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MHC class I-related chain B gene polymorphism is associated with virological response to pegylated interferon plus ribavirin therapy in patients with chronic hepatitis C infection

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Abstract. The outcome of antiviral therapy is associated with viral and host factors. In the present study, the association between MHC class I-related chain B (*MICB*) genotypes and therapeutic response to pegylated interferon plus ribavirin (PEG-IFN/RBV) therapy was investigated in hepatitis C virus (HCV)-infected patients. In total, 107 patients with chronic HCV infection (74 with HCV serotype 1 and 33 with serotype 2) were enrolled. Genotyping of *MICB* single-nucleotide polymorphism (SNP) rs3828913 and interleukin-28B (*IL28B*) SNP rs8099917 was performed using TaqMan® SNP genotyping assays. The genotype distribution of the *MICB* alleles was: CC, 79.4%; CA, 17.8%; and AA, 2.8%. Sustained virological response (SVR) was achieved by 55.1% (59/107) of the HCV patients. The SVR rate of patients with *MICB* major (CC) alleles was 62.3% and this rate was significantly higher than that of the patients with *MICB* minor (CA and AA) alleles (27.2%) (P=0.0068). A multivariate logistic model showed that the *MICB* major genotype was an independent factor contributing to SVR (OR, 4.47; 95% CI, 1.46-13.70; P=0.009). In addition, the *MICB* genotype was identified as the sole independent factor contributing to SVR and non-virological response in HCV serotype 1 patients with the *IL28B* major genotype. In HCV serotype 2 patients, the *MICB* genotype was the sole significant factor contributing to SVR (OR, 30.68; 95% CI, 2.72-346.3; P=0.006). In conclusion, the *MICB* genotype is a strong predictive factor for virological response to PEG-IFN/RBV therapy in HCV patients.

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Introduction

Hepatitis C virus (HCV) is the major cause of chronic liver diseases and ~170 million people are infected worldwide (1-4). Chronic HCV infection leads to life-threatening complications, such as liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (2,5-7). The current standard therapy for chronic HCV infection comprises of a combination of pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) (1,8,9). Successful treatment, termed sustained virological response (SVR), is defined as undetectable HCV RNA 6 months after cessation of treatment (10-12). The SVR rate of PEG-IFN/RBV therapy has been reported as 40% in HCV serotype 1 patients and 75% in serotype 2 patients (9,13,14). In addition, triple combination therapy, including HCV protease inhibitors, has recently been introduced. HCV protease inhibitors block the protease-dependent cleavage of the HCV polyprotein and inhibit an essential step of viral replication (15). Triple combination therapy achieves high antiviral responses (15,16), but HCV protease inhibitors remain unavailable in numerous countries (17).

Innate immunity appears to play an important role in the pathogenesis of viral hepatitis C infection. Of the various subsets of cells involved in innate immunity, natural killer (NK) cells are enriched in the liver and provide inherent defense against a number of pathogens, including HCV (18,19). The prevalence and cytotoxicity of NK cells increase in the early stage of HCV infection, but they are downregulated in number and function in the chronic phase (19). Certain HCV peptides are inhibitory for NK cells, leading to the reduction of their antiviral activity (20).

NK cells exhibit various types of receptors, either inhibitory or activating, that can react with distinct ligands on infected cells. MHC class I-related chain B (*MICB*) is the ligand for the natural killer group 2 member D (NKG2D) activating receptor, which transduces positive intracellular signals in NK cells (21). In addition, dendritic cells (DCs) also express *MICB* upon IFN-α stimulation and gain the ability to activate NK cells. In patients with HCV-infection, the *MICB* induction of DCs is severely impaired, suggesting that this impairment may play a role in HCV infection (22).

Table I. Baseline characteristics of patients with HCV infection.

