

Original Article

Hepatitis C virus (HCV) genotype 2a has a better virologic response to antiviral therapy than HCV genotype 1b

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Received January 31, 2015; Accepted March 27, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: The standard treatment, pegylated interferon (PEG-IFN) plus ribavirin (RBV), for patients with chronic hepatitis C (CHC), does not provide a sustained virologic response (SVR) in a large majority of patients. In the present study, 211 treatment-naïve patients with the hepatitis C virus (HCV) genotype 1b and 2a were recruited and treated weekly with PEG-IFN plus RBV to determine the response of HCV genotype 1b and 2a patients to standard antiviral treatment. Virologic responses were assessed by TaqMan at week 4, 12, 24, 48 and 24 weeks of treatment. Patients with HCV genotype 2a had a significantly higher rapid virologic response (RVR), early virologic response, end-of-treatment response and SVR, and a lower relapse rate than patients with HCV genotype 1b. Multivariate logistic regression analysis showed that the HCV genotype 2a patients had a HCV RNA level $\leq 5.70 \log_{10}$ IU/ml, a fibrosis stage $< S3$, and that HLA-A02 expression and RVR were independent factors of SVR that may improve HCV clearance.

Keywords: Hepatitis C virus, genotype, sustained virologic response, pegylated interferon

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease, affecting 170 million persons world-wide [1]. The standard therapy for the treatment of hepatitis C, up until 2011, was the combination therapy of pegylated interferon (PEG-INF) alfa-2a or alfa-2b and ribavirin (RBV) administered for 24 or 48 weeks [2]. The biomarker that best correlated with a cure was the sustained viral response (SVR) [3].

In recent years, several studies have identified clinical, virologic, histologic, biochemical, and demographic features that can predict a lower response to treatment with PEG-INF plus RBV [4, 5]. For example, changes in serum HCV RNA levels are an important predictor of treatment outcome [6, 7]. In particular, the disappearance or reduction in HCV RNA levels at 4 and 12 weeks after starting therapy is a pivotal cri-

terion in predicting treatment response [8, 9]. Currently, genetic host factors have been reported as becoming important for the pre-treatment prediction of the probability of SVR [10]. Genetic polymorphisms near the interleukin 28B (IL28B) gene have reportedly constituted as a host factor strongly associated with treatment outcome [11, 12]. Thomas et al [13] found that the CC genotype in rs12979860 strongly enhanced resolution of HCV infection among individuals of European or African ancestry.

The effective presentation of viral antigens to CD4⁺ and CD8⁺ T cells by human leukocyte antigen (HLA) class I and class II molecules, respectively, is the key regulator of optimum immune response against viral infection. Both HLA-DQB1*0301 and HLA-DRB1*11 are consistently associated with viral clearance of hepatitis C [14]. Currently, a clear and reproducible associ-

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Table 1. Baseline clinical and demographic data of chronic HCV patients

Variable	Overall	Genotype 1b (n = 112)	Genotype 2a (n = 68)	P value
Sex (M/F)	74/106	46/66	28/40	0.98
Age (y), mean \pm SD	47.0 \pm 13.1	47.0 \pm 12.9	46.9 \pm 13.7	0.97
Body mass index (kg/m ²), mean \pm SD	24.0 \pm 2.6	24.1 \pm 2.7	23.9 \pm 2.5	0.54
ALT (U/L), mean \pm SD	61.7 \pm 44.8	63.2 \pm 43.0	59.3 \pm 47.8	0.58
AST (U/L), mean \pm SD	61.8 \pm 47.6	64.6 \pm 52.4	57.1 \pm 38.4	0.31
GGT (U/L), mean \pm SD	47.0 \pm 37.3	49.6 \pm 38.1	42.9 \pm 35.7	0.25
ALB (g/L), mean \pm SD	42.0 \pm 5.2	41.6 \pm 5.5	42.6 \pm 4.7	0.20
HCV-RNA (log ₁₀ IU/ml), mean \pm SD	6.0 \pm 1.2	6.0 \pm 1.1	6.1 \pm 1.4	0.66
HCV-RNA, n (%)				0.55
\leq 5.70 log ₁₀	77 (42.8)	46 (41.1)	31 (45.6)	
$>$ 5.70 log ₁₀	103 (57.2)	66 (58.9)	37 (54.4)	
Fibrosis stage, n (%)				0.56
$<$ S3	161 (89.4)	99 (88.4)	62 (91.2)	
\geq S3	19 (10.6)	13 (11.6)	6 (8.8)	
HLA-A02, n (%)				0.91
Positive	89 (49.44)	55 (49.1)	34 (50.0)	
Negative	91 (50.56)	57 (50.9)	34 (50.0)	
Source of infection, n (%)				0.11
Maternal-neonatal	4 (2.2)	3 (2.7)	1 (1.5)	
Blood	125 (69.4)	77 (68.8)	48 (70.6)	
Sexual intercourse	6 (3.3)	3 (2.7)	3 (4.4)	
Others	45 (25.0)	29 (25.9)	16 (23.5)	

Legend: M: male, F: female, y: years, BMI: body mass index, ALT: alanine aminotransferase value, SD: standard deviation, AST: aspartate aminotransferase value, GGT: gamma-glutamyltranspeptidase, ALB: albumin, HLA-A02: human leucocyte antigen. A02. P value was given by Chi-square test or Fisher's exact probability test comparing HCV genotype 1b and 2a groups. A 2-sided P value less than 0.05 was considered significant.

ation has not been found between HLA alleles and therapeutic outcome [15].

The HCV genotype is one of the most important predictor of treatment outcome. Recent studies have found that only 40-50% of individuals with genotype 1 experience SVR, however approximately 80-87% patients with other genotypes obtain SVR in the US and in Europe [5, 16]. The SVR of patients with HCV genotype 1b is much higher in the Chinese and Japanese populations than that in Europe and America when treated with the corresponding regimen [17-19]. Currently, it is not clear whether the SVR of HCV genotype 1b and genotype 2a respond differently in the Chinese population.

Various factors might affect the outcomes of the PEG-INF and RBV antiviral therapy. The present study is prospective study on a large cohort of "treatment naïve" HCV patients to determine whether the HCV genotype 1b and genotype 2a respond differently to treatment.

In addition, the relative factors in the PEG-INF plus RBV antiviral therapy were investigated, thus predicting the likelihood that a patient would achieve SVR during early stages of therapy with high reliability.

Materials and methods

Patients

A total of 211 "treatment naïve" HCV patients were recruited in the Department of Infectious Diseases, First Affiliated Hospital of Zhengzhou University between January 2010 and January 2013. Eligible subjects met the EASL Clinical Practice Guidelines of HCV [2]. The exclusion criteria included: co-infection with human immunodeficiency virus; hepatitis B virus; alcohol intake averaging greater than 50 g per day; active drug abuse; chronic systemic disease; psychiatric disorders; autoimmune disease and pregnancy or lactation. The patients' characteristics are listed in **Table 1**. Patients received

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either PEG-INF- α -2a or PEG-INF- α -2b plus RBV according to the EASL Guidelines of HCV treatment [2]. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Zhengzhou University. Written informed consent was obtained from all participants.

Anti-HCV antibody and HCV RNA quantification

Blood samples were collected from each patient at the time of their clinical evaluation, then separated into sera and stored at -80°C until use. The anti-HCV antibody was determined by enhanced chemiluminescence immunoassay (Ortho Clinical Diagnostics, Raritan, NJ, USA). Quantitative determination of HCV RNA (TaqMan Roche Diagnostics) was performed during the different time points of antiviral therapy. The TaqMan value used to determine the response was 25 IU/ml.

HCV genotyping

HCV genotyping was performed using HCV genotyping chip assay (HCV genotyping kit Chip, Roche, Germany) according to the manufacturer's instruction. This assay is based on the reverse-hybridization principle. Specific oligonucleotide probes immobilized on membrane strips were hybridized with an amplified sample material generated by polymerase chain reaction.

Liver function and histological stage

Roche Modular automatic biochemical analyzer (Roche, Germany) and Beckman DXC800 automatic biochemical analyzer (Beckman Coulter, USA) were used for liver function assays. All tests were performed according to the manufacturer's instructions. Histological stages of HCV in patients was determined based on the scales for scoring fibrosis of the METAVIR system [20].

HLA-A02 expression

The peripheral blood mononuclear cells (PBMC) were isolated from 5 ml heparinized blood of HCV patients by density gradient centrifugation. 1×10^5 cells were added to 20 μl FCS and were incubated with anti-CD3 FITC, anti-CD8 APC and anti-HLA-A02 PE antibodies (BD Bioscience, San Jose, CA) for 15 min at room temperature (RT). The IgG2b PE and anti-CD3

PE antibodies (BD Bioscience, San Jose, CA) were used as negative and positive controls, respectively. After 1 ml MACS buffer washing, the pellets were re-suspended in 300 μl buffer and analyzed on a FACS Cano II (BD Bioscience, San Jose, CA).

The HLA-A02 genotyping was carried out by SSO LABType (One Lambda, Canoga Park, CA). Target DNA was amplified by nested PCR using sequence-specific primers (outer primers are: forward 5'-GGTCCGGAGTATTGGGACG-3' and reverse 5'-GTGCTTGGTGGTCTGAGCT-3', inner primers are: forward 5'-GCGCCGTGGAAGAGG-GTCG-3' and reverse 5'-CCCGTCCCAATACTCCCGA-3') followed by hybridization with allele-specific oligodeoxy nucleotides coupled with fluorescent phycoerythrin-labeled microspheres. The fluorescence intensity was determined on a Bio-Plex 200 system (Luminex xMAP, Austin, TX). The HLA allele assignment was performed with HLA-Tools software (Los Angeles, CA, US).

Efficacy assessments

Serum HCV RNA levels, routine hematological workup and biological function were determined at baseline, then at weeks 4, 12, 24 and 48 weeks during treatment and at 24 weeks during follow-up after treatment. End-of-treatment response (ETR) and SVR were defined as a negative qualitative HCV RNA level at the end of treatment and after 24 weeks of follow-up after treatment respectively. Rapid virologic response (RVR) was defined as qualitative HCV RNA negative at week 4 of treatment. Early virologic response (EVR) was defined as qualitative HCV RNA negative or a reduction from baseline HCV RNA level of $> 2 \log_{10}$ IU/ml at week 12 of treatment. All patients with detectable HCV RNA at week 24 stopped treatment and were classified as non-responders. Virologic relapse was defined as reversion to HCV RNA positive status in a patient who had an undetectable HCV RNA level (< 25 IU/ml) at the end of treatment.

Safety assessments

Safety assessments including adverse events, vital signs, and laboratory tests were conducted throughout the treatment period and at follow-up. Information on possible adverse events

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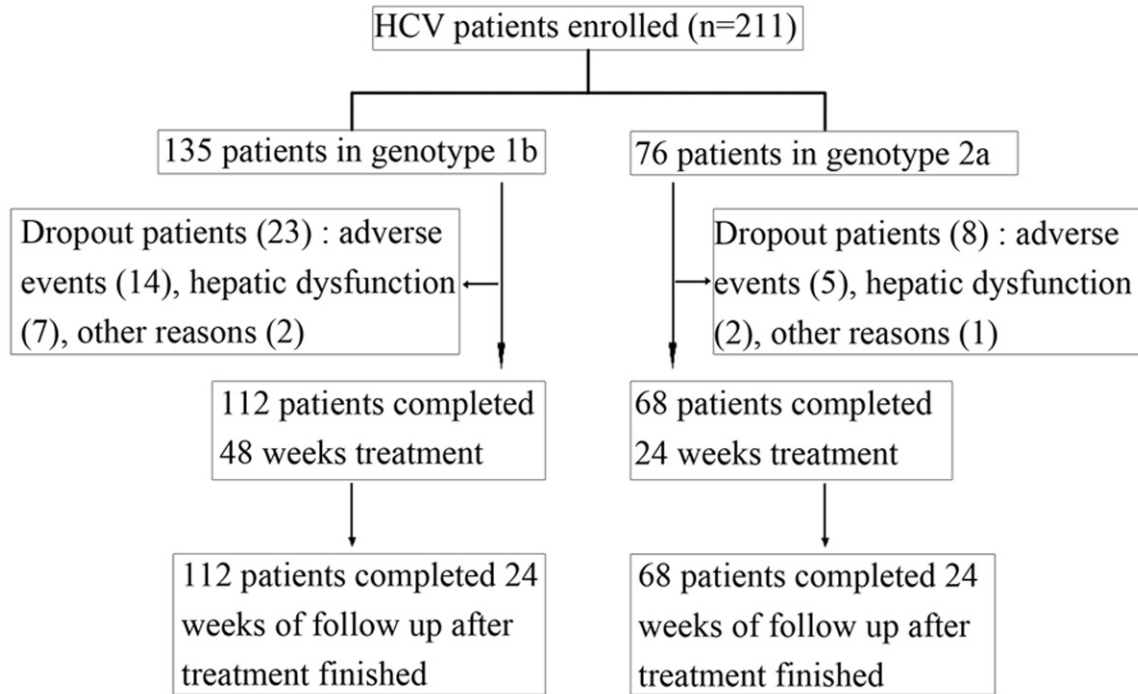


Figure 1. Study flow and disposition of patients. A total of 211 patients were enrolled into the study and assigned to the genotype 1b group (135 patients) and the genotype 2a group (76 patients) by HCV genotypes. The disposition of patients in the study was shown in **Figure 1**. There were 31 dropout patients (14.7%), including 23 patients with HCV genotype 1b and 8 patients with HCV genotype 2a, due to the adverse events (22 patients, 71.0%) or hepatic dysfunction (9 patients, 29.0%). By the end of the study, the data of total 180 patients including 112 genotype 1b patients and 68 genotype 2a patients was collected and analysed.

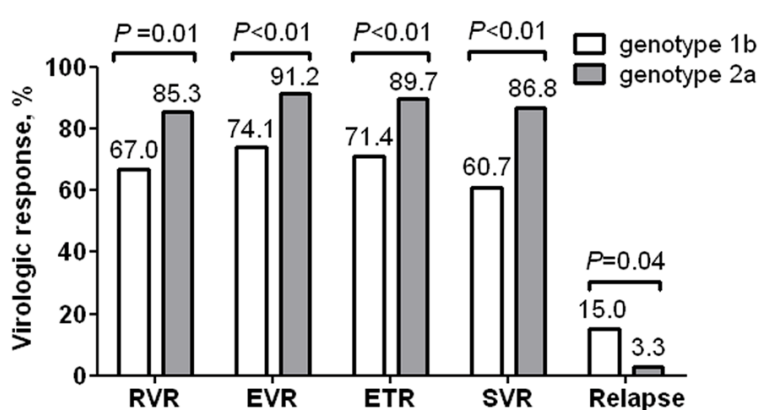


Figure 2. Virologic response in the genotype 1b and genotype 2a patients. Genotype 2a patients had higher RVR (85.3% versus 67.0%), EVR (91.2% versus 74.1%), ETR (89.7% versus 71.4%), SVR (86.8% versus 60.7%) and lower relapse rate (3.3% versus 15.0%) than that of genotype 1b patients. Virologic response was defined as undetectable serum level HCV RNA (< 25 IU/ml). Differences between the two groups were assessed by Chi-square test or Fisher's exact probability test, A 2-sided *P* value less than 0.05 was considered significant.

was obtained by questioning the patients and included: flu-like symptoms; fatigue; nausea; dizziness; depression and hair loss. In addition,

spontaneously reported adverse events were recorded.

Statistical analysis

Statistical analysis was performed using the SPSS software (version 16.0; SPSS Inc, Chicago, IL, USA). Patient characteristics were compared using the Student's *t* test or Mann-Whitney rank sum test for quantitative variables and the Chi-square test or Fisher's exact probability test for qualitative variables when appropriate. Results are expressed as mean \pm standard deviation (SD) or median and range when appropriate. Multiple logistic regression analysis was used to investigate the independent effect of the factors on the likelihood of achieving SVR. A 2-sided *P* value of $P \leq 0.05$ was considered significant.

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Table 2. Baseline factors associated with the likelihood of SVR

Variable	N of pts/ Tot pts	SVR (%)	O.R. (C.I. 95%)	P value
Sex				
M	54/74	72.9	1.22 (0.63-2.36)	0.55
F	73/106	68.9		
Age (year)				
< 50	83/107	77.6	2.28 (1.19-4.38)	0.01
≥ 50	44/73	60.8		
BMI (kg/m²)				
< 25	111/152	73.1	0.49 (0.22-1.13)	0.09
≥ 25	16/28	56.7		
ALT (U/L)				
< 100	95/124	76.8	2.46 (1.25-4.82)	0.01
≥ 100	32/56	57.1		
AST (U/L)				
< 100	100/133	75.6	2.25 (1.12-4.52)	0.02
≥ 100	27/47	56.7		
ALB (g/L)				
< 35	37/63	59.2	0.43 (0.22-0.83)	0.01
≥ 35	90/117	76.7		
HCV-RNA (IU/ml)				
< 5.70 log ₁₀	71/88	80.2	2.69 (1.37-5.27)	< 0.01
≥ 5.70 log ₁₀	56/92	61.3		
HCV Genotype				
1b	68/112	61.1	0.24 (0.11-0.52)	< 0.01
2a	59/68	86.8		
Fibrosis stage				
< S3	100/128	78.1	3.31 (1.66-6.57)	< 0.01
≥ S3	27/52	51.9		
HLA-A02				
Positive	70/89	78.7	2.20 (1.13-4.26)	0.02
Negative	57/91	62.6		
Drug				
PEG-INF-α-2a	84/117	71.6	1.18 (0.61-2.30)	0.62
PEG-INF-α-2b	43/63	68.6		
RVR				
Yes	64/76	84.2	5.82 (2.62-12.91)	< 0.01
No	63/104	60.6		

Legend: SVR: sustained virologic response, N: number of patients who obtained SVR, O.R: odds ratio, C.I: confidence interval, PEG-INF: pegylated interferon, RVR: rapid virologic response. P value was given by using single variable logistic regression models. A 2-sided P value less than 0.05 was considered significant.

Results

Clinical and demographic data

The disposition of 211 patients including 135 genotype 1b patients and 76 genotype 2a

patients is shown in **Figure 1**. By the end of the study, the data of 180 patients including 112 genotype 1b patients and 68 genotype 2a patients was analysed. There were no significant differences in the presence of HCV genotypes by sex, age, BMI, ALT, HCV RNA, liver fibrosis stage, or any other baseline variable ($P > 0.05$; **Table 1**). The HCV genotype 1b group and 2a group were well balanced with respect to patient characteristics.

Virologic response

Undetectable hepatitis C virus RNA rates were significantly higher among patients with HCV genotype 2a versus 1b at week 4 (85.3% versus 67.0%), week 12 (91.2% versus 74.1%), at the end of treatment (89.7% versus 71.4%) and at 24 weeks follow-up (86.8% versus 60.7%). There were 12 genotype 1b patients (15.0%) and 2 genotype 2a patients (3.3%) that relapsed during the 24 week of follow-up ($P = 0.04$; **Figure 2**). These results indicated that the rates of virologic responses during the antiviral therapy were significantly higher in the genotype 2a group compared to the genotype 1b group. The lack of improvement in SVR of the genotype 1b group was partially due to a higher rate of virologic relapse compared to genotype 2a group in the follow-up period after completion of antiviral therapy.

Predictor of SVR

The overall rate of SVR in 180 HCV patients was 70.6%. The pre-treatment clinical data including age, sex, HLA-A02 expression, liver histological stage, HCV genotypes etc. was compared between patients with SVR and non-SVR by single variable logistic regression analyses. The following factors including: age (OR = 2.28; 95% CI, 1.19-4.38); ALT (OR = 2.46; 95% CI, 1.25-4.82); AST (OR = 2.25; 95% CI, 1.12-4.52); ALB (OR = 0.43; 95% CI, 0.22-0.83); serum viral load (OR = 2.69; 95% CI, 1.37-5.27); HCV genotype (OR = 0.24; 95% CI, 0.11-0.52); liver histo-

