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ARTICLE

Genetic variants in chemokine CC subfamily genes influence hepatitis C virus viral clearance

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Abstract

Chemokine genes may influence both hepatitis C virus (HCV) spontaneous clearance in acute infection and treatment response in chronic infection. We conducted this study to evaluate whether the genetic variants in several CC family genes influence HCV spontaneous clearance and treatment response. The current research genotyped eight SNPs, including *CCR1* rs3733096, rs13096371, *CCR5* rs746492, rs1800874, *CCL3* rs1130371, *CCL5* rs3817656, *CCL8* rs1133763, *CCL14* rs854625, to explore their associations with HCV spontaneous clearance and response to treatment in two populations. We identified that the *CCR1* rs3733096 (dominant model: adjusted OR = 2.29, 95% CI = 1.49–3.53, additive model: adjusted OR = 2.21, 95% CI = 1.50–3.25) and *CCL5* rs3817656 (dominant model: OR = 1.37, 95% CI = 1.10–1.70, additive model: OR = 1.33, 95% CI = 1.12–1.58) were associated with HCV spontaneous clearance in Chinese Han population, while we found no association with treatment response. Moreover, the expression quantitative trait loci (eQTL) analysis showed that the risk alleles of rs3817656 were significantly associated with downregulated expression of *CCL5* in whole blood ($P < 0.001$). The polymorphism of *CCR1* rs3733096 and *CCL5* rs3817656 are associated with spontaneous clearance of HCV in Chinese Han population.

Introduction

It was estimated that 71 million individuals worldwide were living with hepatitis C virus (HCV) in 2015. About 1.75 million people newly acquire HCV infection each year. These people are at risk of progressing to liver fibrosis, cirrhosis, even hepatocellular carcinoma, unless they receive regular follow-up and treatment [1]. It was reported that 15–40% of the infected individuals resolved HCV spontaneously and would not be chronic infection [2]. The rate of sustained virological response (SVR) of pegylated (PEG) IFN-α and ribavirin (RBV) therapy is 40–50% for patients infected with HCV genotype 1, and about 80% for genotype 2 and 3 infections [3, 4]. However, this range has a certain degree of uncertainty. Factors such as the immune response determined by environment, viral and host factors can affect both spontaneous clearance and virological response of HCV [5–9]. Genome-wide association studies (GWAS) reported that host genetic factors, such as *IFNλ3* (*IL28B*) and *IFNλ4* SNPs, were associated with the HCV spontaneous clearance and treatment response [10–14].

From the above, we can learn that host genetic variation related to immune response may influence HCV clearance

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and SVR. Th cellular immune response performs an essential role in the process of HCV infection [15]. Chemokine and their receptors expressed by Th cell influence differentiation of T cells. Chemokine receptors can recruit the subgroup of Th cells and migrate to the areas of inflammation by combining to ligands [16]. This process could influence HCV infection and treatment-induced outcome by the immune response of host. Several previous studies have reported that polymorphisms of chemokine and their receptor can affect HCV clearance and treatment response of interferon (IFN) [17–20].

CCR1, *CCR5*, *CCL3*, *CCL5*, *CCL8*, and *CCL14* are members of the chemokine CC subfamily. Grünhage et al. [21] reported that chemokine *CCL3* gene was downregulated in patients with chronic hepatitis C. Woitas et al. [22], Goulding et al. [23], and Ahlenstiel et al. [24] also reported that *CCR5*Δ32 mutation was associated with HCV susceptibility, spontaneous elimination and treatment response of IFN. Thus, the aim of our study is to investigate whether polymorphisms of several chemokine CC family-related genes are associated with the outcomes of HCV infection and treatment response in Chinese Han population. Eight SNPs (*CCR1* rs3733096, rs13096371, *CCR5* rs746492, rs1800874, *CCL3* rs1130371, *CCL5* rs3817656, *CCL8* rs1133763, *CCL14* rs854625) were selected to genotype.

Methods

Ethical approval

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

Participants

Three kinds of high-risk populations from the cross-sectional study were included in this study, including 185 hemodialysis patients recruited from nine hospital hemodialysis centers in southern China, 312 injecting drug users recruited from the Nanjing compulsory detoxification center, and 1095 former paid blood donors from several villages in Jurong of Jiangsu from May 2006 to May 2015, respectively. All participants were tested anti-HCV and HCV-RNA at baseline and followed up for 6–12 months. Spontaneous clearance was defined as anti-HCV positive, HCV-RNA negative for more than 6 months. Persistent infection was defined as anti-HCV positive and HCV-RNA positive for more than 6 months.

