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Association between *human leucocyte antigen-DO* polymorphisms and interferon/ribavirin treatment response in hepatitis C virus type 1 infection in Chinese population: a prospective study

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ABSTRACT

Objective The *human leucocyte antigen-DO* (*HLA-DO*) gene located in the *HLA* non-classical class-II region may play a role in treatment response to hepatitis C virus (HCV). This study was conducted to explore the role of single nucleotide polymorphisms (SNPs) in *HLA-DO* in responding to HCV therapy.

Setting All patients were recruited between January 2011 and September 2016 from the Jurong People's Hospital, Jiangsu Province, China.

Participants A total of 346 chronic hepatitis C (CHC) patients who finished the 48-week pegylated interferon-alpha and ribavirin (PEG IFN-α/RBV) treatment were enrolled in this study. All patients were former remunerated blood donors. The inclusion criteria for patients were as follows: (1) treatment-naive and treated with PEG IFN-α/RBV, (2) HCV RNA was present in serum for over 6 months before treatment, (3) negative for hepatitis B (HBV) or HIV infection and (4) lacked any other hepatic diseases. All participants in this study were Chinese Han population and infected with HCV genotype 1b and treated with subcutaneous PEG IFN-α at a dose of 180 µg once a week with the addition of 800–1000 mg/d RBV according to weight orally for 48 weeks.

Results The SNPs *HLA-DOA* rs1044429 and *HLA-DOB* rs2284191 and rs2856997 of 18 SNPs were correlated with HCV treatment response in the Chinese Han population. The dominant model indicated that patients carrying favourable genotypes at rs1044429 AA and rs2284191 AA were more likely to achieve sustained virological response (SVR) (OR 1.99, 95% CI 1.25 to 3.19; OR 2.71, 95% CI 1.58 to 4.63, respectively), while patients carrying unfavourable genotypes at rs2856997 GG were less likely to achieve SVR (OR 0.48, 95% CI 0.29 to 0.78).

Conclusion Genetic variations at rs1044429, rs2284191 and rs2856997 were independent predictors of HCV treatment response in the Chinese Han population.

Strengths and limitations of this study

- ► It is the first study to demonstrate the relationship between variants in*human leucocyte antigen-DO* (*HLA-DO*) and treatment response among Chinese Han population.
- ► Our sample size is relatively large so that it can provide enough statistical power.
- ► The biological mechanism by which *HLA-DO* affects treatment response has not yet been well established.
- ► Our samples have a relatively poor representation since the participants were all selected from the same hospital within 6 years.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health issue and infects more than 185 million individuals around the world. The estimated prevalence of HCV has increased to 2.8%, and China overall has the most people with HCV.[1 2] If left untreated, infection may result in life-threatening diseases such as liver cirrhosis and hepatocellular carcinoma (HCC), which cause approximately 500 000 related deaths per year.[3–5]

Nowadays is an era of direct acting antiviral (DAAs) drugs, which leads to enhancement of HCV treatment response. However, it has not been approved in many low-income and middle-income countries due to its high costs. A combined treatment of pegylated interferon (PEG-IFN) and ribavirin (RBV) was approved to treat patients with chronic hepatitis C (CHC) for 24 or 48 weeks.[6] It is still the first-line treatment for patients with HCV type 1 infection in China. The rates of sustained virological response (SVR) of this regimen in patients infected with HCV genotypes 1 and 2/3 were 50% and 70%–90%, respectively.[7] Virus and host factors have been shown to associate with long-term treatment outcomes, including age, sex, race, HCV genotype, HCV viral load, cirrhosis, body mass index (BMI),

cytokine polymorphisms and human leucocyte antigen (HLA) type.[8–10]

Single-nucleotide polymorphisms (SNPs) located near the gene *interleukin*-28B (*IL28B*) and the *HLA* region are well studied. The *HLA* genomic region encodes many genes related to antigen processing and presentation, with most residing in the class I (*HLA-A, HLA-B* and *HLA-C*) and class II (*HLA-DR*, *HLA-DQ* and *HLA-DP*) regions.[11] A few studies have shown that host SNPs in these regions were correlated with HCV spontaneous clearance.[12–14] A genome-wide association study reported that *HLA DQB1*03:01* genotypes were related to the spontaneous clearance of HCV infection.[15] Furthermore, recent studies reported that the HLA rs4273729 polymorphism was related to treatment responses of CHC and was a powerful predictor factor for rapid virological response (RVR), early virological response (EVR) and SVR with CHC.[16 17]

These studies suggested that the polymorphism in *HLA*, including SNPs in *HLA-DM* and *HLA-DO* may be potential predictors of treatment efficacy in patients with HCV. *HLA-DM* functions in the assembly and loading of antigenic peptides during antigen presentation, and *HLA-DO* is a protein complex negatively regulating the activity of *DM*.[18] Both *HLA-DM* and *HLA-DO* genes are located in the *HLA* class II genomic region.