Factors	Total	HCV serotype 1	HCV serotype 2
Patients, n	107	74	33
Age, years	58.2±9.9	58.4±10.6	57.6±8.6
Gender, male/female, n	67/40	46/28	21/12
Body mass index, kg/m^2	23.4±4.0	23.0±3.3	24.3±5.2
HCV-RNA levels, log IU/ml	6.25±0.73	6.1±0.72	6.1±0.86
AST, U/l	60.0±42.2	60.2±37.9	59.2±50.7
ALT, U/l	74.2±56.2	73.1±44.8	76.3±76.1
γ-GTP, U/l	63.3±68.0	62.8±54.1	64.0±92.2
Hemoglobin, g/dl	14.0±1.5	14.1±1.5	14.1±1.3
Platelets, x10^4/mm^3	16.4±5.0	15.8±4.4	17.9±5.9
Total cholesterol, mg/dl	174.3±30.0	173.4±28.6	175.7±26.5
PEG-IFN, μg/kg	88.1±47.8	104.5±43.6	50.7±33.1
RBV, mg/kg/day	10.1±2.4	10.1±2.5	10.1±2.1
Histological fibrosis stage: 1-2/3-4[a], n	60/18	39/14	21/4
Histological inflammation grade: 0-1/2-3[a], n	25/50	13/38	12/12

[a]Available sample number only. Data are presented as mean ± standard deviation. HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin.

The outcome of antiviral therapy has been reported to be associated with viral and host factors. Representative viral factors are the HCV genotype, high HCV RNA titers (23) and amino acid substitutions in the NS5A (24) and core region (25). Host factors include age, body mass index, insulin resistance and stage of liver fibrosis (26,27). One host genetic factor, a single-nucleotide polymorphism (SNP) of the interleukin-28B (*IL28B*) gene, is strongly associated with the treatment outcome of IFN and RBV therapy (28-30). *IL28B* genotyping may lead to the development of personalized medicine, where it may aid to identify the candidates most likely to respond to antiviral treatment. *IL28B* genotyping may be particularly important across ethnic divides as *IL28B* genotype differences may account for >50% of ethnic bias (31). However, the *IL28B* polymorphism does not explain all the treatment outcomes, and patients with the non-responder genotype may respond to therapy. Therefore, further studies aimed at finding new genetic factors for prediction of the therapeutic efficacy of antiviral therapy are required.

In the present study, the association between *MICB* genotypes and therapeutic response to PEG-IFN/RBV therapy was investigated in HCV-infected patients. *MICB* genotypes are closely associated with therapeutic outcomes of PEG-IFN/RBV therapy and can be used as predictive factors of treatment outcome.

Patients and methods

Study populations. A total of 107 patients with chronic HCV infection (74 patients with HCV serotype 1 and 33 patients with serotype 2) that were treated with PEG-IFN/RBV therapy at the Hospital of Shiga University of Medical Science (Otsu, Japan), the Notogawa Hospital (Higashioumi, Japan) or the Shiga Hospital of Regional Health Care Promotion Organization were enrolled in the study. Table I shows the baseline features of the patients. The patients received a weekly injection of PEG-IFN-α2b (Peg-Intron®; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg or PEG-IFN-α2a (PEGASYS®; Chugai Pharmaceutical, Tokyo, Japan) at a dose of 90 or 180 μg/subject, and oral administration of RBV (Rebetol®; MSD) for 24-72 weeks. The amount of RBV was adjusted based on the body weight of the patient (600 mg for <60 kg; 800 mg for 60-80 kg; or 1,000 mg for >80 kg). Virological responses were defined as follows: SVR, undetectable HCV RNA at treatment week 24; non-virological response (NVR), continuous detection of HCV RNA throughout the observation period; and transient virological response (TVR), transient undetectable HCV RNA with its reappearance during the follow-up period. Adherence to >80% of the scheduled doses during the first 12 weeks was required for inclusion in the study. Additionally, patients who discontinued treatment within 24 weeks of treatment for reasons other than virological failure were excluded.

Histological features, such as fibrosis and inflammation grade, were determined by pathologists at each hospital according to the Japanese chronic hepatitis classification (New Inuyama classification) (32). Written informed consent was obtained from all the patients. The ethics committee of each hospital approved the present study.

Serotyping of HCV. Serotyping was performed using an enzyme immunoassay kit (Imcheck F-HCV Gr; Kokusai-Shiyaku, Kobe, Japan).

Genotyping. Genomic DNA was extracted from peripheral leukocytes of whole blood samples using the QIAamp® DNA Blood mini kit (Qiagen, Hilden, Germany). The samples were genotyped for *MICB* rs3828913 and *IL28B* rs8099917

Table II. Genotype distribution of *MICB* SNP rs3828913 and *IL28B* SNP rs8099917.

