of the 48-week combination therapy of IFN + ribavirin in 490 patients infected with HCV-1b

Demographic data	
Number of patients	490
Male/female	310/180
Age, years	54 (20–75)
History of blood transfusion	169 (34%)
Family history of liver disease	96 (20%)
Body mass index, kg/m^2	22.6 (15.7–34.7)
Laboratory data	
Level of viremia, log IU/ml	6.4 (2.2–7.7)
Serum AST, IU/l	50 (16–296)
Serum ALT, IU/l	67 (12–836)
Serum albumin, g/dl	3.9 (3.1–4.7)
GGT, IU/l	44 (10–592)
Leukocyte count, n/mm^3	4,700 (1,200–10,900)
Hemoglobin, g/dl	14.4 (10.6–18.1)
Platelet count, $\times 10^4$/mm^3	16.7 (6.4–37.5)
α-Fetoprotein, μg/l	5 (1–459)
Total cholesterol, mg/dl	170 (96–284)
High-density lipoprotein cholesterol, mg/dl	46 (13–95)
Low-density lipoprotein cholesterol, mg/dl	100 (32–190)
Triglycerides, mg/dl	90 (33–416)
Uric acid, mg/dl	5.5 (2.3–9.4)
Treatment	
PEG-IFNα-2b/IFNα-2b	422/68
Ribavirin dose, mg/kg	11.2 (3.7–15.1)
aa substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	266/151
Core aa 91, leucine/methionine	246/169
ISDR of NS5A, 0/1/≥2	227/95/84
IRRDR of NS5A, ≤3/4–5/≥6	73/67/60
Genetic variation near IL28B gene	
rs8099917 genotype, TT/TG/GG	150/65/4

Data represent number of patients with percentages in parentheses, or median (range) values.

Results

Response to Therapy

ETR was achieved by 372 of 487 (76%) patients, SVR by 244 of 448 (54%), and SVR after ETR by 244 of 321 (76%).

Number of aa Substitutions in NS5A-ISDR and NS5A-IRRDR

As a whole, 0, 1, and ≥2 aa substitutions in ISDR were found in 56% (227 of 406), 23% (95 of 406), and 21% (84 of 406) of patients, respectively. Thus, the percentage of patients with ≤1 aa substitution in ISDR (WT) was 79% (322 of 406). Furthermore, ≤3, 4–5, and ≥6 aa substitutions in IRRDR were found in 36% (73 of 200), 34% (67 of 200), and 30% (60 of 200) of patients, respectively (fig. 1).

The correlation between ISDR and IRRDR was analyzed. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR ($r = 0.308$, $p < 0.001$) (fig. 2).

aa Substitutions in the Core Region and NS5A-ISDR/IRRDR

Concerning the substitution of core aa 70, the number of aa substitutions in ISDR of 256 patients with Arg70 (median 0) was not significantly different from that of 146 patients with Gln70 (His70) (median 0) (fig. 3a). Fur-

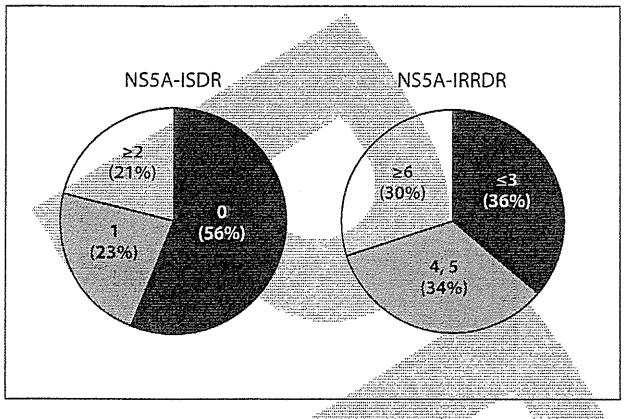

Fig. 1. The number of aa substitutions in NS5A-ISDR and NS5A-IRRDR. The percentage of patients with ≤1 aa substitution in ISDR (WT) was 79%.

