Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Predictive value of the *IL28B* polymorphism on the effect of interferon therapy in chronic hepatitis C patients with genotypes 2a and 2b

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Background & Aims: Common *IL28B* locus polymorphisms (SNPs rs8099917 and rs12979860) have been reported to affect peg-interferon plus ribavirin combination therapy (PEG-RBV) for hepatitis C virus (HCV) genotype 1b, but few reports have examined their effect on other two common genotypes, 2a and 2b.

Methods: We analyzed predictive factors for sustained virological response (SVR) in a retrospective study of 719 patients with either genotype 2a (530) or 2b (189). Of these patients, 160 were treated with PEG-RBV and 559 were treated with interferon monotherapy. We evaluated predictive factors including HCV RNA, histological findings, *IL28B* SNP genotypes (rs8099917, rs12979860, and rs12980275), and the effect of treatment regimen and prior treatment history.

Results: HCV RNA viral load, treatment regimen, and rs8099917 genotypes independently contributed to the effect of the therapy. For patients treated with PEG-RBV, rs8099917 and viral load were independent predictive factors for SVR in genotype 2b but not in genotype 2a. Conversely, in patients treated with interferon monotherapy, viral load and rs8099917 were independent predictive factors for SVR in genotype 2a but not in genotype 2b. The favorable rs8099917 genotype is also associated with a steep decline in viral load by the second week of treatment.

Conclusions: Initial viral load and rs8099917 genotype are significant independent predictors of SVR in genotype 2 patients.

Keywords: Interferon therapy; Single nucleotide polymorphism; Ribavirin; Hepatitis C.

Received 12 March 2010; received in revised form 1 July 2010; accepted 2 July 2010; available online 19 September 2010

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Abbreviations: HCV, hepatitis C virus; IFN, interferon; PEG-IFN, pegylated interferon; RBV, ribavirin; PEG-RBV, pegylated interferon plus ribavirin combination therapy; SNP, single nucleotide polymorphism; SVR, sustained viral responder; NR, non-responder.

Introduction

Hepatitis C virus (HCV) infection is a major worldwide cause of chronic liver diseases, affecting an estimated 170 million people [1]. Chronic HCV infection may progress to hepatocellular carcinoma (HCC) or liver cirrhosis (LC) [2–6], and in Japan, 60–70% of patients with HCC or LC are HCV carriers [7]. There are two major genotypes (1 and 2) and three sub-genotypes (1b, 2a, and 2b) in Japan as well as in many other countries [8]. Although pathological features of these genotypes are similar [9,10], interferon therapy is more effective against genotype 2 than genotype 1 [11,12]. Compared to the less than 50% of genotype 1 patients who respond to therapy [13–19], more than 80% of genotype 2 patients who received 24-week peg-interferon and ribavirin (PEG-RBV) combination therapy achieved sustained virological response (SVR), defined as absence of HCV RNA six months after the cessation of therapy. Because of this otherwise high success rate, the small subset of genotype 2 patients who fail to respond to therapy should be examined more closely. Although treatment-resistant genotype 2 sub-populations have been reported [20–22], the mechanism underlying variable response to treatment is unclear. Multiple viral (e.g., HCV genotype, amino acid substitutions in the NS5A and core region [22–26]) and host factors (e.g., age [14], body mass index [27], and insulin resistance

Table 1. Baseline characteristics of patients with HCV genotypes 2a and 2b.