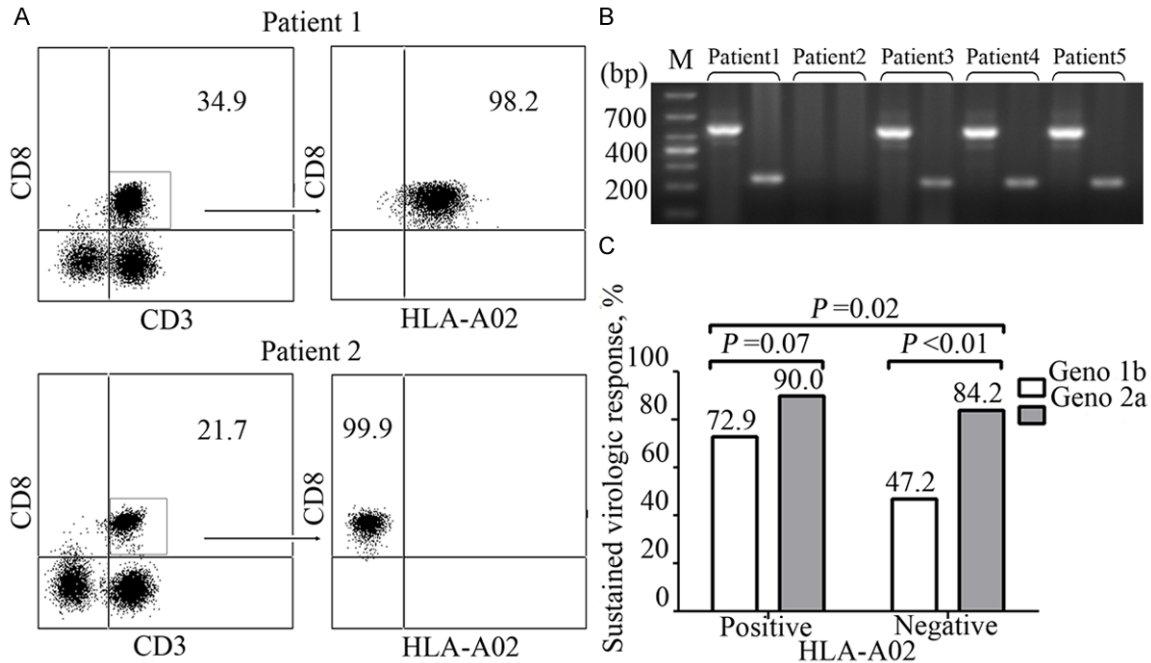


Figure 3. The expression of HLA-A02 in HCV patients. The HLA-A02 expression was tested by FACS and PCR-SSP. A. The HLA-A02 expression on CD3⁺CD8⁺T cells of two HCV patients. B. The HLA-A02 expression was tested by PCR-SSP. The outer pair of primers produced a 511-bp PCR product, the inner pair of primers produced a 236-bp PCR product. C. The SVR of different genotype patients in HLA-A02 expression or non-expression groups. Genotype 2a patients had higher SVR (84.2% versus 47.2%) in HLA-A02 negative group, but had similar SVR (90.0 versus 72.9%) in HLA-A02 positive group. Geno means genotype. Differences between the two groups were assessed by Chi-square test. A 2-sided *P* value less than 0.05 was considered significant.

Table 3. Baseline factors associated with the likelihood of SVR

Variable	β	S.E.	<i>P</i> value	O.R.	C.I. 95.0%
Genotype 2a	1.96	0.49	< 0.01	7.08	2.71-18.54
HCV-RNA < 5.70 log ₁₀	1.19	0.41	< 0.01	3.28	1.47-7.34
Fibrosis stage < S3	1.22	0.43	< 0.01	3.40	1.45-7.96
HLA-A02 positive	0.97	0.41	0.02	2.63	1.19-5.83
RVR	1.68	0.45	< 0.01	5.35	2.24-12.82

Legend: S.E.: standard error. *P* value was given by using multiple variables binary logistic regression analysis. A 2-sided *P* value less than 0.05 was considered significant.

logical stage (OR = 3.31; 95% CI, 1.66-6.57); HLA-A02 (OR = 2.20; 95% CI, 1.13-4.26) and RVR (OR = 5.52; 95% CI, 2.62-12.91) were elucidated as independent factors contributing to SVR (Table 2).

The HLA-A02 allele was expressed in 49.4% patients and 78.7% of these patients achieved SVR. The SVR of the HLA-A02 positive patients was higher than that in the HLA-A02 negative patients (62.6%) (OR = 2.20, 95% CI, 1.13-4.26; Table 2). As seen in Figure 3, the SVR of

genotype 2a patients was 84.2%, significantly higher than that in genotype 1b patients (47.2%), in the HLA-A02 negative group (OR = 0.17, 95% CI, 0.06-0.47). The SVR of HLA-A02 positive patients was 78.7%, significantly higher than that of HLA-A02 negative patients (62.6%) (OR = 2.20, 95% CI, 1.13-4.26). Interestingly the SVR of genotype 2a patients in the HLA-A02 positive group was 90.0%, similar to genotype 1b (72.9%) (*P* = 0.07; Figure 3). These results indicate that HLA-

A02 expression in genotype 1b patients was more important for achieving SVR than in genotype 2a patients.

Multivariate logistic regression analyses indicated that HCV genotype (OR = 7.08, 95% CI, 2.71-18.54), serum viral load (OR = 3.28, 95% CI, 1.47-7.34), liver fibrosis stages (OR = 3.40, 95% CI, 1.45-7.96), HLA-A02 (OR = 2.63, 95% CI, 1.19-5.83) and RVR (OR = 5.35, 95% CI, 2.24-12.82; Table 3) were independent predictors of SVR. Notably, sex, BMI and drug habits

Antiviral therapy than HCV genotype 1b

Table 4. Incidence of adverse clinical and laboratory events during treatment and follow up*

Adverse events	Overall (n = 180)	Genotype 1b (n = 112)	Genotype 2a (n = 68)	P value
Overall n (%)	162 (90.0)	105 (93.8)	57 (83.8)	0.03
Laboratory abnormalities				
Anemia (hemoglobin < 80 g/L)	37 (20.6)	29 (25.9)	8 (11.8)	0.02
Neutropenia (neutrophils < 0.75 × 10 ⁹ /L)	19 (10.6)	13 (11.6)	6 (8.8)	0.56
Thrombocytopenia (platelets < 50 × 10 ⁹ /L)	17 (9.4)	15 (13.4)	2 (2.9)	0.02
Common adverse events (> 10%)				
Pyrexia	128 (71.1)	85 (75.9)	43 (63.2)	0.07
Headache	107 (59.4)	73 (65.2)	34 (50.0)	0.04
Insomnia	61 (33.9)	41 (36.6)	20 (29.4)	0.32
Fatigue	48 (26.7)	32 (28.6)	16 (23.5)	0.46
Irritability or Depression	44 (24.2)	33 (29.5)	11 (15.7)	0.04
Dermatitis or Pruritus	38 (21.1)	30 (26.8)	8 (11.8)	0.02
Nausea	32 (17.8)	21 (18.8)	11 (16.2)	0.66
Arthralgia or Myalgia	28 (15.6)	21 (18.8)	7 (10.3)	0.13

*Data based on all patients who had at least one safety evaluation after baseline. All values are expressed as n (%). P value was given by Chi-square test or Fisher's exact probability test comparing the two different genotype groups. A 2-sided P value less than 0.05 was considered significant.

were not significantly different ($P > 0.05$). The outcomes revealed that dual antiviral therapy was more effective against HCV genotype 2a than against genotype 1b and this difference is independent of other factors that might improve HCV clearance.

Safety

In 180 patients who completed treatment and follow-up, the overall proportion of adverse events was higher in genotype 1b group than genotype 2a group (93.8% versus 83.8%, $P = 0.03$; **Table 4**). Serious anemia (25.9% versus 11.8%, $P = 0.02$) and thrombocytopenia (13.4% versus 2.9%, $P = 0.02$) occurred more often in the genotype 1b group than the genotype 2a group. Patients in the genotype 1b group tended to have higher incidences of common adverse events than patients in the genotype 2a group, including dermatitis (26.8% versus 11.8%, $P = 0.02$), headache (65.2% versus 50.0%, $P = 0.04$) and irritability or depression (29.5% versus 15.7%, $P = 0.04$). The most frequent type of adverse event in both groups was pyrexia (75.9% versus 63.2%, $P = 0.07$). No patients died during the research.

Discussion

Parameters for the prediction of SVR in patients with chronic hepatitis c (CHC) before the initia-

tion of antiviral therapy are important to estimate the potential for treatment success. Such parameters can assist clinicians in the decision of whether or not to commence antiviral therapy and this information can also motivate patients who might have a high chance of virologic response. The present study was a prospective cohort study to assess the influence of HCV genotype 1b and 2a on the virologic response to antiviral treatment in treatment naïve HCV patients. Multivariate logistic analysis showed that HCV genotype 2a, liver fibrosis stage < S3, HCV RNA < 5.7 log₁₀ IU/ml, HLA-A02 expression and RVR were independent predictors of SVR.

There are a number of host and viral factors that influence SVR in patients with CHC [21]. Puoti et al [22] found that individuals with HCV genotype 1 were relatively more difficult to treat than other HCV genotypes. With treatment using PEG-IFN plus RBV, the SVR of genotype 1b patients was 43%-51% in the Pellicelli et al [23] study and the SVR of genotype 2a patients was 58% in Kim's research [24]. In the present study, the SVR of genotype 1b patients (60.7%) and 2a patients (86.8%) were significantly higher than that of the research by Kim and Pellicelli, and were in accordance with the reports of [25], Zhou et al [26] and Sarwar et al [27]. The highlighted differences among these studies may be partially due to racial disparity.

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Compared with Caucasian Americans (CA) and African Americans (AA), SVR was significantly higher in Chinese and Japanese populations with HCV genotype 1 infections [28-30]. These results provide useful information for a personalized treatment regimen.

Recent studies have identified genetic diversity in HLA for the pre-treatment prediction of the probability of SVR [15]. A retrospective cohort study (n = 428) presented evidence that there was a significant association between HLA-A02 and SVR [31]. In the present study, the HLA-A02 positive patients had a significantly higher SVR rate than the HLA-A02 negative patients ($P = 0.02$), and multiple logistic analysis showed that the HLA-A02 allele was an independent predictive factor for SVR. There was no significant difference of HLA-A02 expression between the HCV genotype 1b and 2a patients. Interestingly the SVR of HCV genotype 2a in HLA-A02 positive patients was similar to genotype 1b ($P = 0.07$), however the SVR of genotype 2a in HLA-A02 negative patients was 84.2%, significantly higher than that of genotype 1b patients (47.2%) ($P < 0.01$). These results indicate that HLA-A02 expression in genotype 1b patients is more important for achieving SVR than in genotype 2a patients. The mechanisms behind this phenomenon will be investigated in future studies.