So far, few studies have investigated the relationship between *HLA-DO* genotypes and HCV infection treatment response in the Chinese population. We carried out this study to assess how *HLA-DO* genotypes are associated with SVR, RVR and completely EVR (cEVR) in patients with CHC from the Chinese Han population treated with PEG-IFN/RBV.

MATERIALS AND METHODS

Participants

A total of 346 patients with CHC who finished the 48-week pegylated IFN-alpha and RBV (PEG IFN-α/RBV) treatment were enrolled in this study. All patients were former remunerated blood donors and were recruited between January 2011 and September 2016 from the Jurong People's Hospital, Jiangsu Province, China. The inclusion criteria for patients were as follows: (1) treatment-naive and treated with PEG IFN-α/RBV in this study, (2) HCV RNA was present in serum for over 6 months before treatment, (3) infected with HCV genotype 1b, (4) negative for hepatitis B (HBV) or HIV infection and (5) lacked any other hepatic diseases. The exclusion criteria for patients were as follows: (1) patients received antiviral therapy within 6 months; (2) patients with blood diseases, malignancies, organ transplants or decompensated liver disease and (3) patients with diabetes and thyroid diseases.

All participants in this study were infected with HCV genotype 1b and treated with subcutaneous PEG IFN-α at a dose of 180 µg once a week with the addition of 800–1000 mg/d RBV according to weight orally for 48 weeks. Successful treatment was evaluated according to SVR, which was defined as negative detection of HCV RNA 24 weeks after the end of treatment. RVR was defined as negative detection of HCV RNA at 4 weeks during treatment; cEVR was defined as negative detection of HCV RNA at 12 weeks during treatment. All participants in this study filled out the written informed consent.

Viral testing and SNP genotyping

Blood samples were collected before antiviral therapy for biochemical analysis and SNP determination. For each patient, serum HCV RNA was quantified before treatment and at weeks 4, 12, 24, and 48 and 24 weeks after treatment termination using a CobasAmplicor HCV Monitor Test (V.2.0, Roche, Basel, Switzerland).

We extracted genomic DNA from peripheral blood samples using protease K digestion and phenol/chloroform purification according to standard protocol. According to our previous work, information regarding SNPs in two candidate genes (*HLA-DOA* and *HLA-DOB*) was acquired 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 SNPs were screened according to the following criteria: (1) minor allele frequency ≥0.05 in the Chinese population and (2) the p value of the Hardy-Weinberg equilibrium test was ≥0.05. Tag SNPs were chosen to represent a set of variants with strong linkage disequilibrium (LD).[14] According to the above steps, a total of 18 SNPs in *HLA-DO* gene were selected for genotyping. The TaqMan allelic discrimination technology a 384-well ABI7900HT Sequence Detection system (Applied Biosystems, San Diego, California, USA) was used to polymorphism at the chosen SNPs . The primers and probes used for genotyping are shown in the online Supplementary table 1. Genotyping results were ascertained using SDS V.2.3 software (Applied Biosystems, Foster City, California, USA) and 100% concordance was achieved.

Statistical analysis

All data analysis was operated with Stata/SE (V.12.0 for Windows). Comparisons between individual demographic characteristics were analysed as appropriate with either a Student's t-test (for continuous variables) or a χ^2 test (for categorical variables) with a two-tailed p value. Multivariate logistic regression was used to analyse the association between genotypes and SVR, RVR and cEVR by calculating the OR and 95% CI adjusted for age, gender, baseline HCV RNA level and glucose. Each SNP was analysed using codominant, dominant and additive genetic models. The codominant model considers homozygous type versus wild type and hybrid type versus wild type, respectively. The dominant model considers the homozygous type and heterozygous type together versus the wild type, and the additive model considers the heterozygous type versus the homozygous type versus the wild type. False discovery rate (FDR) corrections were applied for multiple comparisons, and they were carried

Table 1 Characteristics of patients with chronic hepatitis C related with response to interferon/ribavirin treatment