	All (n = 719)	2a (n = 530)	2b (n = 189)
Sex (M/F)	403/316	301/229	102/87
Age	57 (49-64)	56 (48-64)	59 (50-66)
Body weight (kg)	59.8 (51-71.4)	60.15 (53.75-71.65)	57.4 (48.5-70)
BMI (kg/m^2)	23.2 (20.3-25.7)	24.48 (21.43-26.4)	21.78 (19.89-24.79)
Fibrosis (F0-2/F3-4)	484/101	359/68	125/33
Treatment (IFN/PEG-RBV)	559/160	477/53	82/107
Treatment naïve (Y/N)	689/30	523/7	166/23
HCV RNA (log IU/ml)	5.3 (4.7-5.9)	5 (4.6-5.7)	5.9 (5.5-6.5)
rs8099917 (TT/GT/GG)	572/135/11	425/97/7	147/38/4
rs12979860 (CC/TC/TT)	565/137/11	422/98/7	143/39/4
rs12980275 (AA/GA/GG)	543/158/16	402/116/10	141/42/6
SVR/non-SVR	455/264	340/190	115/74

IFN, interferon monotherapy; PEG-RBV, peg-interferon plus ribavirin combination therapy; SVR, sustained viral responder.

[28]) have been reported to affect the outcome of interferon therapy in genotype 1-infected patients but such factors have not been closely examined in genotype 2 patients.

Single nucleotide polymorphisms (SNPs) and other genetic factors have been reported to be useful in predicting the outcome of interferon therapy. Polymorphisms in MxA [29,30], interferon alpha-receptor 1 [31], and osteopontin [32] have also been reported to be associated with interferon response. We also identified a MAPKAPK3 SNP [33] that is a predictive factor for interferon mono-therapy. Recently, several groups have reported an association between several SNPs in the *IL28* locus and the effect of PEG-RBV combination therapy for genotype 1b [34–38] but only a few studies have examined the role of these SNPs in the treatment of other genotypes. In this study, we analyzed predictive factors for SVR in genotype 2a and 2b patients treated with PEG-RBV. Because PEG-RBV was only approved for use in Japan in 2005, we also examined predictive factors in patients who were treated with interferon monotherapy, which is still used in the event of an adverse reaction to ribavirin.

Patients and methods

Patients and study design

We studied 719 Japanese patients with chronic hepatitis C (positive for HCV RNA for more than 6 months) who received interferon therapy with or without ribavirin between 2002 and 2008. Patients were treated at Toranomon Hospital in Tokyo, Hiroshima University Hospital, and hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html). All patients were negative for hepatitis B surface antigen, had no evidence of other liver diseases, such as auto-immune hepatitis or alcoholic liver disease, and had not received immunosuppressive therapy before enrollment in the study. All patients gave written informed consent to participate in the study in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and according to the process approved by the ethical committees of Hiroshima University and the SNP Research Center at the Institute of Physical and Chemical Research (RIKEN) in Yokohama.

PEG-RBV patients received weekly injections of peg-interferon-alpha-2b at 1.5 g/kg body weight for 24 weeks. Ribavirin was administered orally, and the dosage was determined based on the patient's body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients receiving interferon monotherapy were treated daily with 6 million units of IFN intramuscularly for 8 weeks, followed by the same dose three times a week for 16 weeks, for a total of 528 million units. Successful treatment was ascertained based on sustained virological response (SVR), defined as HCV RNA-negative six months after cessation of therapy. Fibrosis stage and activity were diagnosed by pathologists at each hospital according to the criteria of Desmet et al. [39]. Patients were classified as interferon treatment naïve or experienced based on prior interferon treatment but only parameters related to the most recent therapy were used in the analysis.

SNP Genotyping and quality control

We genotyped each patient for three *IL28B* SNPs previously reported to be associated with therapy outcome: rs8099917, rs12979860, and rs12980275. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader assay, as described previously [40,41]. We were unable to determine genotypes for one of the 796 patients for rs8099917, six of the patients for rs12979860, and two for rs12980275.