An important factor that is highly predictive of subsequent SVR is RVR [32]. In patients that achieve RVR, the SVR rates can be as high as 80-90% [33]. The RVR rate in the present study was 67.0% in genotype 1b and 85.3% in genotype 2a, and their SVR rates were 81.3% and 91.4% respectively. There was a strong relationship between RVR and SVR of HCV genotype 1b and 2a patients in the current study indicating that RVR can be a pivotal criterion in predicting treatment response.

The relationship between stage of fibrosis and SVR was particularly striking. Cheng et al [34] found that naïve genotype-1 patients with advanced fibrosis were less likely to achieve SVR than those without advanced fibrosis in accordance with the findings of the present study. In addition, significantly higher SVR rates are noted in younger subjects (< 50 years) compared to older patients. These results highlight the benefit of early treatment in maximizing SVR rates in patients with CHC.

The overall incidence of adverse events in HCV genotype 1b patients (93.8%) was higher than that in genotype 2a patients (83.8%). The incidences of serious thrombocytopenia, serious anemia, headache, irritability and dermatitis in genotype 1b patients were higher than that in genotype 2a patients. The lower incidence of adverse events in the genotype 2a patients may assist these patients in completing antiviral therapy, thus achieving higher rates of SVR in this group of patients.

The interleukin 28B (IL28B) polymorphism has been reported to influence viral kinetics and SVR in HCV genotype 1 patients and genotype 2 patients [11, 12, 35]. The three main IL28B single nucleotide polymorphisms (SNPs), shown to have the strongest association with virologic response are rs12979860, rs8099917 and rs12980275 in genotype 1 patients, and the CC genotype was found to be significantly associated with sustained virologic response in genotype 2 patients [36]. It is noteworthy that a high rate of Chinese and Japanese patients with the IL28B genotype of rs8099917 achieved SVR [30]. Generally speaking, although it was not evaluated in the present study, IL28B polymorphism partially explains why HCV genotype 2a patients have higher rates of SVR than genotype 1b patients and why HCV genotype 1b patients in China have higher rates of SVR.

In conclusion, this study indicates that PEG-INF combined with RBV antiviral therapy is more effective against HCV genotype 2a than genotype 1b and that this difference is independent of other factors which may improve viral clearance in China. However, this study was performed in only one centre, and the findings need to be confirmed and validated in larger, preferably double blind trials.

Acknowledgements

We thank all the clinical staff in the Biotherapy Center for their assistance in experiments. In addition, we thank the staff in the Department of Infectious Disease and the Department of Gastroenterology for their assiduous collecting blood samples and data of patients. Finally, we are grateful to all the participants and their families for generously agreeing to take part in this study. The work was supported by grants from the National Natural Science Foundation of China (Grant no. 81271815), Research Grant

from the Ministry of Public Health (Grant no. 20110110001), the Basic and Advanced Technology Research Foundation from Science and Technology Department of Henan Province (Grant nos. 112300410153 and 122300410-155), Funds for Creative Research Team of Henan Province, Creative Research Team of Higher Education of Henan Province and the Innovation Team of the First Affiliated Hospital of Zhengzhou University.

Disclosure of conflict of interest

None.

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References

- [1] Dienstag JL and McHutchison JG. American Gastroenterological Association technical review on the management of hepatitis C. *Gastroenterology* 2006; 130: 231-264.
- [2] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; 55: 245-264.
- [3] Swain MG, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, Abergel A, Pessôa MG, Lin A, Tietz A, Connell EV and Diago M. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. *Gastroenterology* 2010; 139: 1593-1601.
- [4] Kau A, Vermehren J and Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. *J Hepatol* 2008; 49: 634-651.
- [5] McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS; IDEAL Study Team. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; 361: 580-593.
- [6] Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M and Esteban R. Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. *Hepatology* 2002; 35: 930-936.
- [7] Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zchoval R, Wiedenmann B, Hopf U and Zeuzem S. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003; 37: 600-609.
- [8] Martínez-Bauer E, Crespo J, Romero-Gómez M, Moreno-Otero R, Solá R, Tesei N, Pons F, Forn X and Sánchez-Tapias JM. Development and validation of two models for early prediction of response to therapy in genotype 1 chronic hepatitis C. *Hepatology* 2006; 43: 72-80.
- [9] Poordad F, Reddy KR and Martin P. Rapid virologic response: a new milestone in the management of chronic hepatitis C. *Clin Infect Dis* 2008; 46: 78-84.
- [10] Kawaoka T, Aikata H, Miyaki D, Murakami E, Azakami T, Takaki S, Nagaoki Y, Hashimoto Y, Katamura Y, Hiramatsu A, Waki K, Hiraga N, Miki D, Tsuge M, Imamura M, Kawakami Y, Takahashi S, Ochi H, Tashiro H, Ohdan H and Chayama K. Eradication of hepatitis C virus genotype 1 after liver transplantation by interferon therapy before surgery: Report of three patients with analysis of interleukin-28 polymorphism, hepatitis C virus core region and interferon-sensitivity determining region. *Hepatology Res* 2011; 41: 1126-1131.
- [11] Ge D, Fellay J, Thompson AJ, Simon JS, Shiana KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG and Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399-401.
- [12] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR and George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; 41: 1100-1104.
- [13] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB and Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461: 798-801.
- [14] Singh R, Kaul R, Kaul A and Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol* 2007; 13: 1770-1787.
- [15] Dai CY, Chuang WL, Hsieh MY, Huang JF, Lin YY, Chu PY, Hou NJ, Lin ZY, Chen SC, Hsieh MY, Wang LY and Yu ML. Human leukocyte antigen alleles and the response to pegylated interferon/ribavirin therapy in chronic hepatitis C patients. *Antiviral Res* 2010; 85: 396-402.
- [16] Ghany MG, Strader DB, Thomas DL, Seeff LB; American Association for the Study of Liver