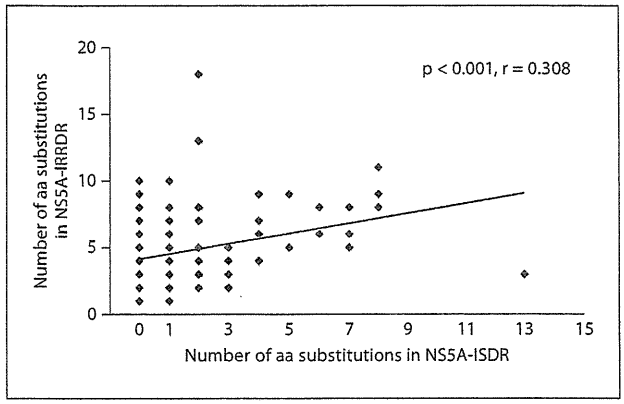

Fig. 2. Correlation between NS5A-ISDR and NS5A-IRRDR. There was a significant positive correlation between the number of aa substitutions in ISDR and that in IRRDR ($r = 0.308$, $p < 0.001$).

thermore, the number of aa substitutions in IRRDR of 123 patients with Arg70 (median 5) was also not significantly different from that of 77 patients with Gln70 (His70) (median 4) (fig. 3b).

Concerning the substitution of core aa 91, the number of aa substitutions in ISDR of 240 patients with Leu91 (median 1) was significantly higher than that of 161 patients with Met91 (median 0) ($p < 0.001$) (fig. 3c). Furthermore, the number of aa substitutions in IRRDR of 111 patients with Leu91 (median 5) was significantly higher than that of 89 patients with Met91 (median 3) ($p < 0.001$) (fig. 3d).

Viremia Level and aa Substitutions in Core Region/ISDR/IRRDR

Concerning the number of substitutions in ISDR, viremia levels of 321 patients with WT (median 6.5) were significantly higher than those of 84 patients with non-WT (median 5.7) ($p < 0.001$) (fig 4a).

Concerning the number of substitutions in IRRDR, viremia levels of 140 patients with ≤5 substitutions (median 6.4) were significantly higher than those of 60 patients with ≥6 (median 6.1) ($p = 0.027$) (fig. 4b).

Concerning the substitution of core aa 70, viremia levels of 265 patients with Arg70 (median 6.4) were not significantly different from those of 151 patients with Gln70 (His70) (median 6.3) (fig. 4c).

Concerning the substitution of core aa 91, viremia levels of 169 patients with Met91 (median 6.5) were significantly higher than those of 245 patients with Leu91 (median 6.2) ($p = 0.028$) (fig. 4d).

Thus, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

Treatment Response according to the Number of aa Substitutions in IRRDR

Concerning the number of aa substitutions in IRRDR, a significantly higher proportion of patients with ≥4 aa substitutions (58%) showed SVR compared to patients with ≤3 (42%) ($p = 0.039$). In contrast, the SVR rate was not significantly different between patients with ≤4 (49%) and those with ≥5 (57%) aa substitutions. Likewise, the SVR rate was not significantly different between patients with ≤5 (51%) and those with ≥6 (55%) aa substitutions (fig. 5a).

The ETR rate was not significantly different between patients with ≤3 (74%) and those with ≥4 (82%) aa substitutions, nor between patients with ≤4 (76%) and those with ≥5 (83%). Likewise, the ETR rate was not significantly different between those with ≤5 (79%) and those with ≥6 (80%) aa substitutions (fig. 5b).

The SVR rate after ETR was not significantly different between patients with ≤3 (61%) and those with ≥4 (74%) aa substitutions, nor between patients with ≤4 (67%) and those with ≥5 (72%). Likewise, they were not significantly different between patients with ≤5 (67%) and those with ≥6 (75%) aa substitutions (fig. 5c).