Variables	N-SVR (n=117)	SVR (n=229)	P values
Mean age, year	53.49±7.91	53.60±8.51	0.903
Age ≥50 (%)	81 (69.23)	156 (68.12)	0.834
Male (%)	28 (23.93)	57 (24.89)	0.845
Baseline HCV-RNA (log_{10})	6.20±0.72	5.84±1.21	0.003
TP (g/L)	78.87±5.78	78.03±6.02	0.216
ALB (g/L)	43.64±3.83	43.28±4.26	0.446
AFP (ng/mL)	7.57±10.00	9.00±24.54	0.544
Haemoglobin (g/L)	134.73±15.45	133.09±17.14	0.386
ALT≥40 U/L (%)	78 (66.67)	137 (59.83)	0.215
AST≥40 U/L (%)	64 (54.70)	125 (54.59)	0.984
GGT≥50 U/L (%)	40 (34.19)	86 (37.55)	0.538
GLU>6 (mmol/L)	48 (41.03)	60 (26.20)	0.005
T3 (nmol/L)	1.60±0.94	1.45±0.42	0.053
T4 (nmol/L)	129.10±37.74	123.38±27.90	0.112
Platelets (10^9/L)	132.07±49.02	132.12±58.91	0.994
Abnormal	36 (30.77)	77 (33.92)	0.555
Normal	81 (69.23)	150 (66.08)	
WBC (10^9/L)	4.97±1.70	4.89±1.76	0.699
Abnormal	35 (29.91)	81 (35.68)	0.284
Normal	82 (70.09)	146 (64.32)	

AFP, alpha fetal protein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; GLU, glucose; HCV, hepatitis C virus; N-SVR, non-sustained virological response; SVR, sustained virological response; TP, total protein; WBC, white blood cell.

out as previously described, considering FDR<0.05 as significant.[19] The combined effect of three independent SNPs (rs1044429, rs2284191 and rs2856997) was analysed using the Cochran-Armitage trend test. A forward elimination stepwise regression analysis containing all variables was used to determine the prediction factors for SVR. A receiver-operating characteristic curve was used to represent the prediction model for SVR, with the area under the curve (AUC) indicating the value of the prediction model. Additionally, a line chart was used to observe the viral load at each follow-up time point. A two-tailed test with a p value <0.05 was regarded as statistically significant in all analyses.

RESULTS

Baseline characteristics of the study population

All participating patients were classified into two groups according to SVR. The baseline demographic and laboratory characteristics of the 346 enrolled patients are shown in table 1. A total of 229 (66.2%) patients achieved SVR overall. Among this group, 24.89% were male, and the average age was 53.60±8.51 years. There was no difference in gender and age between the SVR group and non-SVR group (p>0.05). In addition, the baseline levels of total protein , alpha fetal protein, haemoglobin, alanine transaminase, aspartate transaminase, γ-glutamyl transpeptidase, T3, T4, platelets and white blood cell were similar between two groups (p>0.05).

However, the baseline viral load and glucose levels were different between the SVR and non-SVR group (p<0.05). Individuals with higher baseline viral load and glucose levels were less likely to achieve SVR.

Association between polymorphisms in *HLA-DO* gene and treatment response

All SNPs were in Hardy-Weinberg equilibrium in allele frequency in the non-SVR group except for rs1044429, p=0.048. Codominant, dominant and additive models were analysed for each SNP to confirm the impact on RVR, cEVR and SVR. Factors with p values <0.05 in the univariate analysis were adjusted for age, gender, baseline viral load and glucose. After adjustment, the logistic regression analyses showed that mutations in rs1044429, rs2284191 and rs2856997 were associated with treatment response.

Polymorphisms associated with SVR are presented in table 2. Patients with the AA genotype at rs1044429 or rs2284191 had a higher rate of SVR (80% and 100%, respectively) compared with those carrying the AG (71.82% and 78.07%, respectively) or the GG (58% and 60.17%, respectively) genotypes (dominant model: OR 1.99, 95% CI 1.25 to 3.19; dominant model: OR 2.71, 95% CI 1.58 to 4.63, respectively). For rs2856997, the rate of SVR was higher in patients carrying the TT genotype (75.9%) compared with those with the TG genotype (59.3%) and GG (60%) (dominant model: OR 0.48, 95% CI 0.29 to 0.78). We performed FDR correction for all SNPs as outlined in the online Supplementary table 2. These SNPs at rs1044429, rs2284191 and rs2856997 were also significant after FDR correction for both the dominant model (p=0.024, p=0.005, p=0.024, respectively) and the additive model (p=0.027, p=0.005, p=0.030, respectively).

