Taylor & Francis
Taylor & Francis Group

Immunological Investigations
A Journal of Molecular and Cellular Immunology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iimm20

Association between the Genetic Polymorphisms of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* with Chronic Hepatitis C Virus Infection in the Chinese Han Population

Lin-Nan Shao, Shi-Hang Zhou, Ni Wang, Shu-Ting Zhang & Ming Liu

To cite this article: Lin-Nan Shao, Shi-Hang Zhou, Ni Wang, Shu-Ting Zhang & Ming Liu (2021): Association between the Genetic Polymorphisms of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* with Chronic Hepatitis C Virus Infection in the Chinese Han Population, Immunological Investigations, DOI: 10.1080/08820139.2021.1916524

To link to this article: https://doi.org/10.1080/08820139.2021.1916524

IMMUNOLOGICAL INVESTIGATIONS
https://doi.org/10.1080/08820139.2021.1916524

Taylor & Francis
Taylor & Francis Group

Association between the Genetic Polymorphisms of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* with Chronic Hepatitis C Virus Infection in the Chinese Han Population

Lin-Nan Shao[a], Shi-Hang Zhou[a], Ni Wang[a], Shu-Ting Zhang[a], and Ming Liu[b]

[a]Dalian Blood Center, Dalian, Liaoning, China; [b]Department of Cell Biology, Dalian Medical University, Dalian, Liaoning, China

ABSTRACT

Background: Hepatitis C virus (HCV) infection is a global public health burden. Chronic HCV infection leads to the development of fibrosis, cirrhosis, liver cancer, and liver failure over time.

Methods: A total of 250 patients with chronic HCV infection and 299 healthy blood donors were recruited. Sixteen candidate single nucleotide polymorphisms (SNPs) in chemokine (C-C motif) ligand 2 (*CCL2), CCL5, CCL8*, C-C chemokine receptor 2 (*CCR2*), and *CCR5* were genotyped in all participants.

Results: The rs1024610 AA genotype was significantly associated with decreased susceptibility to chronic HCV infection. Aspartate aminotransferase (AST) levels, AST/platelet ratio index, and the fibrosis 4 score were significantly lower in the *CCL2* rs1024610 T allele and haplotype ATGC carriers. Moreover, expression levels of collagen IV (C–IV) and laminin (LN) were significantly higher in patients with the *CCL5* rs2280788 C allele compared to the non-carriers. Similarly, the expression levels of C–IV, LN, and hyaluronic acid were significantly higher in patients with the *CCL5* haplotype, TGCT. No significant differences were identified between the SNPs/haplotypes and plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* in the healthy controls, and the rs1024610 allele alteration had no effect on *CCL2* promoter activity.

Conclusions: This is the first study to report an association between *CCL2* rs1024610 and the risk of chronic HCV infection in the Chinese Han population. rs1024610 and ATGC haplotype in *CCL2* were reasonable candidate markers of liver abnormalities. rs2280788 and TGCT haplotype in *CCL5* are likely to play a significant role in liver fibrosis during chronic HCV infection.

KEYWORDS
HCV; chemokine; chemokine receptor; SNP; polymorphism

Introduction

Hepatitis C virus (HCV) infection is a global public health burden in both developing and developed countries with an estimated 71 million people infected globally (https://www.who.int/news-room/fact-sheets/detail/hepatitis-c). During acute HCV infections, only approximately 25% of patients spontaneously clear the virus, whereas the majority progress to the chronic phase (Micallef et al. 2006). Over time, chronic HCV infection leads to the development of fibrosis, cirrhosis, liver cancer, and liver failure (Arzumanyan et al. 2013).

CONTACT Ming Liu liuminglinxi@163.com Department of Cell Biology, Dalian Medical University, Dalian, Liaoning 116044, China.

Supplemental data for this article can be accessed on the publisher's website

In 2016, approximately 399 000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer). HCV is also one of the most important infectious diseases in China, with a prevalence of around 0.43%–3.2% in the general population (Chen et al. 2011).

Chemokines are a large family of small-molecule (8–12 kD) chemotactic cytokines that regulate cell trafficking (Viola and Luster 2008). They are commonly referred to as homeostatic or inflammatory depending on the context in which they are expressed. These chemokines are significantly upregulated under inflammatory conditions and may have broad target-cell selectivity (Fahey et al. 2014). The type of recruited leukocytes depends on the chemokines secreted as well as on the chemokine receptors expressed on the surface of target cells. The interactions between chemokines and their receptors help define the immune response and have a major influence on the outcome of HCV infection (Fahey et al. 2014). Several single nucleotide polymorphisms (SNPs) in chemokine and chemokine receptor genes have been reported to be associated with different outcomes of HCV infection. A study by Muhlbauer et al. (Muhlbauer et al. 2003) shows that the well-characterized SNP, rs1024611 (originally designated A-2518G or A-2578G), in the chemokine (C-C motif) ligand 2 (*CCL2*) gene is associated with the severity of fibrosis and the level of inflammation in HCV-infected patients. El-Bendary et al. (2019) demonstrated that the susceptibility to HCV infection is associated with A alleles of both rs743660 and rs1799864 in *CCR2*. Moreover, Hellier et al. (Hellier et al. 2003) screened for 20 polymorphisms in the *CCL2, CCL3, CCL5, CCL8, CCR2, CCR3*, and *CCR5* genes of HCV-positive patients and reported four main findings: (1) rs333 (*i.e., CCR5* △32) was associated with fibrosis and portal inflammation; (2) rs41469351 (*i.e., CCR5*−2132 C/T) was found to be significantly associated with susceptibility to persistent HCV infection; (3) rs2107538 (*i.e., CCL5*−403 G/A) was associated with portal inflammation; and (4) rs1133763 (*i.e., CCL8* Q46 K) mutation was associated with more severe fibrosis.

In this study, we analyzed the association of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* gene SNPs with susceptibility to chronic HCV infection. The influences of these SNPs on the clinical features of HCV infection were also investigated. This information will provide insights into the role of these SNPs in the pathogenesis of chronic HCV infection.

