The association of LMP7 and TAP2 gene polymorphisms with treatment response to interferon/ribavirin in patients with genotype 1 chronic hepatitis C

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Abstract. Previous studies have highlighted the important role of genes related to antigen presentation in the spontaneous clearance of hepatitis C virus. The present study aimed to explore the association between *TAP*, *LMP* and tapasin gene polymorphism and treatment response in chronic hepatitis C virus (CHC) patients. Six single nucleotide polymorphisms in *TAP*, *LMP* and tapasin genes were genotyped among 352 Chinese genotype 1 CHC patients with pegylated interferon-α and ribavirin (pegIFN-α/RBV) treatment. There were 232 cases achieving sustained virological response (SVR), which yielded an SVR rate of 65.9%. *LMP7* rs2071543 variant genotypes [additive model: odds ratio (OR), 0.52; 95% confidence interval (CI), 0.33-0.82; P=0.005] and *TAP2* rs1800454 variant genotypes (additive model: OR, 0.66; 95% CI, 0.45-0.98; P=0.039) were suggested to decrease the possibility of achieving an SVR. After conducting combined effect analysis of rs2071543 and rs1800454, the authors found that the SVR rate was lower among patients carrying more unfavorable rs1800454-A and rs2071543-A alleles, and the SVR rate of carrying 3-4 alleles was 20%. In addition, carrying two unfavorable alleles led to significantly decreased possibility for SVR (OR, 0.30; 95% CI, 0.14-0.61; P=0.001). Multivariate stepwise analysis indicated that rs2071543, rs1800454, glucose, α-fetoprotein, platelets and baseline viral load were risk factors of SVR that were independent of each other. The area under the curve (AUC) consisting of all the above factors produced an AUC of 0.704 (95% CI, 0.647-0.761; P<0.001). The line charts indicated that the drop in viral load was significantly faster in GG patients than in GC/CC patients during the whole therapy, which was in accordance with the decline of viral load in rs2071543. The present study illustrated that the carriage of *LMP7* rs2071543-AA and *TAP2* rs1800454-AA had a negative effect on treatment response to pegIFN-α/RBV among genotype 1 patient with CHC in a Chinese Han population.

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Introduction

Chronic hepatitis C virus (CHC) infection is a major global health problem with ~200 million people infected and 700,000 people dying from hepatitis C-related liver diseases each year (1). China was considered to have a particularly high prevalence of 0.43% in the general population with 29 million hepatitis C virus (HCV) infected individuals (2,3). Over 70% of acutely infected persons progress into chronic HCV infection, that consequently causes progressive liver fibrosis and hepatocellular carcinoma (4,5).

Although successful execution of direct-acting antiviral therapy (DAA) was recently approved in western countries according to the World Health Organization (WHO 2017) (6). The combination therapy of pegylated interferon-α (pegIFN-α) plus ribavirin (RBV) (pegIFN-α/RBV) was still the most effective treatment for patients with HCV infection in developing countries, such as China due to economic reasons and curative concerns (7). Even though progression to severe liver disease could be prevented in 54-63% of patients through antiviral treatment with pegIFN-α/RBV, the fact that some patients treated with pegIFN-α/RBV failing to achieve sustained virological response (SVR) or experienced side effects should not be neglected (8,9). Therefore, it is important to identify the factors that may affect the response to treatment given that interaction between the virus and host genetics has

been theorized to be an important determinant of treatment response and the natural course of hepatitis C (10,11).

A genetic association study clarified that the human leukocyte antigen (HLA) was an essential genetic factor that regulated immune response and may be one of the tactics used by HCV to avoid immune clearance (12). Furthermore, various *HLA* alleles involved in the immune response were demonstrated to be linked to spontaneous clearance of HCV infection and even be potentially predictive for HCV treatment response (13). Studies to date about *HLA* alleles mainly focused on the *HLA* class I and II genes regions, by contrast, the study on non-classic genes located among classic regions was limited, such as *LMP2*/*LMP7* genes, *TAP1*/*TAP2* genes and tapasin genes (14-17). These genes, however, have been found in a previous study of the authors to be related to HCV susceptibility and spontaneous clearance.

In the present study, the authors genotyped the *TAP* gene, *LMP7* gene and tapasin gene to investigate the possible association of *HLA* gene polymorphisms with treatment response to pegIFN-α/RBV in 352 patients with CHC.

Patients and methods

Participants. A total of 352 participants were enrolled in the study from Jurong People's Hospital (Zhenjiang, China). All participants were the patients with genotype 1 CHC identified by the diagnosis of infectious disease in hospital or by doctor visits, and this infection was suspected to come from their former remunerated blood donation behaviors. This study protocol was approved by the institutional review board of Nanjing Medical University (Nanjing, China). All participants provided written informed consent. Interviews for donation history and other risk factors were conducted with signed informed consent from April, 2011 to January, 2016.

Those eligible subjects for the study were included if they received antiviral treatment of pegIFN-α/RBV for the first time and HCV antibody presented positive continually for more than six months. Subjects who were co-infected with hepatitis B virus or human immunodeficiency virus, or suffered from other types of liver diseases, alcoholic diseases, metabolic liver diseases and previous interferon and/or ribavirin therapy during the trial were excluded.

Investigation. Each participant was interviewed face-to-face using a structured and standardized questionnaire administered by trained interviewers. Demographic data, history of common diseases and therapeutic processes were collected for each subject. After the interview, a blood sample of ~10 ml was collected as a source of genomic DNA for serological tests and host DNA genotyping. The authors followed up these participants and detected viral load at on-treatment week 0, 4, 12, 24 and 48 and post-treatment week 24. SVR was defined as HCV RNA below the assay's lower limit of quantitation at post-treatment week 24. Rapid virological response (RVR) was defined as HCV RNA down two logarithmic or below the assay's lower limit of quantitation at post-treatment week 4. Completed early virological response (cEVR) was defined as HCV RNA below the assay's lower limit of quantitation at on-treatment week 12.