Afterwards, we evaluated the combined effect of these three significant SNPs by adding up the unfavourable genotype number. The results indicated that SVR rates declined when patients were carrying the more unfavourable rs1044429 GG, rs2284191 GG and rs2856997 GG genotypes from zero to three, with SVR rates of 84.38%, 67.59%, 58.26% and 45.45%, respectively. The ORs also decreased along with the increase in risk genotypes (OR 0.38, 95% CI 0.17 to 0.83; OR 0.12, 95% CI 0.04 to 0.37, respectively). The risk of treatment failure increased by 62% and 78% when patients carried either one or two risk genotypes. When carrying three risk genotypes, the risk of not achieving SVR increased to 88% risk (figure 1).

In addition, rs1044429, rs2284191 and rs2856997 were also found to be significantly associated with RVR (dominant model: OR 1.62, 95% CI 1.04 to 2.53; OR 2.42,

Table 2 Association of single nucleotide polymorphisms in *human leucocyte antigen-DO* with hepatitis C virus treatment response

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	P values
rs1044429					
GG	63 (53.85)	87 (37.99)	58.00	1.00	–
AG	51 (43.59)	130 (56.77)	71.82	1.92 (1.19 to 3.08)	0.007
AA	3 (2.56)	12 (5.24)	80.00	3.44 (0.91 to 13.04)	0.069
Dominant				1.99 (1.25 to 3.19)	0.004
Additive				1.90 (1.25 to 2.89)	0.003
rs2284191					
GG	92 (78.63)	139 (60.70)	60.17	1.00	–
AG	25 (21.37)	89 (38.86)	78.07	2.67 (1.56 to 4.58)	<0.001
AA	0	1 (0.44)	100	1.00	–
Dominant				2.71 (1.58 to 4.63)	<0.001
Additive				2.70 (1.59 to 4.61)	<0.001
rs2856997					
TT	34 (29.06)	107 (46.72)	75.89	1.00	–
TG	59 (50.43)	86 (37.55)	59.31	0.49 (0.29 to 0.83)	0.008
GG	24 (20.51)	36 (15.73)	60.00	0.44 (0.22 to 0.85)	0.015
Dominant				0.48 (0.29 to 0.78)	0.003
Additive				0.63 (0.46 to 0.87)	0.005
rs408036					
GG	45 (38.46)	80 (34.93)	64.00	1.00	–
AG	57 (48.72)	117 (51.09)	67.24	1.32 (0.80 to 2.18)	0.279
AA	15 (12.82)	32 (13.98)	68.09	1.32 (0.63 to 2.75)	0.463
Dominant				1.32 (0.82 to 2.13)	0.256
Additive				1.19 (0.84 to 1.69)	0.325
rs3128935					
TT	41 (35.04)	89 (38.86)	68.46	1.00	–
CT	59 (50.43)	113 (49.34)	65.70	1.00 (0.60 to 1.66)	0.996
CC	17 (14.53)	27 (11.80)	61.36	0.84 (0.41 to 1.75)	0.645
Dominant				0.96 (0.59 to 1.56)	0.879
Additive				0.94 (0.66 to 1.33)	0.713
rs3129304					
AA	106 (90.60)	207 (90.39)	66.13	1.00	–
AG	10 (8.55)	21 (9.17)	67.74	1.12 (0.50 to 2.51)	0.791
GG	1 (0.85)	1 (0.44)	50.00	0.58 (0.03 to 10.68)	0.714
Dominant				1.07 (0.49 to 2.34)	0.866
Additive				1.02 (0.50 to 2.09)	0.948
rs376892					
CC	72 (61.54)	142 (62.01)	66.36	1.00	–
CT	41 (35.04)	80 (34.93)	66.12	0.92 (0.57 to 1.50)	0.753
TT	4 (3.42)	7 (3.06)	63.64	0.98 (0.27 to 3.59)	0.978
Dominant				0.93 (0.58 to 1.49)	0.763
Additive				0.95 (0.63 to 1.43)	0.796
rs369150					
GG	37 (31.62)	79 (34.50)	68.10	1.00	–