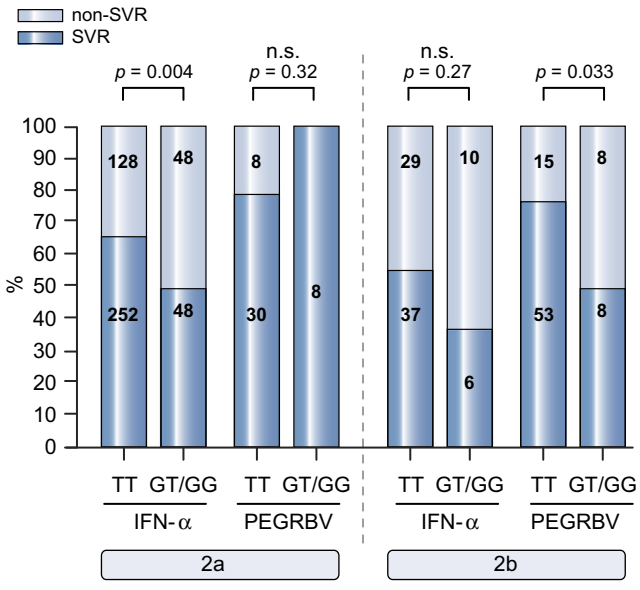

Fig. 1. Effect of interferon therapy on patients with genotype 2a and 2b infection. Sustained viral responders (SVR) and non-responders (non-SVR) were analyzed by IL28B SNP rs8099917 genotype, viral genotype, and treatment type. All patients were interferon-naïve.

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Table 2. Predictors for SVR in treatment-naïve patients treated with peg-interferon plus ribavirin combination therapy.

		Simple		Multiple			
Genotype	Variable	n	*p*	n	OR	(95% CI)	*p*
2a + 2b	Age	130	0.42				
	Sex	130	0.62				
	Genotype	130	0.21				
	Viral load	127	0.002 **	127	0.19	(0.06-0.55)	0.002 **
	Fibrosis	110	0.25				
	rs8099917	130	0.23				
	rs12980275	129	0.79				
2a	Age	46	0.77				
	Sex	46	0.62				
	Viral load	44	0.16				
	Fibrosis	39	0.75				
	rs8099917	46	0.8				
	rs12980275	45	0.77				
2b	Age	84	0.14				
	Sex	84	0.58				
	Viral load	83	0.01 *	83	0.13	(0.03-0.62)	0.01 *
	Fibrosis	71	0.08				
	rs8099917	84	0.03 *	83	0.23	(0.06-0.80)	0.02 *
	rs12980275	84	0.21				

*p <0.05; **p <0.01; ***p <0.001.

HCV RNA levels

HCV RNA levels, corresponding to initial viral load, were measured using one of several RT-PCR-based methods (the original Amplicor method, the high range method, or the TaqMan RT-PCR test). The measurement ranges of these assays were 0.5–850 KIU/ml, 5–5000 KIU/ml, and 1.2–7.8 log IU, respectively. Saturated samples were diluted with PBS and reanalyzed. All values were reported as log IU/ml.

Statistical analysis

Genotype-based associations were tested using the Cochran–Armitage trend test. Combined analysis was performed using the Mantel–Haenszel method. Simple and multiple regression analyses were used to examine the association between viral and clinical factors using p <0.05 as the criterion for inclusion in the multivariate model. HCV RNA was converted into a binary variable based on the median. Multivariate logistic regression analysis was performed using the Design package in *R* (http://www.r-project.org) with fast backward elimination and validation based on AIC score for model construction.

Results

Clinical characteristics are summarized by genotype in Table 1. The SVR rate was slightly but not significantly higher among patients with genotype 2a (340 out of 530; 64%) compared to genotype 2b patients (115 out of 189; 61%) (p = 0.43). Patients who were treated with PEG-RBV had a slightly but not significantly higher rate of SVR (111 out of 160; 69%) than patients treated with interferon monotherapy (344 out of 559, 61%) (p = 0.08). Because the number of patients treated with interferon monotherapy (559) greatly exceeds the number of patients treated with PEG-RBV (160), patients were analyzed separately by treatment type. Because 30 out of the 719 patients (4%) had received prior interferon treatment, only treatment-naïve patients were included in the analyses mentioned below, followed by a separate analysis of the effect of prior interferon treatment on SVR rate.