Finally, 352 cases with HCV infection (anti-HCV positive) were divided into two groups: 232 sustained virological response cases (SVR) and 120 non-sustained virological response cases (non-SVR).

Laboratory testing. Sera and HCV antibody (anti-HCV) were detected by ELISA (S20130002; Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China) under the manufacturers' instructions. Blood biochemical tests were undertaken by Roche Module P800 Automatic Biochemical Analyzer (Roche Diagnostics GmbH, Basel, Switzerland). Total RNA was extracted from the serum using TRIzol LS Reagent, and HCV RNA was detected by RT-PCR with specific primers using PrimeScript RT-PCR kit (DRR014S; Takara Biotechnology Co., Ltd., Dalian, China).

Single nucleotide polymorphisms (SNPs) selection and genotyping assays. The information of SNPs in four candidate genes (*TAP1*, *TAP2*, *LMP7* and *tapasin*) was obtained from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) and the Chinese Han population database of HapMap (http://www.hapmap.org). All the SNPs were filtered with the criteria: MAF (minor allele frequency) ≥0.05. A total of 6 SNPs (*TAP1* rs1135216, TAP2 rs1800454, *LMP7* rs2071543, *tapasin* rs9277972, rs1059288 and rs2282851) were chosen for genotyping.

DNA extraction was performed by protease K digestion and phenol-chloroform purification. Genotyping was performed by using a TaqMan allelic discrimination assay on the ABI PRISM 7900 HT sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). PCR was performed according to recommended thermal profile: 50˚C for 2 min (preheating), 95˚C for 10 min (preincubation) followed by 40 cycles at 95˚C for 15 sec (denaturation) and 60˚C for 1 min (annealing). Table I presents the TaqMan minor groove-binding (TaqMan-MGB) probes and specific forward/reverse PCR primers used in this study (Nanjing BioSteed BioTechnologies Co., Ltd., Nanjing, China). Two blank controls and five repeated samples were assigned to each 384-well format for quality control, and a 100% concordance was achieved.

Statistical analysis. Data were scrutinized and then entered a database using EpiData 3.1 by two different studies for further analysis. Differences in general demographic characteristics were calculated by the Student's t-test or one-way analysis of variance and the Chi-square (χ^2) test. Dominant and additive genetic models were used in the analysis of each SNP. Associations between SNPs and the treatment response of HCV infection were estimated by calculating the odds ratios (ORs) with 95% confidence intervals (CIs). Adjustments for age, sex, baseline viral load, glucose (GLU), α-fetal protein (AFP), albumin (ALB) and platelets were conducted with the use of the regression analysis. To evaluate ability of the genetic and clinical factors to predict HCV treatment response, the area under the curve (AUC) of the receiver operating characteristic was calculated. Line and bar charts were used to present the viral load at each follow-up time-point. All statistical analyses were two sided, and $P<0.05$ was considered to indicate a statistically significant difference. The trend analysis was calculated with Cochran-Armitage trend test. All the statistical analyses were performed using the STATA software (version 12.0; StataCorp LLC, College Station, TX, USA).

Table I. Primers and probes for TaqMan allelic discrimination.

Polymorphism	Sequence (5'-3')
TAP1 rs1135216	
Primer	F: CACACATGTGGCTATACCGTTCTC
	R: TCGCTGACCCCCTGACA
Probe	FAM-TGCAGAGGTAGGCG-MGB
	HEX-TCTGCAGAGGTAGACG-MGB
TAP2 rs1800454	
Primer	F: CCTGGAACGCGCCTTGTA
	R: CCTTTCACAACCACTCTGGTATCTT
Probe	FAM-TGCTCGTAAGGAGG-MGB
	HEX-CTGCTCATAAGGAGG-MGB
LMP7 rs2071543	
Primer	F: GCGACCCTCCACTCCTCA
	R: GGACACTACAGTTTCTCTATGCGATCT
Probe	FAM-CCGACCTTCATTCC-MGB
	HEX-CCGACCTGCATTC-MGB
Tapasin rs9277972	
Primer	F: GTCTAGGTCCTTCAGGTAGAAGTAATCTTT
	R: CTAAGTGAAATTGCATACTGTTTTTACTCTAC
Probe	FAM-CCTATAAGGTTAAACTGTTCT-MGB
	HEX-CCTATAAGGTTTAACTGTTCT-MGB
Tapasin rs1059288	
Primer	F: TGGGCCTTAGGTCCCTATGC
	R: AAGTGATCGTGTGAGTCGTCGTT
Probe	FAM-CAGACAGGCCGGTC-MGB
	HEX-ACAGACAGGCCAGTC-MGB
Tapasin rs2282851	
Primer	F: CCTCATTCTTGAATTATCTGCACAGT
	R: GCCCAGGAGTCAGAAGCTTTT
Probe	FAM-CCACGTCTCAGCCTA-MGB
	HEX-CCACGTCCCAGCCT-MGB

F, forward; R, reverse.

Table II. Baseline characteristics of CHC patients treated with IFN/RBV.