Continued

Table 2 Continued

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	P values
AG	63 (53.85)	121 (52.84)	65.76	0.80 (0.48 to 1.34)	0.396
AA	17 (14.53)	29 (12.66)	63.04	0.71 (0.34 to 1.48)	0.358
Dominant				0.78 (0.48 to 1.28)	0.325
Additive				0.83 (0.59 to 1.18)	0.302
rs86567					
AA	29 (24.79)	65 (28.38)	69.15	1.00	–
AC	67 (57.26)	128 (55.90)	65.64	0.79 (0.46 to 1.36)	0.396
CC	21 (17.95)	36 (15.72)	63.16	0.67 (0.32 to 1.37)	0.267
Dominant				0.76 (0.45 to 1.28)	0.306
Additive				0.81 (0.57 to 1.16)	0.250
rs6913008					
CC	81 (69.23)	161 (70.31)	66.53	1.00	–
CT	35 (29.91)	64 (27.95)	64.65	0.94 (0.57 to 1.56)	0.882
TT	1 (0.86)	4 (1.74)	80.00	1.53 (0.16 to 14.19)	0.708
Dominant				0.96 (0.58 to 1.58)	0.880
Additive				0.99 (0.62 to 1.57)	0.961
rs2582					
CC	69 (58.97)	134 (58.52)	66.01	1.00	–
AC	45 (38.46)	82 (35.81)	64.57	0.94 (0.58 to 1.52)	0.803
AA	3 (2.57)	13 (5.67)	81.25	2.09 (0.56 to 7.83)	0.274
Dominant				1.01 (0.63 to 1.61)	0.963
Additive				1.10 (0.74 to 1.64)	0.650
rs416622					
GG	59 (50.43)	112 (48.91)	65.50	1.00	–
AG	48 (41.03)	101 (44.10)	67.79	1.15 (0.71 to 1.86)	0.571
AA	10 (8.54)	16 (6.99)	61.54	0.97 (0.40 to 2.31)	0.937
Dominant				1.12 (0.71 to 1.77)	0.634
Additive				1.05 (0.73 to 1.52)	0.779
rs453779					
CC	56 (47.86)	115 (50.22)	67.25	1.00	–
CT	53 (45.30)	94 (41.05)	63.95	0.90 (0.56 to 1.46)	0.680
TT	8 (6.84)	20 (8.73)	71.43	1.24 (0.50 to 3.06)	0.637
Dominant				0.95 (0.60 to 1.50)	0.823
Additive				1.02 (0.71 to 1.46)	0.935
rs2857111					
AA	89 (76.07)	170 (74.24)	65.64	1.00	–
AG	28 (23.93)	56 (24.45)	66.67	1.01 (0.59 to 1.74)	0.969
GG	0	3 (1.31)	100.00	1.00	–
Dominant				1.06 (0.62 to 1.82)	0.822
Additive				1.13 (0.68 to 1.88)	0.647
rs1383258					
GG	103 (88.03)	203 (88.65)	66.34	1.00	–
AG	13 (11.11)	25 (10.92)	65.79	0.98 (0.47 to 2.02)	0.955
AA	1 (0.86)	1 (0.43)	50.00	0.80 (0.05 to 14.05)	0.878
Dominant				0.97 (0.48 to 1.96)	0.930

Continued

Table 2 Continued

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	P values
Additive				0.96 (0.50 to 1.85)	0.907
rs2071472					
GG	39 (33.33)	72 (31.44)	64.86	1.00	–
AG	61 (52.14)	118 (51.53)	65.92	1.08 (0.65 to 1.81)	0.760
AA	17 (14.53)	39 (17.03)	69.64	1.35 (0.66 to 2.76)	0.406
Dominant				1.14 (0.70 to 1.86)	0.598
Additive				1.15 (0.82 to 1.61)	0.431
rs7383287					
AA	100 (85.47)	198 (86.46)	66.44	1.00	–
AG	17 (14.53)	31 (13.54)	64.58	1.01 (0.52 to 1.95)	0.975
Dominant				1.01 (0.52 to 1.95)	0.975
Additive				1.01 (0.52 to 1.95)	0.975
rs2071475					
CC	54 (46.15)	91 (39.74)	62.76	1.00	–
CT	54 (46.15)	123 (53.71)	69.49	1.41 (0.87 to 2.27)	0.164
TT	9 (7.70)	15 (6.55)	62.50	1.09 (0.43 to 2.74)	0.852
Dominant				1.36 (0.86 to 2.17)	0.193
Additive				1.21 (0.82 to 1.77)	0.334

Logistic regression analyses adjusted for age, gender, glucose, baseline RNA.
N-SVR, non-sustained virological response.; SVR, sustained virological response.

95% CI 1.50 to 3.90; OR 0.59, 95% CI 0.38 to 0.92, respectively) and cEVR (dominant model: OR 2.05, 95% CI 1.27 to 3.32; OR 2.84, 95% CI 1.62 to 4.96; OR 0.60, 95% CI 0.37 to 0.99, respectively) (see online Supplementary table 3). Patients carrying the mutant alleles rs1044429-A or rs2284191-A or the wild-type allele rs2284191-T were more likely to achieve higher rates of RVR, cEVR and SVR.

