Acta Med. Okayama, 2014
Vol. 68, No. 1, pp. 17–22

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Impact of Comorbid Hepatic Steatosis on Treatment of Chronic Hepatitis C in Japanese Patients and the Relationship with Genetic Polymorphism of IL28B, PNPLA3 and LDL Receptor

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The impact of hepatic steatosis on interferon therapy for patients with chronic hepatitis C (CHC) has been associated with single-nucleotide polymorphisms (SNP) of IL28B, patatin-like phospholipase domain-containing protein 3 (PNPLA3), and low-density lipoprotein (LDL) receptor. Whether this holds true for Japanese patients, however, remains unresolved. The present study prospectively enrolled 226 Japanese patients with CHC, and investigated the impact of hepatic steatosis and its related SNPs, including rs8099917 of IL28B, rs738409 of PNPLA3, and rs14158 of LDL receptor, on outcomes of peg-interferon and ribavirin therapy. In multivariate logistic regression analysis, significant factors affecting the severity of hepatic steatosis were high body mass index and the minor alleles of IL28B SNP ($p = 0.020$ and 0.039, respectively). The risk alleles of PNPLA3 SNP also showed weak association ($p = 0.059$). Severe steatosis and the minor alleles of IL28B SNP were significantly associated with null or partial virological response in patients with HCV genotype 1, as were female gender, and low LDL cholesterol ($p = 0.049$, and < 0.001, respectively). The SNP genotype of PNPLA3 and LDL receptor did not have a significant impact on therapeutic outcomes. With respect to the SNP sites examined, the SNP of PNPLA3 has a weak association with severe hepatic steatosis, but not with the outcome of interferon therapy.

Key words: hepatic steatosis, genetic polymorphism, interferon, HCV

Hepatitis C virus (HCV) infection causes chronic hepatitis, and may progress to liver cirrhosis and hepatocellular carcinoma. More than 170 million people worldwide are infected with HCV, creating a serious global health problem [1]. Combination therapy with pegylated interferon-α (PegIFN) and ribavirin (RBV) achieves a sustained virological response (SVR) in more than 50% of patients with HCV genotype 1 [2]. Recent therapeutic regimens using direct-acting antiviral agents have improved therapeutic outcomes for HCV patients, achieving an SVR of up to 80% [3–5]. Null or partial virological responders still exist, however, due to drug-resistant viruses or other causes [6–8].

Received May 2, 2013; accepted September 2, 2013.
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Single-nucleotide polymorphisms (SNPs) near the IL28B gene are significantly associated with therapeutic outcomes for patients with HCV genotype 1 [9]. In addition, older age, female gender, advanced liver fibrosis, and hepatic steatosis affect therapeutic outcomes. Recently, significant associations have been found between hepatic steatosis and fibrosis and the SNP of rs738409 in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) in patients with fatty liver disease, alcoholic liver disease, and chronic hepatitis C [10, 11]. The impact of this SNP on therapeutic outcomes of PegIFN and RBV has been reported for patients with chronic hepatitis C (CHC). These results were mostly obtained from the analysis of Caucasian patients [12, 13], however, and their applicability to Japanese patients remains uncertain. When human immunodeficiency virus (HIV)/HCV co-infected patients received IFN therapy with PegIFN and RBV, the SNP of rs14158 in the low-density lipoprotein (LDL) receptor may have affected therapeutic outcomes in patients with the major allele of IL28B SNP [14]. Furthermore, the association of this SNP with therapeutic outcome has not been clarified for HCV mono-infected patients.

The present study investigated the impacts of hepatic steatosis and its related SNPs on the outcomes of PegIFN and RBV therapy for Japanese patients with CHC.