Methods

Patients and samples

A total of 250 Chinese Han patients with chronic HCV infection treated at Dalian Infectious Hospital between April 2015 to December 2015 were recruited; 299 healthy blood donors (18–55 years of age) living in the same region of the same ethnicity were recruited as controls. None of the patients had received previous treatment with antiviral drugs. To avoid introducing bias through sample selection, our inclusion criteria were as follows: patients with HCV infection, who did not have ascites, concurrent hepatitis B virus infection, alcohol-related liver disease, and significant pre-existing organ (heart, brain, lung, or kidney) complications. Fasting venous blood sample was collected from each patient for routine full blood count, biochemistry, liver function tests, and serum HCV RNA analysis. All data were anonymized to comply with the provisions of personal data protection legislation. Written informed consent was obtained from all participants. This study was

approved by the Dalian Blood Center Ethics Committee (No: DBC00802011). The demographic and laboratory features of chronic HCV patients are summarized in Table 1.

SNP genotyping

DNA from each patient and control individuals was extracted from peripheral lymphocytes using a DNA isolation kit (RBC Bioscience) following the manufacturer's instructions. Fifteen candidate SNPs of interest were genotyped using TaqMan genotyping assays (Applied Biosystems): *CCL2* (Chr 17) rs1024611, rs1024610, rs2857656, rs13900; *CCL5* (Chr 17) rs1065341, rs2280789, rs2280788, rs2107538; *CCL8* (Chr 17) rs3138036, rs3138037, rs1133763; *CCR2* (Chr 3) rs1799864, rs3138042; *CCR5* (Chr 3) rs1799987, rs1800024. Genotyping was performed using 20 ng of DNA under standard conditions (denaturation step 95°C for 10 min; 40 cycles at 95°C for 15 s, followed by incubation at 60°C for 1 min), with pre-read and post-read at 60°C using an ABI9700 real-time PCR system (Applied Biosystems). Moreover, *CCR5* rs333 genotyping was performed using PCR amplification of genomic DNA with the upstream primer (5′-AGGTCTTCATTACACCTGCAGC-3′) and downstream primer (5′-CTTCTCATTTCGACACCGAAGC-3′). The resulting PCR products were separated using 2% agarose gel electrophoresis (169 bp for the wild-type allele and 137 bp for the 32 bp deletion allele). The details of candidate functional SNPs are listed in Table S1.

Liver fibrosis indices

Serological tests to determine the levels of hyaluronic acid (HA), laminin (LN), collagen IV (C-IV), and amino-terminal pro-peptide of Type-III pro-collagen (PIIINP) were performed by Autobio Diagnostics Co., Ltd. (Zhengzhou, Henan Province, China). All procedures were performed following the manufacturer's instructions. The aspartate aminotransferase (AST)/platelet (PLT) ratio index (APRI) and fibrosis 4 score (FIB-4) were determined using the following equations: APRI = (AST/upper limit of normal)/(PLT×100); FIB-4 = (age×AST)/(PLT×√alanine aminotransferase (ALT)).

Table 1. Demographic and laboratory features of the chronic HCV patients.

		HCV patients
Age	Year	53.6 ± 13.6
Gender	M/F	131/119
Red blood cell	10^{12}/L	4.5 ± 0.6
Hemoglobin	g/L	137.8 ± 18.7
White blood cell	10^9/L	4.24 (3.45 ~ 5.43)
Neutrophil	10^9/L	2.19 (1.66 ~ 3.08)
Lymphocyte	10^9/L	1.67 (1.33 ~ 2.21)
Monocyte	10^9/L	0.19 (0.14 ~ 0.25)
Eosinophil	10^9/L	0.07 (0.04 ~ 0.11)
Basophil	10^9/L	0.01 (0.00 ~ 0.01)
Platelet	10^9/L	156.7 ± 63.9
Mean platelet volume	fL	10.7 ± 1.8
Platelet distribution width	%	13.1 ± 2.8
Platelet large cell ratio	%	30.9 ± 7.8
Alanine aminotransferase	U/L	126.5 (85.6 ~ 163.7)
Aspartate aminotransferase	U/L	80.9 (56.4 ~ 109.8)
Albumin	g/L	44.6 ± 5.5

Determination of chemokine levels and their receptors

ELISA kits were used for measuring the plasma levels of *CCL2, CCL5, CCL8, CCR2* (Cusabio, Wuhan, China, https://www.cusabio.com), and *CCR5* (Mlbio, Enzyme-linked Biotechnology Co., Shanghai, China) of the control subjects according to the manufacturers' instructions.

Transfection and luciferase assays

A−2174 bp to +64 bp fragment in the promotor region of the human *CCL2* gene was prepared using PCR and the two forms (A and T according to genotypes of rs1024610 A/T) of genomic DNA as a template, each of which was separately subcloned into the *Xho* I/*Hind* III site of the pGL3-Basic luciferase reporter vector (Genomeditech Co., Shanghai, China). The orientation of the cloned vectors was confirmed based on direct sequencing analysis. HEK293 (Genomeditech Co., Shanghai, China) cells were used to determine luciferase activity. For each transfection, 450 ng of the reporter constructs, 50 ng of pRL-TK vector, and 1.5 μL of lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were used. The cells were allowed to recover at 37°C for 48 h. The cells were lysed and assayed using the dual-luciferase assay system (Genomeditech Co., Shanghai, China) according to the manufacturer's instructions. Transcriptional activity was determined using a microplate reader (Infinite M1000; Tecan, Mannedorf, Switzerland). The readings were taken in triplicate for each sample. Transcriptional activity is reported as the relative luciferase activity, which is the ratio of firefly luciferase activity to Renilla luciferase activity.