Antiviral therapy than HCV genotype 1b

- Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335-1374.
- [17] Yu ML and Chuang WL. Treatment of chronic hepatitis C in Asia: when east meets west. *J Gastroenterol Hepatol* 2009; 24: 336-345.
- [18] Huang CF, Huang JF, Yang JF, Hsieh MY, Lin ZY, Chen SC, Wang LY, Juo SH, Chen KC, Chuang WL, Kuo HT, Dai CY and Yu ML. Interleukin-28B genetic variants in identification of hepatitis C virus genotype 1 patients responding to 24 weeks peginterferon/ribavirin. *J Hepatol* 2012; 56: 34-40.
- [19] Akuta N, Suzuki F, Fukushima T, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Hara T, Kobayashi M, Saitoh S, Arase Y, Ikeda K and Kumada H. Prediction of treatment efficacy and telaprevir-resistant variants after triple therapy in patients infected with hepatitis C virus genotype 1. *J Clin Microbiol* 2013; 51: 2862-2868.
- [20] Bedossa P and Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289-293.
- [21] Ferenci P. Optimal treatment duration for patients with HCV genotype 1 infection. *J Viral Hepat* 2012; 19: 7-13.
- [22] Puoti C, Guarisco R, Bellis L and Spilabotti L. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2009; 50: 322.
- [23] Pellicelli AM, Romano M, Stroffolini T, Mazzoni E, Mecenate F, Monarca R, Picardi A, Bonaventura ME, Mastropietro C, Vignally P, Andreoli A, Marignani M, D'Ambrosio C, Miglioresi L, Nosotti L, Mitidieri O, Gentilucci UV, Puoti C, Barbaro G, Barlattani A, Furlan C, Barbarini G; CLEO Group. HCV genotype 1a shows a better virological response to antiviral therapy than HCV genotype 1b. *BMC Gastroenterol* 2012; 12: 162.
- [24] Kim IH. [Peginterferon alfa-2a/ribavirin for 48 or 72 weeks in hepatitis C genotype 1 and 4 patients with slow virologic response]. *Korean J Hepatol* 2010; 16: 201-205.
- [25] Ma P, Yang JM, Hou W, Song SD, Wang L and Lu W. A preliminary study on the efficacy and influencing factors of interferon for the treatment of genotype 1 chronic hepatitis C with different dosage forms. *Eur J Gastroenterol Hepatol* 2013; 25: 601-605.
- [26] Zhou JY, Jiang ZA, Zhou DF, Xing WL, Wang W and Wang YD. Virological responses in chronic hepatitis C patients undergoing prolonged therapy with low-dose Peg-IFNalpha-2a. *Hepatology* 2013; 60: 301-304.
- [27] Sarwar S, Ryan EJ, Iqbal M, McCormick PA, O'Farrelly C and Hegarty J. Rapid, early and sustained virological responses in a cohort of Irish patients treated with pegylated interferon and ribavirin for chronic hepatitis C virus infection. *Ir J Med Sci* 2012; 181: 53-58.
- [28] Muir AJ, Bornstein JD, Killenberg PG; Atlantic Coast Hepatitis Treatment Group. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N Engl J Med* 2004; 350: 2265-2271.
- [29] Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, Howell CD; Vira-hep-C Study Group. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology* 2006; 131: 470-477.
- [30] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K and Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41: 1105-1109.
- [31] de Rueda PM, López-Nevot MÁ, Sáenz-López P, Casado J, Martín-Casares A, Palomares P, Quiles R, Gila A, Romero-Gómez M, Pavón EJ, Muñoz JA, Carazo A, Sanz-Cameno P, Moreno-Otero R, Diago M, León J, Ruiz-Extremera A and Salmerón J. Importance of host genetic factors HLA and IL28B as predictors of response to pegylated interferon and ribavirin. *Am J Gastroenterol* 2011; 106: 1246-1254.
- [32] Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Gonçalves FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craix A, Chaneac M and Reddy KR. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* 2005; 43: 425-433.
- [33] Jensen DM, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, Ferenci P, Ackrill AM and Willems B. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* 2006; 43: 954-960.
- [34] Cheng WS, Roberts SK, McCaughan G, Sievert W, Weltman M, Crawford D, Rawlinson W, Marks PS, Thommes J, Rizkalla B, Yoshihara M, Dore GJ; CHARLOTTE Study Group. Low virological response and high relapse rates in hepatitis C genotype 1 patients with advanced fibrosis despite adequate therapeutic dosing. *J Hepatol* 2010; 53: 616-623.
- [35] Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ,

Antiviral therapy than HCV genotype 1b

McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB and McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 2010; 139: 120-129. e18.

[36] Sarrazin C, Susser S, Doehring A, Lange CM, Müller T, Schlecker C, Herrmann E, Lötsch J and Berg T. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 2011; 54: 415-421.