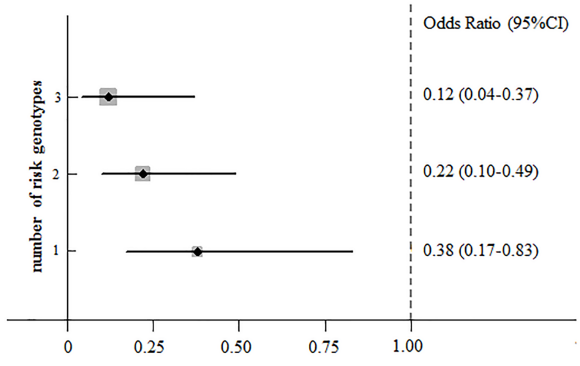

Figure 1 Combined effects of rs1044429, rs2284191 and rs2856997 with sustained virological response. Variables are numbers of combined unfavourable genotypes (rs1044429-GG, rs2284191-GG and rs2856997-GG); logistic regression analyses adjusted for age, gender, glucose, baseline hepatitis C virus RNA.

Interaction analysis

As shown in table 3, the interaction analysis among the meaningful SNPs and potential risk factors was also analysed. A significant multiplicative interaction related to SVR was found between rs2856997 genotypes and gender ($p_{interaction}$=0.019). Compared with individuals carrying the rs2856997 TT genotype, female subjects carrying TG/GG genotypes had a 67% increase of risk for treatment failure (OR 0.33, 95% CI 0.81 to 0.59).

Table 3 Interaction analysis between rs2856997 genotypes and gender

Variables	N-SVR	SVR	OR (95% CI)
Female with TT genotypes	22 (20.75)	84 (79.25)	1.00
Female with TG/GG genotypes	67 (43.23)	88 (56.77)	0.33 (0.18 to 0.59)
Male with TT genotypes	12 (34.29)	23 (65.71)	0.44 (0.18 to 1.04)
Male with TG/GG genotypes	16 (32.00)	34 (68.00)	0.54 (0.25 to 1.19)
P for multiplicative interaction		p=0.019	

Logistic regression analyses adjusted for rs2856997, gender, age, glucose and baseline RNA.

Table 4 Multivariate stepwise regression analysis for independent factors of SVR

Variables	Coef.	SE	95% CI	OR (95% CI)	P values
rs1044429	0.59	0.22	(0.17 to 1.02)	1.80 (1.19 to 2.77)	0.006
rs2284191	0.94	0.28	(0.39 to 1.48)	2.56 (1.48 to 4.39)	0.001
rs2856997	−0.39	0.17	(−0.72 to −0.06)	0.68 (0.49 to 0.94)	0.022
GLU	−0.77	0.26	(−1.28 to −0.26)	0.46 (0.28 to 0.77)	0.003
Baseline HCV-RNA	−0.41	0.14	(−0.69 to −0.13)	0.66 (0.50 to 0.88)	0.004
Cons.	3.10	0.90	(1.34 to 4.86)	22.20 (3.82 to 129.02)	0.001

Coef. coefficient of variation; Cons. constant term; GLU, glucose; HCV, hepatitis C virus; SVR, sustained virological response.

Predictive factors for SVR

A stepwise regression model containing all variables was built. The results showed that rs1044429, rs2284191, rs2856997, baseline glucose and baseline HCV RNA were independent predictors of SVR (table 4). The model yielded approximately parallel AUC when adding one SNP (rs1044429=0.66, rs2284191=0.66 and rs2856997=0.65), which suggests that the predictive value of rs1044429, rs2284191 or rs2856997 are similar. Additionally, adding up these five factors increases the predictive AUC value to 0.71 (figure 2).

Association of SNPs with viral dynamics during treatment

The effect of the three significant SNPs on viral dynamics during treatment was also analysed. The difference between baseline viral load in these SNPs was not significant between patients carrying the wild-type alleles and mutant alleles (p>0.05). Nevertheless, the decline in viral load was significantly quicker in patients carrying rs2284191 AG/AA genotype than in patients carrying GG genotype through the entire therapy. The viral load was significantly declined at weeks 4, 12, 24 and 48 (p<0.05), but not at week 8 (figure 3). Therefore, these results of rs2284191 suggest that individuals with the protective A allele achieve SVR easier. For rs1044429, the viral load decline was statistically significant between AG/AA and GG only at week 12 (p=0.029), but the difference between TG/GG and TT at rs2856997 was not statistically significant.