Variables	N-SVR (n=120)	SVR (n=232)	P-value
Mean age, year	53.41±8.14	53.6±8.50	0.783
Age ≥50	83 (69.2)	159 (68.5)	0.903
Male (%)	29 (24.2)	57 (24.6)	0.934
AST ≥40 U/l (%)	70 (58.3)	121 (52.2)	0.270
ALT ≥40 U/l (%)	79 (65.8)	138 (59.5)	0.245
GGT ≥50 (U/l)	51 (42.5)	77 (33.2)	0.085
GLU > 6.01 (mmol/l)	49 (40.8)	61 (26.3)	0.005[a]
AFP >7.02 (ng/ml)	50 (41.7)	65 (28.0)	0.010[a]
T3 (nmol/l)	1.54±0.49	1.50±0.72	0.593
T4 (nmol/l)	128.67±32.96	123.50±31.01	0.147
Anti-TPO ≥35 I/ml	14 (11.67)	35 (15.09)	0.380
Base HCV-RNA	6.18±0.75	5.82±1.26	0.004[a]
TP (g/l)	77.87±5.92	78.51±5.92	0.335
ALB (g/l)	42.56±4.10	43.87±4.06	0.005[a]
Platelets (109/l)	122.51±59.65	137.19±52.48	0.018[a]
Abnormal	53 (44.2)	61 (26.3)	0.001[a]
Normal	67 (55.8)	171 (73.7)	
WBC (10^9/l)	4.88±2.62	5.02±1.78	0.555
Abnormal	45 (37.5)	73 (31.5)	0.256
Normal	75 (62.5)	159 (68.5)	
Hemoglobin (g/l)	132.83±17.50	134.07±16.02	0.506
Abnormal	22 (18.3)	31 (13.4)	0.216
Normal	98 (81.7)	201 (86.6)	

[a]P<0.05. CHC, chronic hepatitis C virus; IFN-α, interferon-α; RBV, ribavirin; N-SVR, non-sustained virological response; SVR, sustained virological response; AST, aspartate transaminase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; GLU, glucose; AFP, α-fetal protein; TP, total protein; ALB, albumin; WBC, white blood cell.

Results

Demographic characteristics of the study populations. The basic characteristics of 232 cases that achieved an SVR (SVR) and 120 cases who did not achieve an SVR (non-SVR) were available in the study. The rate of SVR was 65.9%. Patients with low viral load and high levels of GLU, AFP, TP, ALB and platelets at baseline were more likely to achieved an SVR, as presented in Table II.

Association of candidate SNPs with SVR. The allelic frequencies of candidate genes (*TAP1* rs1135216, *TAP2* rs1800454, *LMP7* rs2071543, *tapasin* rs9277972, rs1059288 and rs2282851) between SVR and non-SVR groups were compared in Table III. The observed genotype frequencies of these SNPs in the remaining subjects with different HCV status were all in Hardy-Weinberg equilibrium (all P≥0.05). *LMP7* rs2071543-A variant and *TAP2* rs1800454-A variants were related to a decreased possibility of achieving an SVR.

In rs1800454 SNPs, the rate of SVR was significantly higher in patients with the GG genotype compared to those with the GA and AA allele. The additive model analyses indicated that the presence of each additional allele was indicated to reduce the decreased probability of achieving an SVR by ~48% (adjusted OR, 0.52; 95% CI, 0.33-0.82; P=0.005).

In rs2071543 SNPs, for patients with the CC genotype, a higher SVR rate was observed in comparison to the CA and AA genotypes. The additive model indicated that each additional allele contributed to a decreased likelihood of achieving an SVR by ~34% (adjusted OR, 0.66; 95% CI, 0.45-0.98; P=0.039).

Association of rs1800454 and rs2071543 with RVR/cEVR. The association between rs1800454 and rs2071543 with RVR/cEVR was also analyzed, as presented in Table IV. Carrying rs1800454-A allele was showed to be a risk factor

Table III. Association of SNPs in *HLA* with SVR.

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	P-value
rs1135216					
AA	44 (36.7)	85 (36.6)	65.9	1.00	-
AG	29 (24.1)	69 (29.8)	70.4	1.36 (0.74-2.50)	0.328
GG	47 (39.2)	78 (33.6)	62.4	1.14 (0.65-2.01)	0.654
Dominant				1.23 (0.74-2.04)	0.427
Additive				1.06 (0.80-1.41)	0.677
rs1800454					
GG	78 (65.0)	176 (75.9)	69.3	1.00	-
GA	36 (30.0)	52 (22.4)	59.1	0.52 (0.30-0.90)	0.020[a]
AA	6 (5.0)	4 (1.7)	40.0	0.27 (0.07-1.09)	0.066
Dominant				0.49 (0.29-0.82)	0.007[a]
Additive				0.52 (0.33-0.82)	0.005[a]
rs9277972					
AA	91 (75.8)	169 (72.9)	65.0	1.00	-
AT	21 (17.5)	45 (19.4)	68.2	1.10 (0.59-2.04)	0.774
TT	8 (6.7)	18 (7.8)	69.2	1.67 (0.67-4.17)	0.272
Dominant				1.25 (0.73-2.14)	0.422
Additive				1.22 (0.83-1.80)	0.302
rs1059288					
TT	44 (36.7)	82 (35.3)	65.1	1.00	-
TC	58 (48.3)	118 (50.9)	67.0	1.26 (0.75-2.12)	0.382
CC	18 (15.0)	32 (13.8)	64.0	1.02 (0.50-2.12)	0.949
Dominant				1.20 (0.73-1.97)	0.465
Additive				1.06 (0.75-1.51)	0.732
rs2282851					
CC	73 (60.8)	134 (57.8)	43.6	1.00	-
CT	41 (34.2)	90 (38.8)	68.7	1.27 (0.77-2.08)	0.352
TT	6 (5.0)	8 (3.5)	57.1	0.57 (0.18-1.76)	0.327
Dominant				1.16 (0.72-1.88)	0.533
Additive				1.03 (0.68-1.55)	0.891
rs2071543					
CC	68 (56.7)	154 (66.4)	69.4	1.00	-
CA	43 (35.8)	70 (30.2)	61.9	0.77 (0.46-1.28)	0.312
AA	9 (7.5)	8 (3.5)	47.1	0.32 (0.11-0.91)	0.033[a]
Dominant				0.68 (0.42-1.09)	0.112
Additive				0.66 (0.45-0.98)	0.039[a]