A total of 360 chronic hepatitis C (CHC) patients in Jurong People's Hospital from January 2011 to December 2016 were also included in this study. Patients were treated with PEG IFN-α (180 μg) subcutaneously each week and plus 48-week oral RBV 800–1000 mg daily according to the standard guidelines.

Table 1 Baseline characteristics of HCV spontaneous clearance and persistence patients

Variables, *N* (%)	Spontaneous clearance (*N* = 596)	Persistence infection (*N* = 996)	*P*-value
Mean age, year	49.67 ± 13.47	54.01 ± 11.60	<0.001
Sex			
Male	217 (36.41)	336 (33.73)	0.278
Female	379 (63.59)	660 (66.27)	
Population			
Hemodialysis patient	101 (16.95)	84 (8.43)	<0.001
Injecting drug user	162 (27.18)	150 (15.06)	
Paid blood donor	333 (55.87)	762 (76.51)	
ALT			
<40U/L	466 (78.19)	604 (60.64)	<0.001
≥40U/L	130 (21.81)	392 (39.36)	
AST			
<40U/L	471 (79.03)	563 (56.53)	<0.001
≥40U/L	125 (20.97)	433 (43.47)	

ALT alanine aminotransferase, *AST* aspartate transaminase

The eligibility criteria of the two populations included (1) treatment-naive, (2) negative of co-infection with HBV or HIV, (3) infected with HCV genotype 1; (4) lack of other kinds of liver diseases. The exclusion criteria 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. Every participant filled out a questionnaire to collect demographic characteristics, high-risk behavior information and donated 5 mL venous blood for DNA extraction and serological tests before included in the study. Biochemical data of Peg IFN/RBV-treated patients were collected before starting treatment.

Viral testing and SNP genotyping

Genomic DNA of the two populations was both extracted from peripheral blood mononuclear cells using protease K digestion and phenol–chloroform purification. Serum viral load quantitative examination of all treated patients was performed at baseline, weeks 4, 12, 24, 48, and 24 weeks after cessation of treatment. Information regarding SNPs in 6 candidate genes 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). In addition, we also selected SNPs reported in the literatures that are associated with other immune diseases. According to the above steps, *CCR1* rs3733096, rs13096371, *CCR5* rs746492, rs1800874, *CCL3* rs1130371, *CCL5* rs3817656, *CCL8* rs1133763, *CCL14* rs854625 in chemokine *CC* family were chosen for genotyping by the TaqMan allelic discrimination assay on ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, San Diego, CA, USA), with a 100% concordance achieved. The primers and probes used for genotyping were listed in Supplementary Materials Table S1.

Statistical analysis

Chi-squared test and Student's *t*-tests were used to compare demographic characteristics of each group. Multivariate logistic regression analysis adjusted for age, sex, and all significant variables in univariate logistic regression analysis was used to analyze the association between genetic variants with HCV spontaneous clearance and treatment response. They were presented by odds ratio (OR) and 95% confidence interval (95% CI). Co-dominant, dominant, and additive genetic models were performed in each SNP. Cochran–Armitage trend test was used to analyze the combined effect of two independent SNPs (rs3733096-G and rs3817656-C). Variables from 0 to 4 are numbers of combined risk alleles (rs3733096-G and rs3817656-C). Multivariate logistic regression analyses adjusted for age, sex, ALT, AST, and population were performed to compare the patients carrying 1, 2, 3 unfavorable alleles with patients carrying 0 unfavorable allele. Stratified analysis was used to analyze the combined effects of rs3733096 and rs3817656 with HCV-persistent infection. The heterogeneity between the corresponding subgroups was examined by *Q* test. A stepwise regression model comprised of all significant variables was then fit to predict HCV-persistent infection. *P*-values <0.05 were considered as significant in all analysis. Bonferroni corrections were applied for multiple comparisons for SNPs. *P*-values <0.006 (0.05/8) were considered as significant after Bonferroni correction. All the analysis was conducted with Stata/SE (V.12.0 for Windows; StataCorp LP, College Station, TX, USA).