Methods

Patients. This study was a prospective analysis of 226 Japanese CHC patients who received antiviral therapy with standard doses of PegIFN alpha-2a or 2b with RBV between 2005 and 2010 at Okayama University Hospital or Kagawa Prefectural Central Hospital. Hepatocellular carcinoma was ruled out with dynamic computed tomography or magnetic resonance imaging, in combination with serum alpha-fetoprotein. Patients with hepatitis B virus co-infection, HIV co-infection, or autoimmune liver disease were not included in the study. The study was performed in accordance with the Helsinki Declaration, and the protocols were approved by the ethics committees of the participating institutions. All patients provided informed consent before enrollment into the study.

Diagnosis of liver histology. Liver histology was evaluated for all patients prior to the start of therapy. Patients' histological fibrosis stage and hepatitis activity grade were assigned by 2 pathologists according to the criteria outlined in Desmet *et al.* [15]. The severity (grade) of hepatic steatosis was defined according to the criteria used by Valenti *et al.* [13]. Hepatic steatosis occupying less than 5% of the quadrant in the liver was classified as no steatosis; steatosis occupying 5% to less than 30% was classified as mild; and steatosis occupying 30% or more of the quadrant was considered severe.

Genotyping of single nucleotide polymorphisms. Genomic DNA was extracted from whole-blood samples by means of a QIAamp DNA Mini Kit, according to the manufacturer's protocol (Qiagen, Tokyo, Japan). The SNPs rs8099917 of IL28B, rs738409 of PNPLA3, and rs14158 of LDL receptors were genotyped using the TaqMan predesigned SNP genotyping assays in a LightCycler 480 system, as recommended by the manufacturer (Roche Diagnostics, Tokyo, Japan). The SNP genotypes of all the samples were obtained with these systems.

Statistical analysis. Data are expressed as the mean ± standard deviation. Correlations between patient laboratory data and histological findings were evaluated using the Chi-square test and Spearman's rank correlation coefficient. Factors associated with the presence and severity of hepatic steatosis, or the patient characteristics associated with therapeutic outcome, were analyzed by logistic regression, and selected in a stepwise manner among the significant factors in univariate analysis for the multivariate analysis. A value of $p < 0.05$ was considered significant. Statistical analysis was performed with JMP software (SAS Institute, Cary, NC, USA).

Results

Relation between patient characteristics and hepatic steatosis. As shown in Table 1, hepatic steatosis was classified as severe for 34 patients, mild for 92 patients, and as none for 99 patients. We compared patient characteristics at enrollment among these three steatosis-severity groups. Body mass index (BMI), serum triglyceride levels, and homeostasis model assessment insulin resistance (HOMA-IR) were significantly associated with severity of hepatic steatosis ($p < 0.001$, 0.029, and 0.0026, respectively; Spearman's rank correlation coefficient). Severe

Table 1 Patient characteristics and the severity of hepatic steatosis at enrollment

	Severity of hepatic steatosis			
	None (n = 99)	Mild (n = 92)	Severe (n = 34)	*P*
Age (years)	57 ± 10‡	58 ± 9‡	58 ± 10‡	0.46
Sex (male/female)	49/50	47/45	15/19	0.78
Body mass index	23 ± 3‡	24 ± 3‡	25 ± 4‡	0.0002
Liver fibrosis (F1/F2/F3/F4)	46/38/11/3	27/35/23/7	11/8/14/1	0.0008
Hepatitis activity (A1/A2/A3)	77/19/2	68/19/3	19/14/1	0.069
IL28B (TT/TG/GG)	78/20/1	78/14/0	19/14/1	0.0024
PNPLA3 (CC/GC/GG)	23/50/26	27/46/19	3/22/9	0.055
LDL receptor (GG/GA/AA)	38/42/19	36/40/16	11/13/10	0.77
HCV genotype (1/2)	69/30	70/22	22/12	0.94
ALT (IU/L)	61 ± 63‡	74 ± 55‡	67 ± 36‡	0.0089
γGT (IU/L)	48 ± 49‡	57 ± 53‡	71 ± 56‡	0.0003
LDL cholesterol (mg/dL)	102 ± 29‡	99 ± 24‡	96 ± 32‡	0.17
Triglyceride (mg/dL)	106 ± 52‡	113 ± 53‡	136 ± 93‡	0.029
HOMA-IR	4.3 ± 8.8‡	4.6 ± 8.8‡	4.5 ± 6.1‡	0.0026

‡Mean ± standard deviation. PNPLA3, patatin-like phospholipase domain-containing protein 3; LDL, low-density lipoprotein; ALT, alanine aminotransferase; γGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment insulin resistance.

hepatic steatosis was significantly associated with advanced fibrosis stages and the minor alleles (TG or GG) of IL28B SNP ($p < 0.001$, and 0.0024, respectively). The patients with the risk alleles (GC or GG) of PNPLA3 SNP showed a slight tendency toward an association with severe hepatic steatosis ($p = 0.055$), while the SNP genotypes of LDL receptor were not associated with severity of hepatic steatosis ($p = 0.77$). Distributions of age, sex, and HCV genotype did not differ significantly according to severity of hepatic steatosis.

Differences in the relation of the associated factors to the presence and severity of hepatic steatosis. Table 2 shows the results of stepwise logistic regression analysis for the association of patient characteristics with the presence or severity of hepatic steatosis. Advanced fibrosis stages were significantly associated with the presence of hepatic steatosis ($p = 0.0052$), but not with its severity. Higher BMI was significantly associated with both the presence and the severity of hepatic steatosis ($p < 0.001$, and 0.020, respectively). In contrast, the minor alleles of IL28B SNP showed a strong association with the severity but not the presence of hepatic steatosis ($p = 0.0039$). Patients with the risk alleles of PNPLA3 SNP showed a slight tendency toward an association with severe hepatic steatosis ($p = 0.059$).

The impact of hepatic steatosis and its related SNPs on IFN therapeutic outcomes. As for associations between the presence or severity of hepatic steatosis and therapeutic outcomes, SVR was not significantly associated with the presence or severity of hepatic steatosis (Odds 0.69, $p = 0.14$, and Odds 0.63, $p = 0.22$, respectively, by logistic regression analysis), while NVR was significantly associated with the severity, but not the presence of, hepatic steatosis (Odds 3.2, $p = 0.0034$, and odds 1.7, $p = 0.11$, respectively). Among the patients with a null or partial virological response (NVR), a greater percentage had severe hepatic steatosis (41.2%) than no or mild steatosis (16.5% and 19.6%, respectively, $p = 0.0033$). The association of hepatic steatosis and its related SNPs to therapeutic outcomes were further analyzed separately for patients with HCV genotypes 1 and 2, because these patient groups were treated with different IFN regimens. Among the patients with HCV genotype 1, 63 patients (41.1%) obtained SVR, while 44 (28.8%) obtained a null or partial response. Early liver fibrosis stages and the major allele of IL28B SNP were significantly associated with SVR for the patients with HCV genotype 1 ($p = 0.0055$ and 0.024; stepwise logistic regression analysis, Table 3). The minor alleles of IL28B SNP, female gender, low levels of LDL cholesterol, and severe hepatic steatosis were significant predictors for NVR ($p = 0.0001$, 0.011, 0.006, and 0.049, respec-

Table 2 Logistic regression analysis of the factors related to the presence or severity of hepatic steatosis

	Analysis for presence of steatosis				Analysis for severity of steatosis			
	Univariate		Multivariate		Univariate		Multivariate	
Factors	Odds (range†)	*p*	Odds (range†)	*p*	Odds (range†)	*p*	Odds (range†)	*p*
Age	1.0 (0.98–1.0)	0.41			1.0 (0.97–1.1)	0.57		
Sex (male = 1)	1.0 (0.58–1.7)	0.97			0.78 (0.38–1.6)	0.51		
Body mass index	1.2 (1.1–1.3)	0.0003	1.2 (1.1–1.3)	0.0005	1.1 (1.1–1.3)	0.0049	1.2 (1.0–1.3)	0.020
Liver fibrosis	1.7 (1.3–2.4)	0.0007	1.6 (1.2–2.3)	0.0052	2.6 (0.92–2.0)	0.13		
Hepatitis activity	1.3 (0.81–2.2)	0.26			1.9 (1.0–3.5)	0.047	1.6 (0.79–3.2)	0.20
IL28B (TT = 1)	0.9 (0.48–1.7)	0.75			0.28 (0.13–0.61)	0.0014	0.37 (0.15–0.95)	0.039
PNPLA3 (CC = 1)	1.0 (0.56–1.9)	0.92			0.27 (0.08–0.93)	0.038	0.29 (0.081–1.0)	0.059
LDL receptor (GG = 1)	0.96 (0.56–1.6)	0.77			0.76 (0.35–1.6)	0.48		
Genotype (type 1 = 1)	1.2 (0.66–2.1)	0.58			0.69 (0.32–1.5)	0.34		
ALT	1.0 (1.0–1.0)	0.18			1.0 (0.99–1.0)	0.99		
γGT	1.0 (1.0–1.0)	0.052	1.0 (1.0–1.0)	0.70	1.0 (1.0–1.0)	0.089	1.0 (1.0–1.0)	0.44
LDL cholesterol	1.0 (0.99–1.0)	0.32			0.94 (0.98–1.0)	0.31		
Triglyceride	1.0 (1.0–1.0)	0.16			1.0 (1.0–1.0)	0.029	1.0 (1.0–1.0)	0.19
HOMA-IR	1.0 (0.99–1.0)	0.49			0.99 (0.97–1.0)	0.59		

†95% confidence interval; PNPLA3, patatin-like phospholipase domain-containing protein 3; LDL, low-density lipoprotein; ALT, alanine aminotransferase; γGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment insulin resistance.

Table 3 Logistic regression analysis of the factors related to the therapeutic outcomes of interferon therapy for the patients with HCV genotype 1

	Analysis for sustained virological response				Analysis for non-virological response			
	Univariate		Multivariate		Univariate		Multivariate	
Factors	Odds (range†)	*p*	Odds (range†)	*p*	Odds (range†)	*p*	Odds (range†)	*p*
Age	0.95 (0.92–0.99)	0.0064	0.97 (0.93–1.0)	0.12	1.1 (1.0–1.1)	0.0036	1.1 (0.99–1.1)	0.084
Sex (male = 1)	1.4 (0.73–2.6)	0.32			0.51 (0.25–1.0)	0.065	0.27 (0.094–0.74)	0.011
Body mass index	1.0 (0.92–1.1)	0.70			1.0 (0.92–1.1)	0.73		
Liver fibrosis	0.44 (0.29–0.66)	<0.0001	0.50 (0.31–0.82)	0.0055	1.6 (1.1–2.4)	0.018	1.4 (0.77–2.5)	0.28
Hepatitis activity	0.81 (0.45–1.5)	0.48			1.2 (0.64–2.2)	0.59		
Hepatic steatosis	0.50 (0.24–0.87)	0.18			1.8 (0.88–3.8)	0.11		
Severe steatosis	0.76 (0.47–1.2)	0.27			2.2 (1.4–3.6)	0.0009	1.9 (1.0–3.7)	0.049
IL28B (TT = 1)	2.8 (1.2–6.8)	0.019	3.6 (1.2–11)	0.024	0.11 (0.045–0.25)	<0.0001	0.077 (0.021–0.28)	0.0001
PNPLA3 (CC = 1)	0.72 (0.34–1.5)	0.40			1.3 (0.58–2.8)	0.54		
LDL receptor (GG = 1)	1.2 (0.61–2.2)	0.66			1.1 (0.52–2.2)	0.88		
ALT	1.0 (1.0–1.0)	0.28			1.0 (0.99–1.0)	0.80		
γGT	1.0 (1.0–1.0)	0.36			1.0 (1.0–1.0)	0.25		
LDL cholesterol	1.0 (1.0–1.0)	0.0045	1.0 (1.0–1.0)	0.068	0.97 (0.95–0.99)	0.0009	0.97 (0.95–0.99)	0.0060
Triglyceride	1.0 (1.0–1.0)	0.80			1.0 (1.0–1.0)	0.39		
HOMA-IR	1.0 (0.98–1.1)	0.25			0.99 (0.96–1.0)	0.62		

†95% confidence interval; PNPLA3, patatin-like phospholipase domain-containing protein 3; LDL, low-density lipoprotein; ALT, alanine aminotransferase; γGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment insulin resistance.

tively). Unlike in the report for patients co-infected with HCV genotype 1 and HIV [14], the minor allele of rs14158 in the LDL receptor did not affect therapeutic outcomes among the patients with the major allele of IL28B SNP ($p = 0.83$); the prevalence of the minor allele of LDL receptor SNP was 58% among the patients with SVR (35/60), 66% among those with transient virological response (TVR, 29/44), and 59%

among those with NVR (13/22). On the other hand, 34 of the patients with HCV genotype 2 (58.6%) obtained SVR. The stepwise logistic regression analysis on therapeutic outcomes for patients with HCV genotype 2 showed that the major allele of IL28B SNP was significantly associated with SVR ($p = 0.023$). Few patients showed NVR (4/58 (6.9%)), and no predictive factors could be found for NVR.

Discussion

The present study focused on hepatic steatosis and its related SNPs in Japanese patients with CHC, and clarified the impact of factors affecting hepatic steatosis on therapeutic outcomes. The results revealed that severe hepatic steatosis is significantly associated with NVR, but not with SVR. Severe hepatic steatosis was also significantly associated with the minor alleles of IL28B SNP and with high BMI. Our findings suggest that the importance of IL28B SNP on the outcomes of interferon therapy is associated with the severity of hepatic steatosis, reflecting lipid metabolic disorder, although the precise mechanism is unclear. It was reported that several SNPs around the IL28B were significantly associated with therapeutic outcome. However, these associations can be explained by a single SNP for Japanese patients, because the major SNPs of rs8099917 and rs12979860 showed nearly identical results. In terms of the SNPs of the LDL receptor, genetic variants of LDL receptor have been shown to predict SVR to interferon therapy using a combination of several SNPs [16]. The SNP of the LDL receptor we examined in this study did not show any significant associations with hepatic steatosis or the outcome of interferon therapy. The analysis of different SNPs in the LDL receptor might, however, yield various associations with disease progression or prediction.

Recent studies on the SNP of PNPLA3 in patients with CHC revealed that the 148M genotype of PNPLA3 SNP is significantly associated with hepatic steatosis due to its functional involvement in lipid metabolism; the influence of this SNP on SVR remains controversial, however [17, 18]. The present study showed that the SNP genotypes of PNPLA3 have a weak association with severe hepatic steatosis, but not with therapeutic outcomes for Japanese patients. This difference might be due to the high prevalence of the risk allele of rs738409 of PNPLA3 in Japanese patients. Prevalence of this risk allele was 50.2% in the present study, and 43% in the Hap Map study, much higher than in Caucasians (23%). The ethnic differences in the prevalence of the SNP genotype might modulate the impacts of the SNP on hepatic steatosis or IFN therapeutic outcomes.

In conclusion, hepatic steatosis was significantly associated with the outcome of interferon therapy for Japanese patients with CHC. As for the SNP sites examined in the present study, an association was suggested between the SNP of IL28B and the outcome of interferon therapy, but not the SNPs of PNPLA3 or the LDL receptor.

Acknowledgments. This work was supported in part by the Research Program of Intractable Disease sponsored by the Ministry of Health, Labor, and Welfare of Japan, and by a Grant-in-Aid (22590733) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to Y. I.). We thank Chizuru Mori for technical assistance and Toshie Ishi for valuable help with the data collection.

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