Thus, it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. However, the ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

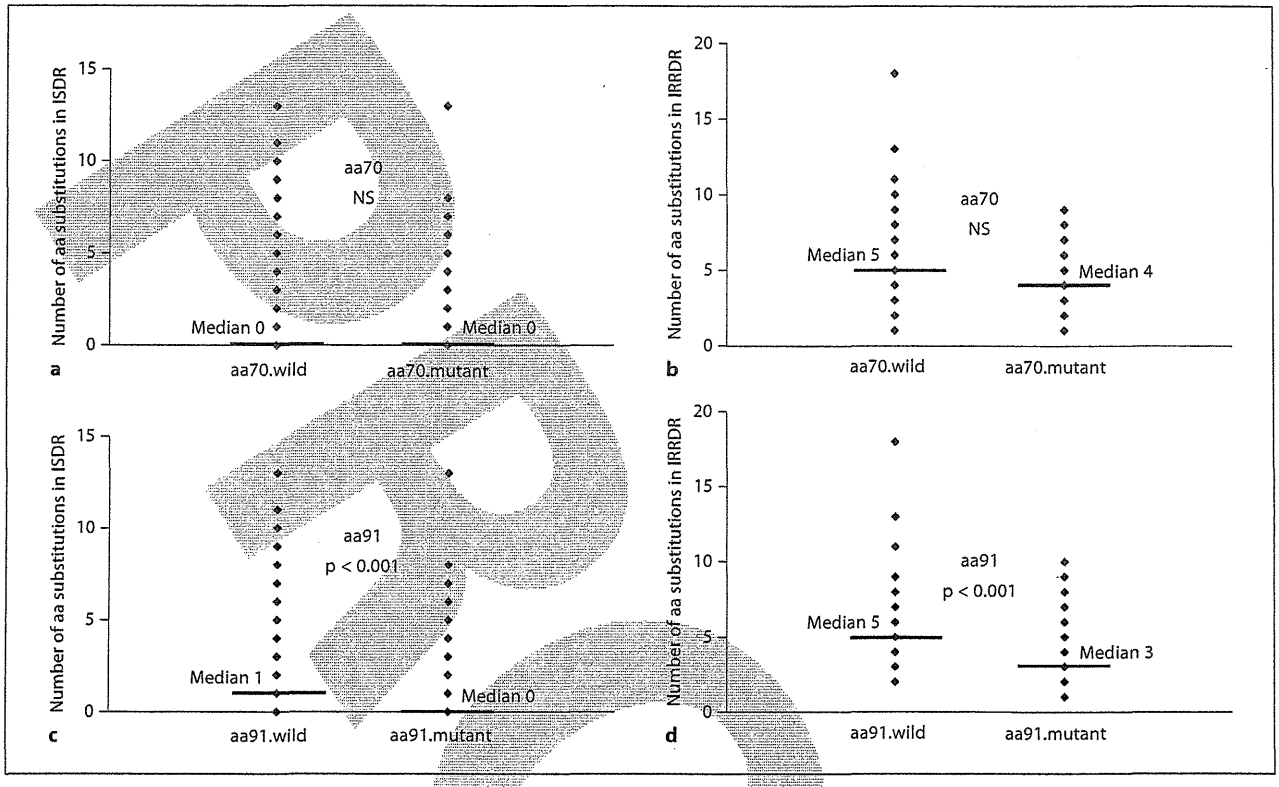

Fig. 3. aa substitutions in the core region and NS5A-ISDR/IRRDR. **a**, **b** Concerning the substitution of core aa 70, the number of aa substitutions in ISDR/IRRDR of patients with Arg70 was not significantly different from that of patients with Gln70 (His70). **c**, **d** Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91 ($p < 0.001$).

Predictors of SVR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 15 parameters that correlate with SVR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.006$), AST (<58 IU/l; $p = 0.039$), leukocyte count (≥4,500/mm^3; $p = 0.043$), hemoglobin (≥14.0 g/dl; $p = 0.001$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), GGT (<50 IU/l; $p = 0.028$), uric acid (≥5.5 mg/dl; $p = 0.005$), level of viremia (<6.0 log IU/ml; $p < 0.001$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), the number of aa substitutions in ISDR (non-WT; $p < 0.001$) and IRRDR (≥4; $p = 0.039$). Figure 6 shows the SVR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 3 parameters that independently influenced SVR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), gender (male sex; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.027$) (table 2).