Results

Baseline characteristics of study subjects

A total of 1592 participants were enrolled in this study, including 185 hemodialysis patients from nine hospital hemodialysis centers, 312 injecting drug users from Nanjing compulsory detoxification center, and 1095 former paid blood donors from Jurong of Jiangsu. The three kinds of high-risk populations from the cross-sectional study were divided into two groups according to HCV-RNA to explore the relationship between SNPs and HCV-persistent infection (596 spontaneous clearance patients and 996 persistent patients). The baseline demographic and clinical characteristics of them were shown in Table 1. Among the persistent carriers, 33.73% were male and the mean age was (54.01 ± 11.60) years. The elder, paid blood donor and with abnormal baseline level of alanine aminotransferase (ALT) and aspartate transaminase (AST) were more likely to be chronic ($P < 0.001$). In addition, there was no difference of sex between these two groups.

Overall, 360 CHC patients treated with PEG IFN-α/ribavirin were all former paid blood donors and 241 patients of them achieved SVR. The baseline demographic and laboratorial characteristics of this treatment cohort were summarized in Table 2. The SVR rate was 66.94%, 25.31% were male and the mean age was (53.68 ± 8.54) years. The difference of age and sex was of no significance between the two groups ($P > 0.05$). Similarly, there was also no

Table 2 Baseline characteristics of chronic hepatitis C patients treated with IFN/RBV

Variables, *N* (%)	N-SVR (*N* = 119)	SVR (*N* = 241)	*P*-value
Mean age, year	53.46 ± 8.05	53.68 ± 8.54	0.820
Male	29 (24.37)	61 (25.31)	0.846
ALT ≥ 40 U/L	69 (57.98)	137 (56.85)	0.838
AST ≥ 40 U/L	62 (52.10)	121 (50.21)	0.735
GLU > 6.1 (mmol/L)	49 (41.18)	63 (26.14)	0.004
Log HCV-RNA	6.20 ± 0.71	5.82 ± 1.25	0.002
AFP (ng/mL)	12.28 ± 29.08	5.99 ± 14.19	0.006
T3 (nmol/L)	1.53 ± 0.49	1.49 ± 0.72	0.583
T4 (nmol/L)	129.18 ± 33.17	122.94 ± 32.50	0.090
TSH (mIU/L)	2.80 ± 1.78	3.86 ± 7.59	0.136
TP (g/L)	79.47 ± 6.10	78.29 ± 6.62	0.102
ALB (g/L)	43.41 ± 4.43	43.84 ± 4.25	0.378
Platelets (10^9/L)	137.63 ± 70.42	143.41 ± 59.38	0.415
WBC (10^9/L)			
Normal	74 (62.18)	165 (68.46)	0.235
Abnormal	45 (37.82)	76 (31.54)	
Hemoglobin (g/L)			
Normal	99 (83.19)	202 (83.82)	0.880
Abnormal	20 (16.81)	39 (16.18)	

N-SVR non-sustained virological response, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AST* aspartate transaminase, *GLU* glucose, *AFP* alpha fetal protein, *TSH* thyroid stimulating hormone, *TP* total protein, *ALB* albumin, *WBC* white blood cell

Table 3 Associations of SNPs in chemokine *CC* family with HCV spontaneous clearance

Genotype	Spontaneous clearance ($N = 596$)	Persistence infection ($N = 996$)	OR (95% CI)	*P*-value
rs3733096				
CC	567 (95.13)	877 (88.05)	1.00	—
CG	28 (4.70)	91 (9.14)	1.90 (1.21–2.98)	0.005
GG	1 (0.17)	28 (2.81)	12.58 (1.69–93.58)	0.013
Dominant			2.29 (1.49–3.53)	<0.001
Additive			2.21 (1.50–3.25)	<0.001
rs3817656				
TT	365 (61.24)	518 (52.01)	1.00	—
CT	199 (33.39)	387 (38.86)	1.28 (1.02–1.60)	0.035
CC	32 (5.37)	91 (9.13)	1.90 (1.23–2.95)	0.004
Dominant			1.37 (1.10–1.70)	0.005
Additive			1.33 (1.12–1.58)	0.001
rs13096371				
AA	501 (84.06)	864 (86.75)	1.00	—
AT	82 (13.76)	123 (12.35)	0.87 (0.63–1.19)	0.378
TT	13 (2.18)	9 (0.90)	0.53 (0.22–1.30)	0.168
Dominant			0.83 (0.61–1.11)	0.212
Additive			0.82 (0.63–1.06)	0.137
rs746492				
AA	204 (34.23)	355 (35.64)	1.00	—
AC	295 (49.50)	464 (46.59)	0.90 (0.71–1.15)	0.403
CC	97 (16.27)	177 (17.77)	1.06 (0.77–1.45)	0.734
Dominant			0.94 (0.75–1.18)	0.597
Additive			1.00 (0.86–1.17)	0.953
rs1800874				
TT	171 (28.69)	264 (26.51)	1.00	—
TG	287 (48.15)	507 (50.90)	1.18 (0.91–1.52)	0.204
GG	138 (23.16)	225 (22.59)	1.15 (0.85–1.55)	0.378
Dominant			1.17 (0.92–1.48)	0.202
Additive			1.08 (0.92–1.25)	0.346
rs1130371				
CC	209 (35.07)	369 (37.05)	1.00	—
CT	271 (45.47)	445 (44.68)	1.00 (0.79–1.27)	0.992
TT	116 (19.46)	182 (18.27)	1.15 (0.84–1.56)	0.385
Dominant			1.04 (0.83–1.30)	0.731
Additive			1.06 (0.91–1.23)	0.446
rs1133763				
AA	265 (44.46)	461 (46.29)	1.00	—
AC	255 (42.79)	424 (42.57)	1.01 (0.80–1.26)	0.960
CC	76 (12.75)	111 (11.14)	1.12 (0.78–1.59)	0.544
Dominant			1.03 (0.83–1.28)	0.809
Additive			1.04 (0.89–1.22)	0.630
rs854625				
GG	276 (46.31)	452 (45.38)	1.00	—
AG	262 (43.96)	409 (41.06)	0.90 (0.72–1.13)	0.365
AA	58 (9.73)	135 (13.56)	1.33 (0.93–1.90)	0.121
Dominant			0.98 (0.79–1.21)	0.840
Additive			1.06 (0.91–1.24)	0.443

Logistic regression analyses adjusted for age, sex, ALT, AST, and population

difference in the baseline levels of ALT, AST, T3, T4, thyroid stimulating hormone (TSH), total protein (TP), albumin (ALB), platelets, white blood cell (WBC), and hemoglobin. At the same time, baseline level of glucose, viral load, and alpha fetal protein (AFP) were different between non-SVR and SVR group ($P < 0.05$). Patients with higher level of glucose, viral load, and AFP were more difficult to achieve SVR.

Association of polymorphisms in chemokine *CC* family with HCV spontaneous clearance

Co-dominant, dominant, and additive genetic models were used to examine the impact of each SNP in chemokine *CC* gene on SVR. The results of all eight SNPs were shown in Table 3. All the variables with *P*-value < 0.05 in univariate analysis were adjusted. After adjusting for age, sex, high-risk population, ALT, and AST, logistic regression analysis discovered that mutations of *CCR1* rs3733096 and *CCL5* rs3817656 were associated with HCV spontaneous clearance ($P < 0.05$).

Patients with mutant G allele in *CCR1* rs3733096 (dominant model: OR = 2.29, 95% CI = 1.49–3.53, additive model: OR = 2.21, 95% CI = 1.50–3.25) and those with mutant C allele in *CCL5* rs3817656 (dominant model: OR = 1.37, 95% CI = 1.10–1.70, additive model: OR = 1.33, 95% CI = 1.12–1.58) were more likely to become persistent infection. They were also significant in dominant and additive models after Bonferroni correction for multiple comparisons ($P < 0.006$). Nevertheless, no significant association was shown between *CCR1* rs13096371, *CCR5* rs746492, rs1800874, *CCL3* rs1130371, *CCL8* rs1133763 and *CCL14* rs854625 variants, and spontaneous clearance in the results ($P > 0.05$).

Table 4 Stepwise regression analysis for independent factors of spontaneous clearance

Variables	Coef.	SE	OR (95%CI)	*P*-value
rs3733096	0.78	0.20	2.19 (1.48–3.22)	<0.001
rs3817656	0.27	0.09	1.32 (1.11–1.56)	0.002
population	0.38	0.09	1.46 (1.23–1.72)	<0.001
Age (≤50 vs. >50)	0.27	0.12	1.31 (1.03–1.67)	0.027
AST	0.91	0.12	2.48 (1.95–3.16)	<0.001
Cons.	−1.11	0.21	0.33 (0.22–0.49)	<0.001

Coef coefficient of variation, *SE* standard error, *OR* odds ratio, *AST* aspartate transaminase

Stepwise regression for HCV-persistent infection

Afterwards, a stepwise regression model consisted of all significant factors was built. The results indicated that rs3733096, rs3817656, high-risk population (paid blood donars), age (>50), and high level of AST were independent factors of HCV-persistent infection (Table 4).

Combined analysis and stratified analysis

We analyzed the combined effect of the two significant SNPs by adding up the number of unfavorable alleles in the next step (Table 5). The results revealed that the spontaneous clearance rate decreased when patients carrying more unfavorable *CCR1* rs3733096-G and *CCL5* rs3817656-C alleles. The spontaneous clearance rates were 43.05%, 34.66%, 25.60%, and 6.45%, respectively. The OR were increased along with the increase of risk alleles (OR = 1.34, 95% CI = 1.07–1.69; OR = 1.93, 95% CI = 1.31–2.84; OR = 8.89, 95% CI = 2.09–37.93, respectively). The risk of CHC increased from 65.34%, 74.40% to 93.55% when patients carrying one, two, and three risk alleles.

Afterwards, stratified analysis of combined effects of rs3733096 and rs3817656 with HCV-persistent infection was conducted for age, sex, population, ALT, and AST (Table 6). We found that the association between persistent infection and unfavorable genotypes was still significant in subgroups of age and sex after stratified analysis. Injecting

Table 5 Combined effects of rs3733096 and rs3817656 with spontaneous clearance

Variables	Spontaneous clearance ($N = 596$)	Persistence infection ($N = 996$)	Spontaneous clearance rate (%)	OR (95% CI)	*P*-value
0	350 (58.72)	463 (46.49)	43.05	1.00	—
1	201 (33.72)	379 (38.05)	34.66	1.34 (1.07–1.69)	0.013
2	43 (7.21)	125 (12.55)	25.60	1.93 (1.31–2.84)	0.001
3	2 (0.34)	29 (2.91)	6.45	8.89 (2.09–37.93)	0.003
Trend					$P < 0.001$[a]

Variables are numbers of combined unfavorable alleles (rs3733096-G and rs3817656-C). Logistic regression analyses adjusted for age, sex, ALT, AST, and population

[a]*P*-value was analyzed by Cochran–Armitage trend test

Table 6 Stratified analysis of combined effects of rs3733096 and rs3817656 with HCV-persistent infection

Variables	Number of unfavorable genotypes 0/1/2-3		OR (95%CI)	P^a	P^b
	Spontaneous clearance	Persistent infection			
Age					
50	161/95/22	150/131/40	1.34 (1.04–1.72)	0.025	0.472
>50	189/106/23	313/248/114	1.51 (1.23–1.86)	<0.001	
Sex					
Male	130/76/11	152/142/42	1.65 (1.23–2.21)	0.001	0.273
Female	220/125/34	311/237/112	1.34 (1.10–1.62)	0.003	
Population					
Hemodialysis patient	61/35/5	44/35/5	1.26 (0.76–2.07)	0.375	0.845
Injecting drug user	97/56/9	73/64/13	1.46 (1.01–2.11)	0.043	
Paid blood donor	192/110/31	346/280/136	1.47 (1.21–1.78)	<0.001	
ALT					
<40	276/156/34	286/214/104	1.46 (1.21–1.75)	<0.001	0.616
≥40	74/45/11	177/165/50	1.33 (0.97–1.83)	0.077	
AST					
<40	285/154/32	278/197/88	1.47 (1.22–1.77)	<0.001	0.471
≥40	65/47/13	185/182/66	1.29 (0.95–1.76)	0.105	

[a]*P*-values after adjusting for age, sex, high-risk population, ALT, and AST in additive model

[b]*P*-values of homogeneity test

drug users (OR = 1.46, 95% CI = 1.01–2.11), paid blood donors (OR = 1.47, 95% CI = 1.21–1.78), and low baseline ALT (OR = 1.46, 95% CI = 1.21–1.75) and AST level patients (OR = 1.47, 95% CI = 1.22–1.77) were significant while there was no significance in hemodialysis patients and high baseline ALT and AST level patients. Moreover, heterogeneity test discovered no statistical significance in all the subgroups ($P > 0.05$) (Table 6).

Association of polymorphisms in chemokine *CC* gene with HCV treatment response

We finally analyzed the relationship between these two significant SNPs and treatment response to evaluate whether it also makes sense in treatment cohort in the same genetic models (co-dominant, dominant, and additive). The results were listed in Table 7. As shown in the table, after adjusting for age, sex, baseline glucose, viral load, and AFP, there was no significance between the two SNPs (*CCR1* rs3733096, *CCL5* rs3817656) with treatment response. They had not been verified in the treatment cohort.

Discussion

Various studies have demonstrated that genetic variations of chemokine CC subfamily have an important role in regulation of immune response to HCV infection [22–24]. GWAS have shown that a single-nucleotide polymorphism (*IL28B* rs12979860) was the strongest and most significant genetic effect associated with natural clearance of HCV and *IL28B* rs8099917 had a primary role in the response to chronic hepatitis C interferon-alpha and ribavirin therapy [10, 11]. Nevertheless, some non-*IL28B* genetic polymorphisms tend to interfere with pathophysiological mechanisms of HCV infection. Chemokine genes do have a significant role in immunological and inflammatory processes in the pathogenesis of chronic hepatitis C as a protein family. Recent studies found that variants of chemokine gene increase the quality of prediction by *IL28B* in patients with chronic hepatitis C genotype 1 [25, 26]. There may be a potential association between genetic variants in IL28B and chemokine region. Our study is the first to discover the associations of rs3733096 and rs3817656 in chemokine *CC* gene with HCV clearance in high-risk Chinese Han populations. The results of this study confirmed that rs3733096-G and rs3817656-C alleles were associated with increased rate of HCV-persistent infection in Chinese Han population. However, we found no association of rs3733096 and rs3817656 with HCV treatment response. The results of stratified analysis showed that there was no significance between combined effect of two SNPs and HCV self-limited clearance in hemodialysis population. One possible explanation was the limited sample size of this population, which was not enough to identify the significant association.

Table 7 Associations of SNPs in gene *CC* with SVR

Genotype	N-SVR ($N = 119$)	SVR ($N = 241$)	SVR rate (%)	OR (95% CI)	*P*-value
rs3733096					
CC	109 (91.60)	228 (94.61)	67.66	1.00	—
CG	10 (8.40)	13 (5.39)	56.52	0.60 (0.21–1.67)	0.326
GG	0	0	0	1.00	—
Dominant				0.59 (0.21–1.66)	0.319
Additive				0.60 (0.21–1.67)	0.326
rs3817656					
TT	69 (57.98)	139 (57.68)	66.83	1.00	—
CT	44 (36.97)	87 (36.10)	66.41	0.93 (0.58–1.51)	0.776
CC	6 (5.05)	15 (6.22)	71.43	1.33 (0.45–3.92)	0.600
Dominant				0.97 (0.61–1.55)	0.914
Additive				1.02 (0.70–1.51)	0.893

Logistic regression analyses adjusted for age, sex, HCV-RNA, glucose, and AFP

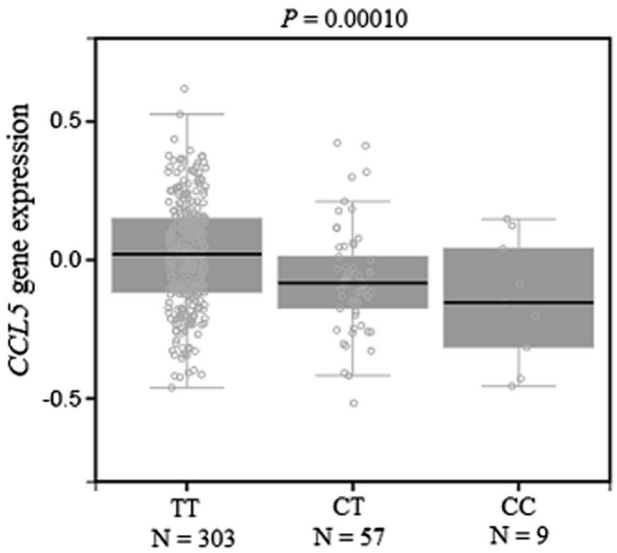

Fig. 1 Rs3817656 eQTL analysis in whole blood

The role of chemokines in viral hepatitis is of great concern, especially in CHC infection. Chemokines are involved in both immune response of liver disease progression and HCV maintenance or resolution [27]. *CCR1* and *CCL5* chemokines are mainly associated with Th1-type immune responses [28]. *CCR1* is one of the chemokine receptors which regulates inflammatory responses, and it was found to be expressed in hepatocellular carcinoma (HCC) [29, 30]. *CCL5*, the ligand of *CCR1*, was a functionally important chemokine pathway in liver fibrosis [27]. Previous studies reported that it was up-regulated in livers of patients with hepatitis C or fibrosis [31, 32]. As for the other SNPs in this study, *CCR5* rs1800874 was found to be associated with severity of rheumatoid arthritis (RA) while no significance difference was found between RA patients and controls in Korean [33]. A GWAS reported that the variant of *CCL3* rs1130371 increased the chance of HIV-associated dementia (HAD) in American, but of no statistical significance [34]. The *CCL8* rs1133763 distribution was not significantly different among patients with Alzheimer's disease (AD) or frontotemporal lobar degeneration (FTLD) and controls in European ancestry [35]. Consistent with these studies, our study has not yet found the significance of these SNPs with HCV clearance and treatment response at present.

CCR1 rs3733096 and *CCL5* rs3817656 located in the three prime untranslated regions (3′-UTR) were found to be related to HCV spontaneous clearance. To further explore the biological significance of rs3733096 and rs3817656, we attempted to find eQTL evidence based on the public GTEx database (http://www.gtexportal.org/). We discovered that there was significant association between rs3817656 genotypes and gene expression of *CCL5* in whole blood. In the boxplot from GTEx portal, we can see that the sample size was 369, including 303 samples carrying rs3817656 TT genotype, 57 samples carrying rs3817656 CT and 9 samples carrying rs3817656 CC genotype, the gene expression of *CCL5* in whole blood among these three genotypes was significantly different ($P = 0.00010$, Fig. 1). Variant of rs3817656-C was associated with downregulation of *CCL5* gene expression. However, we found no evidence of eQTL in rs3733096. On the basis of the UCSC database, we found that rs3817565 located in 3′-UTR region and H3K4Me1 histone modification peak, suggesting that it has enhancer activity. Therefore, rs3817656 T>C may influence transcription regulation.

This study first discovered the association of *CCR1* rs3733096, *CCL5* rs3817656 with HCV spontaneous clearance among high-risk Chinese Han population. We have confidence in our research, although the association

has not been discovered in the CHC treatment cohort. The sample size of our study is relatively large, and CHC individuals recruited in our study were all infected with HCV alone, without co-infection with HIV or other types of liver disease, which ensured the natural history of HCV. It is worth mentioning that many studies were aimed only at HIV/ HCV co-infected people, which were not enough to represent HCV-infected population [36, 37]. Besides, we studied both the association of *CCR1* rs3733096, *CCL5* rs3817656 with HCV spontaneous clearance, and CHC PEG IFN-α/RBV treatment response. Nevertheless, there are still some limitations that need to be taken into consideration in this study. First, the relationship between *CCR1* rs3733096, *CCL5* rs3817656, and CHC treatment response was not discovered in this study. The possible reason may be the initial case selection in the high-risk population and lack of sample sizes of treated patients. On the basis of the existing sample size, the power of the case–control study in the high-risk population obtained over 80%, while the treatment group was only 43%. Second, our study lacked information of *IL28B* genotypes and liver fibrosis, which can affect HCV-1 treatment response. In addition, in the current direct-acting antiviral (DAA) era, our study just aimed at the treatment response of IFN-based therapy. However, in most developing countries like China, the new treatments of DAA have not been widely approved. If DAA treatments are widely used in China in the future, we can carry out further research on the clinical relevance of these results.

In summary, the current research first discovered that genetic mutations of *CCR1* rs3733096 and CCL5 rs3817565 were associated with HCV spontaneous clearance. Chemokine CC-related family polymorphisms might have a vital role in HCV spontaneous clearance in Chinese Han populations.

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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