Statistical analysis

Allele carriage was defined as the number of individuals carrying at least one copy of a specific allele. Allelic frequencies are defined as the number of occurrences of the test allele divided by the total number of alleles in the group. Allelic, genotypic, and haplotypic case-control data were analyzed using χ^2 or Fisher's exact test, and the relative risk estimate was evaluated based on the odds ratio (OR) at the 95% confidence interval (CI). Deviation from Hardy-Weinberg equilibrium (HWE) was assessed using the χ^2 test. Bonferroni-corrected *P*-value (*Pc*) adjustment was made to *P* values when several dependent or independent statistical tests were performed simultaneously on a single dataset. Heterozygosity and the polymorphic information content (PIC) were determined to access the degree of variability for each SNP in terms of their probability of heterozygosity and to estimate their discriminating power, respectively. The Kolmogorov-Smirnov test was used to check the data for normal distribution. If the normal distribution was confirmed, the data were expressed as mean ± standard deviation and compared using Student's *t*-test or ANOVA. In the absence of a normal distribution, data were expressed as median (interquartile range) and analyzed using the Mann-Whitney *U* or Kruskal-Wallis test. $P < .05$ were considered to indicate statistical significance. All statistical analyses were performed using SPSS version 21.0. Haplotype constructions were generated from the observed genotypes using SHEsis (Li et al. 2009) online software (http://analysis.bio-x.cn). Measures of linkage disequilibrium (the normalized disequilibrium constant, D') were calculated using Haploview 4.2 (Broad Institute, Cambridge, MA, USA).

Results

Gene polymorphisms

The genotype distributions of the 16 SNPs investigated in 250 patients with chronic HCV and 299 controls are presented in Figure 1. The distribution of SNPs in both the patients and controls were in accordance with HWE (all $P > .05$). PIC and heterozygosity values of these SNPs in patients with chronic HCV infection and controls are shown in Table S2. For biallelic codominant markers, the highest possible values obtained for PIC and heterozygosity were 0.375 and 0.5, respectively. Thus, some SNPs (such as rs1024611, rs2858656, rs13900, rs2107538, rs1799987, et al.) might be considered as having high genetic variability and reaching close to the highest possible values. The genotype/allele frequencies of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* in patients with chronic HCV infection and in the healthy controls are shown in Table 2. Compared to the healthy controls, the rs1024610 AA genotype was significantly associated with decreased susceptibility to chronic HCV infection (AA vs. AT, $P = .023$, OR = 0.559, 95% CI = 0.338–0.923, $Pc>0.05$; AA vs. AT+TT, $P = .050$, OR = 0.614, 95% CI = 0.376–1.004, $Pc > 0.05$), while no statistically significant associations were identified between other SNPs and susceptibility to chronic HCV infection. In this study, we did not detect rs333 deletion alleles in either the case or control groups; thus, the data for rs333 are not listed in the table. Compound genotype polymorphisms in chronic HCV patients and controls are shown in Table S3. The distribution of compound genotypes was not significant between the two groups. Moreover, we stratified the analyses of patients based on the liver-stiffness values (mild fibrosis < 9.6 kPa and advanced fibrosis > 9.6 kPa), age (≤ 50 years and > 50 years), and gender. We found that the allele/genotype distributions of the 16 SNPs between different groups were not significant (all $P > .05$).

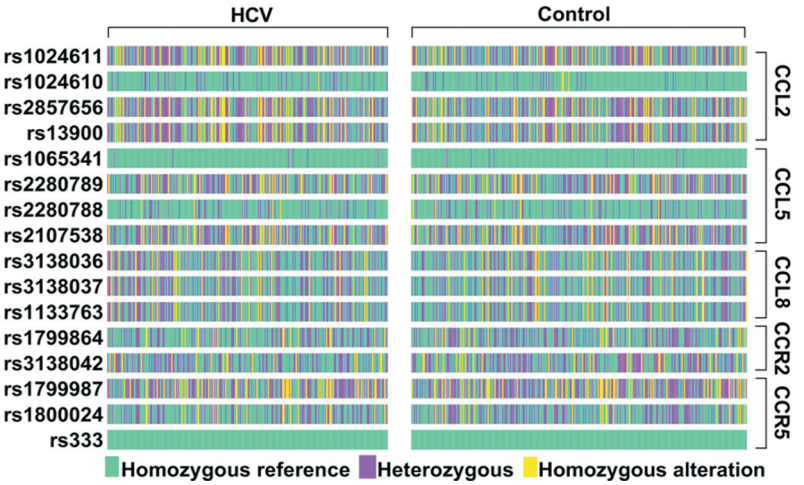

Figure 1. Genotype distributions of *CCL2, CCL5, CCL8, CCR2* and *CCR5* SNPs in patients with HCV and healthy controls. HCV, hepatitis C virus; CCL, C-C motif chemokine ligand; CCR, C-C chemokine receptor.

Table 2. The genotype/allele frequencies of *CCL2, CCL5, CCL8, CCR2* and *CCR5* in chronic HCV patients and control group.