Predictors of ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 14 parameters that correlated with ETR: gender (male sex; $p = 0.001$), age (<55 years; $p = 0.004$), AST (<39 IU/l; $p = 0.027$), hemoglobin (≥14.0 g/dl; $p = 0.035$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), albumin (≥3.9 g/dl; $p = 0.014$), GGT (<50 IU/l; $p < 0.001$), uric acid (≥5.5 mg/dl; $p = 0.003$), level of viremia (<6.0 log IU/ml; $p = 0.001$), low-density lipoprotein cholesterol (≥85 mg/dl; $p = 0.004$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.021$). Figure 7 shows the ETR rate according to aa

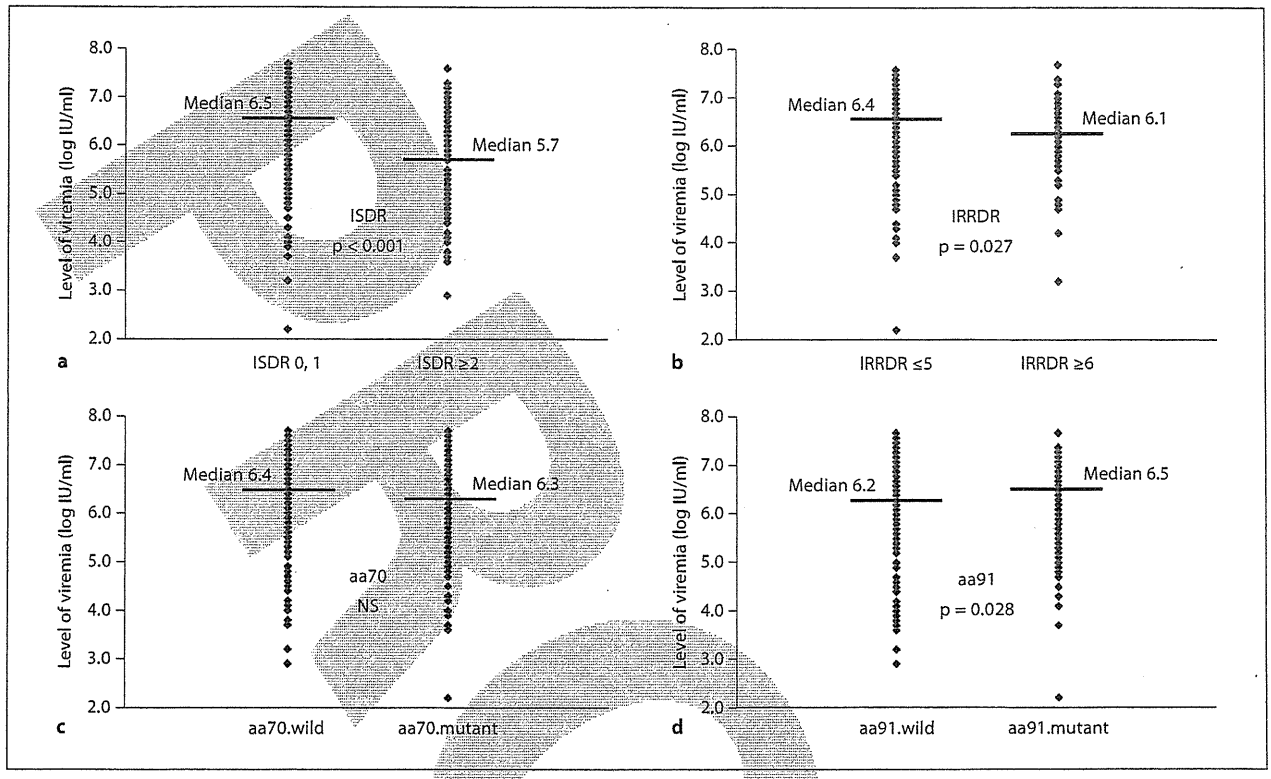

Fig. 4. Viremia level and aa substitutions in core region/ISDR/IRRDR. **a** Concerning the number of substitutions in ISDR, viremia levels of patients with WT were significantly higher than those of patients with non-WT ($p < 0.001$). **b** Concerning the number of substitutions in IRRDR, viremia levels of patients with ≤5 aa substitutions were significantly higher levels than those of patients with ≥6 ($p = 0.027$). **c** Concerning the substitution of core aa 70, viremia levels of patients with Arg70 were not significantly different from those of patients with Gln70 (His70). **d** Concerning the substitution of core aa 91, viremia levels of patients with Met91 were significantly higher than those of patients with Leu91 ($p = 0.028$). Thus, levels of viremia might be influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