	MICB SNP rs3828913			*IL28B* SNP rs8099917		
Variables	CC	CA	AA	TT	TG	GG
HCV serotype 1, n (%)	59 (79.7)	13 (17.6)	2 (2.7)	57 (77.0)	16 (21.6)	1 (1.4)
HCV serotype 2 , n (%)	26 (78.8)	6 (18.2)	1 (3.0)	28 (84.8)	5 (15.2)	0 (0.0)
Total , n (%)	85 (79.4)	19 (17.8)	3 (2.8)	85 (79.4)	21 (19.6)	1 (0.9)
HapMap, %	74.4	23.3	2.3	81.4	18.6	0.0

MICB, MHC class I-related chain B; *IL28B*, interleukin-28B; HCV, hepatitis C virus.

using the TaqMan® SNP Genotyping assay system (Applied Biosystems Inc., Foster City, CA, USA). Locus-specific polymerase chain reaction (PCR) primers and allele-specific TaqMan® probes were purchased from Applied Biosystems. For *MICB* genotyping, homozygosity for the major sequence (CC) was defined as exhibiting the *MICB* major allele, whereas homozygosity (AA) or heterozygosity (CA) was defined as exhibiting the *MICB* minor allele. For the *IL-28* genotypes, homozygosity for the major sequence (TT) was defined as exhibiting the *IL28B* major allele, whereas homozygosity (GG) or heterozygosity (TG) was defined as exhibiting the *IL28B* minor allele. HCV RNA levels were analyzed using the TaqMan reverse transcription PCR (RT-PCR) test (COBAS TaqMan® HCV test ver. 2.0; Roche, Branchburg, NJ, USA). The measurement ranges of these assays were 1.2-7.8 log IU.

Statistical analysis. Hardy-Weinberg equilibrium analysis was performed on these subjects by comparing the detected distribution of allele frequencies to the theoretical distribution estimated from the SNP allelic frequencies. P>0.05 (χ^2 statistics) was considered to indicate an equilibrium. The categorical variables are presented as frequencies and percentages as required. The continuous variables are reported as the means ± standard deviation (range). Statistically significant differences in treatment responses according to patient baseline parameters were determined by the Mann-Whitney U test for numerical variables and by Fisher's exact probability test or the χ^2 test for categorical variables. Variables with a P-value of <0.05 in univariate analysis were included in stepwise multivariate logistic regression analysis. Variables with a P-value of <0.05 in multivariate analysis were considered to indicate a statistically significant difference. The odds ratio was also calculated. All the statistical analyses were carried out with Mac Toukei-Kaiseki Ver. 2 (Esumi Co., Ltd., Tokyo, Japan).

Results

MICB polymorphisms. The frequencies of the *MICB* genotypes in patients with HCV are shown in Table II. HapMap data are shown as a reference. The genotype frequency of the *MICB* alleles in HCV patients (CC, 79.4%; CA, 17.8%; and AA, 2.8%) was almost identical to that determined from the HapMap data (CC, 74.4%; CA, 23.3%; and AA, 2.3%). The frequency of the *MICB* rs3828913 C allele in HCV patients was 88.3%.

Table III. Association between *MICB* genotypes and virological responses.

rs3828913	SVR	NVR	TVR	n
All HCV patients				
CC, n (%)	53 (62.3)	13 (15.2)	19 (22.4)	85
CA, n (%)	6 (31.6)	8 (42.1)	5 (26.3)	19
AA, n (%)	0 (0.0)	0 (0.0)	3 (100.0)	3
Total	59 (55.1)	21 (19.6)	27 (25.2)	107
HCV serotype 1 patients				
CC, n (%)	30 (50.8)	13 (22.0)	16 (27.1)	59
CA, n (%)	4 (30.8)	8 (61.5)	1 (7.6)	13
AA, n (%)	0 (0.0)	0 (0.0)	2 (100.0)	2
Total	34 (45.9)	21 (28.4)	19 (25.6)	74
HCV serotype 2 patients				
CC, n (%)	23 (88.4)	0 (0.0)	3 (11.5)	26
CA, n (%)	2 (33.3)	0 (0.0)	4 (66.6)	6
AA, n (%)	0 (0.0)	0 (0.0)	1 (100.0)	1
Total	25 (75.8)	0 (0.0)	8 (24.2)	33

MICB, MHC class I-related chain B; SVR, sustained virological response; NVR, non virological response; TVR, transient virological response.

The genotype frequencies of *IL28B* polymorphisms were also analyzed. These data were consistent with a recent study of these polymorphisms in the Japanese population reported by Ochi *et al* (33).

Association between the MICB genotypes and treatment responses to PEG-IFN/RBV therapy. Treatment responses to PEG-IFN/RBV therapy of patients with HCV are shown in Table III. SVR was achieved by 55.1% (59/107) of the HCV patients. The SVR rate was significantly lower in HCV serotype 1 patients (45.9%, 34/74) compared to serotype 2 patients (75.8%, 25/33) (P=0.008). NVR was noted in 19.6% (21/107) of the HCV patients. The NVR rate was significantly higher in serotype 1 patients (28.4%, 21/74) compared to serotype 2 patients (0.0%, 0/33) (P=0.0002). TVR was noted in 25.2% (27/107) of the HCV patients. There was no significant

Table IV. Univariate analysis of the factors associated with virological responses in all the HCV patients (n=107).

Factors	SVR	NVR	TVR	P-values	
				SVR vs. non-SVR	NVR vs. non-NVR
Patients, n	59	21	27	-	-
Age, years	57.3±10.2	57.7±11.1	60.4±8.4	0.26	0.54
Gender, male/female, n	37/22	15/6	15/12	0.98	0.50
Body mass index, kg/m^2	23.4±3.7	23.2±2.5	23.8±5.4	0.53	0.52
HCV-RNA level, log IU/ml	6.08±0.74	6.36±0.69	6.52±0.75	0.0062[b]	0.51
AST, U/l	63.4±49.3	59.2±31.5	52.8±31.2	0.52	0.50
ALT, U/l	81.8±67.2	66.8±39.3	62.7±35.1	0.40	0.52
γ-GTP, U/l	64.8±80.1	79.8±62.8	46.5±29.3	0.36	0.039[*]
Hemoglobin, g/dl	14.1±1.5	13.8±1.6	14.1±1.1	0.53	0.51
Platelets, x10^4/mm^3	16.7±4.7	16.6±5.3	15.8±5.3	0.51	0.53
Total cholesterol, mg/dl	172.8±30.9	171.1±27.2	179.4±30.3	0.49	0.52
PEG-IFN, μg/kg	82.3±48.8	103.0±52.2	89.5±40.6	0.091	0.14
RBV, mg/kg/day	10.0±2.3	10.4±2.8	9.9±2.4	0.53	0.44
MICB genotype: major/minor, n	53/6	13/8	19/8	0.0068[b]	0.055
IL28B genotype: major/minor, n	53/6	11/10	21/6	0.0068[b]	0.00062[b]
Fibrosis stage: 1-2/3-4[a], n	35/9	11/6	14/3	0.72	0.30
Inflammation grade: 0-1/2-3[a], n	12/30	5/11	8/9	0.32	0.92

[a]Available sample number only. [b]P=0.004. Data are presented as mean ± standard deviation. *MICB*, MHC class I-related chain B; *IL28B*, interleukin-28B; HCV, hepatitis C virus; SVR, sustained virological response; NVR, non virological response; TVR, transient virological response; AST, aspartate transaminase; ALT, alanine transaminase; GTP, glutamyl transpeptidase; PEG-IFN, pegylated interferon; RBV, ribavirin.

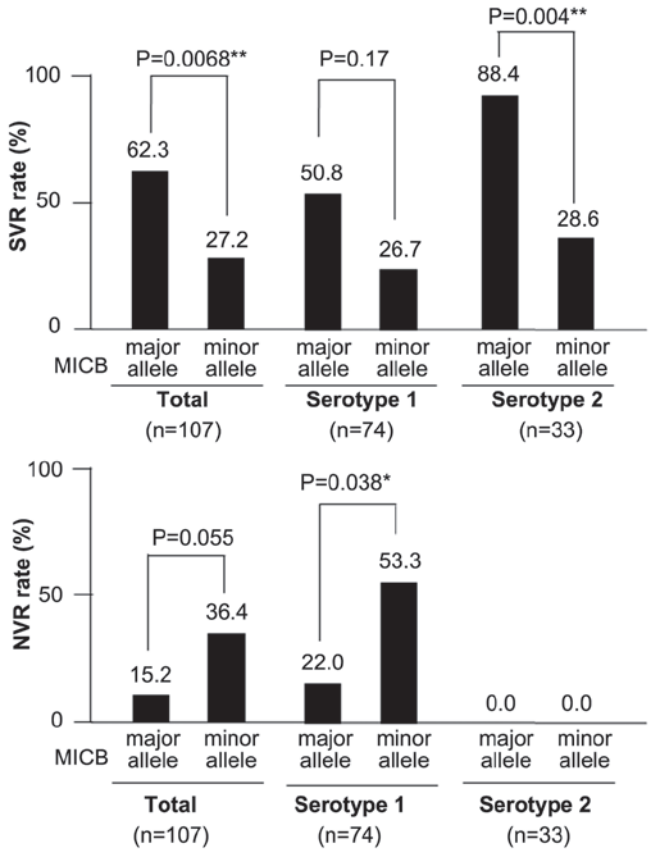

Figure 1. Association between the sustained virological response (SVR) rate (upper panel) and non-virological response (NVR) rate (lower panel) in hepatitis C virus (HCV) patients. [*]P<0.05 and [**]P<0.01.

difference in the TVR rate between serotype 1 (25.6%, 19/74) and 2 (24.2%, 8/33) patients.

Association of the SVR rate with *MICB* genotypes of HCV patients was analyzed (Fig. 1). Of the total HCV patients, SVR was noted in 62.3% of patients with *MICB* major (CC) alleles and this rate was significantly higher than that of the patients with *MICB* minor (CA and AA) alleles (27.2%). In HCV serotype 1 patients, the SVR rate (50.8%) tended to be higher in patients with *MICB* major alleles compared to patients with *MICB* minor alleles (26.7%), but this difference was not significant. In HCV serotype 2 patients, 88.4% of patients with *MICB* major alleles achieved SVR and this rate was significantly higher than that of the patients with *MICB* minor alleles (28.6%).

By contrast, of the total HCV patients the NVR rate (15.2%) tended to be lower in patients with *MICB* major alleles compared to the patients with *MICB* minor alleles (36.4%). In particular, in HCV serotype 1 patients the NVR rate (22.0%) was significantly lower in patients with *MICB* major alleles compared to patients with *MICB* minor alleles (53.3%).

Factors contributing to virological responses in HCV patients. When pretreatment clinical data of all the HCV patients were analyzed and data of patients with SVR (n=59) and non-SVR (TVR plus NVR, n=48) were compared, a significant difference was found in the distribution of serum HCV RNA levels and in *IL28B* and *MICB* genotypes (Table IV). Factors identified as significantly different between patients with NVR

Table V. Multivariate analysis of the factors associated with SVR and NVR in the HCV patients (n=107).

Variables	OR	95% CI	P-value
Predicting SVR			
IL-28B major genotype	7.14	2.19-23.22	0.001
MICB major genotype	4.47	1.46-13.70	0.009
Lower HCV-RNA	2.39	1.19-4.78	0.014
Predicting NVR			
IL28B minor genotype	5.63	1.94-16.38	0.002
Higher γ-GTP levels	1	1.00-1.01	0.121

SVR, sustained virological response; NVR, non virological response; OR, odds ratio; CI, confidence interval; *IL28B*, interleukin-28B; *MICB*, MHC class I-related chain B; HCV, hepatitis C virus; GTP, glutamyl transpeptidase.

Table VI. Multivariate analysis of the factors associated with SVR and NVR in the HCV serotype 1 patients (n=74).

Variables	OR	95% CI	P-value
Predicting SVR			
IL28B major genotype	13.8	2.58-74.0	0.002
Lower HCV-RNA	3.4	1.26-9.11	0.016
Predicting NVR			
IL28B minor genotype	7.9	2.20-28.4	0.002
MICB minor genotype	5.8	1.5-21.8	0.01

SVR, sustained virological response; NVR, non virological response; OR, odds ratio; CI, confidence interval; *IL28B*, interleukin-28B; *MICB*, MHC class I-related chain B; HCV, hepatitis C virus.

Table VII. Multivariate analysis of the factors associated with SVR and NVR in the *IL28B* major type of HCV serotype 1 patients (n=57).

Variables	OR	95% CI	P-value
Predicting SVR			
MICB major genotype	6.74	1.19-38.2	0.031
Lower HCV-RNA	2.77	0.88-8.72	0.082
Predicting NVR			
MICB minor genotype	5.89	1.22-28.5	0.027
Lower hemoglobin levels	0.6	0.34-1.06	0.079

SVR, sustained virological response; NVR, non virological response; OR, odds ratio; CI, confidence interval; *IL28B*, interleukin-28B; *MICB*, MHC class I-related chain B; HCV, hepatitis C virus.

(n=21) and non-NVR (SVR and TVR, n=86) were γ-glutamyl transpeptidase (GTP) and the *IL28B* genotype.

A multivariate logistic model was applied for analysis of the three variables that were significantly different between patients with SVR and non-SVR to determine independent predictive factors (Table V). Among the 107 patients, the *IL28B* major (TT) genotype (OR, 7.14; 95% CI, 2.19-23.22), lower HCV RNA levels (OR, 2.39; 95% CI, 1.19-4.78) and the *MICB* major genotype (OR, 4.47; 95% CI, 1.46-13.70) were identified as independent factors contributing to SVR. The *IL28B* minor genotype (TG and GG), but not γ-GTP levels, was identified as an independent factor contributing to NVR (OR, 5.63; 95% CI, 1.94-16.38) (Table V).

Factors contributing to virological responses in HCV serotype 1 patients. In the 74 patients with HCV serotype 1, the *IL28B* major genotype and lower HCV-RNA level were identified as factors contributing to SVR. Multivariate logistic analysis showed that these were independent factors contributing to SVR (Table VI). Similar analytical processes identified that *MICB* and the *IL28B* minor genotype are independent factors contributing to NVR (OR, 5.8; 95% CI, 1.5-21.8 for *MICB* minor genotype; and OR, 7.9; 95% CI, 2.2-28.4 for *IL28B* minor genotype) (Table VI).

HCV serotype 1 patients were divided into 57 patients with the *IL28B* major (TT) genotype and 17 patients with the *IL28B* minor (TG and GG) genotype. When the study population was limited to HCV serotype 1 patients with the *IL28B* major genotype, the *MICB* genotype was identified as the sole independent factor contributing to SVR and NVR (Table VII).

Factors contributing to virological responses in HCV serotype 2 patients. In the 33 patients with HCV serotype 2, the *MICB* genotype was the sole significant factor contributing to SVR, whereas the *IL28B* genotype did not affect SVR (Table VIII). Using the *IL28B*, HCV-RNA and *MICB* genotypes as variables for simultaneous multivariate logistic regression analysis, the OR of *MICB* genotypes for prediction of SVR was 30.68 (95% CI, 2.72-346.3; P=0.006).

Discussion

Combined PEG-IFN and RBV therapy has been the main treatment for patients with chronic hepatitis C in recent decades. However, the SVR rate is ~40 and 75% in patients with HCV serotype 1 and 2 infection, respectively (1,13,14). In addition, PEG-IFN/RBV therapy is poorly tolerated due to the side-effects and requires long-term treatment (48 weeks). Therefore, pretreatment prediction of therapeutic response would be of great clinical benefit. In previous studies, host factors including genetic background (age, gender, ethnicity, platelets, liver fibrosis, obesity and *IL28B* polymorphisms) and viral factors (genotype, viral load and viral genetic polymorphisms) were reported to be associated with the outcome of PEG-IFN/RBV therapy (1,34). In particular, a polymorphism upstream of the *IL28B* gene is strongly associated with a favorable response in HCV serotype 1 patients (6,28). However, the SVR rate of HCV serotype 1 patients possessing favorable *IL28B* genotypes is as high as 50% (14), and thus, half of patients cannot achieve SVR (14). In addition, it remains unclear whether the *IL28B* genotype aids in predicting virological response in HCV serotype 2 patients (35). For these reasons, identification of new

Table VIII. Univariate analysis of the factors associated with SVR and TVR in HCV serotype 2 patients.

Factors	SVR	TVR	P-value SVR vs. non-SVR
Patients, n	25	8	-
Age, years	56.5±8.9	60.6±7.0	0.28
Gender, male/female, n	16/9	5/3	1
Body mass index, kg/m^2	23.7±3.7	26.3±8.3	0.53
HCV-RNA level, log IU/ml	6.01±0.77	6.19±1.15	0.26
AST, U/l	63.4±56.6	46.1±23.2	0.53
ALT, U/l	83.6±84.9	53.5±31.1	0.51
γ-GTP, U/l	70.8±104.4	42.5±28.3	0.51
Hemoglobin, g/dl	14.1±1.41	14.2±0.99	0.55
Platelets, x10^4/mm^3	18.6±5.6	15.6±6.3	0.40
Total cholesterol, mg/dl	173.7±27.0	181.9±25.7	0.51
PEG-IFN, μg/kg	49.1±32.1	55.7±38.0	0.54
RBV, mg/kg/day	10.5±1.9	8.6±2.4	0.52
MICB genotype: major/minor, n	23/2	3/5	0.004[b]
IL28B genotype: major/minor, n	21/4	7/1	1
Fibrosis stage: 1-2/3-4[a], n	18/3	3/1	0.83

[a]Available sample number only. [b]P=0.004. Data are presented as mean ± SD. SVR, sustained virological response; TVR, transient virological response; HCV, hepatitis C virus; SVR, sustained virological response; AST, aspartate transaminase; ALT, alanine transaminase; GTP, glutamyl transpeptidase; PEG-IFN, pegylated interferon; RBV, ribavirin; *IL28B*, interleukin-28B; *MICB*, MHC class I-related chain B; SD, standard deviation.

determinants for response to treatment is considered a high priority.

MICB is a ligand for the NKG2D activating receptor expressed on NK cells, natural killer T (NKT) cells, cluster of differentiation 8$^+$ T cells and γδT cells (36). NKG2D ligand expression is stress-related and upregulated by infected or oncogenic cells leading to cytolysis. *MICB* genes exhibit considerable polymorphism among individuals and studies have investigated allelic association with disease (37-39). The *MICB* SNP rs3828913 (position -176) is located adjacent to the heat-shock response element and the GC box in the *MICB* promoter (40), and plays a role in transcriptional activation of the *MICB* gene. Previous studies reported that IFN-α stimulates *MICB* expression in DCs, leading to activation of NK cells, which play an important role in host antiviral activity. In addition, in patients with HCV-infection the *MICB* induction of DCs is severely impaired and this impairment may play a specific role in HCV infection (22). These observations prompted the investigation of the association between *MICB* SNP rs3828913 and treatment outcome of PEG-IFN/RBV therapy in patients with HCV infection.

In the present study, the *MICB* genotype, as well as the *IL28B* major genotype and lower HCV-RNA level, were significantly associated with SVR in HCV patients. Although the predictive power of the *MICB* major genotype was not as strong as that of the *IL28B* major genotype (OR, 7.14; 95% CI, 2.19-23.22), it appeared to be sufficient for clinical use (OR, 4.47; 95% CI, 1.46-13.70). When limited to HCV serotype 1 patients, the predictive power of the *MICB* major genotype for SVR was not significant, but the *MICB* minor genotype was useful for prediction of NVR in these patients (OR, 5.8; 95% CI, 1.5-21.8). As mentioned above, a considerable number of HCV serotype 1 patients with the *IL28B* major genotype (~50%) are resistant to PEG-IFN/RBV therapy (14). Therefore, the predictive power of the *MICB* genotype was analyzed for achievement of SVR in HCV serotype 1 patients with the *IL-28* major genotype. In HCV serotype 1 patients with the *IL-28* major genotype, the achievement of SVR by patients with the *MICB* major genotype was 6.74-fold higher than that of the patients with the *MICB* minor genotype (Table VII). Conversely, the *MICB* minor genotype appeared to be useful for prediction of NVR in HCV serotype 1 patients with the *IL28B* major genotype (OR, 5.89; 95% CI; 1.22-28.5). When limited to HCV serotype 2 patients, the *MICB* major genotype is the sole factor associated with SVR.

The combination of the HCV protease inhibitor with PEG-IFN/RBV was recently introduced for anti-HCV therapy and was reported to achieve higher antiviral responses (15). However, the SVR rate of non-responders who previously failed to respond to IFN-based therapy was only 40-50% (15,16). Therefore, the present data regarding the use of *MICB* genotyping for the prediction of response to therapy should contribute to a higher SVR rate in selected treated patients. In the future, the clinical utility of *MICB* genotyping should be evaluated as a predictive factor of the efficacy of the HCV protease inhibitor combined to PEG-IFN/RBV.

In conclusion, the *MICB* genotype is a strong predictive factor of virological response to PEG-IFN/RBV therapy in HCV patients. *MICB* genotyping may become part of the

clinical assessment prior to standard antiviral therapy in individuals chronically infected with HCV.

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