Gene	SNP	Genotype or allele	Case (%)	Control (%)	*P*		*P*	OR (95% CI)
CCL2	rs1024611	GG	85 (34.0)	114 (38.1)	0.536	GG+AG vs. AA	0.427	0.831 (0.525–1.314)
		AG	122 (48.8)	141 (47.2)		GG vs. AG+AA	0.316	0.836 (0.589–1.187)
		AA	43 (17.2)	44 (14.7)		GG vs. AA	0.294	0.763 (0.460–1.265)
						AG vs. AA	0.623	0.885 (0.545–1.438)
		G	292 (58.4)	369 (61.7)		GG vs. AG	0.432	1.160 (0.801–1.682)
		A	208 (41.6)	229 (38.3)		G vs. A	0.265	0.871 (0.684–1.110)
	rs1024610	AA	208 (83.2)	266 (89.0)	**0.020**	AA+AT vs. TT	0.255	-
		AT	42 (16.8)	30 (10.0)		AA vs. AT+TT	**0.050***	0.614 (0.376–1.004)
		TT	0 (0.0)	3 (0.1)		AA vs. TT	0.261	-
						AT vs. TT	0.081	-
		A	457 (91.4)	563 (94.1)		AA vs. AT	**0.023***	0.559 (0.338–0.923)
		T	43 (8.6)	35 (5.9)		A vs. T	0.157	0.699 (0.440–1.109)
	rs2857656	CC	85 (34.0)	114 (38.1)	0.536	CC+GC vs. GG	0.427	0.831 (0.525–1.314)
		GC	122 (48.8)	141 (47.2)		CC vs. GC+GG	0.316	0.836 (0.589–1.187)
		GG	43 (17.2)	44 (14.7)		CC vs. GG	0.294	0.763 (0.460–1.265)
						GC vs. GG	0.623	0.885 (0.545–1.438)
		C	292 (58.4)	369 (61.7)		CC vs. GC	0.432	1.160 (0.801–1.682)
		G	208 (41.6)	229 (38.3)		C vs. G	0.265	0.871 (0.684–1.110)
	rs13900	TT	85 (34.0)	115 (38.5)	0.500	TT+TC vs. CC	0.427	0.831 (0.525–1.314)
		TC	122 (48.8)	140 (46.8)		TT vs. TC+CC	0.279	0.824 (0.581–1.170)
		CC	43 (17.2)	44 (14.7)		TT vs. CC	0.278	0.756 (0.456–1.253)
						TC vs. CC	0.643	0.892 (0.549–1.449)
		T	292 (58.4)	370 (61.9)		TT vs. TC	0.384	1.179 (0.814–1.708)
		C	208 (41.6)	228 (38.1)		T vs. C	0.241	0.865 (0.679–1.103)
CCL5	rs1065341	TT	244 (97.6)	291 (97.3)	0.838	TT+TC vs. CC	-	-
		TC	6 (2.4)	8 (2.7)		TT vs. TC+CC	0.838	1.118 (0.383–3.266)
		CC	0 (0.0)	0 (0.0)		TT vs. CC	-	-
						TC vs. CC	-	-
		T	494 (98.8)	590 (98.7)		TT vs. TC	0.838	1.118 (0.383–3.266)
		C	6 (1.2)	8 (1.3)		T vs. C	0.839	1.116 (0.385–3.239)
	rs2280789	AA	107 (42.8)	124 (41.5)	0.804	AA+AG vs. GG	0.657	0.894 (0.546–1.465)
		AG	108 (43.2)	137 (45.8)		AA vs. AG+GG	0.754	1.056 (0.752–1.484)
		GG	35 (14.0)	38 (12.7)		AA vs. GG	0.808	0.937 (0.553–1.587)
						AG vs. GG	0.560	0.856 (0.507–1.445)
		A	322 (64.4)	385 (64.4)		AA vs. AG	0.624	0.914 (0.637–1.311)
		G	178 (35.6)	213 (35.6)		A vs. G	0.995	1.001 (0.781–1.283)
	rs2280788	GG	201 (80.4)	234 (78.3)	0.811	GG+GC vs. CC	0.762	1.259 (0.351–4.514)
		GC	45 (18.0)	59 (19.7)		GG vs. GC+CC	0.538	1.139 (0.752–1.727)
		CC	4 (1.6)	6 (2.0)		GG vs. CC	0.759	1.288 (0.359–4.630)
						GC vs. CC	1.000	1.144 (0.305–4.297)
		G	447 (89.4)	527 (88.1)		GG vs. GC	0.661	0.888 (0.577–1.367)
		C	53 (10.6)	71 (11.9)		G vs. C	0.507	1.136 (0.779–1.657)
	rs2107538	CC	101 (40.4)	115 (38.5)	0.843	CC+TC vs. TT	0.848	0.953 (0.581–1.562)
		TC	115 (46.0)	145 (48.5)		CC vs. TC+TT	0.643	1.085 (0.769–1.529)
		TT	34 (13.6)	39 (13.0)		CC vs. TT	0.978	1.007 (0.592–1.715)
						TC vs. TT	0.722	0.910 (0.540–1.532)
		C	317 (63.4)	375 (62.7)		CC vs. TC	0.581	0.903 (0.629–1.297)
		T	183 (36.6)	223 (37.3)		C vs. T	0.813	1.030 (0.805–1.318)
CCL8	rs3138036	AA	125 (50.0)	164 (54.8)	0.460	AA+AG vs. GG	0.902	1.039 (0.569–1.895)
		AG	104 (41.6)	109 (36.5)		AA vs. AG+GG	0.257	0.823 (0.588–1.153)
		GG	21 (8.4)	26 (8.7)		AA vs. GG	0.855	0.944 (0.507–1.755)
						AG vs. GG	0.607	1.181 (0.626–2.228)
		A	354 (70.8)	437 (73.1)		AA vs. AG	0.215	1.252 (0.877–1.786)
		G	146 (29.2)	161 (26.9)		A vs. G	0.402	0.893 (0.686–1.163)
	rs3138037	TT	125 (50.0)	164 (54.8)	0.460	TT+TC vs. CC	0.902	1.039 (0.569–1.895)
		TC	104 (41.6)	109 (36.5)		TT vs. TC+CC	0.257	0.823 (0.588–1.153)
		CC	21 (8.4)	26 (8.7)		TT vs. CC	0.855	0.944 (0.507–1.755)
						TC vs. CC	0.607	1.181 (0.626–2.228)
		T	354 (70.8)	437 (73.1)		TT vs. TC	0.215	1.252 (0.877–1.786)
		C	146 (29.2)	161 (26.9)		T vs. C	0.402	0.893 (0.686–1.163)

(*Continued*)

Table 2. (Continued).