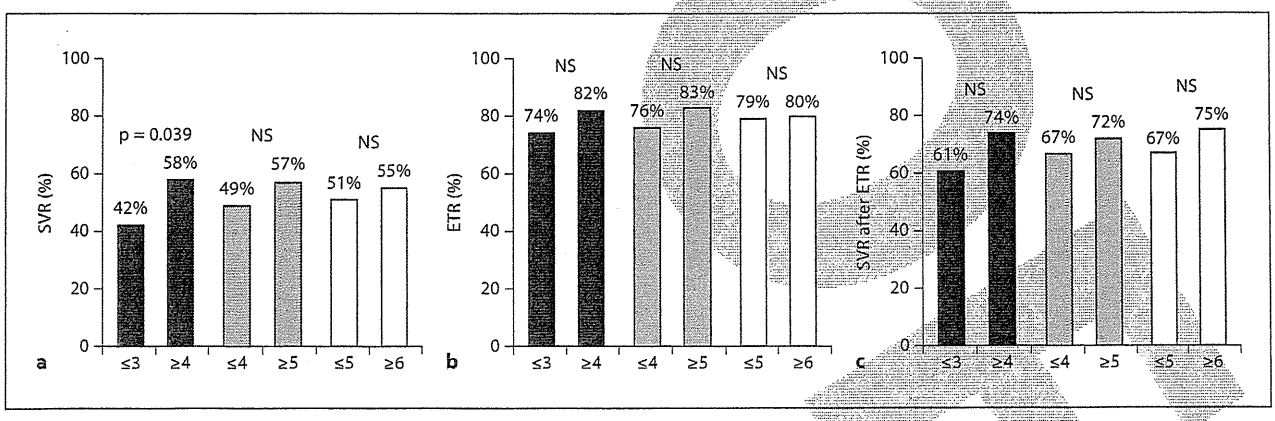

Fig. 5. Treatment response according to the number of aa substitutions in NS5A-IRRDR. **a** A significantly higher proportion of patients with ≥4 (58%) aa substitutions showed SVR compared to patients with ≤3 (42%) ($p = 0.039$), and it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. **b**, **c** ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