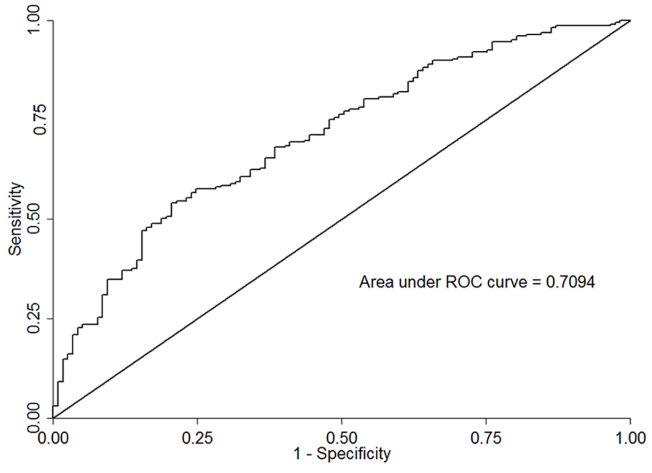

Figure 2 Predictors of hepatitis C virus (HCV) treatment response. The response variable is sustained virological response and the diagnostic test variable is a combination of rs1044429, rs2284191, rs2856997, glucose and baseline HCV RNA with the coefficients taken from the regression analysis. ROC, receiver-operating characteristic.

DISCUSSION

Currently, HCV infection is no longer considered an incurable disease. Therefore, plenty of studies have been conducted to investigate the relationship between genetic polymorphism and treatment response.[20 21] Several studies have revealed that *HLA* class II genotypes are important in immune system response to HCV infection and are associated with the spontaneous elimination of HCV.[13 22 23] *HLA* class II genotypes are also related to HCV treatment response.[24] Our previous study showed that *HLA-DOA* rs2284191 and *HLA-DOB* rs7383287 are independent factors predicting HCV treatment outcomes.[14] The current study was conducted to investigate the correlation between the candidate SNPs in *HLA-DO* gene and HCV treatment outcomes.

A total of 18 tagging SNPs involved in antigen processing and presentation in *HLA-DO* were selected and analysed. The results showed that the polymorphisms *HLA-DOA* rs1044429 and rs2284191 and *HLA-DOB* rs28546997 were correlated with HCV treatment response. The mutant alleles rs1044429-A and rs2284191-A and the wild-type allele rs2856997-T were protective factors for HCV treatment. The combined analysis of these three significant SNPs showed that as an individual carried more unfavourable rs1044429, rs2284191 and rs2856997 GG genotypes, their SVR rates would gradually decrease. From the stepwise regression analysis, we determined that rs1044429, rs2284191, rs2856997, baseline glucose and baseline viral load were independent predictors of SVR, with a predictive AUC value of 0.71. This prediction model is similar to previous research and may contribute to the prediction of HCV prognosis and the adjustment of therapeutic regimens accordingly.[25 26] In addition, the association of SNPs with viral dynamics during treatment suggested that individuals carrying the protective rs2284191-A allele achieve SVR easier almost throughout the course of treatment. But the difference between rs1044429,