Gene	SNP	Genotype or allele	Case (%)	Control (%)	*P*		*P*	OR (95% CI)
	rs1133763	AA	125 (50.0)	164 (54.8)	0.460	AA+AC vs. CC	0.902	1.039 (0.569–1.895)
		AC	104 (41.6)	109 (36.5)		AA vs. AC+CC	0.257	0.823 (0.588–1.153)
		CC	21 (8.4)	26 (8.7)		AA vs. CC	0.855	0.944 (0.507–1.755)
						AC vs. CC	0.607	1.181 (0.626–2.228)
		A	354 (70.8)	437 (73.1)		AA vs. AC	0.215	1.252 (0.877–1.786)
		C	146 (29.2)	161 (26.9)		A vs. C	0.402	0.893 (0.686–1.163)
CCR2	rs1799864	GG	144 (57.6)	159 (53.2)	0.484	GG+AG vs. AA	0.796	0.918 (0.482–1.750)
		AG	87 (34.8)	119 (39.8)		GG vs. AG+AA	0.299	1.196 (0.853–1.678)
		AA	19 (7.6)	21 (7.0)		GG vs. AA	0.998	1.001 (0.517–1.937)
						AG vs. AA	0.538	0.808 (0.410–1.594)
		G	375 (75.0)	437 (73.1)		GG vs. AG	0.239	1.239 (0.867–1.770)
		A	125 (25.0)	161 (26.9)		G vs. A	0.470	1.105 (0.843–1.450)
	rs3138042	AA	131 (52.4)	140 (46.8)	0.394	AA+AG vs. GG	0.436	1.279 (0.687–2.382)
		AG	101 (40.4)	132 (44.1)		AA vs. AG+GG	0.193	1.250 (0.893–1.750)
		GG	18 (7.2)	27 (9.0)		AA vs. GG	0.299	1.404 (0.738–2.668)
						AG vs. GG	0.678	1.148 (0.599–2.199)
		A	363 (72.6)	412 (68.9)		AA vs. AG	0.262	0.818 (0.575–1.163)
		G	137 (27.4)	186 (31.1)		A vs. G	0.180	1.196 (0.921–1.554)
CCR5	rs1799987	GG	82 (32.8)	90 (30.1)	0.744	GG+AG vs. AA	0.903	0.974 (0.632–1.499)
		AG	121 (48.4)	154 (51.5)		GG vs. AG+AA	0.497	1.133 (0.790–1.627)
		AA	47 (18.8)	55 (18.4)		GG vs. AA	0.798	1.066 (0.653–1.742)
						AG vs. AA	0.718	0.919 (0.583–1.451)
		G	285 (57.0)	335 (56.0)		GG vs. AG	0.448	0.862 (0.588–1.264)
		A	215 (43.0)	263 (44.0)		G vs. A	0.744	1.041 (0.819–1.322)
	rs1800024	CC	143 (57.2)	159 (53.2)	0.488	CC+TC vs. TT	0.679	0.872 (0.454–1.672)
		TC	88 (35.2)	120 (40.1)		CC vs. TC+TT	0.345	1.177 (0.839–1.650)
		TT	19 (7.6)	20 (6.7)		CC vs. TT	0.872	0.947 (0.486–1.845)
						TC vs. TT	0.458	0.772 (0.389–1.532)
		C	374 (74.8)	438 (73.2)		CC vs. TC	0.261	0.815 (0.571–1.164)
		T	126 (25.2)	160 (26.8)		C vs. T	0.559	1.084 (0.827–1.422)

rs333 is not listed in the table. The *P* values showed in the sixth column correspond to the differences in genotypic distribution. *Not significant after Bonferroni correction.

Distribution of haplotypes

Table 3 shows the haplotypes of the *CCL2, CCL5, CCL8, CCR2*, and *CCR5* genes in patients with chronic HCV infections and healthy controls. Only haplotypes with a frequency greater than 1% are shown. We did not find a statistically significant association between the haplotypes and susceptibility to chronic HCV infection.

Linkage disequilibrium (LD) and haplotype block analysis

Results of LD and haplotype analyses in chronic HCV patients and controls are presented in Figure 2. All SNPs within the blocks were in high LD with a pairwise D' of at least 0.95. In Figure 2a, markers 1, 3, and 4 of *CCL2* (block 1) were in strong LD and the haplotype GCT was predominant, with a total prevalence of 60.2%. Moreover, the most frequent haplotype composed of markers 5, 6, and 7 of *CCL8* (block 2) was ATA, occurring with a frequency of 72.0%. The most common haplotype in block 3 was AGC (prevalence 62.7%). In Figure 2b, haplotype GGGC of *CCR5* and *CCR2* on chromosome 3 was the most frequent (29.1%).

Table 3. Haplotype frequencies of *CCL2, CCL5, CCL8, CCR2* and *CCR5* in chronic HCV patients and controls.

		Case		Control			
Gene	Haplotype	n	%	n	%	OR (95% CI)	*P*
CCL2	AAGC	168	33.5	196	32.8	1.034 (0.804–1.331)	0.794
	ATGC	41	8.1	32	5.3	1.555 (0.962–2.514)	0.069
	GACT	290	58.1	366	61.2	0.878 (0.689–1.119)	0.293
CCL5	CAGT	6	1.2	8	1.3	0.896 (0.309–2.599)	0.839
	TAGC	315	63.0	374	62.5	1.020 (0.798–1.304)	0.876
	TGCT	52	10.4	72	12.0	0.848 (0.581–1.238)	0.392
	TGGT	124	24.8	140	23.4	1.079 (0.817–1.424)	0.592
CCL8	ATA	354	70.8	437	73.1	0.893 (0.686–1.163)	0.402
	GCC	146	29.2	161	26.9	1.119 (0.860–1.458)	0.402
CCR2	AA	125	25.0	161	26.9	0.905 (0.690–1.187)	0.470
	GA	238	47.6	251	42.0	1.256 (0.989–1.595)	0.062
	GG	137	27.4	186	31.1	0.836 (0.643–1.086)	0.180
CCR5	AC	89	17.8	104	17.4	1.029 (0.753–1.405)	0.859
	AT	126	25.2	160	26.8	0.922 (0.703–1.210)	0.559
	GC	285	57.0	334	55.9	1.048 (0.825–1.331)	0.703

Haplotypes represent the following SNPs for *CCL2*: rs1024611, rs1024610, rs2857656, rs13900; *CCL5*: rs1065341, rs2280789, rs2280788, rs2107538; *CCL8*: rs3138036, rs3138037, rs1133763; *CCR2*: rs1799864, rs3138042; *CCR5*: rs1799987, rs1800024. Only haplotypes with frequency greater than 1% are shown. rs333 is not included in haplotype analysis.