IL28B polymorphisms

Minor allele frequencies for rs8099917, rs12979860, and rs12980275 were 0.109, 0.112, and 0.132, respectively. The frequency of the rs8099917 risk allele was lower in SVR patients than non-SVR patients (0.089 vs. 0.14; p = 1.03e-05). The risk allele frequency among all patients was slightly higher than in the HapMap-JPT population (0.109 vs. 0.093; p = 0.01) but lower than in the HapMap-CEU population (0.109 vs. 0.183; p = 1.6e-05). We compared rs8099917 allele and genotype frequencies with 900 healthy Japanese subjects but found no significant differences. 67% of patients (372 out of 552) with the favorable rs8099917 TT genotype achieved SVR, compared to 51% (70 out of 136) of patients with GT or GG genotypes. Fig. 1 shows the joint effects of treatment type, viral genotype, and rs8099917 genotype. In every case results for rs8099917 and rs12979860 are the same, but both factors cannot be included in a multivariate model simultaneously due to multicollinearity, so results for rs8099917 are presented due to the higher genotyping success rate.

Predictive factors for SVR in patients treated with PEG-RBV

Among treatment-naïve patients treated with PEG-RBV, 78% (83 out of 106) of patients with rs8099917 TT achieved SVR compared

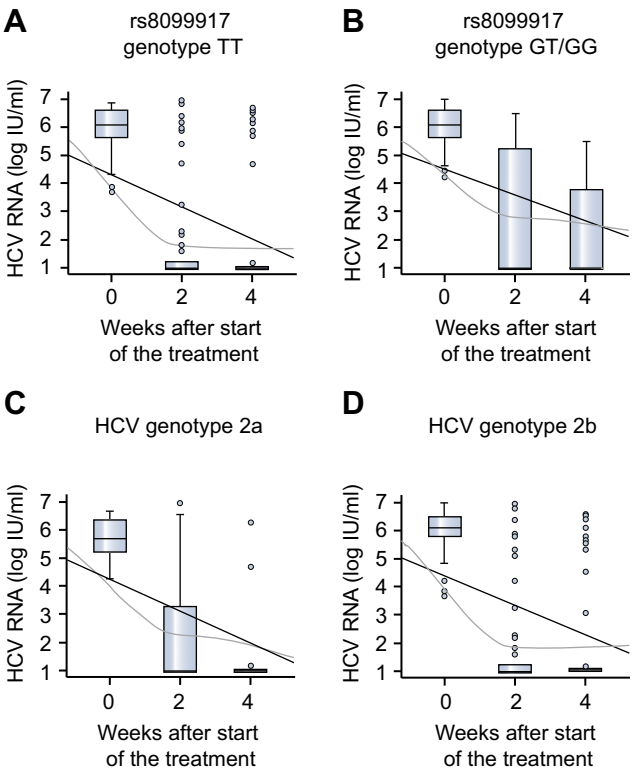

Fig. 2. Effect of rs8099917 genotype and HCV genotype on change in HCV RNA levels. HCV RNA levels at 0, 2, and 4 weeks after the start of peg-interferon plus ribavirin combination therapy in treatment-naïve patients. (A and B) Change in viral load for patients with the protective TT genotype for rs8099917 (A) compared to patients with the GT or GG genotypes (B). (C and D) Change in viral load for patients with HCV genotype 2a (A) versus genotype 2b (B).

to 67% (16 out of 24) of patients with non-TT genotypes ($p = 0.29$). In univariate and multivariate analyses, only viral load was an independent predictive factor for SVR ($p = 0.002$; Table 2), but when we examined genotypes 2a and 2b separately, rs8099917 genotype ($p = 0.02$) and viral load ($p = 0.01$) were both significant independent predictors of SVR for patients with genotype 2b, whereas no significant univariate or multivariate predictors were found for patients with genotype 2a. Notably, however, all 8 patients with genotype 2a with rs8099917 GT/GG achieved SVR (Fig. 1). The same pattern held for patients with rs12979860 TC/TT (9 SVR, 0 non-SVR) and rs12980275 GA/GG (11 SVR, 0 non-SVR) genotypes. Moreover, none of these patients was homozygous for the risk allele at each SNP.

Change in HCV RNA levels for patients treated with PEG-RBV

HCV RNA levels at the start of PEG-RBV therapy and after 2 and 4 weeks of treatment are plotted by rs8099917 genotype and viral genotype in Fig. 2. Under multivariate analysis, rs8099917 genotype was an independent predictive factor for change in HCV RNA level by week 2 ($p = 0.036$) but viral genotype was not significant ($p = 0.15$). For changes in HCV RNA levels by week 4, neither the rs8099917 genotype nor the viral genotype was significant ($p = 0.17$ and $p = 0.22$, respectively).

Predictive factors for SVR in patients treated with interferon monotherapy

Among patients treated with interferon monotherapy, 65% of patients with rs8099917 TT achieved SVR, compared to only 48% of patients with GT or GG genotypes ($p = 0.002$). Viral load and the rs8099917 and rs12980275 genotypes were significant univariate predictors of SVR, and under multivariate analysis viral load and rs8099917 remained as independent predictors (Table 3). When genotypes 2a and 2b were analyzed separately, viral load ($p = 0.001$) and rs8099917 genotype ($p = 0.014$) were independent predictive factors for SVR in patients with genotype 2a but no significant univariate of multivariate terms were found for genotype 2b.

Effect of prior interferon treatment

Thirty out of the 719 patients (4%) had previously received treatment with interferon. Among these patients, only 40% achieved SVR, compared to the 64% SVR rate among treatment-naïve patients. Initial viral load was the only independent predictor of SVR in these patients, whereas in treatment-naïve patients, viral load, rs8099917 genotype, and treatment type (PEG-RBV vs interferon monotherapy) were independent predictors of SVR (Table 4).

Development of resistance to interferon therapy

Over the course of therapy five patients developed resistance to PEG-RBV treatment. In each case the patient showed an initial drop in viremia followed by viral breakthrough. Three out of the five patients were heterozygous (T/G) for the rs8099917 genotype and two out of the five were homozygous for the favorable allele (T/T).

Discussion

As the effect of *IL28B* polymorphism has not been reported separately for genotype 2 and its subtypes so far, we investigated whether the polymorphism influences treatment outcome in patients with HCV genotype 2a and 2b infections. In addition to previously reported effects for genotypes 1 and 4, our results demonstrate that polymorphisms in the *IL28B* locus are also predictive for SVR in genotype 2 (Table 2). We also showed that the favorable *IL28B* SNP genotype is associated with a rapid decrease in HCV RNA levels, which is itself a predictive factor for SVR [42]. Several studies have reported that polymorphisms at the *IL28B* locus affect the outcome of peg-interferon and ribavirin combination therapy in patients with HCV genotype 1b [34–36,38]. In particular, associations with therapy outcome have been reported for two SNPs in strong linkage disequilibrium, rs8099917 (T/G), and rs12979860 (C/T). Only a few studies have examined the effect of the SNP on the treatment outcome for other genotypes. Rallón et al. reported that the rs12979860 genotype is associated with treatment outcome for genotypes 1 and 4 but not genotype 3 in patients with HIV/HCV co-infection [43]. Similarly Rauch et al. reported an association between rs8099917 polymorphism and NVR for genotypes 1 and 4 (difficult-to-treat) but not for genotypes 2 and 3 (easier-to-treat) but the effect due to genotype 2 alone is unclear [38]. In a recent study, Mangia et al. also exam-

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Table 3. Predictors for SVR in treatment-naïve patients treated with IFN monotherapy.

		Simple			Multiple			
Genotype	Variable	n	*p*		n	OR	(95% CI)	*p*
2a + 2b	Age	559	0.35					
	Sex	559	0.17					
	Genotype	559	0.068					
	Viral load	507	0.0002	***	506	0.59	(0.45-0.77)	0.0001 ***
	Fibrosis	450	0.61					
	rs8099917	558	0.001	**	506	0.52	(0.33-0.82)	0.005 **
	rs12980275	558	0.009	**				
2a	Age	477	0.19					
	Sex	477	0.2					
	Viral load	425	0.001	**	424	0.6	(0.44-0.81)	0.001 ***
	Fibrosis	382	0.37					
	rs8099917	476	0.003	**	424	0.53	(0.32-0.88)	0.014 *
	rs12980275	476	0.01	**				
2b	Age	82	0.67					
	Sex	82	0.56					
	Viral load	82	0.47					
	Fibrosis	68	0.53					
	rs8099917	82	0.19					
	rs12980275	82	0.44					

*p <0.05; **p <0.01; ***p <0.001.

ined genotypes 2 and 3 and found a significant association between rs12979860 genotype and rapid virological response (RVR) at week 4 for genotype 2 [44]. While rs12979860 was not directly associated with SVR in their study, rs12979860 genotype was significantly associated with SVR among those patients who failed to achieve RVR. In this study, we found a significant association between rs8099917 genotype and RVR in multivariate analysis for genotype 2b ($p = 0.028$, data not shown) but not for genotype 2a. When RVR was included as a factor in multivariate logistic regression analysis for genotype 2b, RVR and rs8099917 genotype were both retained in the final model but only RVR was significant (RVR: $p = 4.9e\text{-}05$; rs8099917: $p = 0.0850$; data not shown). When only non-RVR patients were included, no factors were significant; however, there were only six patients who achieved SVR without RVR and only one patient who achieved RVR but then failed to achieve SVR.

Although SVR rate was generally higher for genotype 2a, as reported previously [20,21], we found few differences between genotypes 2a and 2b. However, when analyzed separately, the results suggest an interesting interaction between the *IL28B* genotype, the viral genotype, and treatment type. In particular, we found that rs8099917 was a predictive factor for genotype 2a treated with IFN but not PEG-RBV, and conversely for genotype 2b treated with PEG-RBV but not IFN. This result is likely due to the relatively small sample sizes, but nonetheless all 8 (100%) of the genotype 2a PEG-RBV patients lacking the favorable rs8099917 genotype achieved SVR, compared to less than 50% for IFN therapy or either type of treatment with genotype 2b. In fact, each patient was heterozygous for each of the three *IL28B* SNPs examined. A further complication is that each of the five patients who developed resistance to interferon therapy was infected with genotype 2a, and two of these patients had the favorable rs8099917 TT genotype while the others were heterozygous (GT). More detailed analysis will be required to interpret these results.

Because PEG-RBV therapy was not covered by insurance in Japan until 2005, we also present data comparing the effects of *IL28B* polymorphisms on treatment with the older IFN monotherapy versus the more recent PEG-RBV combination therapy. Although the small sample sizes within each patient group likely underestimate the effect of SNP genotype, we found that rs8099917 influences response to IFN monotherapy in patients with genotype 2a and also influences the response to PEG-RBV therapy in patients with genotype 2b. Although PEG-RBV is currently the standard treatment for chronic hepatitis C infection, interferon monotherapy may still be used in the case of intolerance to ribavirin; therefore, it is important to understand the direct effects of interferon with and without ribavirin. Moreover, even with the advent of protease inhibitors and other antiviral drugs undergoing clinical trials, they are likely to be co-administered with interferon to prevent the otherwise rapid emergence of resistant quasispecies [45].

In summary, we showed that the *IL28B* SNP genotype is an important predictive factor for SVR and early viral dynamics in patients with HCV genotypes 2a and 2b.

Conflict of interest

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

Table 4. Comparison of predictive factors for SVR based on prior treatment with interferon.