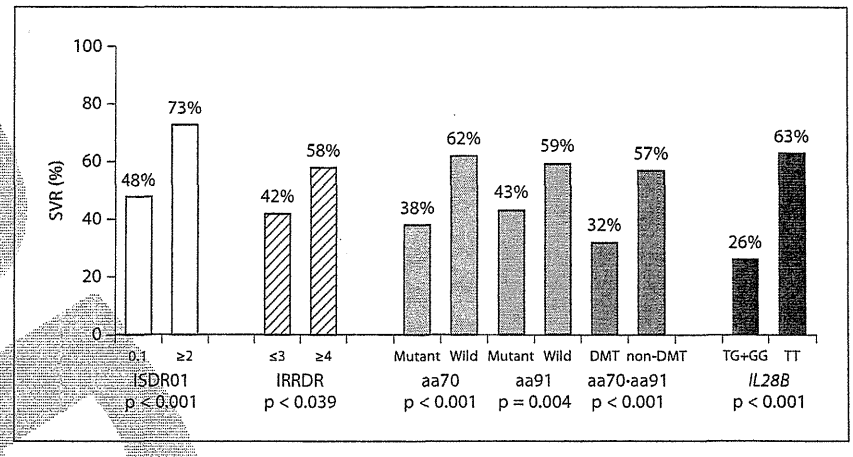

Fig. 6. SVR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

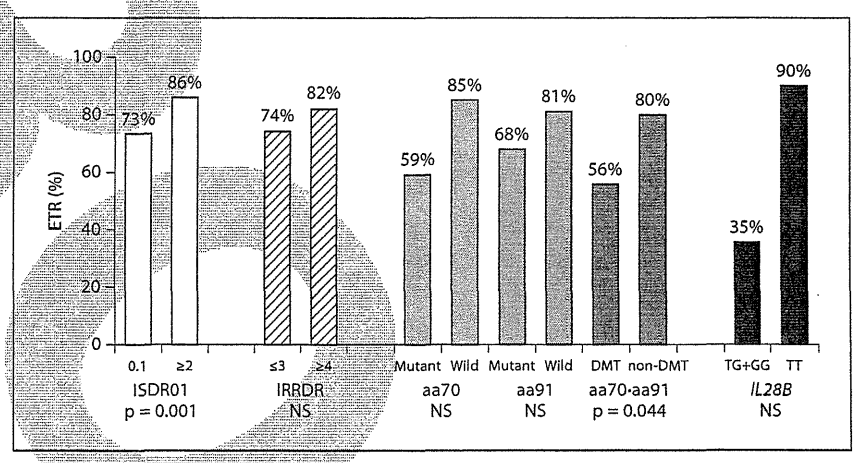

Fig. 7. ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Table 2. Factors associated with SVR to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 16.7 (4.54–61.3)	<0.001
Gender	1: Female 2: Male	1 10.5 (3.47–32.3)	<0.001
ISDR of NS5A	1: WT 2: Non-WT	1 5.68 (1.22–26.3)	0.027

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Factors associated with ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 18.2 (6.29–52.6)	<0.001
Level of viremia log IU/ml	1: ≥6.0 2: <6.0	1 9.20 (2.59–32.6)	0.001
Core aa 70	1: Gln70 (His70) 2: Arg70	1 4.68 (1.65–13.3)	0.004
Serum albumin g/dl	1: <3.9 2: ≥3.9	1 3.08 (1.11–8.47)	0.030

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

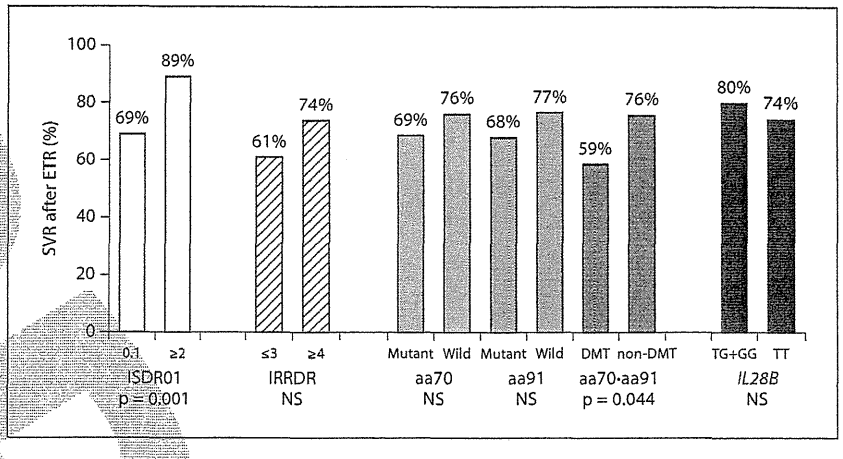

Fig. 8. SVR after ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 4 parameters that independently influenced ETR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.001$), substitution of aa 70 (Arg70; $p = 0.004$), and albumin (≥3.9 g/dl; $p = 0.030$) (table 3).

Predictors of SVR after ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 11 parameters that influenced SVR after ETR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.025$), leukocyte count (≥4,500/mm^3; $p = 0.033$), hemoglobin (≥14.0 g/dl; $p = 0.025$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.020$), total cholesterol (<170 mg/dl; $p = 0.017$), α-fetoprotein (<10 μg/l; $p = 0.004$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.044$), and the number of aa substitutions in ISDR (non-WT; $p = 0.001$). Figure 8 shows the SVR after ETR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 6 parameters that independently influenced the SVR after ETR: gender (male sex; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.002$), the number of aa substitutions in ISDR (non-WT; $p = 0.012$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.023$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.033$), and α-fetoprotein (<10 μg/l; $p = 0.042$) (table 4).

Comparison of Factors Associated with Treatment Efficacy Identified by Multivariate Analysis

Table 5 shows the variables that achieved statistical significance on multivariate logistic regression for each evaluation of treatment efficacy. Rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core region was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. Level of viremia was an important predictor of ETR. Thus, genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia) were important predictors of treatment efficacy. Furthermore, gender, α-fetoprotein, albumin, and platelet count were also identified as other important predictors of treatment efficacy, in addition to genetic variation near *IL28B* and viral factors.