Clinical parameters of chronic HCV patients and studied polymorphisms

We analyzed the relationships between the clinical parameters and polymorphisms investigated in this study (Table S4). Compared to the non-carriers, AST, APRI, and FIB-4 were significantly lower in *CCL2* rs1024610 T allele patients ($P = .003$, 0.002, and 0.027, respectively), as well as in *CCL2* haplotype ATGC carriers ($P = .004$, 0.005, and 0.023, respectively). Moreover, patients with the *CCL5* rs2280788 C allele had significantly increased the expression levels of C-IV and LN compared with those in the non-carriers ($P = .013$ and 0.021, respectively). Similarly, patients with the *CCL5* haplotype TGCT had significantly higher levels of C-IV, LN, and HA ($P = .012$, 0.014, and 0.044, respectively). The relationships between the clinical parameters and compound genotype polymorphisms are shown in Table S5. No significant differences were found between any of the compound genotypes and clinical parameters.

Plasma levels of chemokines and their receptors in controls

Plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* in the healthy controls are listed in Table 4 according to the SNPs investigated. No significant differences were identified. Results of the haplotype (frequency > 1%) analyses for plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* are listed in Table 5. We found no evidence of a significant association between haplotypes and plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5*. Plasma levels of circulating *CCL2, CCL5, CCR2*, and *CCR5* in the healthy controls were stratified according to compound genotype polymorphisms and are depicted in Table S6. No significant differences were identified.

Effects of rs1024610 A/T on promoter transcriptional activity

The potential association of rs1024610 A/T with altered promoter activity was investigated using dual-luciferase reporter gene assays. Alteration in alleles containing rs1024610 had no effect on *CCL2* promoter activity (Figure 3).

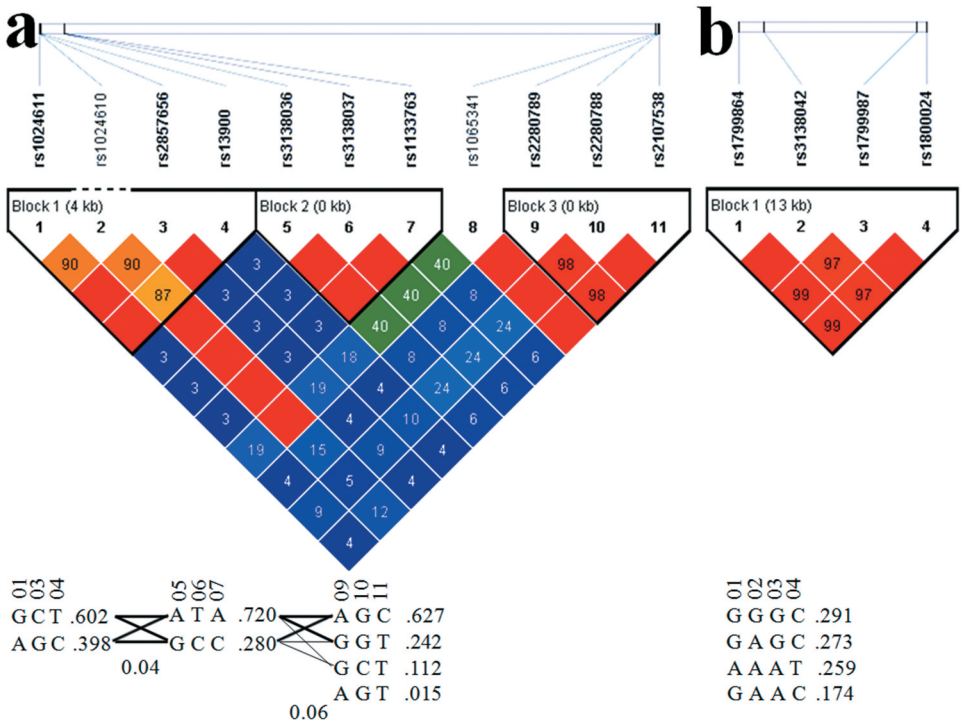

Figure 2. The linkage disequilibrium and haplotype analysis in HCV patients and controls. The figure was generated using Haploview 4.2 software. The numbers in the squares (0–100) refer to pairwise linkage disequilibrium measured as D′. Haplotype blocks are defined with D′ higher than 0.95. Haplotype frequencies in the haplotype blocks are shown under the matrix. Marker numbers are given above the haplotypes and the frequency of each haplotype within a block is given to the right of the haplotype. The thickness of the lines connecting the haplotypes across blocks represents the relative frequency with which a given haplotype is associated with the haplotype in the neighboring block. (a) Haplotype analysis of *CCL2, CCL8* and *CCL5* on chromosome 17. *CCL2* SNPs include rs1024611, rs1024610, rs2857656 and rs13900; *CCL8* SNPs include rs3138036, rs3138037 and rs1133763; and *CCL5* SNPs include rs1065341, rs2280789, rs2280788 and rs2107538. (b) Haplotype analysis of *CCR5* and *CCR2* on chromosome 3. *CCR5* SNPs include rs1799987, rs1800024 and rs333, but rs333 is not shown in the figure. *CCR2* SNPs include rs1799864 and rs3138042.

Discussion

HCV is a non-cytopathic, hepatotropic, enveloped particle measuring about 40–70 nm in diameter (Nielsen et al. 2008). It belongs to the genus *Hepacivirus* of the Flaviviridae family and has a host range limited to humans and chimpanzees (Chisari 2005). Chemokines/chemokine receptors have been shown to play an important role in the pathogenesis of HCV infection.

In this study, we investigated the correlations between 16 SNPs located in five genes (*CCL2, CCL5, CCL8, CCR2*, and *CCR5*) and the risk of chronic HCV infection. We demonstrated, for the first time, a possible role of the *CCL2* rs1024610 AA genotype in the decreased susceptibility of the Chinese Han population to chronic HCV infection. Therefore, the rs1024610 polymorphism is likely associated with spontaneous virus clearance. On the other hand, the remaining SNPs in this study, including the most well-characterized SNPs, rs1024611,

Table 4. Plasma circulating levels of *CCL2, CCL5, CCL8, CCR2* and *CCR5* in health controls stratifed according to SNPs.

Gene	Contents							
CCL2	rs1024611		rs1024610		rs2857656		rs13900	
	AA	64.7 (46.0 ~ 100.2)	AA	58.1 (27.2 ~ 92.6)	GG	59.9 (36.3 ~ 100.3)	CC	64.7 (46.0 ~ 100.2)
	AG	57.0 (24.2 ~ 89.5)	AT+TT	73.2 (50.6 ~ 89.8)	GC	57.0 (23.7 ~ 89.3)	TC	57.9 (24.2 ~ 89.7)
	GG	53.1 (35.2 ~ 99.7)			CC	54.4 (35.9 ~ 101.3)	TT	53.1 (36.1 ~ 99.7)
	P	0.332	*P*	0.141	*P*	0.26	*P*	0.317
CCL5	rs1065341		rs2280789		rs2280788		rs2107538	
	TT	33.2 (24.8 ~ 39.4)	GG	33.7 (28.0 ~ 40.0)	GG	32.8 (24.2 ~ 38.4)	TT	33.4 (28.5 ~ 39.1)
	TC	25.7 (16.8 ~ 35.8)	AG	32.8 (24.1 ~ 40.2)	GC	33.5 (28.3 ~ 40.8)	TC	33.3 (23.9 ~ 40.2)
			AA	33.2 (24.4 ~ 38.1)	CC	32.1 (26.4 ~ 54.6)	CC	33.1 (24.5 ~ 38.1)
	P	0.14	*P*	0.641	*P*	0.707	*P*	0.702
CCL8	rs3138036		rs3138037		rs1133763			
	AA	32.7 (5.4 ~ 52.5)	TT	32.7 (5.4 ~ 52.5)	AA	32.7 (5.4 ~ 52.5)		
	AG	27.4 (3.7 ~ 52.9)	TC	27.4 (3.7 ~ 52.9)	AC	27.4 (3.7 ~ 52.9)		
	GG	43.8 (18.2 ~ 63.9)	CC	43.8 (18.2 ~ 63.9)	CC	43.8 (18.2 ~ 63.9)		
	P	0.26	*P*	0.26	*P*	0.26		
CCR2	rs1799864		rs3138042					
	GG	18.9 (9.2 ~ 38.9)	AA	22.3 (13.0 ~ 37.7)				
	AG	21.9 (13.6 ~ 38.8)	AG	21.3 (10.6 ~ 41.1)				
	AA	27.3 (16.2 ~ 41.4)	GG	10.3 (4.7 ~ 30.4)				
	P	0.187	*P*	0.069				
CCR5	rs1799987		rs1800024					
	GG	68.4 (42.9 ~ 127.6)	TT	45.3 (32.6 ~ 81.7)				
	AG	65.4 (44.7 ~ 119.4)	TC	66.4 (44.8 ~ 115.5)				
	AA	69.8 (38.5 ~ 119.2)	CC	69.2 (44.1 ~ 138.9)				
	P	0.856	*P*	0.458				

Data are presented as median (interquartile range) and analyzed using Kruskal Wallis or Mann–Whitney *U* test. The unit is ng/mL for *CCL5* and pg/mL for *CCL2, CCL8, CCR2* and *CCR5*. rs333 is not included.

Table 5. Plasma circulating levels of *CCL2, CCL5, CCL8, CCR2* and *CCR5* in control subjects stratifed according to haplotypes.

Gene	Haplotype	Contents		*P*
		Carriers	Noncarriers	
CCL2	AAGC	59.7 (23.2 ~ 90.1)	57.7 (37.0 ~ 98.8)	0.147
	ATGC	74.2 (51.4 ~ 90.3)	57.6 (27.4 ~ 92.6)	0.117
	GACT	57.0 (30.4 ~ 92.0)	64.7 (48.7 ~ 96.4)	0.308
CCL5	CAGT	25.7 (16.8 ~ 35.8)	33.2 (24.7 ~ 39.3)	0.142
	TAGC	33.2 (24.3 ~ 39.3)	33.0 (27.0 ~ 39.0)	0.612
	TGCT	33.5 (28.3 ~ 41.1)	32.8 (24.2 ~ 38.4)	0.408
	TGGT	33.3 (24.2 ~ 40.2)	32.7 (24.5 ~ 38.8)	0.553
CCL8	ATA	30.1 (3.4 ~ 52.7)	43.8 (18.2 ~ 63.9)	0.125
	GCC	30.5 (3.7 ~ 55.5)	32.7 (5.4 ~ 52.5)	0.983
CCR2	AA	23.0 (13.9 ~ 38.8)	18.9 (9.2 ~ 38.9)	0.082
	GA	22.0 (11.1 ~ 38.6)	20.1 (9.5 ~ 40.0)	0.832
	GG	18.9 (9.2 ~ 40.6)	22.5 (13.6 ~ 37.8)	0.279
CCR5	AC	73.5 (45.9 ~ 136.8)	63.5 (43.2 ~ 112.9)	0.221
	AT	63.6 (43.3 ~ 111.3)	68.8 (44.1 ~ 139.7)	0.210
	GC	66.3 (44.4 ~ 125.3)	69.8 (38.5 ~ 119.2)	0.693

Data are presented as median (interquartile range) and analyzed using Mann–Whitney U test. The unit is ng/mL for *CCL5* and pg/mL for *CCL2, CCL8, CCR2* and *CCR5*. Only haplotypes with frequency greater than 1% are shown. rs333 is not included in haplotype analysis.