		Simple			Multiple				
	Variable	n	*p*		n	OR	(95% CI)	*p*	
All	Age	719	0.70						
	Sex	719	0.28						
	Genotype	719	0.42						
	Viral load	663	6.00E-02	***	662	0.63	(0.51-0.79)	4.30E-05	***
	Fibrosis	585	0.83						
	rs8099917	718	0.002	**	662	0.57	(0.38-0.85)	0.0055	**
	rs12980275	717	0.03	*					
	Treatment	719	0.054						
Naïve	Age	689	0.58						
	Sex	689	0.18						
	Genotype	689	0.62						
	Viral load	634	0.0011	**	633	0.53	(0.41-0.69)	2.00E-06	***
	Fibrosis	560	0.95						
	rs8099917	688	0.00059	***	633	0.5	(0.33-0.77)	0.0015	**
	rs12980275	687	0.013	*					
	Treatment	689	0.0013	**	633	3.01	(1.82-4.99)	1.80E-05	***
Experienced	Age	30	0.91						
	Sex	30	0.75						
	Genotype	30	0.14						
	Viral load	29	0.032	*	29	0.21	(0.05-0.87)	0.032	*
	Fibrosis	25	0.53						
	rs8099917	30	0.12						
	rs12980275	30	0.1						
	Treatment	30	N/A						

*p <0.05; **p <0.01; ***p <0.001.

Acknowledgments

This work was supported in part by Grants-in-Aid for scientific research and development from the Ministry of Health, Labor and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology, of the Government of Japan. Part of this work was carried out at the Analysis Center of Life Science, Hiroshima University. The authors thank Yasufumi Hayashida, Rie Akiyama, Yoshie Yoshida, Kazuyo Hattori, Mariko Shiota, Hiromi Ishino, and Takako Yokogi for excellent technical assistance, and Yuko Nagai, Junko Sakamiya and Aya Furukawa for clerical assistance.

References

[1] Alter MJ. Epidemiology of hepatitis C in the West. Semin Liver Dis 1995;15:5–14.
[2] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349:825–832.
[3] Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 1990;12:671–675.
[4] Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–1801.
[5] Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology 1995;21:650–655.
[6] Shiratori Y, Shiina S, Imamura M, Kato N, Kanai F, Okudaira T, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C-viral infection in Japan. Hepatology 1995;22:1027–1033.
[7] Tomimatsu M, Ishiguro N, Taniai M, Okuda H, Saito A, Obata H, et al. Hepatitis C virus antibody in patients with primary liver cancer (hepatocellular carcinoma, cholangiocarcinoma, and combined hepatocellular–cholangiocarcinoma) in Japan. Cancer 1993;72:683–688.
[8] Takada N, Takase S, Takada A, Date T. Differences in the hepatitis C virus genotypes in different countries. J Hepatol 1993;17:277–283.
[9] Zeuzem S, Franke A, Lee JH, Herrmann G, Ruster B, Roth WK. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. Hepatology 1996;24:1003–1009.
[10] Adinolfi LE, Utili R, Andreana A, Tripodi MF, Rosario P, Mormone G, et al. Relationship between genotypes of hepatitis C virus and histopathological manifestations in chronic hepatitis C patients. Eur J Gastroenterol Hepatol 2000;12:299–304.
[11] Martinot-Peignoux M, Marcellin P, Pouteau M, Castelnau C, Boyer N, Poliquin M, et al. Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alfa therapy in chronic hepatitis C. Hepatology 1995;22:1050–1056.
[12] Tsubota A, Chayama K, Arase Y, Koida I, Saitoh S, Ikeda K, et al. Factors useful in predicting the response to interferon therapy in chronic hepatitis C. J Gastroenterol Hepatol 1993;8:535–539.
[13] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

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[14] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.

[15] Mangia A, Minerva N, Bacca D, Cozzolongo R, Agostinacchio E, Sogari F, et al. Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. Hepatology 2009;49:358–363.

[16] von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40 kDa) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129:522–527.

[17] Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609–2617.

[18] Fujiwara K, Yokosuka O, Komine F, Moriyama M, Kato N, Yoshida H, et al. Twenty-four weeks of interferon alpha-2b in combination with ribavirin for Japanese hepatitis C patients: sufficient treatment period for patients with genotype 2 but not for patients with genotype 1. Liver Int 2006;26:520–528.

[19] Kawaoka T, Kawakami Y, Tsuji K, Ito H, Kitamoto M, Aimitsu S, et al. Dose comparison study of pegylated interferon-alpha-2b plus ribavirin in naive Japanese patients with hepatitis C virus genotype 2: a randomized clinical trial. J Gastroenterol Hepatol 2009;24:366–371.

[20] Akuta N, Suzuki F, Tsubota A, Suzuki Y, Someya T, Kobayashi M, et al. Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interaction of viral, host and interferon treatment-related factors. J Hepatol 2002;37:831–836.

[21] Akuta N, Suzuki F, Tsubota A, Suzuki Y, Hosaka T, Someya T, et al. Association of amino acid substitution pattern in nonstructural protein 5A of hepatitis C virus genotype2a low viral load and response to interferon monotherapy. J Med Virol 2003;69:376–383.

[22] Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. Association of Amino Acid Substitution Pattern in Core Protein of Hepatitis C Virus Genotype 2a High Viral Load and Virological Response to Interferon-Ribavirin Combination Therapy. Intervirology 2009;52:301–309.

[23] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 2007;46:1357–1364.

[24] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–1695.

[25] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.

[26] Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, et al. Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. J Med Virol 2009;81:640–649.

[27] Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. Hepatology 2003;38:639–644.

[28] Romero-Gomez M, Del Mar Viloria M, Andrade RJ, Salmeron J, Diago M, Fernandez-Rodriguez CM, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology 2005;128:636–641.

[29] Hijikata M, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt −88) correlated with the response of hepatitis C patients to interferon. Intervirology 2000;43:124–127.

[30] Knapp S, Yee LJ, Frodsham AJ, Hennig BJ, Hellier S, Zhang L, et al. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. Genes Immun 2003;4:411–419.

[31] Matsuyama N, Mishiro S, Sugimoto M, Furuichi Y, Hashimoto M, Hijikata M, et al. The dinucleotide microsatellite polymorphism of the IFNAR1 gene promoter correlates with responsiveness of hepatitis C patients to interferon. Hepatol Res 2003;25:221–225.

[32] Naito M, Matsui A, Inao M, Nagoshi S, Nagano M, Ito N, et al. SNPs in the promoter region of the osteopontin gene as a marker predicting the efficacy of interferon-based therapies in patients with chronic hepatitis C. J Gastroenterol 2005;40:381–388.

[33] Tsukada H, Ochi H, Maekawa T, Abe H, Fujimoto Y, Tsuge M, et al. A polymorphism in MAPKAPK3 affects response to interferon therapy for chronic hepatitis C. Gastroenterology 2009;136:1796–1805, e1796.

[34] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.

[35] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.

[36] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104.

[37] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.

[38] Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138:1338–1345, e1331–1337.

[39] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis – diagnosis, grading and staging. Hepatology 1994;19:1513–1520.

[40] Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 2001;46:471–477.

[41] Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidy-larginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395–402.

[42] Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, et al. IL28B polymorphism improves viral kinetics and is the strongest pre-treatment predictor of SVR in HCV-1 patients. Gastroenterology 2010;139:120–129.

[43] Rallon NI, Naggie S, Benito JM, Medrano J, Restrepo C, Goldstein D, et al. Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus-coinfected patients. AIDS 2010;24:F23–29.

[44] Mangia A, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, et al. IL28B polymorphism determines treatment response of patients with hepatitis C genotypes 2 or 3 who do not achieve a rapid virologic response. Gastroenterology 2010;139:821–827.

[45] Lange CM, Sarrazin C, Zeuzem S. Review article: HCV – STAT-C era of therapy. Aliment Pharmacol Ther 2010;31:31.