[a]P<0.05. Logistic regression analyses adjusted for age, sex, GLU, AFP, ALB, platelets, baseline RNA. SVR, sustained virological response; N-SVR, non-sustained virological response; OR, odds ratio; CI, confidence interval; SNPs, single nucleotide polymorphisms; HLA, human leukocyte antigen.

of both achieving a RVR (dominant model: OR, 0.41; 95% CI, 0.25-0.70; P=0.001) and a cEVR (additive model: OR, 0.53; 95% CI, 0.34-0.83; P=0.006). Subjects with rs2071543-A allele were less prone to achieve a RVR (dominant model: OR, 0.59; 95% CI, 0.37-0.93; P=0.023). However, no significant correlation was observed between rs2071543-A allele and cEVR (all P>0.05).

Combined effect of rs1800454 and rs2071543. The evaluation of the combined effects of rs1800454-A and rs2071543-A was performed to test the association with SVR. The results showed that SVR rate was lower when patients carried more unfavorable rs1800454-A and rs2071543-A alleles, and the SVR rate of carrying 3-4 alleles was 20%. Carrying two unfavorable alleles appeared to have a negative dangerous effect on the SVR (OR, 0.30; 95% CI, 0.14-0.61; P=0.001), as showed in Fig. 1 and Table V.

Multivariate stepwise regression analysis. A stepwise regression model comprising all statistically significant variables was established. The final model included the rs1800454, rs2071543, baseline HCV RNA level, baseline platelet level,

Table IV. Association of rs1800454 and rs2071543 in *HLA* with RVR/cEVR.

Genotype	N-RVR	RVR	OR (95% CI)	P-value	N-cEVR	cEVR	OR (95% CI)	P-value
rs1800454								
GG	111 (64.9)	143 (79.0)	1.00	-	71 (65.1)	183 (75.3)	1.00	-
GA	54 (31.6)	34 (18.8)	0.41 (0.24-0.69)	0.001	31(28.4)	57 (23.5)	0.64 (0.37-1.10)	0.107
AA	6 (3.5)	4 (2.2)	0.60 (0.15-2.37)	0.464	7 (6.4)	3 (1.2)	0.15 (0.03-0.65)	0.011[a]
Dominant			0.42 (0.25-0.70)	0.001			0.55 (0.33-0.92)	0.024[a]
Additive			0.50 (0.32-0.79)	0.003			0.53 (0.34-0.83)	0.006[a]
rs2071543								
CC	97 (56.7)	124 (68.5)	1.00	-	62 (56.9)	159 (65.4)	1.00	-
CA	65 (38.0)	48 (26.5)	0.55 (0.34-0.89)	0.015	43 (39.5)	70 (28.8)	0.67 (0.40-1.11)	0.117
AA	9 (5.3)	9 (5.0)	0.90 (0.33-2.47)	0.831	4 (3.7)	14 (5.8)	1.79 (0.55-5.90)	0.335
Dominant			0.59 (0.37-0.93)	0.023			0.76 (0.47-1.23)	0.262
Additive			0.71 (0.48-1.03)	0.073			0.92 (0.62-1.36)	0.662

[a]P<0.05. Logistic regression analyses adjusted for age, sex, glucose, α-fetal protein, albumin, platelets, baseline RNA. RVR, rapid virological response; N-RVR, non-rapid virological response; cEVR, completed early virological response; N-cEVR, non-completed early virological response; OR, odds ratio; CI, confidence interval; HLA, human leukocyte antigen.

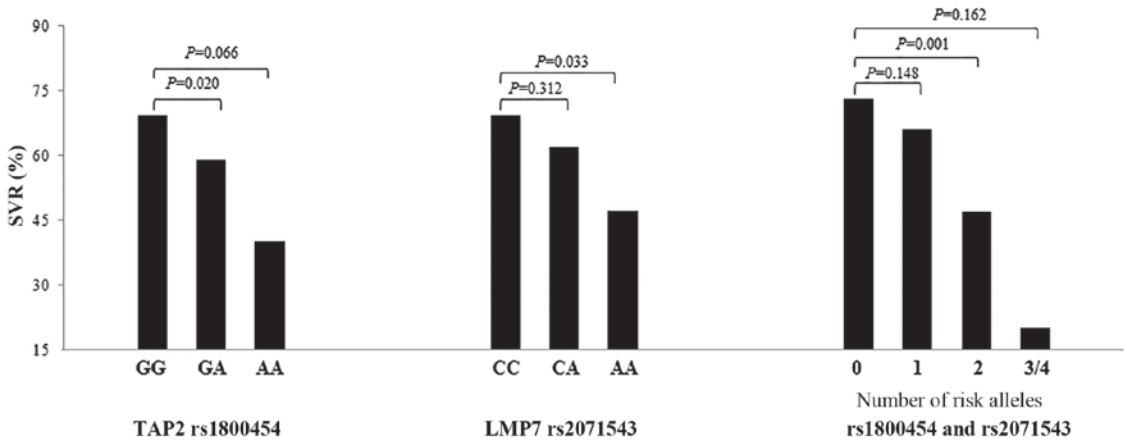

Figure 1. Combined effects of *TAP2* rs1800454 and *LMP7* rs2071543. Rate of SVR was compared among patients with different genetic variants. The combined effect of two independent SNPs was analyzed by Cochran-Armitage trend test. Variables are numbers of combined unfavorable genotypes (rs1800454-A and rs2071543-A). Logistic regression analyses were adjusted for age, sex, glucose, α-fetal protein, albumin, platelets, baseline RNA. SVR, sustained virological response.