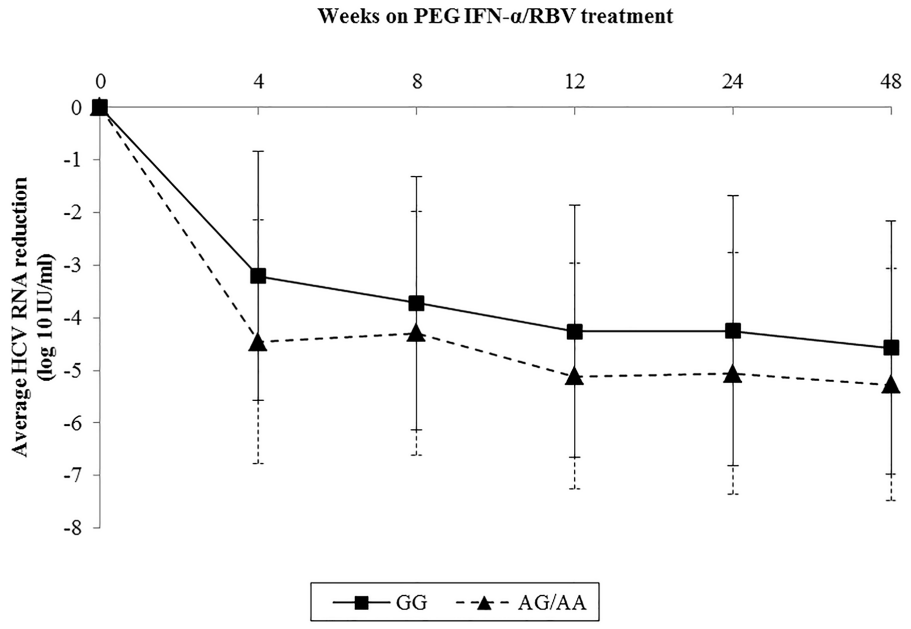

Figure 3 Effect of HLA-DOA rs2284191 variants on hepatitis C virus viral kinetics during therapy. The fold of viral decline was compared among patients with the GG genotype and the AG/AA genotype. The fold of viral decline was calculated as the viral load at follow-up time point divided by the initial viral load. PEG IFN, pegylated interferon; RBV, ribavirin.

rs2856997 wild type and mutant type was not statistically significant during the entire course of treatment. The mechanism of the difference among these three SNPs remains to be elucidated.

This study is the first to demonstrate a relationship between variants in *HLA-DO* and HCV treatment response in the Chinese Han population. *HLA-DOA* rs1044429 (G>A) is located in the three prime untranslated regions (3′UTR) of *HLA-DO*. *HLA-DOA* rs2284191 (G>A) and *HLA-DOB* rs2856997 (T>G) are in the intron region, and rs2284191 is a transcription factor binding site. The mutation at rs2284191 may influence transcription and transform the encoding protein's function, ultimately affecting antigen processing and presentation. The associations between these three SNPs and SVR were significant in codominant, dominant and additive models. In addition, the relationship between rs2856997 and SVR seemed to be stronger in females according to the interaction analysis. It is well known that the occurrence of HCV and other chronic inflammatory diseases such as mellitus type 2 and HIV is often correlated with host immune response.[27 28] *HLA-DO* is also involved in the host immune response. It mainly operates in the negative regulation of antigen processing and presentation by regulating DM molecules.[18] Few studies have investigated the association between *HLA-DO* polymorphism and inflammatory diseases. However, previous studies have reported that *DM* gene polymorphisms were associated with systemic lupus erythematous and HIV-related Kaposi's sarcoma.[29 30] Therefore, more attention should be given to the structure and function of *HLA-DO* and *DM* molecules.

Our study also has some potential limitations. First, the biological mechanism by which *HLA-DO* affects treatment response has not yet been well established. Stepwise regression model showed that rs1044429, rs2284191, rs2856997, baseline glucose and baseline HCV RNA were independent predictors of SVR. Previous studies reported that HCV genotypes and ethnicities were also predictors of SVR rate in naive patients with CHC.[31–33] In the current study, we only focused on HCV-1b genotype in the Chinese population without taking other genotypes and ethnicities into consideration. Therefore, further studies are required in diverse HCV genotypes and populations. Besides, treatment of CHC currently is a triple DAA epoch. Predicting treatment response to an IFN-based regimen is still far from enough. However, the new therapy has not been used extensively because of its adverse effects and expensive costs in low-income and middle-income countries like China. As it was before, PEG-IFN/RBV regimen is still the first-line treatment for patients with HCV type 1 infection in China. Additionally, our samples are a relatively poor representation of the larger population since they were all selected from the same hospital within 6 years. A multicentre study may be more suitable for representing the Chinese Han population. Meanwhile, our study lacked information of liver fibrosis and cirrhosis, which can affect HCV treatment response. And this study also lacked information of trial registration, which may affect the credibility of our study. We will pay attention to collecting this information in future research. In contrast, our study also has some advantages which should not be ignored. This study validated the relationship between *HLA-DO* gene and HCV treatment response for the first time. Our previous study had found that *HLA-DOA* rs2284191

and *HLA-DOB* rs7383287 played a significant role in HCV susceptibility.[14] We performed this study to further explore the function of *HLA-DO* gene in HCV treatment response in the same population. This treatment cohort is credible since all patients were only infected with HCV and were enrolled from the same area at the same time. Our results indicated that mutation of *HLA-DOA* rs2284191 is significant for both HCV susceptibility and treatment response.

In conclusion, this research first showed that genetic mutations in *HLA-DO* may be important for HCV treatment outcomes in the Chinese Han population. *HLA-DO* rs1044429, rs2284191, rs2856997, baseline glucose and baseline viral load were all independent predictors of HCV treatment response.

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Contributors YY, PH and RY designed the study. YY, ML and FZ performed the experiment and wrote the draft manuscript. MY and HF conducted the statistical analysis. XX, YF and YZ provided materials and analysis tools. PH revised the manuscript. All authors accepted the final manuscript.

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