Discussion

Using multivariate analysis, the present study identified viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene) that influenced treatment efficacy to 48-week IFN/ribavirin combination therapy, which is in agreement with recent findings [22, 23]. Identification of these viral and host factors before the start of IFN/ribavirin combination therapy should help to select better therapeutic regimens, including triple therapy of telaprevir/PEG-IFN/ribavirin [24–26], for those patients who are less likely to achieve SVR.

According to the number of substitutions in ISDR, a previous report showed that levels of viremia were sig-

Table 4. Factors associated with SVR in patients who achieved ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
Gender	1: Female 2: Male	1 4.27 (2.15–8.55)	<0.001
Ribavirin dose, mg/kg	1: <11.0 2: ≥11.0	1 2.95 (1.48–5.86)	0.002
ISDR of NS5A	1: WT 2: Non-WT	1 4.00 (1.35–11.8)	0.012
Core aa 70 and 91	1: Gln70 (His70) and Met91 2: Arg70 and/or Leu91	1 2.96 (1.16–7.52)	0.023
Platelet count $\times 10^4/mm^3$	1: <15.0 2: ≥15.0	1 2.19 (1.07–4.50)	0.033
α-Fetoprotein µg/l	1: ≥10 2: <10	1 2.66 (1.04–6.80)	0.042

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 5. Comparison of factors associated with efficacy of 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	ETR response (at 48 weeks)	SVR after ETR response	SVR
IL28B	rs8099917 p < 0.001, 18.2 (6.29–52.6)[a]		rs8099917 p < 0.001, 16.7 (4.54–61.3)[a]
Virus	Core aa 70 p = 0.004, 4.68 (1.65–13.3)[a]	Core aa 70 and 91 p = 0.023, 2.96 (1.16–7.52)[a]	
	Level of viremia p = 0.001, 9.20 (2.59–32.6)[a]	ISDR p = 0.012, 4.00 (1.35–11.8)[a]	ISDR p = 0.027, 5.68 (1.22–26.3)[a]
Others	Albumin p = 0.030, 3.08 (1.11–8.47)[a]	α-Fetoprotein p = 0.042, 2.66 (1.04–6.80)[a]	
		Platelet count p = 0.033, 2.19 (1.07–4.50)[a]	
		Gender p < 0.001, 4.27 (2.15–8.55)[a]	Gender p < 0.001, 10.5 (3.47–32.3)[a]
		Ribavirin dose p = 0.002, 2.95 (1.48–5.86)[a]	

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.
[a] OR (95% CI).

nificantly lower in patients with non-WT of ISDR than in those with WT [8]. The present study indicated that substitution of IRRDR and core aa 91, in addition to substitution of ISDR, also significantly influenced levels of viremia. Furthermore, there was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR, and the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. To our knowledge, this is the first report of the relationship between viremia levels and aa substitutions in core region/ISDR/IRRDR. This result might be interpreted to mean

that core aa 91/ISDR/IRRDR might be associated with viremia levels involved in resistance to combination therapy. Further studies that examine the functional impact of aa substitutions to combination therapy should be conducted to confirm the above finding.

The present results showed that α-fetoprotein, albumin, platelet count, and gender were predictors of virological response to IFN/ribavirin combination therapy. Previous data indicated that absence of advanced liver fibrosis was a positive predictor of SVR to IFN monotherapy and IFN/ribavirin combination therapy [2, 3, 13, 27–29], and that advanced liver fibrosis was usually associated with higher levels of α-fetoprotein, and lower levels of albumin and platelet count [1, 3, 30–32]. Furthermore, gender is also a predictor of treatment response to IFN/ribavirin combination therapy [2, 3, 14]. In the present study based on a large number of patients, histopathological changes in the liver and gender were identified as independent predictors of virological response, in addition to genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia).

