Association of *IL28B* Gene Polymorphism with Development of Hepatocellular Carcinoma in Japanese Patients with Chronic Hepatitis C Virus Infection

Satoru Joshita,[1] Takeji Umemura,[1] Yoshihiko Katsuyama,[2] Yuki Ichikawa,[1] Takefumi Kimura,[1] Susumu Morita,[1] Atsushi Kamijo,[1] Michiharu Komatsu,[1] Tetsuya Ichijo,[1] Akihiro Matsumoto,[1] Kaname Yoshizawa,[1] Nozomi Kamijo,[1] Masao Ota,[3] and Eiji Tanaka[1]

1: Department of Medicine, Division of Gastroenterology and Hepatology, Shinshu University School of Medicine, Matsumoto, Japan

2: Department of Pharmacy, Shinshu University Hospital, Matsumoto, Japan

3: Department of Legal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

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Corresponding author: Takeji Umemura, MD, PhD, Department of Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan
E-mail: tumemura@shinshu-u.ac.jp
Telephone: +81-263-37-2634; Fax: +81-263-32-9412

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List of abbreviations used

SNPs: single nucleotide polymorphisms

HCV: hepatitis C virus

HCC: hepatocellular carcinoma

IFN : interferon

PEG: pegylated

SVR: sustained virological response

Abstract

IL28B single nucleotide polymorphisms (SNPs) are associated with spontaneous and treatment-induced elimination of hepatitis C virus (HCV). To assess whether the *IL28B* rs8099917 SNP also affects the progression of chronic HCV infection, we genotyped 511 Japanese HCV patients, including 69 with hepatocellular carcinoma (HCC). The T/T genotype of rs8099917 was not associated with the development of HCC (P = 0.623), although stepwise logistic regression analysis showed liver cirrhosis, age greater than 68 years, and serum albumin < 4.2 mg/dL were associated with HCC onset. It appears that the *IL28B* SNP does not directly influence hepato-carcinogenesis in chronic HCV infection.

1. Introduction

Nearly 2 million individuals in Japan and 170 million people worldwide are infected with the hepatitis C virus (HCV). Persistent infection with HCV causes chronic hepatitis and eventually leads to liver cirrhosis and/or hepatocellular carcinoma (HCC) [1]. HCC is one of the major causes of cancer-related death, and patients with HCV-associated HCC account for 70-80% of HCC cases in Japan [2]. It is generally considered that several factors, including viral, host, and environmental elements, are involved in the development of HCV-associated HCC. Recently, single nucleotide polymorphisms (SNPs) around the *IL28B* gene have been identified as strong predictors of spontaneous [3] and treatment-induced HCV clearance (reviewed in [4]). Although such studies have confirmed an association of *IL28B* SNPs with the outcome of antiviral therapy, only a few have investigated whether this genetic variant also affects the progression of chronic HCV infection [5-7]. Furthermore, it remains unclear whether *IL28B* SNPs influence the development of HCV-associated HCC. The aim of the present cross-sectional study was to determine the association between the *IL28B* rs8099917 SNP and HCC onset in Japanese patients with chronic HCV infection.

2. Material and Methods

2.1 Subjects

A total of 511 consecutively treated Japanese patients with chronic HCV infection who were seen at Shinshu University Hospital (Matsumoto, Japan) between April 2004 and December 2010 were enrolled. The diagnosis of chronic hepatitis C was based on the following criteria: 1) the presence of both anti-HCV antibody and HCV RNA in the serum for at least 6 months; 2) the absence of detectable hepatitis B surface

antigen and antibody to the human immunodeficiency virus; and 3) the exclusion of other causes of chronic liver disease, including Wilson's disease and hemochromatosis. Liver cirrhosis was diagnosed by histological examination and/or characteristic clinical signs of advanced liver disease. HCC was diagnosed by histological examination and/or imaging studies [8]. Hypertension was defined according to the Seventh Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of Hypertension. Diabetes mellitus was defined according to the American Diabetes Association diagnostic criteria. All participants provided written informed consent for this study, which has been approved by our institutional ethics committee.

Antibodies to HCV were measured in serum samples via third-generation EIA (Abbott Laboratories, North Chicago, IL). Serum HCV RNA was determined using Cobas Amplicor assays (sensitivity 50 IL/ml; Roche Diagnostic Systems, Tokyo, Japan). HCV genotypes were determined using INNO-LiPA HCV II assays (Innogenetics, Gent, Belgium). Alanine aminotransferase, albumin, and other relevant blood and biochemical tests were performed using standard methods [9] .

Among the 511 patients, 306 (59.9%) underwent interferon (IFN)-based therapy prior to the study period (Table 1). Of these, 238 were treated with pegylated (PEG)-IFN and ribavirin combination therapy and 75 were treated with either PEG-IFN or IFN monotherapy. A sustained virological response (SVR) was defined as undetectable serum HCV RNA 24 weeks after the discontinuation of IFN-based therapy.

2.2. Genotyping of *IL28* SNP (rs8099917)

Genomic DNA from patients was isolated by phenolic extraction of sodium

dodecyl sulfate-lyzed and proteinase K-treated cells and adjusted to 10-15 ng/μl. Genotyping of the rs8099917 SNP was performed using the ABI TaqMan allelic discrimination kit and an ABI 7500 Real Time PCR System (Applied Biosystems) [10].

2.3. Statistical analysis

Statistical analysis of data was performed using StatFlex ver. 6 software (Artech Co., Ltd. Osaka, Japan). Continuous variables were presented as the median (range) and were analyzed using the Mann-Whitney *U* test. Categorical variables were presented as frequency (%) and were analyzed using the chi-square test with Yate's correction. Fisher's exact probability test was used for groups with fewer than 5 samples. The model was checked by regression diagnostic plots to verify normality, linearity of the data, and constant variance. Stepwise logistic regression analysis with a forward approach was performed to identify independent factors associated with HCV-induced HCC after continuous variables were separated into two categorical variables by each median. A *P* value of less than 0.05 was considered statistically significant.

3. Results

The demographic, virological, and clinical characteristics of our cohort are summarized in Table 1. Most patients were infected with HCV genotype 1b (76.7%), and the majority (76.3%) had chronic hepatitis rather than liver cirrhosis (23.7%). Forty-five (8.8%) and 126 (24.7%) patients were complicated with diabetes mellitus and hypertension, respectively. Ninety patients (17.6%) had a history of regular alcohol intake of more than 20 g/day. The median follow up period between first visit and final

follow up was 6.9 years. Among the 511 patients studied for the *IL28B* rs8099917 SNP, 365 had the T/T genotype (71.4%), 139 had T/G (27.2%), and 7 had G/G (1.4%). Of the 392 patients infected with HCV genotype 1b, 273 had the T/T genotype (69.6%), 113 had T/G (28.8%), and 6 had G/G (1.6%). Ninety-two of the 119 patients infected with HCV genotype 2 had the T/T genotype (77.3%), 26 had T/G (21.8%), and 1 had G/G (0.9%). With regard to gender, 176 males had the T/T genotype (74.3%), 57 had T/G (24.1%), and 4 had G/G (1.6%), compared to 189 females who had the T/T genotype (69.0%), 82 who had T/G (29.9%), and 3 who had G/G (1.1%). There were no significant differences related to genotype (P = 0.359) or gender (P = 0.393). There were also no remarkable differences found between the clinical state of LC and non-LC (73%, 87 of 120 vs. 71%, 274 of 384; P = 0.808) groups among patients with the T/T or T/G or G/G genotypes.

The clinical features of patients with and without HCC are shown in Table 2. Patients with HCC were significantly older and had a higher frequency of liver cirrhosis than those without HCC (both P < 0.0001). HCC development in patients with HCV genotype 1b was higher than in those with genotype 2a or 2b (P = 0.03). Patients with HCC who had been treated with IFN-based therapy were statistically fewer than those without (P < 0.0001), as was the case for SVR rate (P < 0.0001). More HCC patients were complicated with diabetes mellitus and hypertension than those without HCC (P = 0.002 and P = 0.003, respectively). These patients also showed statistically higher alcohol consumption (P = 0.020). Regarding laboratory findings, albumin and platelet levels were significantly lower and alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transpeptidase levels were significantly higher in patients with HCC than in those without. However, there was no significant difference

between HCC and non-HCC groups (74%, 51 of 69 vs. 71%, 314 of 442; *P* = 0.623) among patients with the T/T genotype and those with the T/G or G/G genotypes. There was also no significant difference between the groups according to allele frequencies (T vs. G) (p = 0.495). We further analyzed the IL-28B SNP among 205 patients who had never received IFN-based therapy. No significant association was found between HCC and non-HCC groups (74%, 34 of 46 vs. 71%, 113 of 159; *P* = 0.701) among patients with the T/T genotype or T/G or G/G genotypes.

We used a stepwise logistic regression model to determine the relationship between the above-described explanatory factors and complicating HCC in HCV patients. In agreement with the linear model, significant variables were found for liver cirrhosis (odds ratio [OR] = 57.3; *P* < 0.0001), age greater than 68 years (OR = 3.9; *P* = 0.0018), and serum albumin <4.2 mg/dL (OR = 3.6; *P* = 0.021). *IL28B* gene polymorphisms had no effect on the estimated risk of complication with HCC.

4. Discussion

The allelic frequency of the *IL28B* rs8099917 T/T polymorphism was significantly associated with treatment response in patients with chronic hepatitis C [3, 4]. However, the influence of *IL28B* polymorphisms on the severity and progression of liver disease remains unclear; it was reported that the rs8099917 T/T genotype was associated with inflammatory activity and fibrosis in patients with chronic hepatitis C in Japan [5], and Fabris et al. reported that this polymorphism was significantly associated with development of HCC in the patients with liver cirrhosis, regardless of etiology [6]. Conversely, Marabita et al. found that the host genetic background at the *IL28B* locus was not associated with the risk of developing advanced fibrosis in an Italian

prospective study [7]. In the current study, the frequency of patients with the rs8099917 T/T genotype did not differ between HCC and non-HCC groups, suggesting that the *IL28B* SNP may play little or no role in HCC development among Japanese HCV patients.

Stepwise logistic regression analysis confirmed that liver cirrhosis, advanced age (>68 years), and low serum albumin (<4.2 mg/dL) were significant predictors of HCC. These risk factors were in agreement with earlier studies [5, 11-13]. No associations of *IL28B* SNPs with HCC development were found.

It may be considered that some HCV patients in our study who were complicated with HCC did not achieve an SVR because they were untreatable with IFN-based therapy. Accordingly, clinicians may opt for early therapeutic intervention in patients with HCV possessing traits that are positively associated with an SVR, such as *IL-28B* polymorphisms. Furthermore, elderly patients should be carefully monitored for HCC once liver cirrhosis is detected, regardless of *IL-28B* genotype.

Very recently, two genome-wide association studies from different Japanese groups showed that an SNP in the 5' flanking region of the MHC class I polypeptide-related sequence A on chromosome 6 [14] and an SNP within isoform 1 in the DEP domain-containing protein 5 on chromosome 22 [15] were associated with HCC risk in HCV patients. Further association studies of such gene polymorphisms are needed both in Japan and abroad.

In summary, our findings showed that the *IL28B* rs8099917 genotype did not affect the development of HCC in Japanese patients with chronic HCV infection, whereas liver cirrhosis, advanced age, and low serum albumin were confirmed to

influence disease progression.

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6. References

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Table 1. Demographic, Virological, and Clinical Characteristics of 511 Patients with Chronic Hepatitis C Virus (HCV) Infection

Characteristics		Range
Median age, yrs	68	(18-96)
Gender (male / female)	237 / 274	
HCV genotype 1 / 2	392 / 119	
Median BMI (kg/m^2)	22.2	(15.8-32.3)
Clinical state, n (%)		
CH without HCC	386 (75.5)	
LC without HCC	56 (11.0)	
HCC	69 (13.5)	
IFN Therapy, n (%)	306 (59.9)	
Diabetes mellitus, n (%)	45 (8.8)	
Hypertension, n (%)	126 (24.7)	
Alcohol intake, n (%)	90 (17.6)	
Median serum value		
Albumin (mg/dL)	4.2	(1.5-4.8)
ALT (IU/L)	25	(3-199)
AST (IU/L)	31	(3-237)
GGTP (IU/L)	22	(7-295)
PLT ($10^4/\mu L$)	16.0	(1.0-43.1)
Median follow-up, yrs	6.9	(0.2-41.6)
IL28B SNPs (rs8099917)		
T/T / T/G / G/G	365 / 139 / 7	
Clinical state of LC	87 / 33 / 0	

Abbreviations: BMI, body mass index; IFN, interferon; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGTP, γ-glutamyl transpeptidase; PLT, platelet count

Table 2. Clinical Features of HCC-present and HCC-absent Patients with Chronic Hepatitis C Virus (HCV) Infection

Characteristics	HCC-present (n = 69)	HCC-absent (n = 442)	*P*
Median age, yrs	77	66	<0.0001
Gender (male / female)	36 / 33	201 / 241	0.299
Clinical state at LC, n (%)	65 (94.2)	56 (12.7)	<0.0001
HCV genotype 1 / 2	60 / 9	332 / 110	0.030
Median BMI (kg/m^2)	22.4	22.2	0.124
Treatment			
IFN therapy, n (%)	23 (33.3)	283 (64.0)	<0.0001
SVR, n (%)	6 (8.7)	176 (39.8)	<0.0001
Diabetes mellitus, n (%)	13 (18.8)	32 (7.2)	0.002
Hypertension, n (%)	27 (39.1)	99 (22.4)	0.003
Alcohol intake, n (%)	19 (27.5)	71 (16.1)	0.020
Median serum value			
Albumin (mg/dL)	3.4	4.3	<0.0001
ALT (IU/L)	40	27	0.0005
AST (IU/L)	56	29	<0.0001
GGTP (IU/L)	37	24	0.0003
PLT ($10^4/\mu L$)	9.6	16.4	<0.0001
IL28B SNPs (rs8099917)			
T/T / T/G + G/G	51 / 18	314 / 128	0.623

Abbreviations: LC, liver cirrhosis; BMI, body mass index; IFN, interferon; SVR, sustained virological response; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGTP, γ-glutamyl transpeptidase; PLT, platelet count