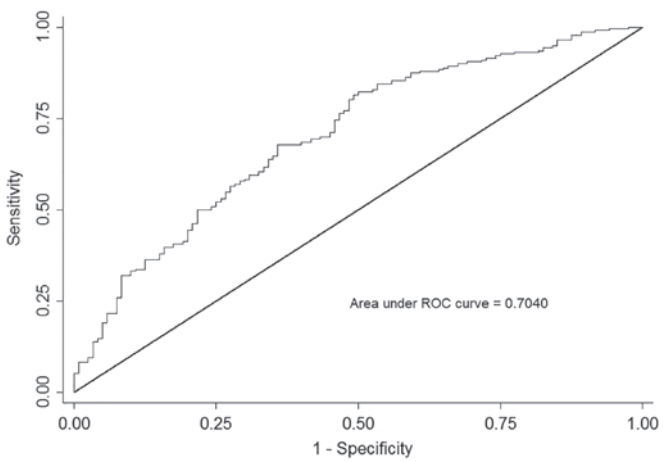

Figure 2. Predictors of hepatitis C virus treatment response. ROC, receiver operating characteristic.

baseline GLU level and baseline AFP level as independent predictors of SVR in Table VI (all P<0.05). Subsequently, the receiver-operating characteristic analysis for SVR was performed to estimate the predicted value of the independent factors. The AUC based on this model, including the above factors, produced an AUC of 0.704 (95% CI, 0.647-0.761; P<0.001), as presented in Fig. 2. An approximately parallel AUC was yielded when adding up one SNP of rs1800454 or rs2071543, suggesting that the predicted value of rs1800454 and rs2071543 was at the similar level.

Association of rs1800454 and rs2071543 with viral kinetics during treatment. Sample data on viral kinetics of rs1800454 and rs2071543 were collected and further analyzed by line charts. Baseline viral load of rs1800454 was higher in patients with the GG genotype than in those carrying the A allele (mean $\log_{10}$ HCV RNA: 6.03 for GG, 5.70 for GC/CC,

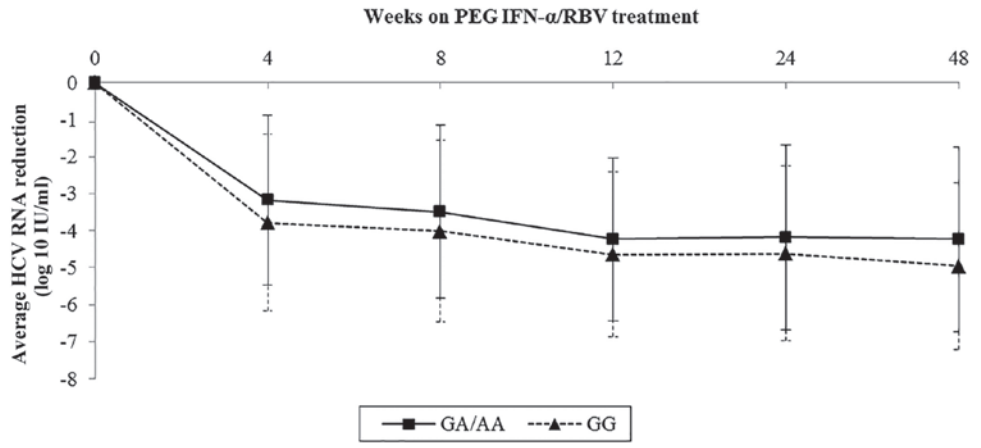

Figure 3. Effect of rs1800454 variants on HCV viral kinetics during therapy. The fold of viral decline was compared among patients with the CC genotype (triangle) and the GA/AA genotype (square). The fold of viral decline was calculated as the viral load at follow-up time-point divided by the initial viral load. The error bars were plotted according to the mean standard deviation of viral load at each time-point. HCV, hepatitis C virus; PEG IFN-α/RBV, pegylated interferon-α and ribavirin.

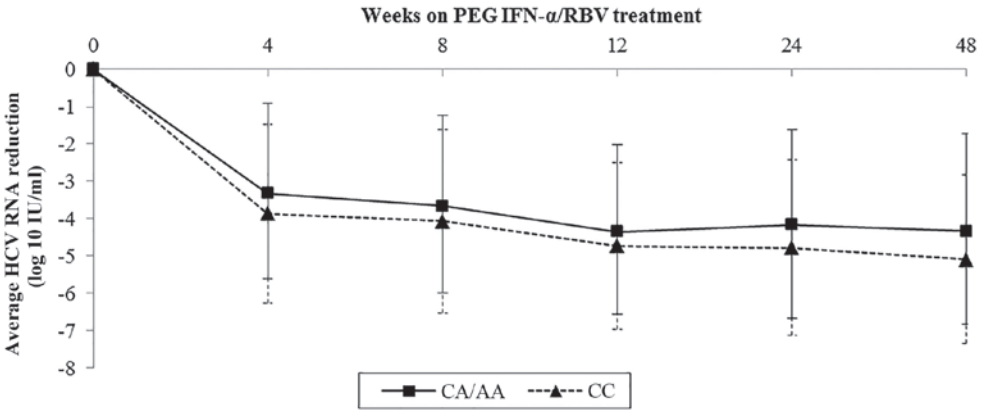

Figure 4. Effect of rs2074543 variants on HCV viral kinetics during therapy. The fold of viral decline was compared among patients with the CC genotype (triangle) and the CA/AA genotype (square). The fold of viral decline was calculated as the viral load at follow-up time-point divided by the initial viral load. The error bars were plotted according to the mean standard deviation of viral load at each time-point. HCV, hepatitis C virus; PEG IFN-α/RBV, pegylated interferon-α and ribavirin.