In a previous study, multivariate analysis identified core region, gender, and stage of liver fibrosis as parameters that independently influenced the SVR of patients who achieved early virological response, but ISDR was not entered into uni- and multivariate analysis [3]. To our knowledge, the present study based on multivariate analysis is the first report to identify ISDR as pretreatment predictor of SVR after ETR to combination therapy. Interestingly, ISDR was not a predictor of ETR, but was a significant predictor of SVR to combination therapy. Thus, the underlying mechanisms of failure to develop SVR in those patients who achieve HCV-RNA negativity remain unclear. Further studies that examine the impact of aa substitutions of ISDR to combination therapy should be conducted to confirm the above finding.

One limitation of the present study was that aa substitutions in areas other than the core region and NS5A-ISDR/IRRDR of the HCV genome were not examined. Other limitations were differences in host factors including race [24, 33, 34] and differences in viral factors, such as the distribution of HCV-1a or -1b, and geographic diversities of HCV-1b [35]. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of 48-week IFN/ribavirin combination therapy, and further understanding of the complex interaction between virus- and host- related factors should facilitate the development of more effective therapeutic regimens.

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Amino Acid Substitution in HCV Core Region and Genetic Variation near the *IL28B* Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment

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Key Words
Hepatitis C virus · Core region · *IL28B* · Telaprevir · Peginterferon · Ribavirin · Viral dynamics

Abstract
Objectives: Genetic variation near the *IL28B* gene and substitution of aa 70 and 91 in the core region of HCV-1b are useful as predictors of treatment efficacy to telaprevir/pegylated interferon (PEG-IFN)/ribavirin, but its impact on viral dynamics is not clear. ***Methods:*** This study investigated predictive factors of viral dynamics during 12- or 24-week regimen of triple therapy in 80 Japanese adults infected with HCV-1b. ***Results:*** After 24 h of commencement of treatment, the proportion of patients with Arg70 and Leu91 substitutions in the core region who showed ≥3.0 log drop in HCV RNA level was significantly higher than that of patients with Gln70 (His70) and/or Met91. At 8 and 12 weeks, HCV RNA loss rate of patients with rs8099917 genotype TT near *IL28B* gene was significantly higher than that of patients with non-TT. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and SVR (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and SVR. ***Conclusions:*** This study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 kIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis

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C [3]. Such a background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads [4, 5]. Recently, a new strategy was introduced in the treatment of chronic HCV infection by means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [6]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity [7, 8]. Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin could achieve sustained virological response rates of 35–60 and 61–69% in patients infected with HCV-1, respectively [9, 10]. Furthermore, a recent study (PROVE3) also showed that the 24- and 48-week regimen of triple therapy could achieve sustained virological response rates of 51 and 53% in HCV-1 infected patients in whom initial PEG-IFN/ribavirin treatment failed, respectively [11].

Amino acid (aa) substitutions at positions 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy [12–14], and also affect clinical outcome, including hepatocarcinogenesis [15, 16]. Furthermore, genetic variations near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 as host-related factor, which encodes IFN-λ-3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1 [17–20], and also affect clinical outcome, including spontaneous clearance of HCV [21]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of sustained virological response to triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b [22]. However, it is not clear at this stage whether genetic variation near the *IL28B* gene and aa substitution of the core region can be used before therapy to predict viral dynamics during triple therapy.

The present study included 80 patients with HCV-1b and high viral loads, who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict viral dynamics during treatment, including viral-(aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near *IL28B* gene).

Patients and Methods

Study Population

Between May 2008 and September 2009, 81 patients infected with HCV were recruited to this study at the Department of Hepatology in Toranomon Hospital in metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN and ribavirin] (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

Eighty of the 81 patients met the following inclusion and exclusion criteria: (1) Diagnosis of chronic hepatitis C. (2) HCV-1b confirmed by sequence analysis. (3) HCV RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20–65 years. (6) Body weight ≥35 kg and ≤120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of ≤50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of ≥110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of ≥12 g/dl, neutrophil count ≥1,500/mm^3, and platelet count of ≥100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. In this study, all of the 80 patients were evaluated for the pretreatment predictors for viral dynamics during triple therapy, and 77 of the 80 patients were followed up for at least 24 weeks after the completion of treatment. The treatment efficacy was evaluated by 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 or 500 mg three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, N.J., USA) was injected subcutaneously at a median dose of 1.5 μg/kg (range 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose 600–1,000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutro-