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Association between *HLA-DO* polymorphisms and interferon/ribavirin treatment response in hepatitis C virus type 1 infection in Chinese population

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Abstract

Objective: The human gene *leukocyteantigen-DO* (*HLA-DO*) 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 became infected through former remunerated blood donation. The inclusion criteria for patients were as follows: (1) treatment-naïve 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 1 and treated with subcutaneous PEG IFN-α at a dose of 180 μg once a week with the addition of 600-1000 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 favorable genotypes at rs1044429 AA and rs2284191 AA were more likely to achieve sustained virological

response (SVR) (Odds ratio (OR) = 1.99, 95% confidence interval (CI) = 1.25-3.19; OR = 2.71, 95% CI = 1.58-4.63, respectively), while patients carrying unfavorable genotypes at rs2856997GG were less likely to achieve SVR (OR = 0.48, 95% CI = 0.29-0.78).

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

Key words: *HLA-DO*; chronic hepatitis C; gene polymorphism; treatment; virological response.

Article summary

Strengths and limitations of this study

1) It is the first study to demonstrate the relationship between variants in *HLA-DO* and treatment response among Chinese Han population.
2) The results indicated that *HLA-DOA* rs1044429, rs2284191 and *HLA-DOB* rs28546997 polymorphisms were correlated with treatment response of HCV.
3) Our sample size is relatively large so that it can provide enough statistical power.
4) The biological mechanism by which *HLA-DO* affects treatment response has not yet been well established.
5) Our samples have a relatively poor representation since the participants were all selected from the same hospital within 6 years.

1. 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].

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]. The rates of sustained virological response (SVR) of this regimen in patients infected with HCV genotype 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 leukocyte 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, -B* and *-C*) and class II (*HLA-DR, -DQ* and *-DP*) regions [11]. A few studies have shown that host SNPs in these regions were correlated with HCV spontaneous clearance [12-14]. Furthermore, a recent genome-wide association study (GWAS) reported that *HLA DQB1*03:01* genotypes were related to the spontaneous clearance

of HCV infection [15].

These studies reported that the polymorphism in *HLA*, including SNPs in *HLA-DM* and *-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* [16]. Both *HLA-DM* and *-DO* genes are located in the *HLA* class □ 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, rapid virological response (RVR) and completely early virological response (cEVR) in CHC patients from the Chinese Han population treated with PEG-IFN/RBV.

2. Materials and methods

2.1 Participants

A total of 346 CHC patients who finished the 48-week pegylated interferon-alpha and ribavirin (PEG IFN-α/RBV) treatment were enrolled in this study. All patients became infected through former remunerated blood donation. The inclusion criteria for patients were as follows: (1) treatment-naïve 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 infected with HCV genotype 1 and treated with

subcutaneous PEG IFN-α at a dose of 180 μg once a week with the addition of 600-1000 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.

2.2Viral 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 (v2.0, Roche, Basel, Switzerland)..

We extracted genomic DNA from peripheral blood samples using protease K digestion and phenol/chloroform purification according to standard protocol. Information regarding SNPs in *HLA-DO* 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 (MAF) ≥ 0.05 in the Chinese population; and (2) the *P* value of the Hardy-Weinberg equilibrium (HWE) test was ≥ 0.05. Tag SNPs were chosen to represent a set of variants with strong linkage disequilibrium (LD). 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, CA, USA) was used to polymorphism at the chosen SNPs. The primers and probes used for genotyping are shown in Supplemental Table 1. Genotyping results were ascertained using SDS 2.3 software (Applied Biosystems, Foster City, CA, USA), and 100% concordance was achieved.

2.3 Statistical analysis

All data analysis was operated with Stata/SE (V.12.0 for Windows; StataCorp LP, College Station, TX, USA). Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's *t* test (for continuous variables) or a chi-square (χ^2) test (for categorical variables) with a two-tailed *P* value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR) and 95% confidence interval (95% CI) adjusted for age, gender, baseline HCV RNA level and glucose. Each SNP was analyzed using dominant and additive genetic models. The dominant model considers the homozygous type and heterozygous type together *vs*. the wild type, and the additive model considers the heterozygous type *vs*. the homozygous type *vs.* the wild type. False discovery rate (FDR) corrections were applied for multiple comparisons, and they were carried out as previously described, considering FDR < 0.05 as significant [17]. The combined effect of three independent SNPs (rs1044429, rs2284191 and rs2856997) was analyzed 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 (ROC) 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.

2.4 Ethical approval and informed consent

Our study protocol was approved by the Institutional Ethics Review Committee of Nanjing Medical University. All participants in this study filled out the written informed consent.

3. Results

3.1 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 (TP), alpha fetal protein (AFP), hemoglobin, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), T3, T4, platelets and WBC 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.

Table1. Characteristics of chronic hepatitis C patients related with IFN/RBV

Variables	N-SVR (n=117)	SVR (n=229)	*P* value
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
Hemoglobin (g/L)	134.73±15.45	133.09±17.14	0.386
ALT ≥ 40U/L (%)	78 (66.67)	137 (59.83)	0.215
AST ≥ 40U/L (%)	64 (54.70)	125 (54.59)	0.984
GGT≥50U/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)	

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

3.2 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, $P = 0.048$. Dominant and additive models were analyzed

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 = 1.25-3.19; Dominant model: OR = 2.71, 95% CI = 1.58-4.63, respectively). For rs2856997, the rate of SVR was higher in patients carrying the TT genotype (75.9%) compared to those with the TG genotype (59.3%) and GG (60%) (Dominant model: OR = 0.48, 95%CI = 0.29-0.78). We performed FDR correction for all SNPs as outlined in Supplemental 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).

In addition, rs1044429, rs2284191 and rs2856997 were also found to be significantly associated with RVR (Dominant model: OR = 1.62, 95%CI = 1.04-2.53; OR = 2.42, 95% CI = 1.50-3.90; OR = 0.59, 95% CI = 0.38-0.92, respectively) and cEVR (Dominant model: OR = 2.05, 95% CI = 1.27-3.32; OR = 2.84, 95% CI = 1.62-4.96; OR = 0.60, 95% CI = 0.37-0.99, respectively) (Supplemental 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.

Table 2. Association of SNPs in HLA-DO with SVR

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	*P* value
rs1044429					
GG	63 (53.85)	87 (37.99)	58.00	1.00	--
AG	51 (43.59)	130 (56.77)	71.82	1.92 (1.19-3.08)	0.007
AA	3 (2.56)	12 (5.24)	80.00	3.44 (0.91-13.04)	0.069
Dominant				1.99 (1.25-3.19)	0.004
Additive				1.90 (1.25-2.89)	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 (1.56-4.58)	<0.001
AA	0	1 (0.44)	100	1.00	--
Dominant				2.71 (1.58-4.63)	<0.001
Additive				2.70 (1.59-4.61)	<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 (0.29-0.83)	0.008
GG	24 (20.51)	36 (15.75)	60.00	0.44 (0.22-0.85)	0.015
Dominant				0.48 (0.29-0.78)	0.003
Additive				0.63 (0.46-0.87)	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 (0.80-2.18)	0.279
AA	15 (12.82)	32 (13.98)	68.09	1.32 (0.63-2.75)	0.463
Dominant				1.32 (0.82-2.13)	0.256
Additive				1.19 (0.84-1.69)	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 (0.60-1.66)	0.996
CC	17 (14.53)	27 (11.80)	61.36	0.84 (0.41-1.75)	0.645
Dominant				0.96 (0.59-1.56)	0.879
Additive				0.94 (0.66-1.33)	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 (0.50-2.51)	0.791
GG	1 (0.85)	1 (0.44)	50.00	0.58 (0.03-10.68)	0.714

Dominant				1.07 (0.49-2.34)	0.866
Additive				1.02 (0.50-2.09)	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 (0.57-1.50)	0.753
TT	4 (3.42)	7 (3.06)	63.64	0.98 (0.27-3.59)	0.978
Dominant				0.93 (0.58-1.49)	0.763
Additive				0.95 (0.63-1.43)	0.796
rs369150					
GG	37 (31.62)	79 (34.50)	68.10	1.00	--
AG	63 (53.85)	121 (52.84)	65.76	0.80 (0.48-1.34)	0.396
AA	17 (14.53)	29 (12.66)	63.04	0.71 (0.34-1.48)	0.358
Dominant				0.78 (0.48-1.28)	0.325
Additive				0.83 (0.59-1.18)	0.302
rs86567					
AA	29 (24.79)	65 (28.38)		1.00	--
AC	67 (57.26)	128 (55.90)		0.79 (0.46-1.36)	0.396
CC	21 (17.95)	36 (15.72)		0.67 (0.32-1.37)	0.267
Dominant				0.76 (0.45-1.28)	0.306
Additive				0.81 (0.57-1.16)	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 (0.57-1.56)	0.882
TT	1 (0.86)	4 (1.74)	80.00	1.53 (0.16-14.19)	0.708
Dominant				0.96 (0.58-1.58)	0.880
Additive				0.99 (0.62-1.57)	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 (0.58-1.52)	0.803
AA	3 (2.57)	13 (5.68)	81.25	2.09 (0.56-7.83)	0.274
Dominant				1.01 (0.63-1.61)	0.963
Additive				1.10 (0.74-1.64)	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 (0.71-1.86)	0.571
AA	10 (8.54)	16 (6.99)	61.54	0.97 (0.40-2.31)	0.937
Dominant				1.12 (0.71-1.77)	0.634
Additive				1.05 (0.73-1.52)	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 (0.56-1.46)	0.680

TT	8 (6.84)	20 (8.73)	71.43	1.24 (0.50-3.06)	0.637
Dominant				0.95 (0.60-1.50)	0.823
Additive				1.02 (0.71-1.46)	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 (0.59-1.74)	0.969
GG	0	3 (1.31)	100.00	1.00	--
Dominant				1.06 (0.62-1.82)	0.822
Additive				1.13 (0.68-1.88)	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 (0.47-2.02)	0.955
AA	1 (0.86)	1 (0.43)	50.00	0.80 (0.05-14.05)	0.878
Dominant				0.97 (0.48-1.96)	0.930
Additive				0.96 (0.50-1.85)	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 (0.65-1.81)	0.760
AA	17 (14.53)	39 (17.03)	69.64	1.35 (0.66-2.76)	0.406
Dominant				1.14 (0.70-1.86)	0.598
Additive				1.15 (0.82-1.61)	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 (0.52-1.95)	0.975
Dominant				1.01 (0.52-1.95)	0.975
Additive				1.01 (0.52-1.95)	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 (0.87-2.27)	0.164
TT	9 (7.70)	15 (6.55)	62.50	1.09 (0.43-2.74)	0.852
Dominant				1.36 (0.86-2.17)	0.193
Additive				1.21 (0.82-1.77)	0.334

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

Abbreviation: SVR, sustained virological response; N-SVR, non-sustained virological response.

Afterward, we evaluated the combined effect of these three significant SNPs by adding up the unfavorable genotype number. The results indicated that SVR rates declined when patients were carrying the more unfavorable 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 odds ratios also decreased along with the increase in risk genotypes (OR = 0.38, 95% CI = 0.17-0.83; OR = 0.22, 95% CI = 0.10-0.49; OR = 0.12, 95% CI = 0.04-0.37, 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).

3.3 Interaction analysis

As shown in Table 3, the interaction analysis among the meaningful SNPs and potential risk factors was also analyzed. A significant multiplicative interaction related to SVR was found between rs2856997 genotypes and gender ($P_{interaction}$= 0.019). Compared to 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 = 0.81-0.59).

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 (0.18-0.59)
Male with TT genotypes	12 (34.29)	23 (65.71)	0.44 (0.18-1.04)
Male with TG/GG genotypes	16 (32.00)	34 (68.00)	0.54 (0.25-1.19)
P for multiplicative interaction			*P* = 0.019

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

3.4 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).

Table 4. **Multivariate Stepwise regression analysis for independent factors of SVR**

Variables	Coef.	SE	95% CI	*p*-Value
rs1044429	0.59	0.22	(0.17–1.02)	0.006
rs2284191	0.94	0.28	(0.39–1.48)	0.001
rs2856997	-0.39	0.17	(-0.72–-0.06)	0.022
GLU	-0.77	0.26	(-1.28–-0.26)	0.003
baseline HCV-RNA	-0.41	0.14	(-0.69–-0.13)	0.004
Cons.	3.10	0.90	(1.34–4.86)	0.001

Abbreviation: SVR, sustained virological response; Coef. coefficient of variation; SE, standard error; CI, confidence interval; GLU, glucose; Cons. Constant term.

3.5 Association of SNPs with viral dynamics during treatment

The effect of the three significant SNPs on viral dynamics during treatment was also analyzed. The difference between baseline viral load in these SNPs was not significant between patients carrying the wild-type and mutant alleles (P>0.05). Nevertheless, the decline in viral load was significantly quicker in rs2284191 AG/AA patients than in GG patients 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.

4. 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 [18, 19]. Several studies have revealed that *HLA* class □ genotypes are important in immune system response to HCV infection and are associated with the spontaneous elimination of HCV [13, 20, 21]. *HLA* class □ genotypes are also related to HCV treatment response [22]. 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 analyzed. 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 unfavorable 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 [23, 24].

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 (TFBS). The mutation at rs2284191 may influence transcription and transform the encoding protein's function, ultimately affecting antigen processing 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 [25, 26]. *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 [16]. 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 (SLE) and HIV-related Kaposi's sarcoma [27, 28]. Therefore, more attention should be given to the structure and function of *HLA-DO* and *DM* molecules.

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.

Contributorship statement

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. YZ, XX and YF provided materials and analysis tools. PH revised the manuscript. All authors accepted the final manuscript.

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Conflicts of Interest

There is no conflict of interest.

Data sharing statement

No additional data is available.

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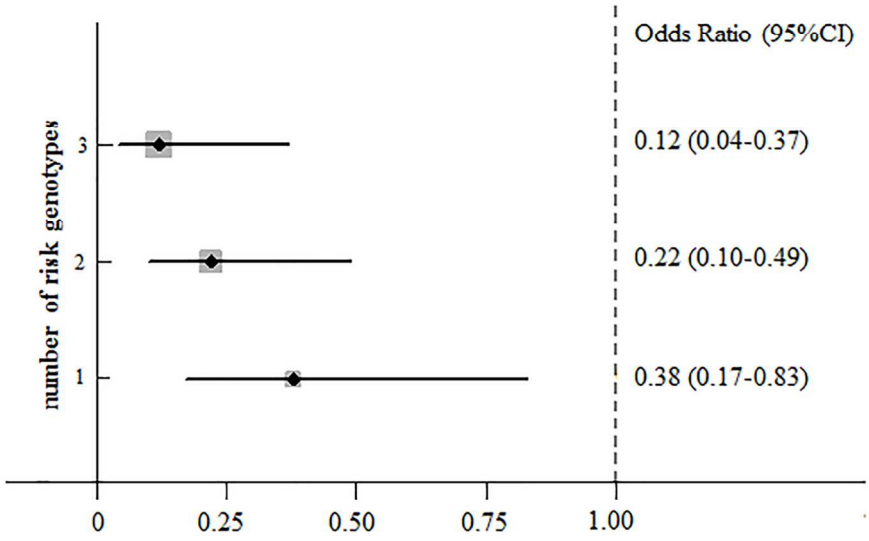

Figure 1. Combined effects of rs1044429, rs2284191 and rs2856997 with SVR. Variables are numbers of combined unfavorable genotypes (rs1044429-GG, rs2284191-GG and rs2856997-GG); logistic regression analyses adjusted for age, gender, glucose, baseline HCV RNA; OR, odds ratio; CI, confidence interval.

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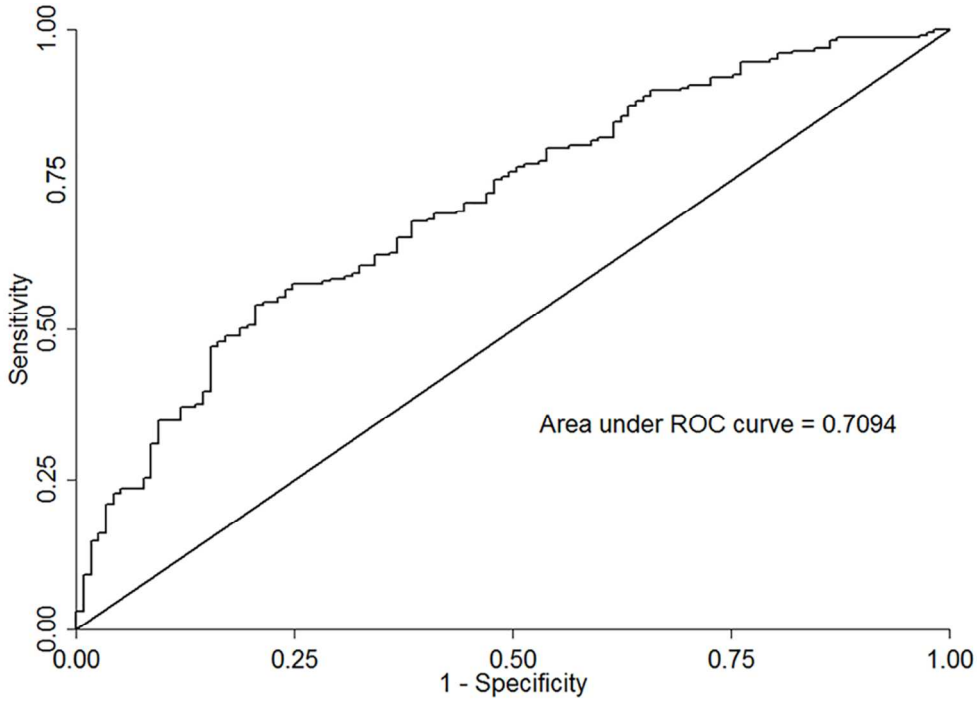

Figure 2.Predictors of HCV treatment response

105x76mm (300 x 300 DPI)

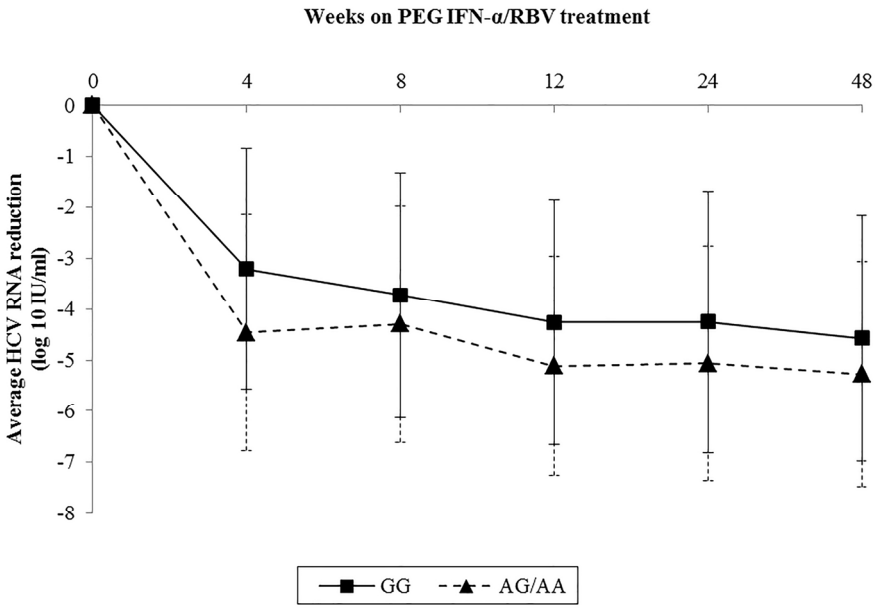

Figure 3.Effect of HLA-DOA rs2284191 variants on HCV viral kinetics during therapy. The fold of viral decline was compared among patients with the GG genotype and the AG/AA genotype. The fold of viral decline was calculated as the viral load at follow up time point divided by the initial viral load.

173x109mm (300 x 300 DPI)

Supplemental Table 1. Information of primers and probes for TaqMan allelic discrimination

Polymorphism		Sequence(5'-3')
DOA rs1044429	Primer	F: TCACACAAAGAGGGTTTCTGTTACTG
		R: GAATAAGTTGAAATCAATGACCAGAAGA
	Probe	FAM-TGAGATGATTCTCCTCCAC-MGB
		HEX-TGAGATGATTTTCCTCCAC-MGB
DOA rs2284191	Primer	F: TCCTCCATCTCAGAGCATTATGAC
		R: TGTTGCTCAAACAACTTCATAGAGTTC
	Probe	FAM-CTTCCATAACTGTTGTCTAG-MGB
		HEX-TAACTGTTATCTAGTTTTCTGG-MGB
DOB rs2856997	Primer	F: CCAAATCCAATGCTAGCTAGAGAAA
		R: ATGGGCTGTGAGAATCTGTAACC
	Probe	FAM-CATGGAGTTACCCCC-MGB
		HEX-CCATGGAGTTACCACC-MGB
DOA rs408036	Primer	F: CCAGGCCTTGGCCAGTT
		R: GTAACACACAATGGGCCAAATG
	Probe	FAM-TTGGCAGCCGTCCT-MGB
		HEX-ATTGGCAGCCATC-MGB
DOA rs3128935	Primer	F: TGTCGGGTGGACATGTTCAC
		R: GGATCCACATGGTCTGTGTTCTC
	Probe	FAM-AGAACACCGCTAACA-MGB
		HEX-AGAACACCGCCAACA-MGB
DOA rs3129304	Primer	F: AAAACATACAAAGAGATAAATCACCATACC
		R: TGAAAACCGTAATCTGTATTGCTCAT
	Probe	FAM-CATAGTTTATGTCAGGACC-MGB
		HEX-CATAGTTTATGTCAAGACC-MGB
DOA rs376892	Primer	F: CTTGGCTGTGGTCTGGTAACTG
		R: CCTTCCTAGTCCACCTCAGACCTT
	Probe	FAM-TAATCAGGTGCCATTGG-MGB
		HEX-TAATCAGGTGCCATCGG-MGB
DOA rs369150	Primer	F: GAAAGAAAGGAACAGGGCATGAC
		R: GGCGGGAAGGTCCAGAGA
	Probe	FAM-TGATGGGAACCTAGG-MGB
		HEX-TGATGGGAGCCTAGG-MGB
DOA rs86567	Primer	F: GGTGCGGGTCTACAGATGGTT
		R: GAGCAACAGTTATTGAGGAACTAGCAT
	Probe	FAM-TGGCCCCCCATTG-MGB
		HEX-TGGCCCACCATTG-MGB
DOA rs6913008	Primer	F: GTCCTGTTCAGAGTCATCCACTTT
		R: TCCTCATCATCATGGGCACAT
	Probe	FAM-CCCAGACTCCCGG-MGB
		HEX-CCCAGACTCCTGG-MGB
DOA rs2582	Primer	F: TGATCCTTCTGAGAGAAATGACTTGT
		R: CACAGCGGGATGCACTTAAA

	Probe	FAM-TGTGACAGACCCTGC-MGB HEX-TGTGACAGCCCCTG-MGB
DOA rs416622	Primer	F: CAGCCTGGTGACAGAGTGAGA R: TCACCCAGACCTACTGAATTAGAATCT
	Probe	FAM-AGACAGCCCCCCTGT-MGB HEX-AGACAGCCTCCCTGTT-MGB
DOA rs453779	Primer	F: GTCACCCGTGGAGGCACTA R: AACGTCCCTTAATCCCAGTCCTA
	Probe	FAM-AGGAACAGGCCCTG-MGB HEX-AGGAACGGGCCCTG-MGB
DOB rs2857111	Primer	F: TCTCTTGCCTCCGTTCTCATTC R: TGCTACATATTTCTAAAAGCCACTCTCATA
	Probe	FAM-TCCCCTCCCTGGAGA-MGB HEX-CTCCCCTCCCTAGAG-MGB
DOB rs1383258	Primer	F: TTACCAGACACGTTTAGAATGGATTC R: GAGTTCACAGCACATTGTAATTATTGG
	Probe	FAM-AGAAGAGATGAGAGAGTC-MGB HEX-CAAGAGAAGAGACGAGAG-MGB
DOB rs2071472	Primer	F: GACTGGATTCCTCCATGACTCAA R: CATGCCAATTCTTGCATACACA
	Probe	FAM-AACAGAGCAATTGTT-MGB HEX-AACAGAGCAATTATT-MGB
DOB rs7383287	Primer	F: CGTAATTTACCAGGCATGGGTTT R: CAGTCAGCCTTTGCCTGAATC
	Probe	FAM-TTCCAGAAGATTTTG-MGB HEX-TTTCCAGAAGACTTTG-MGB
DOB rs2071475	Primer	F: GGTCCTCTCTGGGTACACTGTCA R: GGTTTTCTTTCACGGTGTCTCAT
	Probe	FAM-CTAGGAAGGGAGGAAA-MGB HEX-ACTAGGAAGAGAGGAAA-MGB

Supplemental Table 2. Results of SNPs distribution in dominant, recessive, and additive models

SNPs	Location	Dominant		Additive	
		P Value*	FDR*	*P* Value*	FDR*
DOA rs1044429	3'UTR(G>A)	4.00×10^{-3}	0.024	3.00×10^{-3}	0.027
DOA rs2284191	intron(G>A)	2.83×10^{-4}	0.005	2.52×10^{-4}	0.005
DOB rs2856997	intron(T>G)	3.00×10^{-3}	0.024	5.00×10^{-3}	0.030
DOA rs408036	3'UTR(G>A)	0.256	0.836	0.325	0.859
DOA rs3128935	3'UTR(T>C)	0.879	0.975	0.713	0.975
DOA rs3129304	3'UTR(A>G)	0.866	0.975	0.948	0.975
DOA rs376892	3'UTR(C>T)	0.763	0.975	0.796	0.975
DOA rs369150	intron(G>A)	0.325	0.836	0.302	0.859
DOA rs86567	intron(A>C)	0.306	0.836	0.250	0.859
DOA rs6913008	intron(C>T)	0.880	0.975	0.961	0.975
DOA rs2582	3'UTR(C>A)	0.963	0.975	0.650	0.975
DOA rs416622	3'UTR(G>A)	0.634	0.975	0.779	0.975
DOA rs453779	intron(C>T)	0.823	0.975	0.935	0.975
DOB rs2857111	intron(A>G)	0.822	0.975	0.647	0.975
DOB rs1383258	intron(G>A)	0.930	0.975	0.907	0.975
DOB rs2071472	intron(G>A)	0.598	0.975	0.431	0.970
DOB rs7383287	synonymous(A>G)	0.975	0.975	0.975	0.975
DOB rs2071475	intron(C>T)	0.193	0.836	0.334	0.859

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

Supplemental Table 3. Association of SNPs in *HLA-DO* with RVR/ cEVR

Genotype	N-RVR(n=178)	RVR (n=168)	OR (95% CI)	*P* value	N-cEVR (n=106)	cEVR (n=235)	OR (95% CI)	*P* value
rs1044429								
GG	88 (49.44)	62 (36.90)	1.00	--	58 (54.72)	90 (38.30)	1.00	--
AG	82 (46.07)	99 (58.93)	1.66(1.05-2.60)	0.029	43 (40.57)	136 (57.87)	2.13(1.30-3.48)	0.003
AA	8 (4.49)	7 (4.17)	1.22 (0.40-3.67)	0.727	5 (4.71)	9 (3.83)	1.37 (0.43-4.39)	0.593
Dominant			1.62 (1.04-2.53)	0.034			2.05 (1.27-3.32)	0.003
Additive			1.42 (0.97-2.10)	0.074			1.73 (1.12-2.65)	0.013
rs2284191								
GG	134 (75.28)	97 (57.74)	1.00	--	84 (79.25)	143 (60.85)	1.00	--
AG	44 (24.72)	70 (41.67)	2.37 (1.47-3.83)	<0.001	22 (20.75)	91 (38.72)	2.81 (1.60-4.91)	<0.001
AA	0	1 (0.59)	1	--	0	1 (0.43)	1.00	--
Dominant			2.42 (1.50-3.90)	<0.001			2.84 (1.62-4.96)	<0.001
Additive			2.44 (1.52-3.91）	<0.001			2.83 (1.63-4.94)	<0.001
rs2856997								
TT	61 (34.27)	80 (47.62)	1.00	--	34 (32.08)	106 (45.11)	1.00	--
TG	84 (47.19)	61 (36.31)	0.60 (0.37-0.96)	0.035	49 (46.23)	92 (39.15)	0.66 (0.39-1.12)	0.122
GG	33 (18.54)	27 (16.07)	0.58 (0.31-1.10)	0.093	23 (21.69)	37 (15.74)	0.49 (0.25-0.96)	0.038
Dominant			0.59 (0.38-0.92)	0.021			0.60 (0.37-0.99)	0.045
Additive			0.72 (0.53-0.98)	0.040			0.70 (0.50-0.96)	0.029
rs408036								
GG	61 (34.27)	64 (38.10)	1.00	--	40 (37.74)	82 (34.89)	1.00	--
AG	94 (52.81)	80 (47.62)	0.86 (0.53-1.38)	0.528	52 (49.06)	121 (51.49)	1.28 (0.76-2.14)	0.351

AA	23 (12.92)	24 (14.28)	1.06 (0.53-2.12)	0.866	14 (13.20)	32 (13.62)	1.18 (0.56-2.50)	0.663
Dominant			0.90 (0.57-1.41)	0.642			1.26 (0.77-2.05)	0.361
Additive			0.98 (0.71-1.36)	0.922			1.13 (0.79-1.62)	0.489
rs3128935								
TT	78 (43.82)	52 (30.95)	1.00	--	45 (42.45)	83 (35.32)	1.00	--
CT	88 (49.44)	84 (50.00)	1.84 (1.12-3.02)	0.016	51 (48.11)	118 (50.21)	1.49 (0.89-2.48)	0.130
CC	12 (6.74)	32 (19.05)	5.59 (2.55-12.26)	<0.001	10 (9.44)	34 (14.47)	2.22 (0.99-5.01)	0.054
Dominant			2.27 (1.41-3.67)	0.001			1.61 (0.98-2.64)	0.058
Additive			2.20 (1.54-3.13)	<0.001			1.49 (1.03-2.15)	0.034
rs3129304								
AA	166 (93.26)	147 (87.50)	1.00	--	96 (90.57)	212 (90.21)	1.00	--
AG	11 (6.18)	20 (11.90)	2.17 (0.99-4.78)	0.054	9 (8.49)	22 (9.36)	1.15 (0.50-2.64)	0.739
GG	1 (0.56)	1 (0.60)	1.47 (0.08-25.49)	0.792	1 (0.94)	1 (0.43)	0.46 (0.03-8.21)	0.594
Dominant			2.12 (0.99-4.55)	0.054			1.08 (0.49-2.41)	0.846
Additive			1.91 (0.95-3.87)	0.070			1.02 (0.49-2.11)	0.962
rs376892								
CC	103 (57.87)	111 (66.07)	1.00	--	64 (60.38)	148 (62.98)	1.00	--
CT	69 (38.76)	52 (30.95)	0.62 (0.38-0.98)	0.043	38 (35.85)	80 (34.04)	0.83 (0.51-1.38)	0.479
TT	6 (3.37)	5 (2.98)	0.81 (0.23-2.85)	0.746	4 (3.77)	7 (2.98)	0.84 (0.23-3.06)	0.796
Dominant			0.63 (0.40-0.99)	0.048			0.84 (0.52-1.36)	0.467
Additive			0.70 (0.47-1.04)	0.080			0.86 (0.57-1.31)	0.493
rs369150								
GG	58 (32.58)	58 (34.52)	1.00	--	33 (31.13)	81 (34.47)	1.00	--
AG	96 (53.93)	88 (52.38)	0.81 (0.50-1.31)	0.396	56 (52.83)	126 (53.62)	0.82 (0.49-1.40)	0.473

AA	24 (13.49)	22 (13.10)	0.94 (0.46-1.90)	0.863	17 (16.04)	28 (11.91)	0.60 (0.28-1.27)	0.179
Dominant			0.84 (0.53-1.33)	0.448			0.77 (0.47-1.28)	0.317
Additive			0.93 (0.66-1.29)	0.657			0.78 (0.55-1.13)	0.187
rs86567								
AA	46 (25.84)	48 (28.57)	1.00	--	24 (22.64)	68 (28.94)	1.00	--
AC	107 (60.11)	88 (52.38)	0.74 (0.45-1.23)	0.246	65 (61.32)	128 (54.47)	0.64 (0.36-1.12)	0.118
CC	25 (14.05)	32 (19.05)	1.23 (0.62-2.43)	0.559	17 (16.04)	39 (16.59)	0.73 (0.34-1.56)	0.417
Dominant			0.83 (0.51-1.35)	0.452			0.66 (0.38-1.14)	0.132
Additive			1.04 (0.75-1.46)	0.800			0.82 (0.57-1.19)	0.299
rs6913008								
CC	119 (66.85)	123 (73.21)	1.00	--	74 (69.81)	166 (70.64)	1.00	--
CT	57 (32.02)	42 (25.00)	0.68 (0.42-1.11)	0.122	32 (30.19)	64 (27.23)	0.91 (0.55-1.53)	0.734
TT	2 (1.13)	3 (1.79)	1.01 (0.16-6.36)	0.989	0	5 (2.13)	1.00	--
Dominant			0.69 (0.43-1.12)	0.135			0.98 (0.59-1.64)	0.936
Additive			0.74 (0.47-1.15)	0.177			1.06 (0.66-1.72)	0.798
rs2582								
CC	101 (56.74)		1.00	--	65 (61.32)	136 (57.87)	1.00	--
AC	67 (37.64)		0.88 (0.56-1.40)	0.589	38 (35.85)	87 (37.02)	1.09 (0.66-1.79)	0.732
AA	10 (5.62)		0.46 (0.15-1.41)	0.175	3 (2.83)	12 (5.11)	1.77 (0.47-6.67)	0.400
Dominant			0.82 (0.53-1.29)	0.395			1.14 (0.70-1.84)	0.595
Additive			0.79 (0.54-1.16)	0.235			1.17 (0.77-1.77)	0.462
rs416622								
GG	94 (52.81)	77 (45.83)	1.00	--	54 (50.94)	115 (48.94)	1.19 (0.73-1.96)	0.485
AG	68 (38.20)	81 (48.21)	1.44 (0.91-2.28)	0.115	42 (39.62)	104 (44.26)	0.85 (0.36-2.05)	0.724

AA	16 (8.99)	10 (5.96)	0.85 (0.36-2.03)	0.721	10 (9.44)	16 (6.80)	1.13 (0.71-1.81)	0.614
Dominant			1.33 (0.86-2.07)	0.196			0.79 (0.34-1.84)	0.583
Additive			1.13 (0.80-1.60)	0.481				
rs453779								
CC	87 (48.88)	84 (50.00)	1.00	--	52 (49.06)	116 (49.36)	1.00	--
CT	78 (43.82)	69 (41.07)	0.94 (0.59-1.48)	0.775	46 (43.40)	100 (42.55)	1.01 (0.62-1.65)	0.794
TT	13 (7.30)	15 (8.93)	1.22 (0.53-2.80)	0.632	8 (7.54)	19 (8.09)	1.07 (0.43-2.65)	0.886
Dominant			0.98 (0.63-1.51)	0.914			1.02 (0.64-1.63)	0.943
Additive			1.03 (0.73-1.45)	0.872			1.02 (0.70-1.48)	0.907
rs2857111								
AA	135 (75.84)	124 (73.81)	1.00	--	84 (79.25)	170 (72.34)	1.00	--
AG	43 (24.16)	41 (24.40)	0.94 (0.56-1.57)	0.818	22 (20.75)	62 (26.38)	1.39 (0.79-2.46)	0.253
GG	0	3 (1.79)	1.00	--	0	3 (1.28)	1.00	--
Dominant			1.00 (0.61-1.67)	0.987			1.46 (0.83-2.57)	0.189
Additive			1.08 (0.67-1.75)	0.740			1.51 (0.87-2.60)	0.139
rs1383258								
GG	155 (87.08)	151 (89.88)	1.00	--	89 (83.96)	213 (90.64)	1.00	--
AG	22 (12.36)	16 (9.52)	0.75 (0.37-1.49)	0.406	17 (16.04)	20 (8.51)	0.49 (0.24-0.99)	0.047
AA	1 (0.56)	1 (0.60)	1.85 (0.11-31.36)	0.669	0	2 (0.85)	1.00	--
Dominant			0.78 (0.40-1.53)	0.470			0.55 (0.28-1.11)	0.094
Additive			0.83 (0.44-1.57)	0.563			0.66 (0.34-1.26)	0.205
rs2071472								
GG	50 (28.09)	61 (36.31)	1.00	--	36 (33.96)	74 (31.49)	1.00	--
AG	100 (56.18)	79 (47.02)	0.62 (0.38-1.02)	0.058	56 (52.83)	119 (50.64)	1.05 (0.62-1.78)	0.850

AA	28 (15.73)	28 (16.67)	0.89 (0.46-1.74)	0.743	14 (13.21)	42 (17.87)	1.52 (0.72-3.19)	0.269
Dominant			0.68 (0.42-1.08)	0.105			1.14 (0.69-1.89)	0.597
Additive			0.88 (0.63-1.21)	0.430			1.19 (0.84-1.69)	0.324
rs7383287								
AA	156 (87.64)	142 (84.52)	1.00	--	94 (88.68)	201 (85.53)	1.00	--
AG	22 (12.36)	26 (15.48)	1.47 (0.78-2.76)	0.237	12 (11.32)	34 (14.47)	1.44 (0.70-2.95)	0.320
Dominant			1.47 (0.78-2.76)	0.237			1.44 (0.70-2.95)	0.320
Additive			1.47 (0.78-2.76)	0.237			1.44 (0.70-2.95)	0.320
rs2071475								
CC	66 (37.08)	79 (47.02)	1.00	--	42 (39.62)	101 (42.98)	1.00	--
CT	99 (55.62)	78 (46.43)	0.67 (0.42-1.06)	0.084	58 (54.72)	116 (49.36)	0.82 (0.50-1.34)	0.423
TT	13 (7.30)	11 (6.55)	0.81 (0.33-1.99)	0.651	6 (5.66)	18 (7.66)	1.31 (0.48-3.62)	0.601
Dominant			0.68 (0.44-1.07)	0.095			0.86 (0.53-1.39)	0.545
Additive			0.78 (0.54-1.12)	0.179			0.97 (0.66-1.43)	0.874

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

Abbreviation: RVR, rapid virological response; N-RVR, non-rapid virological response; cEVR, complete early virological response, N-cEVR, non-complete early virological response.

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Association between *human leukocyte 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 leukocyte 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-naïve 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 1 and treated with subcutaneous PEG IFN-α at a dose of 180 μg once a week with the addition of 600-1000 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 favorable genotypes at rs1044429 AA and rs2284191 AA were more likely to achieve sustained virological

response (SVR) (Odds ratio (OR) = 1.99, 95% confidence interval (CI) = 1.25-3.19; OR = 2.71, 95% CI = 1.58-4.63, respectively), while patients carrying unfavorable genotypes at rs2856997 GG were less likely to achieve SVR (OR = 0.48, 95% CI = 0.29-0.78).

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

Key words: *HLA-DO*; chronic hepatitis C; gene polymorphism; treatment; virological response.

Article summary

Strengths and limitations of this study

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

1. 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 developing 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 genotype 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 leukocyte 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, -B* and *-C*) and class II (*HLA-DR, -DQ* and *-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 (GWAS) 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 *-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 *-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 CHC patients from the Chinese Han population treated with PEG-IFN/RBV.

2. Materials and methods

2.1 Participants

A total of 346 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 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-naïve 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 1; (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; (3) patients with diabetes, thyroid diseases.

All participants in this study were infected with HCV genotype 1 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.

2.2Viral 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 (v2.0, 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 2 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 (MAF) ≥ 0.05 in the Chinese population; and (2) the *P* value of the Hardy-Weinberg equilibrium (HWE) 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, CA, USA) was used to polymorphism at the chosen SNPs. The primers and probes used for genotyping are shown in Supplemental Table 1. Genotyping results were ascertained using SDS 2.3 software (Applied Biosystems, Foster City, CA, USA), and 100% concordance was achieved.

2.3 Statistical analysis

All data analysis was operated with Stata/SE (V.12.0 for Windows; StataCorp LP, College Station, TX, USA). Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's *t* test (for

continuous variables) or a chi-square (χ^2) test (for categorical variables) with a two-tailed *P* value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR) and 95% confidence interval (95% CI) adjusted for age, gender, baseline HCV RNA level and glucose. Each SNP was analyzed using codominant, dominant and additive genetic models. The codominant model considers homozygous type *vs.* wild type and hybrid type *vs.* wild type, respectively. The dominant model considers the homozygous type and heterozygous type together *vs*. the wild type, and the additive model considers the heterozygous type *vs*. the homozygous type *vs.* the wild type. False discovery rate (FDR) corrections were applied for multiple comparisons, and they were carried out as previously described, considering FDR < 0.05 as significant [19]. The combined effect of three independent SNPs (rs1044429, rs2284191 and rs2856997) was analyzed 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 (ROC) 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.

2.4 Ethical approval and informed consent

Our study protocol was approved by the Institutional Ethics Review Committee of

Nanjing Medical University (approval number: 2009-161). All participants in this study filled out the written informed consent.

3. Results

3.1 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 (TP), alpha fetal protein (AFP), hemoglobin, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), T3, T4, platelets and WBC 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.

Table1. Characteristics of chronic hepatitis C patients related with response to IFN/RBV treatment

Variables	N-SVR (n=117)	SVR (n=229)	*P* value
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
Hemoglobin (g/L)	134.73±15.45	133.09±17.14	0.386
ALT ≥ 40U/L (%)	78 (66.67)	137 (59.83)	0.215
AST ≥ 40U/L (%)	64 (54.70)	125 (54.59)	0.984
GGT≥50U/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)	

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

3.2 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 analyzed 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 = 1.25-3.19; Dominant model: OR = 2.71, 95% CI = 1.58-4.63, respectively). For rs2856997, the rate of SVR was higher in patients carrying the TT genotype (75.9%) compared to those with the TG genotype (59.3%) and GG (60%) (Dominant model: OR = 0.48, 95%CI = 0.29-0.78). We performed FDR correction for all SNPs as outlined in Supplemental 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).

In addition, rs1044429, rs2284191 and rs2856997 were also found to be significantly associated with RVR (Dominant model: OR = 1.62, 95%CI = 1.04-2.53; OR = 2.42, 95% CI = 1.50-3.90; OR = 0.59, 95% CI = 0.38-0.92, respectively) and cEVR

(Dominant model: OR = 2.05, 95% CI = 1.27-3.32; OR = 2.84, 95% CI = 1.62-4.96; OR = 0.60, 95% CI = 0.37-0.99, respectively) (Supplemental 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.

Table 2. Association of SNPs in *HLA-DO* with HCV treatment response

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	*P* value
rs1044429					
GG	63 (53.85)	87 (37.99)	58.00	1.00	--
AG	51 (43.59)	130 (56.77)	71.82	1.92 (1.19-3.08)	0.007
AA	3 (2.56)	12 (5.24)	80.00	3.44 (0.91-13.04)	0.069
Dominant				1.99 (1.25-3.19)	0.004
Additive				1.90 (1.25-2.89)	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 (1.56-4.58)	<0.001
AA	0	1 (0.44)	100	1.00	--
Dominant				2.71 (1.58-4.63)	<0.001
Additive				2.70 (1.59-4.61)	<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 (0.29-0.83)	0.008
GG	24 (20.51)	36 (15.75)	60.00	0.44 (0.22-0.85)	0.015
Dominant				0.48 (0.29-0.78)	0.003
Additive				0.63 (0.46-0.87)	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 (0.80-2.18)	0.279
AA	15 (12.82)	32 (13.98)	68.09	1.32 (0.63-2.75)	0.463
Dominant				1.32 (0.82-2.13)	0.256
Additive				1.19 (0.84-1.69)	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 (0.60-1.66)	0.996
CC	17 (14.53)	27 (11.80)	61.36	0.84 (0.41-1.75)	0.645
Dominant				0.96 (0.59-1.56)	0.879
Additive				0.94 (0.66-1.33)	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 (0.50-2.51)	0.791
GG	1 (0.85)	1 (0.44)	50.00	0.58 (0.03-10.68)	0.714
Dominant				1.07 (0.49-2.34)	0.866
Additive				1.02 (0.50-2.09)	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 (0.57-1.50)	0.753
TT	4 (3.42)	7 (3.06)	63.64	0.98 (0.27-3.59)	0.978
Dominant				0.93 (0.58-1.49)	0.763
Additive				0.95 (0.63-1.43)	0.796
rs369150					
GG	37 (31.62)	79 (34.50)	68.10	1.00	--
AG	63 (53.85)	121 (52.84)	65.76	0.80 (0.48-1.34)	0.396
AA	17 (14.53)	29 (12.66)	63.04	0.71 (0.34-1.48)	0.358
Dominant				0.78 (0.48-1.28)	0.325
Additive				0.83 (0.59-1.18)	0.302
rs86567					
AA	29 (24.79)	65 (28.38)		1.00	--
AC	67 (57.26)	128 (55.90)		0.79 (0.46-1.36)	0.396
CC	21 (17.95)	36 (15.72)		0.67 (0.32-1.37)	0.267
Dominant				0.76 (0.45-1.28)	0.306
Additive				0.81 (0.57-1.16)	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 (0.57-1.56)	0.882
TT	1 (0.86)	4 (1.74)	80.00	1.53 (0.16-14.19)	0.708
Dominant				0.96 (0.58-1.58)	0.880
Additive				0.99 (0.62-1.57)	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 (0.58-1.52)	0.803
AA	3 (2.57)	13 (5.68)	81.25	2.09 (0.56-7.83)	0.274
Dominant				1.01 (0.63-1.61)	0.963
Additive				1.10 (0.74-1.64)	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 (0.71-1.86)	0.571
AA	10 (8.54)	16 (6.99)	61.54	0.97 (0.40-2.31)	0.937
Dominant				1.12 (0.71-1.77)	0.634

Additive				1.05 (0.73-1.52)	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 (0.56-1.46)	0.680
TT	8 (6.84)	20 (8.73)	71.43	1.24 (0.50-3.06)	0.637
Dominant				0.95 (0.60-1.50)	0.823
Additive				1.02 (0.71-1.46)	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 (0.59-1.74)	0.969
GG	0	3 (1.31)	100.00	1.00	--
Dominant				1.06 (0.62-1.82)	0.822
Additive				1.13 (0.68-1.88)	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 (0.47-2.02)	0.955
AA	1 (0.86)	1 (0.43)	50.00	0.80 (0.05-14.05)	0.878
Dominant				0.97 (0.48-1.96)	0.930
Additive				0.96 (0.50-1.85)	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 (0.65-1.81)	0.760
AA	17 (14.53)	39 (17.03)	69.64	1.35 (0.66-2.76)	0.406
Dominant				1.14 (0.70-1.86)	0.598
Additive				1.15 (0.82-1.61)	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 (0.52-1.95)	0.975
Dominant				1.01 (0.52-1.95)	0.975
Additive				1.01 (0.52-1.95)	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 (0.87-2.27)	0.164
TT	9 (7.70)	15 (6.55)	62.50	1.09 (0.43-2.74)	0.852
Dominant				1.36 (0.86-2.17)	0.193
Additive				1.21 (0.82-1.77)	0.334

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

Abbreviation: SVR, sustained virological response; N-SVR, non-sustained virological response.

Afterward, we evaluated the combined effect of these three significant SNPs by adding up the unfavorable genotype number. The results indicated that SVR rates declined when patients were carrying the more unfavorable 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 odds ratios also decreased along with the increase in risk genotypes (OR = 0.38, 95% CI = 0.17-0.83; OR = 0.22, 95% CI = 0.10-0.49; OR = 0.12, 95% CI = 0.04-0.37, 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).

3.3 Interaction analysis

As shown in Table 3, the interaction analysis among the meaningful SNPs and potential risk factors was also analyzed. A significant multiplicative interaction related to SVR was found between rs2856997 genotypes and gender ($P_{interaction}$= 0.019). Compared to 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 = 0.81-0.59).

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 (0.18-0.59)
Male with TT genotypes	12 (34.29)	23 (65.71)	0.44 (0.18-1.04)
Male with TG/GG genotypes	16 (32.00)	34 (68.00)	0.54 (0.25-1.19)
P for multiplicative interaction			*P* = 0.019

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

3.4 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).

Table 4. **Multivariate Stepwise regression analysis for independent factors of SVR**

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

Abbreviation: SVR, sustained virological response; Coef. coefficient of variation; SE, standard error; CI, confidence interval; GLU, glucose; Cons. Constant term.

3.5 Association of SNPs with viral dynamics during treatment

The effect of the three significant SNPs on viral dynamics during treatment was also analyzed. The difference between baseline viral load in these SNPs was not significant between patients carrying the wild-type and mutant alleles (P>0.05). Nevertheless, the decline in viral load was significantly quicker in rs2284191 AG/AA patients than in GG patients 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.

4. 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 analyzed. 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 unfavorable 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, 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 (TFBS). The mutation at rs2284191 may influence transcription and transform the encoding protein's function, ultimately affecting antigen processing 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 (SLE) 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 CHC patients[31-33]. In the current study, we only focused on HCV-1 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 direct-acting antiviral (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 developing 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 multi-center 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. 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.

Contributorship statement

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. YZ, XX and YF provided materials and analysis tools. PH revised the manuscript. All authors accepted the final manuscript.

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Conflicts of Interest

There is no conflict of interest.

Data sharing statement

No additional data is available.

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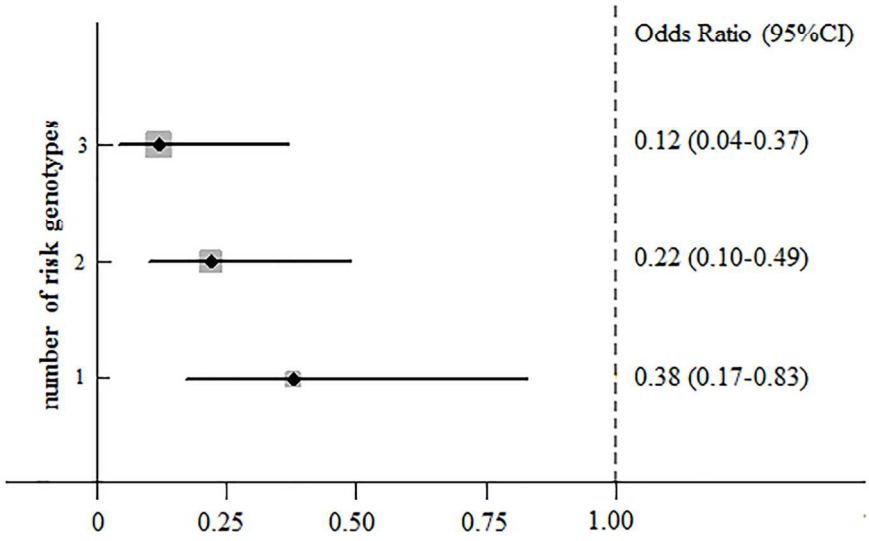
Figure legends

Figure 1. Combined effects of rs1044429, rs2284191 and rs2856997 with SVR. Variables are numbers of combined unfavorable genotypes (rs1044429-GG, rs2284191-GG and rs2856997-GG); logistic regression analyses adjusted for age, gender, glucose, baseline HCV RNA; OR, odds ratio; CI, confidence interval.

Figure 2.Predictors of HCV treatment response. The response variable is SVR 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.

Figure 3.Effect of HLA-DOA rs2284191 variants on HCV 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.



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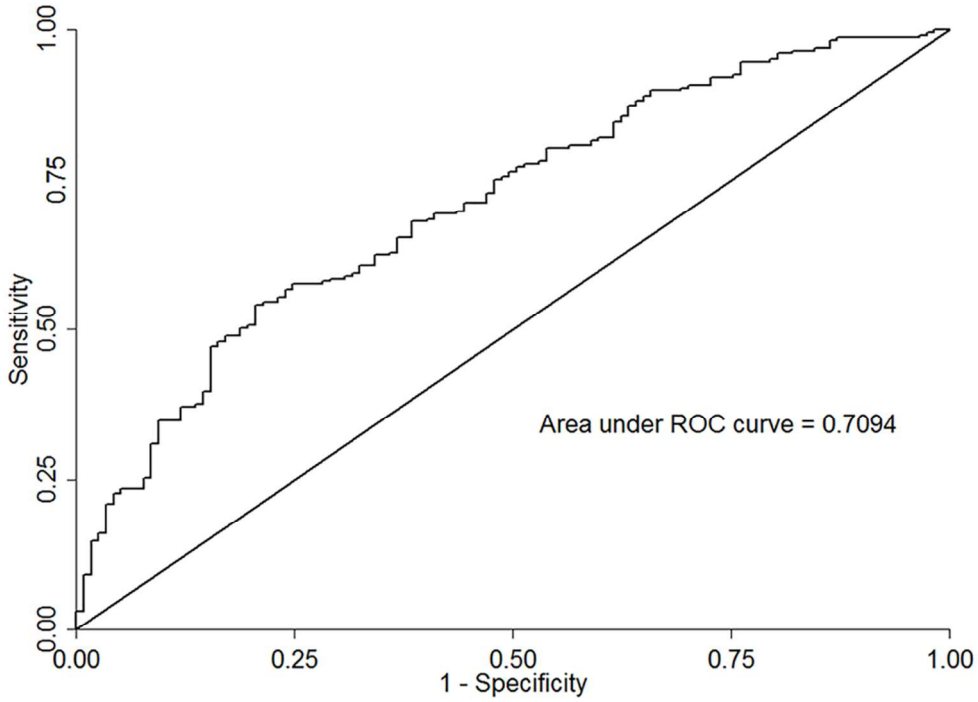

Figure 2.Predictors of HCV treatment response. The response variable is SVR and the diagnostic test variable is a combination of rs1044429, rs2284191, rs2856997, glucose and baseline HCV RNA with the coefficients taken from the regression analysis.

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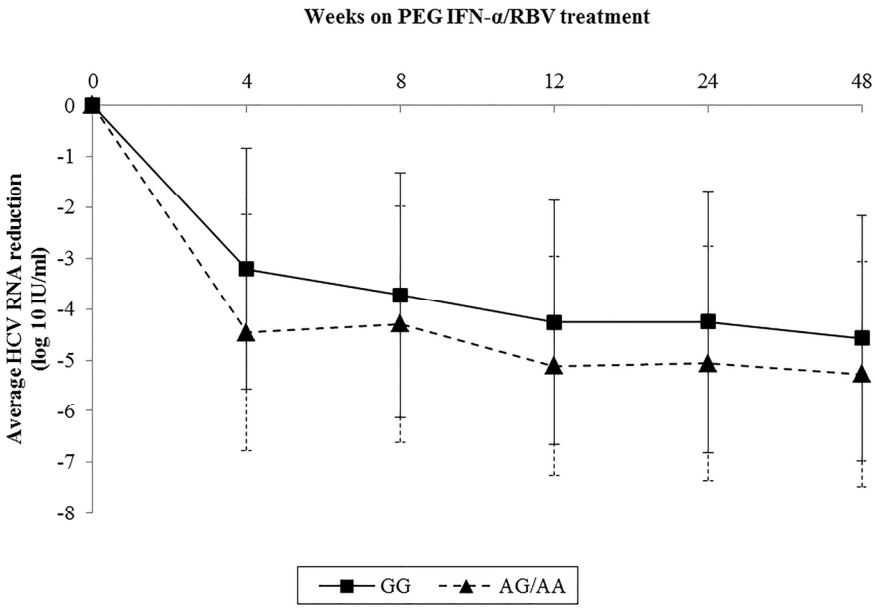

Figure 3.Effect of HLA-DOA rs2284191 variants on HCV viral kinetics during therapy. The fold of viral decline was compared among patients with the GG genotype and the AG/AA genotype. The fold of viral decline was calculated as the viral load at follow up time point divided by the initial viral load.

173x109mm (300 x 300 DPI)

Supplemental Table 1. Information of primers and probes for TaqMan allelic discrimination

Polymorphism		Sequence(5'-3')
DOA rs1044429	Primer	F: TCACACAAAGAGGGTTTCTGTTACTG
		R: GAATAAGTTGAAATCAATGACCAGAAGA
	Probe	FAM-TGAGATGATTCTCCTCCAC-MGB
		HEX-TGAGATGATTTTCCTCCAC-MGB
DOA rs2284191	Primer	F: TCCTCCATCTCAGAGCATTATGAC
		R: TGTTGCTCAAACAACTTCATAGAGTTC
	Probe	FAM-CTTCCATAACTGTTGTCTAG-MGB
		HEX-TAACTGTTATCTAGTTTTCTGG-MGB
DOB rs2856997	Primer	F: CCAAATCCAATGCTAGCTAGAGAAA
		R: ATGGGCTGTGAGAATCTGTAACC
	Probe	FAM-CATGGAGTTACCCCC-MGB
		HEX-CCATGGAGTTACCACC-MGB
DOA rs408036	Primer	F: CCAGGCCTTGGCCAGTT
		R: GTAACACACAATGGGCCAAATG
	Probe	FAM-TTGGCAGCCGTCCT-MGB
		HEX-ATTGGCAGCCATC-MGB
DOA rs3128935	Primer	F: TGTCGGGTGGACATGTTCAC
		R: GGATCCACATGGTCTGTGTTCTC
	Probe	FAM-AGAACACCGCTAACA-MGB
		HEX-AGAACACCGCCAACA-MGB
DOA rs3129304	Primer	F: AAAACATACAAAGAGATAAATCACCATACC
		R: TGAAAACCGTAATCTGTATTGCTCAT
	Probe	FAM-CATAGTTTATGTCAGGACC-MGB
		HEX-CATAGTTTATGTCAAGACC-MGB
DOA rs376892	Primer	F: CTTGGCTGTGGTCTGGTAACTG
		R: CCTTCCTAGTCCACCTCAGACCTT
	Probe	FAM-TAATCAGGTGCCATTGG-MGB
		HEX-TAATCAGGTGCCATCGG-MGB
DOA rs369150	Primer	F: GAAAGAAAGGAACAGGGCATGAC
		R: GGCGGGAAGGTCCAGAGA
	Probe	FAM-TGATGGGAACCTAGG-MGB
		HEX-TGATGGGAGCCTAGG-MGB
DOA rs86567	Primer	F: GGTGCGGGTCTACAGATGGTT
		R: GAGCAACAGTTATTGAGGAACTAGCAT
	Probe	FAM-TGGCCCCCCATTG-MGB
		HEX-TGGCCCACCATTG-MGB
DOA rs6913008	Primer	F: GTCCTGTTCAGAGTCATCCACTTT
		R: TCCTCATCATCATGGGCACAT
	Probe	FAM-CCCAGACTCCCGG-MGB
		HEX-CCCAGACTCCTGG-MGB
DOA rs2582	Primer	F: TGATCCTTCTGAGAGAAATGACTTGT
		R: CACAGCGGGATGCACTTAAA

	Probe	FAM-TGTGACAGACCCTGC-MGB HEX-TGTGACAGCCCCTG-MGB
DOA rs416622	Primer	F: CAGCCTGGTGACAGAGTGAGA R: TCACCCAGACCTACTGAATTAGAATCT
	Probe	FAM-AGACAGCCCCCCTGT-MGB HEX-AGACAGCCTCCCTGTT-MGB
DOA rs453779	Primer	F: GTCACCCGTGGAGGCACTA R: AACGTCCCTTAATCCCAGTCCTA
	Probe	FAM-AGGAACAGGCCCTG-MGB HEX-AGGAACGGGCCCTG-MGB
DOB rs2857111	Primer	F: TCTCTTGCCTCCGTTCTCATTC R: TGCTACATATTTCTAAAAGCCACTCTCATA
	Probe	FAM-TCCCCTCCCTGGAGA-MGB HEX-CTCCCCTCCCTAGAG-MGB
DOB rs1383258	Primer	F: TTACCAGACACGTTTAGAATGGATTC R: GAGTTCACAGCACATTGTAATTATTGG
	Probe	FAM-AGAAGAGATGAGAGAGTC-MGB HEX-CAAGAGAAGAGACGAGAG-MGB
DOB rs2071472	Primer	F: GACTGGATTCCTCCATGACTCAA R: CATGCCAATTCTTGCATACACA
	Probe	FAM-AACAGAGCAATTGTT-MGB HEX-AACAGAGCAATTATT-MGB
DOB rs7383287	Primer	F: CGTAATTTACCAGGCATGGGTTT R: CAGTCAGCCTTTGCCTGAATC
	Probe	FAM-TTCCAGAAGATTTTG-MGB HEX-TTTCCAGAAGACTTTG-MGB
DOB rs2071475	Primer	F: GGTCCTCTCTGGGTACACTGTCA R: GGTTTTCTTTCACGGTGTCTCAT
	Probe	FAM-CTAGGAAGGGAGGAAA-MGB HEX-ACTAGGAAGAGAGGAAA-MGB

Supplemental Table 2. Results of SNPs distribution in dominant, recessive, and additive models

SNPs	Location	Dominant		Additive	
		P* Value	**FDR***	***P* Value***	**FDR***
DOA rs1044429	3'UTR(G>A)	4.00×10^{-3}	0.024	3.00×10^{-3}	0.027
DOA rs2284191	intron(G>A)	2.83×10^{-4}	0.005	2.52×10^{-4}	0.005
DOB rs2856997	intron(T>G)	3.00×10^{-3}	0.024	5.00×10^{-3}	0.030
DOA rs408036	3'UTR(G>A)	0.256	0.836	0.325	0.859
DOA rs3128935	3'UTR(T>C)	0.879	0.975	0.713	0.975
DOA rs3129304	3'UTR(A>G)	0.866	0.975	0.948	0.975
DOA rs376892	3'UTR(C>T)	0.763	0.975	0.796	0.975
DOA rs369150	intron(G>A)	0.325	0.836	0.302	0.859
DOA rs86567	intron(A>C)	0.306	0.836	0.250	0.859
DOA rs6913008	intron(C>T)	0.880	0.975	0.961	0.975
DOA rs2582	3'UTR(C>A)	0.963	0.975	0.650	0.975
DOA rs416622	3'UTR(G>A)	0.634	0.975	0.779	0.975
DOA rs453779	intron(C>T)	0.823	0.975	0.935	0.975
DOB rs2857111	intron(A>G)	0.822	0.975	0.647	0.975
DOB rs1383258	intron(G>A)	0.930	0.975	0.907	0.975
DOB rs2071472	intron(G>A)	0.598	0.975	0.431	0.970
DOB rs7383287	synonymous(A>G)	0.975	0.975	0.975	0.975
DOB rs2071475	intron(C>T)	0.193	0.836	0.334	0.859

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

Supplemental Table 3. Association of SNPs in *HLA-DO* with RVR/ cEVR

Genotype	N-RVR(n=178)	RVR (n=168)	OR (95% CI)	*P* value	N-cEVR (n=106)	cEVR (n=235)	OR (95% CI)	*P* value
rs1044429								
GG	88 (49.44)	62 (36.90)	1.00	--	58 (54.72)	90 (38.30)	1.00	--
AG	82 (46.07)	99 (58.93)	1.66(1.05-2.60)	0.029	43 (40.57)	136 (57.87)	2.13(1.30-3.48)	0.003
AA	8 (4.49)	7 (4.17)	1.22 (0.40-3.67)	0.727	5 (4.71)	9 (3.83)	1.37 (0.43-4.39)	0.593
Dominant			1.62 (1.04-2.53)	0.034			2.05 (1.27-3.32)	0.003
Additive			1.42 (0.97-2.10)	0.074			1.73 (1.12-2.65)	0.013
rs2284191								
GG	134 (75.28)	97 (57.74)	1.00	--	84 (79.25)	143 (60.85)	1.00	--
AG	44 (24.72)	70 (41.67)	2.37 (1.47-3.83)	<0.001	22 (20.75)	91 (38.72)	2.81 (1.60-4.91)	<0.001
AA	0	1 (0.59)	1	--	0	1 (0.43)	1.00	--
Dominant			2.42 (1.50-3.90)	<0.001			2.84 (1.62-4.96)	<0.001
Additive			2.44 (1.52-3.91）	<0.001			2.83 (1.63-4.94)	<0.001
rs2856997								
TT	61 (34.27)	80 (47.62)	1.00	--	34 (32.08)	106 (45.11)	1.00	--
TG	84 (47.19)	61 (36.31)	0.60 (0.37-0.96)	0.035	49 (46.23)	92 (39.15)	0.66 (0.39-1.12)	0.122
GG	33 (18.54)	27 (16.07)	0.58 (0.31-1.10)	0.093	23 (21.69)	37 (15.74)	0.49 (0.25-0.96)	0.038
Dominant			0.59 (0.38-0.92)	0.021			0.60 (0.37-0.99)	0.045
Additive			0.72 (0.53-0.98)	0.040			0.70 (0.50-0.96)	0.029
rs408036								
GG	61 (34.27)	64 (38.10)	1.00	--	40 (37.74)	82 (34.89)	1.00	--
AG	94 (52.81)	80 (47.62)	0.86 (0.53-1.38)	0.528	52 (49.06)	121 (51.49)	1.28 (0.76-2.14)	0.351

AA	23 (12.92)	24 (14.28)	1.06 (0.53-2.12)	0.866	14 (13.20)	32 (13.62)	1.18 (0.56-2.50)	0.663
Dominant			0.90 (0.57-1.41)	0.642			1.26 (0.77-2.05)	0.361
Additive			0.98 (0.71-1.36)	0.922			1.13 (0.79-1.62)	0.489
rs3128935								
TT	78 (43.82)	52 (30.95)	1.00	--	45 (42.45)	83 (35.32)	1.00	--
CT	88 (49.44)	84 (50.00)	1.84 (1.12-3.02)	0.016	51 (48.11)	118 (50.21)	1.49 (0.89-2.48)	0.130
CC	12 (6.74)	32 (19.05)	5.59 (2.55-12.26)	<0.001	10 (9.44)	34 (14.47)	2.22 (0.99-5.01)	0.054
Dominant			2.27 (1.41-3.67)	0.001			1.61 (0.98-2.64)	0.058
Additive			2.20 (1.54-3.13)	<0.001			1.49 (1.03-2.15)	0.034
rs3129304								
AA	166 (93.26)	147 (87.50)	1.00	--	96 (90.57)	212 (90.21)	1.00	--
AG	11 (6.18)	20 (11.90)	2.17 (0.99-4.78)	0.054	9 (8.49)	22 (9.36)	1.15 (0.50-2.64)	0.739
GG	1 (0.56)	1 (0.60)	1.47 (0.08-25.49)	0.792	1 (0.94)	1 (0.43)	0.46 (0.03-8.21)	0.594
Dominant			2.12 (0.99-4.55)	0.054			1.08 (0.49-2.41)	0.846
Additive			1.91 (0.95-3.87)	0.070			1.02 (0.49-2.11)	0.962
rs376892								
CC	103 (57.87)	111 (66.07)	1.00	--	64 (60.38)	148 (62.98)	1.00	--
CT	69 (38.76)	52 (30.95)	0.62 (0.38-0.98)	0.043	38 (35.85)	80 (34.04)	0.83 (0.51-1.38)	0.479
TT	6 (3.37)	5 (2.98)	0.81 (0.23-2.85)	0.746	4 (3.77)	7 (2.98)	0.84 (0.23-3.06)	0.796
Dominant			0.63 (0.40-0.99)	0.048			0.84 (0.52-1.36)	0.467
Additive			0.70 (0.47-1.04)	0.080			0.86 (0.57-1.31)	0.493
rs369150								
GG	58 (32.58)	58 (34.52)	1.00	--	33 (31.13)	81 (34.47)	1.00	--
AG	96 (53.93)	88 (52.38)	0.81 (0.50-1.31)	0.396	56 (52.83)	126 (53.62)	0.82 (0.49-1.40)	0.473

AA	24 (13.49)	22 (13.10)	0.94 (0.46-1.90)	0.863	17 (16.04)	28 (11.91)	0.60 (0.28-1.27)	0.179
Dominant			0.84 (0.53-1.33)	0.448			0.77 (0.47-1.28)	0.317
Additive			0.93 (0.66-1.29)	0.657			0.78 (0.55-1.13)	0.187
rs86567								
AA	46 (25.84)	48 (28.57)	1.00	--	24 (22.64)	68 (28.94)	1.00	--
AC	107 (60.11)	88 (52.38)	0.74 (0.45-1.23)	0.246	65 (61.32)	128 (54.47)	0.64 (0.36-1.12)	0.118
CC	25 (14.05)	32 (19.05)	1.23 (0.62-2.43)	0.559	17 (16.04)	39 (16.59)	0.73 (0.34-1.56)	0.417
Dominant			0.83 (0.51-1.35)	0.452			0.66 (0.38-1.14)	0.132
Additive			1.04 (0.75-1.46)	0.800			0.82 (0.57-1.19)	0.299
rs6913008								
CC	119 (66.85)	123 (73.21)	1.00	--	74 (69.81)	166 (70.64)	1.00	--
CT	57 (32.02)	42 (25.00)	0.68 (0.42-1.11)	0.122	32 (30.19)	64 (27.23)	0.91 (0.55-1.53)	0.734
TT	2 (1.13)	3 (1.79)	1.01 (0.16-6.36)	0.989	0	5 (2.13)	1.00	--
Dominant			0.69 (0.43-1.12)	0.135			0.98 (0.59-1.64)	0.936
Additive			0.74 (0.47-1.15)	0.177			1.06 (0.66-1.72)	0.798
rs2582								
CC	101 (56.74)		1.00	--	65 (61.32)	136 (57.87)	1.00	--
AC	67 (37.64)		0.88 (0.56-1.40)	0.589	38 (35.85)	87 (37.02)	1.09 (0.66-1.79)	0.732
AA	10 (5.62)		0.46 (0.15-1.41)	0.175	3 (2.83)	12 (5.11)	1.77 (0.47-6.67)	0.400
Dominant			0.82 (0.53-1.29)	0.395			1.14 (0.70-1.84)	0.595
Additive			0.79 (0.54-1.16)	0.235			1.17 (0.77-1.77)	0.462
rs416622								
GG	94 (52.81)	77 (45.83)	1.00	--	54 (50.94)	115 (48.94)	1.19 (0.73-1.96)	0.485
AG	68 (38.20)	81 (48.21)	1.44 (0.91-2.28)	0.115	42 (39.62)	104 (44.26)	0.85 (0.36-2.05)	0.724

AA	16 (8.99)	10 (5.96)	0.85 (0.36-2.03)	0.721	10 (9.44)	16 (6.80)	1.13 (0.71-1.81)	0.614
Dominant			1.33 (0.86-2.07)	0.196			0.79 (0.34-1.84)	0.583
Additive			1.13 (0.80-1.60)	0.481				
rs453779								
CC	87 (48.88)	84 (50.00)	1.00	--	52 (49.06)	116 (49.36)	1.00	--
CT	78 (43.82)	69 (41.07)	0.94 (0.59-1.48)	0.775	46 (43.40)	100 (42.55)	1.01 (0.62-1.65)	0.794
TT	13 (7.30)	15 (8.93)	1.22 (0.53-2.80)	0.632	8 (7.54)	19 (8.09)	1.07 (0.43-2.65)	0.886
Dominant			0.98 (0.63-1.51)	0.914			1.02 (0.64-1.63)	0.943
Additive			1.03 (0.73-1.45)	0.872			1.02 (0.70-1.48)	0.907
rs2857111								
AA	135 (75.84)	124 (73.81)	1.00	--	84 (79.25)	170 (72.34)	1.00	--
AG	43 (24.16)	41 (24.40)	0.94 (0.56-1.57)	0.818	22 (20.75)	62 (26.38)	1.39 (0.79-2.46)	0.253
GG	0	3 (1.79)	1.00	--	0	3 (1.28)	1.00	--
Dominant			1.00 (0.61-1.67)	0.987			1.46 (0.83-2.57)	0.189
Additive			1.08 (0.67-1.75)	0.740			1.51 (0.87-2.60)	0.139
rs1383258								
GG	155 (87.08)	151 (89.88)	1.00	--	89 (83.96)	213 (90.64)	1.00	--
AG	22 (12.36)	16 (9.52)	0.75 (0.37-1.49)	0.406	17 (16.04)	20 (8.51)	0.49 (0.24-0.99)	0.047
AA	1 (0.56)	1 (0.60)	1.85 (0.11-31.36)	0.669	0	2 (0.85)	1.00	--
Dominant			0.78 (0.40-1.53)	0.470			0.55 (0.28-1.11)	0.094
Additive			0.83 (0.44-1.57)	0.563			0.66 (0.34-1.26)	0.205
rs2071472								
GG	50 (28.09)	61 (36.31)	1.00	--	36 (33.96)	74 (31.49)	1.00	--
AG	100 (56.18)	79 (47.02)	0.62 (0.38-1.02)	0.058	56 (52.83)	119 (50.64)	1.05 (0.62-1.78)	0.850

AA	28 (15.73)	28 (16.67)	0.89 (0.46-1.74)	0.743	14 (13.21)	42 (17.87)	1.52 (0.72-3.19)	0.269
Dominant			0.68 (0.42-1.08)	0.105			1.14 (0.69-1.89)	0.597
Additive			0.88 (0.63-1.21)	0.430			1.19 (0.84-1.69)	0.324
rs7383287								
AA	156 (87.64)	142 (84.52)	1.00	--	94 (88.68)	201 (85.53)	1.00	--
AG	22 (12.36)	26 (15.48)	1.47 (0.78-2.76)	0.237	12 (11.32)	34 (14.47)	1.44 (0.70-2.95)	0.320
Dominant			1.47 (0.78-2.76)	0.237			1.44 (0.70-2.95)	0.320
Additive			1.47 (0.78-2.76)	0.237			1.44 (0.70-2.95)	0.320
rs2071475								
CC	66 (37.08)	79 (47.02)	1.00	--	42 (39.62)	101 (42.98)	1.00	--
CT	99 (55.62)	78 (46.43)	0.67 (0.42-1.06)	0.084	58 (54.72)	116 (49.36)	0.82 (0.50-1.34)	0.423
TT	13 (7.30)	11 (6.55)	0.81 (0.33-1.99)	0.651	6 (5.66)	18 (7.66)	1.31 (0.48-3.62)	0.601
Dominant			0.68 (0.44-1.07)	0.095			0.86 (0.53-1.39)	0.545
Additive			0.78 (0.54-1.12)	0.179			0.97 (0.66-1.43)	0.874

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

Abbreviation: RVR, rapid virological response; N-RVR, non-rapid virological response; cEVR, complete early virological response, N-cEVR, non-complete early virological response.

STREGA guidance, extended from STROBE Statement

	Item number		Extension for genetic association studies
TITLE	1	Association between *human leukocyte antigen-DO* polymorphisms and interferon/ribavirin treatment response in hepatitis C virus type 1 infection in Chinese population: a prospective study	
ABSTRACT	2	HLA-DO may play a role in treatment response to HCV. This study was conducted to explore the role of SNPs in HLA-DO in responding to HCV therapy. A total of 346 CHC patients undergoing PEG IFN-α/RBV reatment were enrolled in this study. We genotyped 18 SNPs in HLA-DO using the ABI TaqMan allelic discrimination assay. The SNPs HLA-DOA rs1044429 and HLA-DOB rs2284191 and rs2856997 were correlated with HCV treatment response in the Chinese Han population.	
INTRODUCTION			
Background	4	The polymorphism in HLA-DO may be potential predictors of treatment efficacy in patients with HCV.	
Objectives	5	This study was conducted to assess how HLA-DO genotypes are associated with SVR, RVR and completely EVR (cEVR) in CHC patients from the Chinese Han population treated with PEG-IFN/RBV.	This study is the first to demonstrate a relationship between variants in HLA-DO and HCV treatment response in the Chinese Han population.
METHODS			
Study design	6	This was a prospective study followed up of HCV treatment response for one year and a half.	
Setting	6	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.	
Participants	6	A total of 346 chronic hepatitis C (CHC) patients who finished the 48-week pegylated interferon-alpha and	Inclusion criteria: (1) treatment-naïve and treated with PEG IFN-α/RBV in

		ribavirin (PEG IFN-α/RBV) treatment were enrolled in this study.	this study; (2) HCV RNA was present in serum for over 6 months before treatment; (3) infected with HCV genotype 1; (4) negative for hepatitis B (HBV) or HIV infection; and (5) lacked any other hepatic diseases. Exclusion criteria: (1) patients received antiviral therapy within 6 months; (2) patients with blood diseases, malignancies, organ transplants, or decompensated liver disease; (3) patients with diabetes, thyroid diseases.
Variables	6	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.	
Data sources measurement	5-7	All participating patients were classified into two groups according to SVR. Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's t test (for continuous variables) or a chi-square ($\chi 2$) test (for categorical variables) with a two-tailed P value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR).	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. We extracted genomic DNA from peripheral blood samples using protease K digestion and phenol/chloroform

			purification according to standard protocol. Genotyping results were ascertained using SDS 2.3 software
Bias	7	Measurement bias	Genotyping results were ascertained using SDS 2.3 software and 100% concordance was achieved.
Study size	5	A total of 346 CHC patients who finished the 48-week pegylated interferon-alpha and ribavirin (PEG IFN-α/RBV) treatment were enrolled in this study.	A total of 427 patients were enrolled in the treatment cohort. After one month of treatment, 8 patients were lost to follow-up. After three months, another 7 patients were lost to follow-up. By the time the study began, another 17 patients were lost to follow-up and 51 patients had not finished treatment. Ultimately, a total of 346 patients with CHC who finished the 48-week treatment and 24-week follow-up were enrolled in this study.
Quantitative variables			
Statistical methods	7-8	Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's t test (for continuous variables) or a chi-square ($\chi 2$) test (for categorical variables) with a two-tailed P value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR) and 95% confidence interval (95% CI) adjusted for age, gender, baseline HCV RNA level and glucose. Each SNP was analyzed using codominant, dominant and additive	All data analysis was operated with Stata/SE (V.12.0 for Windows; StataCorp LP, College Station, TX, USA).All SNPs were in Hardy-Weinberg equilibrium in allele frequency in the non-SVR group except for rs1044429, P = 0.048.

		genetic models. False discovery rate (FDR) corrections were applied for multiple comparisons, and they were carried out as previously described, considering FDR < 0.05 as significant. The combined effect of three independent SNPs (rs1044429, rs2284191 and rs2856997) was analyzed 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 (ROC) 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			
Participants	9	The baseline demographic and laboratory characteristics of the 346 enrolled patients are shown in Table 1.	
Descriptive data	9	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 (TP), alpha fetal protein (AFP), hemoglobin, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyltranspeptidase (GGT), T3, T4, platelets and WBC were similar between two groups (P>0.05). However, the baseline viral load and glucose levels were different between	All patients were infected with HCV genotype 1.

		the SVR and non-SVR group (P < 0.05). Individuals with higher baseline viral load and glucose levels were less likely to achieve SVR.	
Outcome data	10	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. For rs2856997, the rate of SVR was higher in patients carrying the TT genotype (75.9%) compared to those with the TG genotype (59.3%) and GG (60%).	
Main results	11-14	Factors with P values < 0.05 in the univariate analysis were adjusted for age, gender, baseline viral load and glucose. The dominant model indicated that patients carrying favorable genotypes at rs1044429 AA and rs2284191 AA were more likely to achieve sustained virological response (SVR) (Odds ratio (OR) = 1.99, 95% confidence interval (CI) = 1.25-3.19; OR = 2.71, 95% CI = 1.58-4.63, respectively), while patients carrying unfavorable genotypes at rs2856997 GG were less likely to achieve SVR (OR = 0.48, 95% CI = 0.29-0.78). In addition, rs1044429, rs2284191 and rs2856997 were also found to be significantly associated with RVR (Dominant model: OR = 1.62, 95%CI = 1.04-2.53; OR = 2.42, 95% CI = 1.50-3.90; OR = 0.59, 95% CI = 0.38-0.92, respectively) and cEVR (Dominant model: OR = 2.05, 95% CI = 1.27-3.32; OR = 2.84, 95% CI = 1.62-4.96; OR = 0.60, 95% CI = 0.37-0.99, respectively). Patients carrying the mutant alleles rs1044429-A or rs2284191-A or the wild-type allele rs2284191-T were more likely to	We performed FDR correction for all SNPs as outlined in Supplemental 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).

		achieve higher rates of RVR, cEVR and SVR.	
Other analyses	15-17	Combined effect analysis: the results indicated that SVR rates declined when patients were carrying the more unfavorable 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 odds ratios also decreased along with the increase in risk genotypes (OR = 0.38, 95% CI = 0.17-0.83; OR = 0.22, 95% CI = 0.10-0.49; OR = 0.12, 95% CI = 0.04-0.37, 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. Interaction analysis: A significant multiplicative interaction related to SVR was found between rs2856997 genotypes and gender (Pinteraction= 0.019). Compared to 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 = 0.81-0.59). Stepwise regression analysis: The results showed that rs1044429, rs2284191, rs2856997, baseline glucose and baseline HCV RNA were independent predictors of SVR. Adding up these five factors, the predictive AUC value was 0.71. Association of SNPs with viral dynamics during treatment: Nevertheless, the decline in viral load was significantly quicker in rs2284191 AG/AA patients than in GG patients 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. 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.	
DISCUSSION			
Key results	18-19	A total of 18 tagging SNPs involved in antigen processing and presentation in HLA-DO were selected and analyzed. 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 unfavorable 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 accordingly25 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, 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.	
Limitations	20	First, the biological mechanism by which HLA-DO affects treatment response has not yet been well established. This may be related to the wide variety of ethnicities and HCV genotypes. In the current study, we only focused on HCV-1 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 direct-acting antiviral (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 developing 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 multi-center 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. We will pay attention to collecting this information in future research.	
Interpretation	19-20	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 (TFBS). The mutation at rs2284191 may influence transcription and transform the encoding protein's function, ultimately affecting antigen processing 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. 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. 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 (SLE) and HIV-related Kaposi's sarcoma. Therefore, more attention should be given to the structure and function of HLA-DO and DM molecules.	
Generalizability	21	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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Association between *human leukocyte 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 leukocyte 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-naïve 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 favorable genotypes at rs1044429 AA and rs2284191 AA were more likely to achieve sustained virological

response (SVR) (Odds ratio (OR) = 1.99, 95% confidence interval (CI) = 1.25-3.19; OR = 2.71, 95% CI = 1.58-4.63, respectively), while patients carrying unfavorable genotypes at rs2856997 GG were less likely to achieve SVR (OR = 0.48, 95% CI = 0.29-0.78).

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

Key words: *HLA-DO*; chronic hepatitis C; gene polymorphism; treatment; virological response.

Article summary

Strengths and limitations of this study

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

1. 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 developing 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 genotype 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 leukocyte 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, -B* and *-C*) and class II (*HLA-DR, -DQ* and *-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 (GWAS) 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 *-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 *-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 CHC patients from the Chinese Han population treated with PEG-IFN/RBV.

2. Materials and methods

2.1 Participants

A total of 346 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 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-naïve 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; (3) patients with diabetes, 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.

2.2Viral 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 (v2.0, 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 2 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 (MAF) ≥ 0.05 in the Chinese population; and (2) the *P* value of the Hardy-Weinberg equilibrium (HWE) 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, CA, USA) was used to polymorphism at the chosen SNPs. The primers and probes used for genotyping are shown in Supplemental Table 1. Genotyping results were ascertained using SDS 2.3 software (Applied Biosystems, Foster City, CA, USA), and 100% concordance was achieved.

2.3 Statistical analysis

All data analysis was operated with Stata/SE (V.12.0 for Windows; StataCorp LP, College Station, TX, USA). Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's *t* test (for

continuous variables) or a chi-square (χ^2) test (for categorical variables) with a two-tailed *P* value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR) and 95% confidence interval (95% CI) adjusted for age, gender, baseline HCV RNA level and glucose. Each SNP was analyzed using codominant, dominant and additive genetic models. The codominant model considers homozygous type *vs.* wild type and hybrid type *vs.* wild type, respectively. The dominant model considers the homozygous type and heterozygous type together *vs*. the wild type, and the additive model considers the heterozygous type *vs*. the homozygous type *vs.* the wild type. False discovery rate (FDR) corrections were applied for multiple comparisons, and they were carried out as previously described, considering FDR < 0.05 as significant [19]. The combined effect of three independent SNPs (rs1044429, rs2284191 and rs2856997) was analyzed 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 (ROC) 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.

2.4 Ethical approval and informed consent

Our study protocol was approved by the Institutional Ethics Review Committee of

Nanjing Medical University (approval number: 2009-161). All participants in this study filled out the written informed consent.

3. Results

3.1 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 (TP), alpha fetal protein (AFP), hemoglobin, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), T3, T4, platelets and WBC 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.

Table1. Characteristics of chronic hepatitis C patients related with response to IFN/RBV treatment

Variables	N-SVR (n=117)	SVR (n=229)	*P* value
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
Hemoglobin (g/L)	134.73±15.45	133.09±17.14	0.386
ALT ≥ 40U/L (%)	78 (66.67)	137 (59.83)	0.215
AST ≥ 40U/L (%)	64 (54.70)	125 (54.59)	0.984
GGT≥50U/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)	

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

3.2 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 analyzed 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 = 1.25-3.19; Dominant model: OR = 2.71, 95% CI = 1.58-4.63, respectively). For rs2856997, the rate of SVR was higher in patients carrying the TT genotype (75.9%) compared to those with the TG genotype (59.3%) and GG (60%) (Dominant model: OR = 0.48, 95%CI = 0.29-0.78). We performed FDR correction for all SNPs as outlined in Supplemental 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).

In addition, rs1044429, rs2284191 and rs2856997 were also found to be significantly associated with RVR (Dominant model: OR = 1.62, 95%CI = 1.04-2.53; OR = 2.42, 95% CI = 1.50-3.90; OR = 0.59, 95% CI = 0.38-0.92, respectively) and cEVR (Dominant model: OR = 2.05, 95% CI = 1.27-3.32; OR = 2.84, 95% CI = 1.62-4.96; OR = 0.60, 95% CI = 0.37-0.99, respectively) (Supplemental 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.

Table 2. Association of SNPs in *HLA-DO* with HCV treatment response

Genotype	N-SVR	SVR	SVR rate (%)	OR (95% CI)	*P* value
rs1044429					
GG	63 (53.85)	87 (37.99)	58.00	1.00	--
AG	51 (43.59)	130 (56.77)	71.82	1.92 (1.19-3.08)	0.007
AA	3 (2.56)	12 (5.24)	80.00	3.44 (0.91-13.04)	0.069
Dominant				1.99 (1.25-3.19)	0.004
Additive				1.90 (1.25-2.89)	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 (1.56-4.58)	<0.001
AA	0	1 (0.44)	100	1.00	--
Dominant				2.71 (1.58-4.63)	<0.001
Additive				2.70 (1.59-4.61)	<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 (0.29-0.83)	0.008
GG	24 (20.51)	36 (15.75)	60.00	0.44 (0.22-0.85)	0.015
Dominant				0.48 (0.29-0.78)	0.003
Additive				0.63 (0.46-0.87)	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 (0.80-2.18)	0.279
AA	15 (12.82)	32 (13.98)	68.09	1.32 (0.63-2.75)	0.463
Dominant				1.32 (0.82-2.13)	0.256
Additive				1.19 (0.84-1.69)	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 (0.60-1.66)	0.996
CC	17 (14.53)	27 (11.80)	61.36	0.84 (0.41-1.75)	0.645
Dominant				0.96 (0.59-1.56)	0.879
Additive				0.94 (0.66-1.33)	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 (0.50-2.51)	0.791
GG	1 (0.85)	1 (0.44)	50.00	0.58 (0.03-10.68)	0.714
Dominant				1.07 (0.49-2.34)	0.866
Additive				1.02 (0.50-2.09)	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 (0.57-1.50)	0.753
TT	4 (3.42)	7 (3.06)	63.64	0.98 (0.27-3.59)	0.978
Dominant				0.93 (0.58-1.49)	0.763
Additive				0.95 (0.63-1.43)	0.796
rs369150					
GG	37 (31.62)	79 (34.50)	68.10	1.00	--
AG	63 (53.85)	121 (52.84)	65.76	0.80 (0.48-1.34)	0.396
AA	17 (14.53)	29 (12.66)	63.04	0.71 (0.34-1.48)	0.358
Dominant				0.78 (0.48-1.28)	0.325
Additive				0.83 (0.59-1.18)	0.302
rs86567					
AA	29 (24.79)	65 (28.38)		1.00	--
AC	67 (57.26)	128 (55.90)		0.79 (0.46-1.36)	0.396
CC	21 (17.95)	36 (15.72)		0.67 (0.32-1.37)	0.267
Dominant				0.76 (0.45-1.28)	0.306
Additive				0.81 (0.57-1.16)	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 (0.57-1.56)	0.882
TT	1 (0.86)	4 (1.74)	80.00	1.53 (0.16-14.19)	0.708
Dominant				0.96 (0.58-1.58)	0.880
Additive				0.99 (0.62-1.57)	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 (0.58-1.52)	0.803
AA	3 (2.57)	13 (5.68)	81.25	2.09 (0.56-7.83)	0.274
Dominant				1.01 (0.63-1.61)	0.963
Additive				1.10 (0.74-1.64)	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 (0.71-1.86)	0.571
AA	10 (8.54)	16 (6.99)	61.54	0.97 (0.40-2.31)	0.937
Dominant				1.12 (0.71-1.77)	0.634
Additive				1.05 (0.73-1.52)	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 (0.56-1.46)	0.680
TT	8 (6.84)	20 (8.73)	71.43	1.24 (0.50-3.06)	0.637
Dominant				0.95 (0.60-1.50)	0.823

Additive				1.02 (0.71-1.46)	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 (0.59-1.74)	0.969
GG	0	3 (1.31)	100.00	1.00	--
Dominant				1.06 (0.62-1.82)	0.822
Additive				1.13 (0.68-1.88)	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 (0.47-2.02)	0.955
AA	1 (0.86)	1 (0.43)	50.00	0.80 (0.05-14.05)	0.878
Dominant				0.97 (0.48-1.96)	0.930
Additive				0.96 (0.50-1.85)	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 (0.65-1.81)	0.760
AA	17 (14.53)	39 (17.03)	69.64	1.35 (0.66-2.76)	0.406
Dominant				1.14 (0.70-1.86)	0.598
Additive				1.15 (0.82-1.61)	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 (0.52-1.95)	0.975
Dominant				1.01 (0.52-1.95)	0.975
Additive				1.01 (0.52-1.95)	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 (0.87-2.27)	0.164
TT	9 (7.70)	15 (6.55)	62.50	1.09 (0.43-2.74)	0.852
Dominant				1.36 (0.86-2.17)	0.193
Additive				1.21 (0.82-1.77)	0.334

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

Abbreviation: SVR, sustained virological response; N-SVR, non-sustained virological response.

Afterward, we evaluated the combined effect of these three significant SNPs by adding up the unfavorable genotype number. The results indicated that SVR rates declined when patients were carrying the more unfavorable 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 odds ratios also decreased along with the increase in risk genotypes (OR = 0.38, 95% CI = 0.17-0.83; OR = 0.22, 95% CI = 0.10-0.49; OR = 0.12, 95% CI = 0.04-0.37, 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).

3.3 Interaction analysis

As shown in Table 3, the interaction analysis among the meaningful SNPs and potential risk factors was also analyzed. A significant multiplicative interaction related to SVR was found between rs2856997 genotypes and gender ($P_{interaction}$= 0.019). Compared to 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 = 0.81-0.59).

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 (0.18-0.59)
Male with TT genotypes	12 (34.29)	23 (65.71)	0.44 (0.18-1.04)
Male with TG/GG genotypes	16 (32.00)	34 (68.00)	0.54 (0.25-1.19)
P for multiplicative interaction		*P* = 0.019	

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

3.4 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).

Table 4. **Multivariate Stepwise regression analysis for independent factors of SVR**

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

Abbreviation: SVR, sustained virological response; Coef. coefficient of variation; SE, standard error; CI, confidence interval; GLU, glucose; Cons. Constant term.

3.5 Association of SNPs with viral dynamics during treatment

The effect of the three significant SNPs on viral dynamics during treatment was also analyzed. The difference between baseline viral load in these SNPs was not significant between patients carrying the wild-type and mutant alleles (P>0.05). Nevertheless, the decline in viral load was significantly quicker in rs2284191 AG/AA patients than in GG patients 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.

4. 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 analyzed. 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 unfavorable 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, 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 (TFBS). The mutation at rs2284191 may influence transcription and transform the encoding protein's function, ultimately affecting antigen processing 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 (SLE) 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 CHC patients[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 direct-acting antiviral (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 developing 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 multi-center 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.

Contributorship statement

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. YZ, XX and YF provided materials and analysis tools. PH revised the manuscript. All authors accepted the final manuscript.

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Conflicts of Interest

There is no conflict of interest.

Data sharing statement

No additional data is available.

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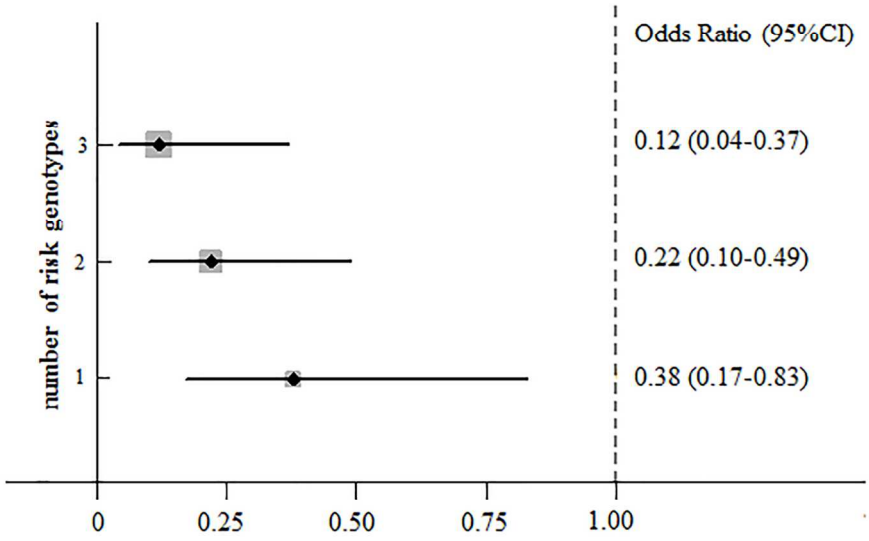
Figure legends

Figure 1. Combined effects of rs1044429, rs2284191 and rs2856997 with SVR. Variables are numbers of combined unfavorable genotypes (rs1044429-GG, rs2284191-GG and rs2856997-GG); logistic regression analyses adjusted for age, gender, glucose, baseline HCV RNA; OR, odds ratio; CI, confidence interval.

Figure 2.Predictors of HCV treatment response. The response variable is SVR 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.

Figure 3.Effect of HLA-DOA rs2284191 variants on HCV 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.



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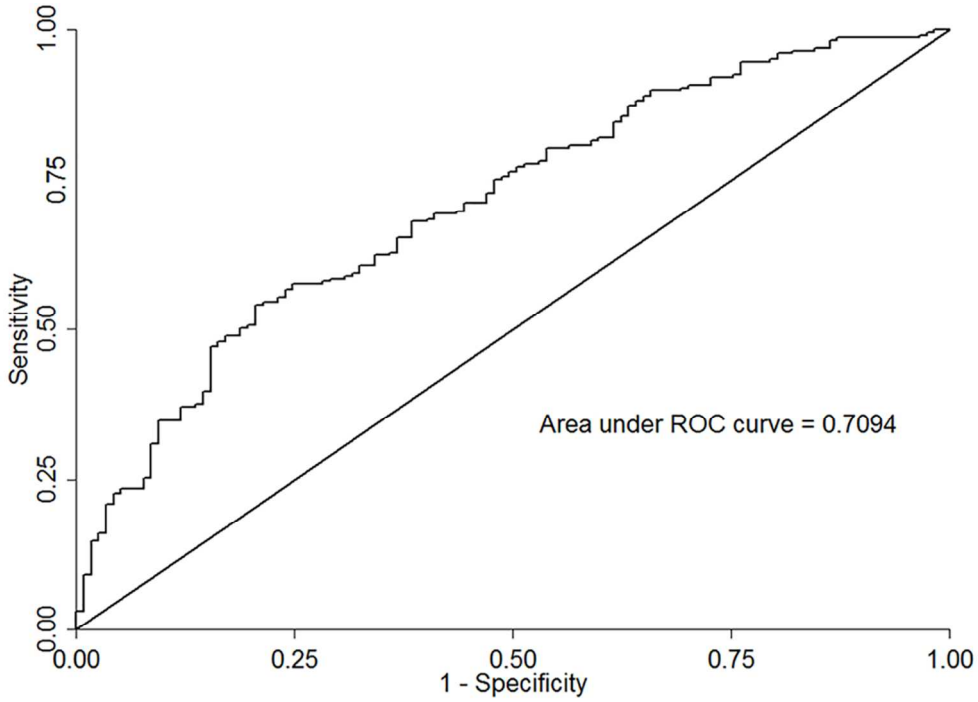

Figure 2.Predictors of HCV treatment response. The response variable is SVR and the diagnostic test variable is a combination of rs1044429, rs2284191, rs2856997, glucose and baseline HCV RNA with the coefficients taken from the regression analysis.

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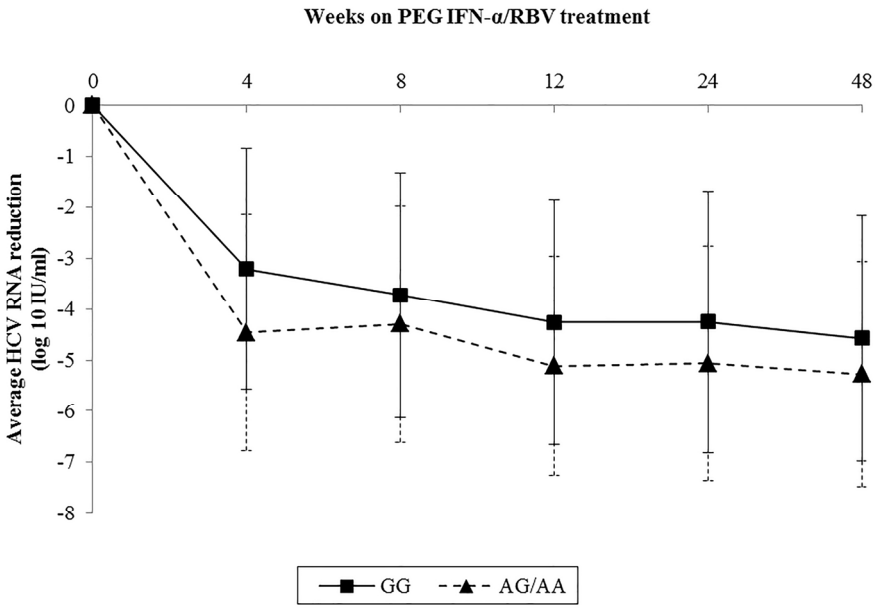

Figure 3.Effect of HLA-DOA rs2284191 variants on HCV viral kinetics during therapy. The fold of viral decline was compared among patients with the GG genotype and the AG/AA genotype. The fold of viral decline was calculated as the viral load at follow up time point divided by the initial viral load.

173x109mm (300 x 300 DPI)

Supplemental Table 1. Information of primers and probes for TaqMan allelic discrimination

Polymorphism		Sequence(5'-3')
DOA rs1044429	Primer	F: TCACACAAAGAGGGTTTCTGTTACTG
		R: GAATAAGTTGAAATCAATGACCAGAAGA
	Probe	FAM-TGAGATGATTCTCCTCCAC-MGB
		HEX-TGAGATGATTTTCCTCCAC-MGB
DOA rs2284191	Primer	F: TCCTCCATCTCAGAGCATTATGAC
		R: TGTTGCTCAAACAACTTCATAGAGTTC
	Probe	FAM-CTTCCATAACTGTTGTCTAG-MGB
		HEX-TAACTGTTATCTAGTTTTCTGG-MGB
DOB rs2856997	Primer	F: CCAAATCCAATGCTAGCTAGAGAAA
		R: ATGGGCTGTGAGAATCTGTAACC
	Probe	FAM-CATGGAGTTACCCCC-MGB
		HEX-CCATGGAGTTACCACC-MGB
DOA rs408036	Primer	F: CCAGGCCTTGGCCAGTT
		R: GTAACACACAATGGGCCAAATG
	Probe	FAM-TTGGCAGCCGTCCT-MGB
		HEX-ATTGGCAGCCATC-MGB
DOA rs3128935	Primer	F: TGTCGGGTGGACATGTTCAC
		R: GGATCCACATGGTCTGTGTTCTC
	Probe	FAM-AGAACACCGCTAACA-MGB
		HEX-AGAACACCGCCAACA-MGB
DOA rs3129304	Primer	F: AAAACATACAAAGAGATAAATCACCATACC
		R: TGAAAACCGTAATCTGTATTGCTCAT
	Probe	FAM-CATAGTTTATGTCAGGACC-MGB
		HEX-CATAGTTTATGTCAAGACC-MGB
DOA rs376892	Primer	F: CTTGGCTGTGGTCTGGTAACTG
		R: CCTTCCTAGTCCACCTCAGACCTT
	Probe	FAM-TAATCAGGTGCCATTGG-MGB
		HEX-TAATCAGGTGCCATCGG-MGB
DOA rs369150	Primer	F: GAAAGAAAGGAACAGGGCATGAC
		R: GGCGGGAAGGTCCAGAGA
	Probe	FAM-TGATGGGAACCTAGG-MGB
		HEX-TGATGGGAGCCTAGG-MGB
DOA rs86567	Primer	F: GGTGCGGGTCTACAGATGGTT
		R: GAGCAACAGTTATTGAGGAACTAGCAT
	Probe	FAM-TGGCCCCCCATTG-MGB
		HEX-TGGCCCACCATTG-MGB
DOA rs6913008	Primer	F: GTCCTGTTCAGAGTCATCCACTTT
		R: TCCTCATCATCATGGGCACAT
	Probe	FAM-CCCAGACTCCCGG-MGB
		HEX-CCCAGACTCCTGG-MGB
DOA rs2582	Primer	F: TGATCCTTCTGAGAGAAATGACTTGT
		R: CACAGCGGGATGCACTTAAA

	Probe	FAM-TGTGACAGACCCTGC-MGB HEX-TGTGACAGCCCCTG-MGB
DOA rs416622	Primer	F: CAGCCTGGTGACAGAGTGAGA R: TCACCCAGACCTACTGAATTAGAATCT
	Probe	FAM-AGACAGCCCCCCTGT-MGB HEX-AGACAGCCTCCCTGTT-MGB
DOA rs453779	Primer	F: GTCACCCGTGGAGGCACTA R: AACGTCCCTTAATCCCAGTCCTA
	Probe	FAM-AGGAACAGGCCCTG-MGB HEX-AGGAACGGGCCCTG-MGB
DOB rs2857111	Primer	F: TCTCTTGCCTCCGTTCTCATTC R: TGCTACATATTTCTAAAAGCCACTCTCATA
	Probe	FAM-TCCCCTCCCTGGAGA-MGB HEX-CTCCCCTCCCTAGAG-MGB
DOB rs1383258	Primer	F: TTACCAGACACGTTTAGAATGGATTC R: GAGTTCACAGCACATTGTAATTATTGG
	Probe	FAM-AGAAGAGATGAGAGAGTC-MGB HEX-CAAGAGAAGAGACGAGAG-MGB
DOB rs2071472	Primer	F: GACTGGATTCCTCCATGACTCAA R: CATGCCAATTCTTGCATACACA
	Probe	FAM-AACAGAGCAATTGTT-MGB HEX-AACAGAGCAATTATT-MGB
DOB rs7383287	Primer	F: CGTAATTTACCAGGCATGGGTTT R: CAGTCAGCCTTTGCCTGAATC
	Probe	FAM-TTCCAGAAGATTTTG-MGB HEX-TTTCCAGAAGACTTTG-MGB
DOB rs2071475	Primer	F: GGTCCTCTCTGGGTACACTGTCA R: GGTTTTCTTTCACGGTGTCTCAT
	Probe	FAM-CTAGGAAGGGAGGAAA-MGB HEX-ACTAGGAAGAGAGGAAA-MGB

Supplemental Table 2. Results of SNPs distribution in dominant, recessive, and additive models

SNPs	Location	Dominant		Additive	
		P* Value	**FDR***	***P* Value***	**FDR***
DOA rs1044429	3'UTR(G>A)	4.00×10^{-3}	0.024	3.00×10^{-3}	0.027
DOA rs2284191	intron(G>A)	2.83×10^{-4}	0.005	2.52×10^{-4}	0.005
DOB rs2856997	intron(T>G)	3.00×10^{-3}	0.024	5.00×10^{-3}	0.030
DOA rs408036	3'UTR(G>A)	0.256	0.836	0.325	0.859
DOA rs3128935	3'UTR(T>C)	0.879	0.975	0.713	0.975
DOA rs3129304	3'UTR(A>G)	0.866	0.975	0.948	0.975
DOA rs376892	3'UTR(C>T)	0.763	0.975	0.796	0.975
DOA rs369150	intron(G>A)	0.325	0.836	0.302	0.859
DOA rs86567	intron(A>C)	0.306	0.836	0.250	0.859
DOA rs6913008	intron(C>T)	0.880	0.975	0.961	0.975
DOA rs2582	3'UTR(C>A)	0.963	0.975	0.650	0.975
DOA rs416622	3'UTR(G>A)	0.634	0.975	0.779	0.975
DOA rs453779	intron(C>T)	0.823	0.975	0.935	0.975
DOB rs2857111	intron(A>G)	0.822	0.975	0.647	0.975
DOB rs1383258	intron(G>A)	0.930	0.975	0.907	0.975
DOB rs2071472	intron(G>A)	0.598	0.975	0.431	0.970
DOB rs7383287	synonymous(A>G)	0.975	0.975	0.975	0.975
DOB rs2071475	intron(C>T)	0.193	0.836	0.334	0.859

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

Supplemental Table 3. Association of SNPs in *HLA-DO* with RVR/ cEVR

Genotype	N-RVR(n=178)	RVR (n=168)	OR (95% CI)	*P* value	N-cEVR (n=106)	cEVR (n=235)	OR (95% CI)	*P* value
rs1044429								
GG	88 (49.44)	62 (36.90)	1.00	--	58 (54.72)	90 (38.30)	1.00	--
AG	82 (46.07)	99 (58.93)	1.66(1.05-2.60)	0.029	43 (40.57)	136 (57.87)	2.13(1.30-3.48)	0.003
AA	8 (4.49)	7 (4.17)	1.22 (0.40-3.67)	0.727	5 (4.71)	9 (3.83)	1.37 (0.43-4.39)	0.593
Dominant			1.62 (1.04-2.53)	0.034			2.05 (1.27-3.32)	0.003
Additive			1.42 (0.97-2.10)	0.074			1.73 (1.12-2.65)	0.013
rs2284191								
GG	134 (75.28)	97 (57.74)	1.00	--	84 (79.25)	143 (60.85)	1.00	--
AG	44 (24.72)	70 (41.67)	2.37 (1.47-3.83)	<0.001	22 (20.75)	91 (38.72)	2.81 (1.60-4.91)	<0.001
AA	0	1 (0.59)	1	--	0	1 (0.43)	1.00	--
Dominant			2.42 (1.50-3.90)	<0.001			2.84 (1.62-4.96)	<0.001
Additive			2.44 (1.52-3.91）	<0.001			2.83 (1.63-4.94)	<0.001
rs2856997								
TT	61 (34.27)	80 (47.62)	1.00	--	34 (32.08)	106 (45.11)	1.00	--
TG	84 (47.19)	61 (36.31)	0.60 (0.37-0.96)	0.035	49 (46.23)	92 (39.15)	0.66 (0.39-1.12)	0.122
GG	33 (18.54)	27 (16.07)	0.58 (0.31-1.10)	0.093	23 (21.69)	37 (15.74)	0.49 (0.25-0.96)	0.038
Dominant			0.59 (0.38-0.92)	0.021			0.60 (0.37-0.99)	0.045
Additive			0.72 (0.53-0.98)	0.040			0.70 (0.50-0.96)	0.029
rs408036								
GG	61 (34.27)	64 (38.10)	1.00	--	40 (37.74)	82 (34.89)	1.00	--
AG	94 (52.81)	80 (47.62)	0.86 (0.53-1.38)	0.528	52 (49.06)	121 (51.49)	1.28 (0.76-2.14)	0.351

AA	23 (12.92)	24 (14.28)	1.06 (0.53-2.12)	0.866	14 (13.20)	32 (13.62)	1.18 (0.56-2.50)	0.663
Dominant			0.90 (0.57-1.41)	0.642			1.26 (0.77-2.05)	0.361
Additive			0.98 (0.71-1.36)	0.922			1.13 (0.79-1.62)	0.489
rs3128935								
TT	78 (43.82)	52 (30.95)	1.00	--	45 (42.45)	83 (35.32)	1.00	--
CT	88 (49.44)	84 (50.00)	1.84 (1.12-3.02)	0.016	51 (48.11)	118 (50.21)	1.49 (0.89-2.48)	0.130
CC	12 (6.74)	32 (19.05)	5.59 (2.55-12.26)	<0.001	10 (9.44)	34 (14.47)	2.22 (0.99-5.01)	0.054
Dominant			2.27 (1.41-3.67)	0.001			1.61 (0.98-2.64)	0.058
Additive			2.20 (1.54-3.13)	<0.001			1.49 (1.03-2.15)	0.034
rs3129304								
AA	166 (93.26)	147 (87.50)	1.00	--	96 (90.57)	212 (90.21)	1.00	--
AG	11 (6.18)	20 (11.90)	2.17 (0.99-4.78)	0.054	9 (8.49)	22 (9.36)	1.15 (0.50-2.64)	0.739
GG	1 (0.56)	1 (0.60)	1.47 (0.08-25.49)	0.792	1 (0.94)	1 (0.43)	0.46 (0.03-8.21)	0.594
Dominant			2.12 (0.99-4.55)	0.054			1.08 (0.49-2.41)	0.846
Additive			1.91 (0.95-3.87)	0.070			1.02 (0.49-2.11)	0.962
rs376892								
CC	103 (57.87)	111 (66.07)	1.00	--	64 (60.38)	148 (62.98)	1.00	--
CT	69 (38.76)	52 (30.95)	0.62 (0.38-0.98)	0.043	38 (35.85)	80 (34.04)	0.83 (0.51-1.38)	0.479
TT	6 (3.37)	5 (2.98)	0.81 (0.23-2.85)	0.746	4 (3.77)	7 (2.98)	0.84 (0.23-3.06)	0.796
Dominant			0.63 (0.40-0.99)	0.048			0.84 (0.52-1.36)	0.467
Additive			0.70 (0.47-1.04)	0.080			0.86 (0.57-1.31)	0.493
rs369150								
GG	58 (32.58)	58 (34.52)	1.00	--	33 (31.13)	81 (34.47)	1.00	--
AG	96 (53.93)	88 (52.38)	0.81 (0.50-1.31)	0.396	56 (52.83)	126 (53.62)	0.82 (0.49-1.40)	0.473

AA	24 (13.49)	22 (13.10)	0.94 (0.46-1.90)	0.863	17 (16.04)	28 (11.91)	0.60 (0.28-1.27)	0.179
Dominant			0.84 (0.53-1.33)	0.448			0.77 (0.47-1.28)	0.317
Additive			0.93 (0.66-1.29)	0.657			0.78 (0.55-1.13)	0.187
rs86567								
AA	46 (25.84)	48 (28.57)	1.00	--	24 (22.64)	68 (28.94)	1.00	--
AC	107 (60.11)	88 (52.38)	0.74 (0.45-1.23)	0.246	65 (61.32)	128 (54.47)	0.64 (0.36-1.12)	0.118
CC	25 (14.05)	32 (19.05)	1.23 (0.62-2.43)	0.559	17 (16.04)	39 (16.59)	0.73 (0.34-1.56)	0.417
Dominant			0.83 (0.51-1.35)	0.452			0.66 (0.38-1.14)	0.132
Additive			1.04 (0.75-1.46)	0.800			0.82 (0.57-1.19)	0.299
rs6913008								
CC	119 (66.85)	123 (73.21)	1.00	--	74 (69.81)	166 (70.64)	1.00	--
CT	57 (32.02)	42 (25.00)	0.68 (0.42-1.11)	0.122	32 (30.19)	64 (27.23)	0.91 (0.55-1.53)	0.734
TT	2 (1.13)	3 (1.79)	1.01 (0.16-6.36)	0.989	0	5 (2.13)	1.00	--
Dominant			0.69 (0.43-1.12)	0.135			0.98 (0.59-1.64)	0.936
Additive			0.74 (0.47-1.15)	0.177			1.06 (0.66-1.72)	0.798
rs2582								
CC	101 (56.74)		1.00	--	65 (61.32)	136 (57.87)	1.00	--
AC	67 (37.64)		0.88 (0.56-1.40)	0.589	38 (35.85)	87 (37.02)	1.09 (0.66-1.79)	0.732
AA	10 (5.62)		0.46 (0.15-1.41)	0.175	3 (2.83)	12 (5.11)	1.77 (0.47-6.67)	0.400
Dominant			0.82 (0.53-1.29)	0.395			1.14 (0.70-1.84)	0.595
Additive			0.79 (0.54-1.16)	0.235			1.17 (0.77-1.77)	0.462
rs416622								
GG	94 (52.81)	77 (45.83)	1.00	--	54 (50.94)	115 (48.94)	1.19 (0.73-1.96)	0.485
AG	68 (38.20)	81 (48.21)	1.44 (0.91-2.28)	0.115	42 (39.62)	104 (44.26)	0.85 (0.36-2.05)	0.724

AA	16 (8.99)	10 (5.96)	0.85 (0.36-2.03)	0.721	10 (9.44)	16 (6.80)	1.13 (0.71-1.81)	0.614
Dominant			1.33 (0.86-2.07)	0.196			0.79 (0.34-1.84)	0.583
Additive			1.13 (0.80-1.60)	0.481				
rs453779								
CC	87 (48.88)	84 (50.00)	1.00	--	52 (49.06)	116 (49.36)	1.00	--
CT	78 (43.82)	69 (41.07)	0.94 (0.59-1.48)	0.775	46 (43.40)	100 (42.55)	1.01 (0.62-1.65)	0.794
TT	13 (7.30)	15 (8.93)	1.22 (0.53-2.80)	0.632	8 (7.54)	19 (8.09)	1.07 (0.43-2.65)	0.886
Dominant			0.98 (0.63-1.51)	0.914			1.02 (0.64-1.63)	0.943
Additive			1.03 (0.73-1.45)	0.872			1.02 (0.70-1.48)	0.907
rs2857111								
AA	135 (75.84)	124 (73.81)	1.00	--	84 (79.25)	170 (72.34)	1.00	--
AG	43 (24.16)	41 (24.40)	0.94 (0.56-1.57)	0.818	22 (20.75)	62 (26.38)	1.39 (0.79-2.46)	0.253
GG	0	3 (1.79)	1.00	--	0	3 (1.28)	1.00	--
Dominant			1.00 (0.61-1.67)	0.987			1.46 (0.83-2.57)	0.189
Additive			1.08 (0.67-1.75)	0.740			1.51 (0.87-2.60)	0.139
rs1383258								
GG	155 (87.08)	151 (89.88)	1.00	--	89 (83.96)	213 (90.64)	1.00	--
AG	22 (12.36)	16 (9.52)	0.75 (0.37-1.49)	0.406	17 (16.04)	20 (8.51)	0.49 (0.24-0.99)	0.047
AA	1 (0.56)	1 (0.60)	1.85 (0.11-31.36)	0.669	0	2 (0.85)	1.00	--
Dominant			0.78 (0.40-1.53)	0.470			0.55 (0.28-1.11)	0.094
Additive			0.83 (0.44-1.57)	0.563			0.66 (0.34-1.26)	0.205
rs2071472								
GG	50 (28.09)	61 (36.31)	1.00	--	36 (33.96)	74 (31.49)	1.00	--
AG	100 (56.18)	79 (47.02)	0.62 (0.38-1.02)	0.058	56 (52.83)	119 (50.64)	1.05 (0.62-1.78)	0.850

AA	28 (15.73)	28 (16.67)	0.89 (0.46-1.74)	0.743	14 (13.21)	42 (17.87)	1.52 (0.72-3.19)	0.269
Dominant			0.68 (0.42-1.08)	0.105			1.14 (0.69-1.89)	0.597
Additive			0.88 (0.63-1.21)	0.430			1.19 (0.84-1.69)	0.324
rs7383287								
AA	156 (87.64)	142 (84.52)	1.00	--	94 (88.68)	201 (85.53)	1.00	--
AG	22 (12.36)	26 (15.48)	1.47 (0.78-2.76)	0.237	12 (11.32)	34 (14.47)	1.44 (0.70-2.95)	0.320
Dominant			1.47 (0.78-2.76)	0.237			1.44 (0.70-2.95)	0.320
Additive			1.47 (0.78-2.76)	0.237			1.44 (0.70-2.95)	0.320
rs2071475								
CC	66 (37.08)	79 (47.02)	1.00	--	42 (39.62)	101 (42.98)	1.00	--
CT	99 (55.62)	78 (46.43)	0.67 (0.42-1.06)	0.084	58 (54.72)	116 (49.36)	0.82 (0.50-1.34)	0.423
TT	13 (7.30)	11 (6.55)	0.81 (0.33-1.99)	0.651	6 (5.66)	18 (7.66)	1.31 (0.48-3.62)	0.601
Dominant			0.68 (0.44-1.07)	0.095			0.86 (0.53-1.39)	0.545
Additive			0.78 (0.54-1.12)	0.179			0.97 (0.66-1.43)	0.874

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

Abbreviation: RVR, rapid virological response; N-RVR, non-rapid virological response; cEVR, complete early virological response, N-cEVR, non-complete early virological response.

STREGA guidance, extended from STROBE Statement

	Item number		Extension for genetic association studies
TITLE	1	Association between *human leukocyte antigen-DO* polymorphisms and interferon/ribavirin treatment response in hepatitis C virus type 1 infection in Chinese population: a prospective study	
ABSTRACT	2	HLA-DO may play a role in treatment response to HCV. This study was conducted to explore the role of SNPs in HLA-DO in responding to HCV therapy. A total of 346 CHC patients undergoing PEG IFN-α/RBV reatment were enrolled in this study. We genotyped 18 SNPs in HLA-DO using the ABI TaqMan allelic discrimination assay. The SNPs HLA-DOA rs1044429 and HLA-DOB rs2284191 and rs2856997 were correlated with HCV treatment response in the Chinese Han population.	
INTRODUCTION			
Background	4	The polymorphism in HLA-DO may be potential predictors of treatment efficacy in patients with HCV.	
Objectives	5	This study was conducted to assess how HLA-DO genotypes are associated with SVR, RVR and completely EVR (cEVR) in CHC patients from the Chinese Han population treated with PEG-IFN/RBV.	This study is the first to demonstrate a relationship between variants in HLA-DO and HCV treatment response in the Chinese Han population.
METHODS			
Study design	6	This was a prospective study followed up of HCV treatment response for one year and a half.	
Setting	6	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.	
Participants	6	A total of 346 chronic hepatitis C (CHC) patients who finished the 48-week pegylated interferon-alpha and	Inclusion criteria: (1) treatment-naïve and treated with PEG IFN-α/RBV in

		ribavirin (PEG IFN-α/RBV) treatment were enrolled in this study.	this study; (2) HCV RNA was present in serum for over 6 months before treatment; (3) infected with HCV genotype 1; (4) negative for hepatitis B (HBV) or HIV infection; and (5) lacked any other hepatic diseases. Exclusion criteria: (1) patients received antiviral therapy within 6 months; (2) patients with blood diseases, malignancies, organ transplants, or decompensated liver disease; (3) patients with diabetes, thyroid diseases.
Variables	6	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.	
Data sources measurement	5-7	All participating patients were classified into two groups according to SVR. Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's t test (for continuous variables) or a chi-square ($\chi 2$) test (for categorical variables) with a two-tailed P value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR).	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. We extracted genomic DNA from peripheral blood samples using protease K digestion and phenol/chloroform

			purification according to standard protocol. Genotyping results were ascertained using SDS 2.3 software
Bias	7	Measurement bias	Genotyping results were ascertained using SDS 2.3 software and 100% concordance was achieved.
Study size	5	A total of 346 CHC patients who finished the 48-week pegylated interferon-alpha and ribavirin (PEG IFN-α/RBV) treatment were enrolled in this study.	A total of 427 patients were enrolled in the treatment cohort. After one month of treatment, 8 patients were lost to follow-up. After three months, another 7 patients were lost to follow-up. By the time the study began, another 17 patients were lost to follow-up and 51 patients had not finished treatment. Ultimately, a total of 346 patients with CHC who finished the 48-week treatment and 24-week follow-up were enrolled in this study.
Quantitative variables			
Statistical methods	7-8	Comparisons between individual demographic characteristics were analyzed as appropriate with either a student's t test (for continuous variables) or a chi-square ($\chi 2$) test (for categorical variables) with a two-tailed P value. Multivariate logistic regression was used to analyze the association between genotypes and SVR, RVR and cEVR by calculating the odds ratio (OR) and 95% confidence interval (95% CI) adjusted for age, gender, baseline HCV RNA level and glucose. Each SNP was analyzed using codominant, dominant and additive	All data analysis was operated with Stata/SE (V.12.0 for Windows; StataCorp LP, College Station, TX, USA).All SNPs were in Hardy-Weinberg equilibrium in allele frequency in the non-SVR group except for rs1044429, P = 0.048.

		genetic models. False discovery rate (FDR) corrections were applied for multiple comparisons, and they were carried out as previously described, considering FDR < 0.05 as significant. The combined effect of three independent SNPs (rs1044429, rs2284191 and rs2856997) was analyzed 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 (ROC) 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			
Participants	9	The baseline demographic and laboratory characteristics of the 346 enrolled patients are shown in Table 1.	
Descriptive data	9	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 (TP), alpha fetal protein (AFP), hemoglobin, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyltranspeptidase (GGT), T3, T4, platelets and WBC were similar between two groups (P>0.05). However, the baseline viral load and glucose levels were different between	All patients were infected with HCV genotype 1.

		the SVR and non-SVR group (P < 0.05). Individuals with higher baseline viral load and glucose levels were less likely to achieve SVR.	
Outcome data	10	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. For rs2856997, the rate of SVR was higher in patients carrying the TT genotype (75.9%) compared to those with the TG genotype (59.3%) and GG (60%).	
Main results	11-14	Factors with P values < 0.05 in the univariate analysis were adjusted for age, gender, baseline viral load and glucose. The dominant model indicated that patients carrying favorable genotypes at rs1044429 AA and rs2284191 AA were more likely to achieve sustained virological response (SVR) (Odds ratio (OR) = 1.99, 95% confidence interval (CI) = 1.25-3.19; OR = 2.71, 95% CI = 1.58-4.63, respectively), while patients carrying unfavorable genotypes at rs2856997 GG were less likely to achieve SVR (OR = 0.48, 95% CI = 0.29-0.78). In addition, rs1044429, rs2284191 and rs2856997 were also found to be significantly associated with RVR (Dominant model: OR = 1.62, 95%CI = 1.04-2.53; OR = 2.42, 95% CI = 1.50-3.90; OR = 0.59, 95% CI = 0.38-0.92, respectively) and cEVR (Dominant model: OR = 2.05, 95% CI = 1.27-3.32; OR = 2.84, 95% CI = 1.62-4.96; OR = 0.60, 95% CI = 0.37-0.99, respectively). Patients carrying the mutant alleles rs1044429-A or rs2284191-A or the wild-type allele rs2284191-T were more likely to	We performed FDR correction for all SNPs as outlined in Supplemental 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).

		achieve higher rates of RVR, cEVR and SVR.	
Other analyses	15-17	Combined effect analysis: the results indicated that SVR rates declined when patients were carrying the more unfavorable 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 odds ratios also decreased along with the increase in risk genotypes (OR = 0.38, 95% CI = 0.17-0.83; OR = 0.22, 95% CI = 0.10-0.49; OR = 0.12, 95% CI = 0.04-0.37, 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. Interaction analysis: A significant multiplicative interaction related to SVR was found between rs2856997 genotypes and gender (Pinteraction= 0.019). Compared to 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 = 0.81-0.59). Stepwise regression analysis: The results showed that rs1044429, rs2284191, rs2856997, baseline glucose and baseline HCV RNA were independent predictors of SVR. Adding up these five factors, the predictive AUC value was 0.71. Association of SNPs with viral dynamics during treatment: Nevertheless, the decline in viral load was significantly quicker in rs2284191 AG/AA patients than in GG patients 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. 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.	
DISCUSSION			
Key results	18-19	A total of 18 tagging SNPs involved in antigen processing and presentation in HLA-DO were selected and analyzed. 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 unfavorable 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 accordingly25 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, 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.	
Limitations	20	First, the biological mechanism by which HLA-DO affects treatment response has not yet been well established. This may be related to the wide variety of ethnicities and HCV genotypes. In the current study, we only focused on HCV-1 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 direct-acting antiviral (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 developing 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 multi-center 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. We will pay attention to collecting this information in future research.	
Interpretation	19-20	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 (TFBS). The mutation at rs2284191 may influence transcription and transform the encoding protein's function, ultimately affecting antigen processing 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. 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. 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 (SLE) and HIV-related Kaposi's sarcoma. Therefore, more attention should be given to the structure and function of HLA-DO and DM molecules.	
Generalizability	21	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.	
OTHER INFORMATION			
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