P=0.015) (Fig. 3). However, the drop in viral load was significantly faster in GG patients than in GC/CC patients during the whole therapy, which was in accordance with the decline of viral load in rs2071543 (Fig. 4). The results highlighted that the mutation of rs1800454-G and rs2071543-C may reduce the chance to achieve an SVR.

Discussion

In the present study, the authors attempted to investigate the viral (HCV viral load) and host (*HLA* SNPs, HCV RNA, platelets, GLU and AFP) factors affecting the treatment response of pegIFN-α/RBV, and to evaluate whether the SNPs were associated with SVR, RVR and cEVR in Chinese patients with CHC genotype 1 infection.

MHC class I and II antigens are the central to the host immune response, which make them ideal candidate genes to investigate their association with HCV infection (18). *HLA* is a crucial genetic factor that initiates and regulates immune response through presenting endogenous and exogenous antigen to T lymphocytes (12). An increased and broadly multi-specific T-cell response is critical to get a favorable outcome. $CD8^+$ T-cell response to HCV is important to the occurrence of successful immune response and spontaneous infection clearance. HLA class II presents viral peptides to $CD8^+$ T-cells to permit detection of infected cells (19). *TAP*, *LMP* and *tapasin* located in the human *MHC* class II DNA-binding loci are playing a crucial role in the *HLA* class I-restricted endogenous antigen presenting system (20). A previous study identified some genomic variants of *TAP* and *LMP* which were associated with chronic hepatitis B and hepatitis C (21). A previous study of the authors also showed that *TAP*, *LMP* and *tapasin* affected HCV susceptibility and spontaneous clearance. However, the relationship between variants of these genes and treatment response of HCV infection has not been fully studied. Therefore, the present study was performed to elucidate whether these antigen-presenting gene polymorphisms could influence the response to pegIFN-α/RBV treatment in CHC patients.

The results demonstrated that two tagging SNPs of *tapasin* (rs1059288 T>C and rs2282851 C>T) had no relationship with the treatment response of HCV infection, which contradicted the results from the study in a European Caucasian

Table V. Combined effects of rs1800454 and rs2071543 with SVR.

Variable	N-SVR	SVR	SVR rate (%)	OR (95% CI)	P-value
0	41 (34.2)	112 (48.3)	73.2	1	-
1	50 (41.7)	97 (41.8)	66.0	0.68 (0.40-1.15)	0.148
2	25 (20.8)	22 (9.5)	46.8	0.30 (0.14-0.61)	0.001[a]
3-4	4 (3.3)	1 (0.4)	20.0	0.17 (0.01-2.02)	0.162
Trend					P<0.001[a]

[a]P<0.05. Variables are numbers of combined favorable genotypes (rs1800454-A and rs2071543-A). Logistic regression analyses adjusted for age, sex, glucose, α-fetal protein, albumin, platelets, baseline RNA [a]P-value was analyzed by Cochran-Armitage trend test. SVR, sustained virological response; N-SVR, non-sustained virological response; OR, odds ratio; CI, confidence interval.

Table VI. Multivariate Stepwise regression analysis for independent factors of SVR.

Variable	Coef.	SE	95% CI	P-value
rs1800454	-0.15	0.05	(-0.24, -0.05)	0.002[a]
rs2071543	-0.10	0.04	(-0.18, -0.02)	0.016[a]
GLU	-0.12	0.05	(-0.23, -0.02)	0.021[a]
RNA level	-0.07	0.02	(-0.11, -0.02)	0.002[a]
Platelets (10^9/l)	-0.14	0.05	(-0.24, -0.03)	0.009[a]
AFP	-0.11	0.05	(-0.21, -0.005)	0.041[a]

[a]P<0.05. SVR, sustained virological response; Coef, coefficient of variation; SE, standard error; GLU, glucose; AFP, α-fetoprotein.

population (22). Another study found that mutation of *tapasin* rs9277972 A>T increased the risk of HCV chronicity, which was not observed in the present study (23). In line with other studies, *TAP2* rs1800454 was shown to be significantly associated with increased risk for progression of HCV infection (19,23). *TAP2* rs1800454 G>A makes a missense mutation and may be an independent risk factor for failing to achieve SVR, RVR and cEVR. However, another two studies in European and Japan population respectively failed to delineate a strong association between *TAP* gene polymorphism and response to interferon treatment (19,24). Mutation may alter the activity of the encoding protein and have an impact on antigen presentation process. Studies around the world have suggested that *LMP7* gene polymorphism had an influence on the outcomes of HCV infection, which was consistent with the finding of the authors' previous study (20,25,26). The present study indicated that *LMP7* rs2071543 C>A also renders a missense mutation, influencing the treatment response in CHC patients (19,27). Of note, few studies have explored the role of *TAP2*, *LMP7* gene in the treatment response of CHC patients, with inconsistent study designs, as well as participants of different physical conditions and genetic background, which led to a marked knowledge gap in this field (19,24,27).

In the analysis of rs1800454 and rs2071543 with those factors in model (baseline HCV RNA level, baseline platelets level, baseline GLU level and baseline AFP level), the interaction effect is not significant (data not shown), which illustrated that all factors above may be independent factors of hepatitis C treatment response. In addition, when including six factors of rs1800454, rs2071543, baseline HCV RNA level, baseline platelets level, baseline GLU level and baseline AFP level, the AUC was improved to 0.704. The similar rise in AUC could be found in an Egyptian research where an AUC of 0.68 including serum AFP and viral load was calculated (28). The results of the study have a potential implication that making full use of collected routine data can take effect in the determination of prognosis and the adjustment for treatment procedures.

There are several potential limitations that need to be considered and discussed. In the present study, only six SNPs of three genes were selected in the HLA class II region, whereas there are many genes involved in the MHC region. Some small molecular compounds known as DAAs, have been developed in recent years. The preliminary results have shown promising clinical application, however, this study only predicted the response to pegIFN-α/RBV therapy. PegIFN-α/RBV combination therapy is still the predominant treatment for hepatitis C in light of the economic burden in developing countries, such as China. However, the DAA will be a major direction for the future research. Finally, there are other well-known predictive factors that the authors did not adjust for in this study, such as HCV core amino acid 70 and IL28B, which may influence the results. Therefore, the authors' future research will focus on the following aspects. First of all, the types of hospital patients will be considered to test the validity and generalizability of results of the present study. Secondly, the authors intend to expand the sample size and include more known predictive factors, for example HCV core amino acid 70 and IL28B. Thirdly, the biological information of these genes will be utilized for further functional studies, exploring the mechanism of the association between these genes and hepatitis C virus. The benefits of conducting such research would be barely doubted given good representativeness, since all patients were remunerated blood donation population and from the same district. Furthermore, the patients were exposed during the same period and their infection outcomes were steady after decades. More importantly, earlier study of the authors suggested that genetic variants in *HLA* were relevant to HCV infection susceptibility and viral clearance, the present study thoroughly discussed and determined the association between *LMP7*, *TAP2* gene and treatment response in CHC patients.

In conclusion, the present study demonstrated that *LMP7* and *TAP2* loci were candidate regions that had some novel SNPs for treatment response to pegIFN-α/RBV in the Chinese CHC patients.

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References

1. Chen SR, Wang AQ, Lin LG, Qiu HC, Wang YT and Wang Y: In vitro study on anti-hepatitis C virus activity of *Spatholobus suberectus* Dunn. Molecules 21: E1367, 2016.
2. Cui Y and Jia J: Update on epidemiology of hepatitis B and C in China. J Gastroenterol Hepatol 28 (Suppl 1): 7-10, 2013.
3. Cai L, Gao C, Tang S, Wang J, Xue X, Yue M, Deng X, Su J, Peng Z, Lu Y, *et al*: Sex-specific association of estrogen receptor 2 polymorphisms with hepatitis C virus infection outcomes in a high-risk Chinese Han population. Infect Genet Evol 28: 118-124, 2014.
4. Bandiera S, Billie Bian C, Hoshida Y, Baumert TF and Zeisel MB: Chronic hepatitis C virus infection and pathogenesis of hepatocellular carcinoma. Curr Opin Virol 20: 99-105, 2016.
5. Matsuura K and Tanaka Y: Natural history of hepatitis C virus infection. Nihon Rinsho 73: 195-200, 2015 (In Japanese).
6. Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB and Sulkowski MS: Oral direct-acting agent therapy for hepatitis C virus infection: A systematic review. Ann Intern Med 166: 637-648, 2017.
7. Fateh A, Aghasadeghi MR, Keyvani H, Mollaie HR, Yari S, Hadizade Tasbiti AR, Ghazanfari M and Monavari SH: High resolution melting curve assay for detecting rs12979860 IL28B polymorphisms involved in response of Iranian patients to chronic hepatitis C treatment. Asian Pac J Cancer Prev 16: 1873-1880, 2015.
8. Ragheb MM, Nemr NA, Kishk RM, Mandour MF, Abdou MM, Matsuura K, Watanabe T and Tanaka Y: Strong prediction of virological response to combination therapy by IL28B gene variants rs12979860 and rs8099917 in chronic hepatitis C genotype 4. Liver Int 34: 890-895, 2014.
9. Aziz H, Raza A, Ali K, Khattak JZ, Irfan J and Gill ML: Polymorphism of the IL28B gene (rs8099917, rs12979860) and virological response of Pakistani hepatitis C virus genotype 3 patients to pegylated interferon therapy. Int J Infect Dis 30: 91-97, 2015.
10. Garcia RF, Moreira S, de Araújo Ramos AL, Ferreira LE, de Mattos AA, Tovo CV, Nader LA, Ramos JA, Rondinelli E, de Jesus Dominici A, *et al*: Interleukin 28B-related polymorphisms: A pathway for understanding hepatitis C virus infection? World J Gastroenterol 19: 7399-7404, 2013.
11. Chowdhry M, Makroo RN, Singh M, Agrawal S, Kumar M and Thakur Y: Human leucocyte antigen class I and II alleles associated with anti-hepatitis C virus-positive patients of North India. Indian J Med Microbiol 34: 299-302, 2016.
12. El-Bendary M, Neamatallah M, Esmat G, Kamel E, Elalfy H, Besheer T, Eldeib D, Eladl AH, El-Setouhy M, El-Gilany AH, *et al*: Associations of human leucocyte antigen class II-DQB1 alleles with hepatitis C virus infection in Egyptian population: A multicentre family-based study. J Viral Hepat 23: 961-970, 2016.
13. Huang J, Huang K, Xu R, Wang M, Liao Q, Xiong H, Li C, Tang X, Shan Z, Zhang M, *et al*: The associations of HLA-A*02:01 and DRB1*11:01 with hepatitis C virus spontaneous clearance are independent of IL28B in the chinese population. Sci Rep 6: 31485, 2016.
14. Waldron PR, Belitskaya-Lévy I, Chary A, Won J, Winters M, Monto A, Ryan J, Lazzeroni LC and Holodniy M: Genetic variation in the IL-6 and HLA-DQB1 genes is associated with spontaneous clearance of hepatitis C virus infection. J Immunol Res 2016: 6530436, 2016.
15. Sedighimehr P, Irani S, Sakhaee F, Vaziri F, Aghasadeghi M, Sadat SM, Jamnani FR, Fateh A and Siadat SD: IL28B rs12980275 and HLA rs4273729 genotypes as a powerful predictor factor for rapid, early, and sustained virologic response in patients with chronic hepatitis C. Arch Virol 162: 181-189, 2017.
16. McKiernan SM, Hagan R, Curry M, McDonald GS, Kelly A, Nolan N, Walsh A, Hegarty J, Lawlor E and Kelleher D: Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. Hepatology 40: 108-114, 2004.
17. Huang P, Zhang Y, Lu X, Xu Y, Wang J, Zhang Y, Yu R and Su J: Association of polymorphisms in HLA antigen presentation-related genes with the outcomes of HCV infection. PLoS One 10: e0123513, 2015.
18. Duggal P, Thio CL, Wojcik GL, Goedert JJ, Mangia A, Latanich R, Kim AY, Lauer GM, Chung RT, Peters MG, *et al*: Genome-wide association study of spontaneous resolution of hepatitis C virus infection: data from multiple cohorts. Ann Intern Med 158: 235-245, 2013.
19. Sugimoto Y, Kuzushita N, Takehara T, Kanto T, Tatsumi T, Miyagi T, Jinushi M, Ohkawa K, Horimoto M, Kasahara A, *et al*: A single nucleotide polymorphism of the low molecular mass polypeptide 7 gene influences the interferon response in patients with chronic hepatitis C. J Viral Hepat 9: 377-384, 2002.
20. Cui Q, Zhang Y, Su J, Shi C, Lei N, Ding K, Li J, Yu R, Wang L and Wang N: The association between the genetic polymorphisms of LMP2/LMP7 and the outcomes of HCV infection among drug users. J Biomed Res 24: 374-380, 2010.
21. Huang P, Dong L, Lu X, Zhang Y, Chen H, Wang J, Zhang Y, Su J and Yu R: Genetic variants in antigen presentation-related genes influence susceptibility to hepatitis C virus and viral clearance: A case control study. BMC Infect Dis 14: 716, 2014.
22. Ashraf S, Nitschke K, Warshow UM, Brooks CR, Kim AY, Lauer GM, Hydes TJ, Cramp ME, Alexander G, Little AM, *et al*: Synergism of tapasin and human leukocyte antigens in resolving hepatitis C virus infection. Hepatology 58: 881-889, 2013.
23. Kuzushita N, Hayashi N, Kanto T, Takehara T, Tatsumi T, Katayama K, Ohkawa K, Ito A, Kasahara A, Moribe T, *et al*: Involvement of transporter associated with antigen processing 2 (TAP2) gene polymorphisms in hepatitis C virus infection. Gastroenterology 116: 1149-1154, 1999.
24. Airoldi A, Zavaglia C, Silini E, Tinelli C, Martinetti M, Asti M, Rossini A, Vangeli M, Salvaneschi L and Pinzello G: Lack of a strong association between HLA class II, tumour necrosis factor and transporter associated with antigen processing gene polymorphisms and virological response to alpha-interferon treatment in patients with chronic hepatitis C. Eur J Immunogenet 31: 259-265, 2004.
25. El Awady MK, Omran MH, Ibrahim MK, Moustafa AM, Dawood RM, Bader El Din NG, Elsharkawy A, Abdel Aziz MS, El Shenawy R, El Abd YS, *et al*: Low Molecular Mass Polypeptide 7 single nucleotide polymorphism is associated with the progression of liver fibrosis in patients infected with hepatitis C virus genotype 4. Clin Lab 62: 381-387, 2016.
26. Ibrahim MK, Salama H, Abd El Rahman M, Dawood RM, Bader El Din NG, Salem HF, Abdelrahim ME, Omran D, Omran MH, El-Wakeel KH, *et al*: Three gene signature for predicting the development of hepatocellular carcinoma in chronically infected hepatitis C virus patients. J Interferon Cytokine Res 36: 698-705, 2016.
27. Ibrahim MK, Salama H, Abd El Rahman M, Dawood RM, Bader El Din NG, Salem HF, Abdelrahim ME, Omran D, Omran MH, El-Wakeel KH, *et al*: Three gene signature for predicting the development of hepatocellular carcinoma in chronically infected hepatitis C virus patients. J Interferon Cytokine Res 36: 698-705, 2016.
28. El Raziky M, Fathalah WF, Zakaria Z, Eldeen HG, Abul-Fotouh A, Salama A, Awad A, Esmat G and Mabrouk M: Predictors of virological response in 3,235 chronic HCV Egyptian patients treated with peginterferon alpha-2a compared with peginterferon alpha-2b using statistical methods and data mining techniques. J Interferon Cytokine Res 36: 338-346, 2016.