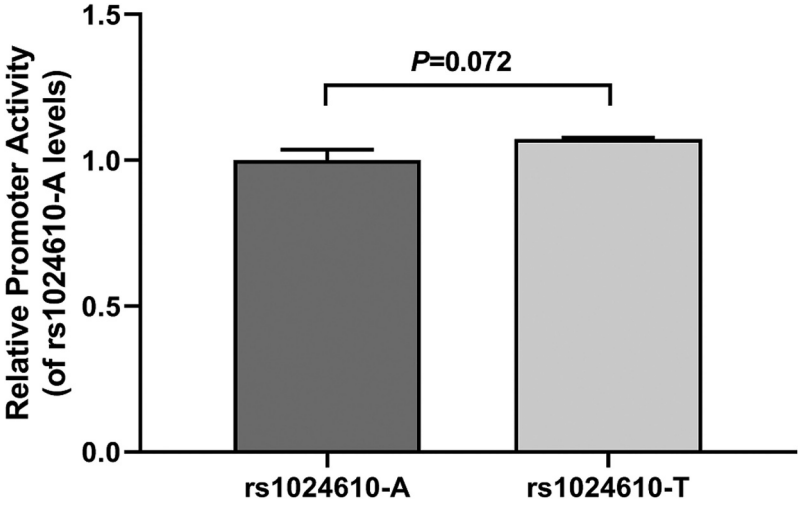

Figure 3. The influence of rs1024610 A/T on *CCL2* promoter transcriptional activity. HEK293 cells were transfected with rs1024611-A, rs1024611-T and transcriptional activity was assessed using a dual-luciferase reporter assay. The data represent mean values with standard deviations for the entire data set (three independent experiments, each in triplicate) and are expressed as relative activity of rs1024610-A.

rs2107538, rs2280788, rs2280789, rs1799864, and rs333, which have been associated with several diseases (Colobran et al. 2007; Guergnon and Combadiere 2012; Qidwai 2016), showed no associations with susceptibility to chronic HCV infection. Our results are consistent with other studies showing that rs1024611, rs1799987, rs333 (Rebbani et al. 2014), and rs1799864 (Promrat et al. 2003) are not significantly correlated with susceptibility to chronic HCV. However, contradictory results have also been reported. Several studies on genetic

polymorphism indicate that *CCR2* rs1799864 is significantly different in patients with HCV compared to the healthy controls (El-Bendary et al. 2019; Ksiaa Cheikh Rouhou et al. 2011). This discrepancy may be due to different genetics and environmental factors that contribute to disease susceptibility. For instance, in accordance with the results of our study, the 32 bp deletion polymorphism of rs333 is extremely rare in Asians; however, in other populations, genetic variations in rs333 were found to be associated with systemic lupus erythematosus (Baltus et al. 2016) and rheumatoid arthritis (Toson et al. 2017).

Haplotype analysis can be used to clarify the genetic contribution to disease susceptibility. We did not find any significant differences in haplotypes between patients with chronic HCV and healthy controls although haplotype-based analysis is generally believed to have greater power than SNP genotyping. We analyzed all participants who showed very strong or even complete LD of rs1024611, rs2857656, and rs13900 in *CCL2*, as well as rs3138036, rs3138037, and rs1133763 in *CCL8*, and our findings indicated that these SNPs can be used as proxies for each other in our population.

In our analysis of the relationships between clinical parameters and chemokine/chemokine receptor SNPs and haplotypes in chronic HCV infection, we found that carriers of the *CCL2* rs1024610 T allele and ATGC haplotype (including the rs1024610 T allele) had significantly lower levels of AST and its derivative markers, APRI and FIB-4. This finding is consistent with reports suggesting that genetic factors can influence the levels of liver enzymes (Van Beek et al. 2013). Chiraunyanann et al. (Chiraunyanann et al. 2019). Previous reports have established that chemokine *CXCL12* G801A SNP is significantly correlated with AST, APRI, and FIB-4 levels in HIV-infected Thai individuals with liver complications. Moreover, a series of reports indicate that certain HLA genes may play a protective role against HCV-induced liver injury in cases of lower ALT levels (Asti et al. 1999; Renou et al. 2002; Yu et al. 2008). Although the mechanisms responsible for these findings are not completely clear, the *CCL2* rs1024610 and ATGC haplotype are implicated as candidate markers based on their potential involvement in liver abnormalities associated with chronic HCV infection. *CCL5* is one of the best-characterized chemokines during hepatic fibrogenesis. Previous studies have demonstrated that *CCL5* mediates hepatic fibrogenesis in murine and human hepatic disease (Castera 2015; Mohs et al. 2017; Seki et al. 2009). In the current study, we identified significant associations between the rs2280788 C allele in *CCL5* and severe fibrosis (P = .013 and 0.021 for C–IV and LN, respectively). Moreover, the TGCT haplotypes (including the rs2280788 C allele) showed significantly increased expression of C–IV, LN, and HA (P = .012, 0.014, and 0.044, respectively). C–IV, LN, and HA are considered to be direct biomarkers of liver fibrosis reflecting the turnover of the extracellular matrix (Kim et al. 2014). Therefore, rs2280788 and the TGCT haplotype in *CCL5* are likely to play a significant role in the progression of liver fibrosis. Although other SNPs, such as rs1024611 in *CCL2* (Muhlbauer et al. 2003) and rs1133763 in *CCL8* (Hellier et al. 2003), have been reported to be significantly correlated with liver fibrosis in HCV infection, our results are not consistent with these reports.

We measured the plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* in healthy controls. No significant differences were identified between the SNPs/haplotypes and plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* suggesting that these SNPs/haplotypes do not influence the plasma levels of *CCL2, CCL5, CCL8, CCR2*, and *CCR5* in healthy individuals. However, contradictory findings have been reported from other studies regarding the influence of rs1024611 on the serum/plasma levels of *CCL2* in healthy controls

(Alegret et al. 2013; He et al. 2017; Joven et al. 2006; Wei et al. 2015). We speculated that this discrepancy may be owing to the following: (1) variations in the frequencies of alleles and haplotypes in different ethnic populations, which provide a potential reason that results may not always be replicated in other populations; (2) limited sample size and different inclusion/exclusion criteria of the enrolled subjects; and (3) other factors that also contribute to the plasma levels of these components.

Transcriptional factors regulate gene expression by binding to the promoter region (Sun et al. 2017). To further clarify the association between rs1024610 polymorphism in the *CCL2* promoter region and *CCL2* production, we performed transient transfection studies. In dual-luciferase reporter assays, the luciferase activity associated with the rs1024610 allele T construct was higher than that associated with the A allele construct, although this difference was not statistically significant (P = .072). This finding is consistent with our previous results showing that rs1024610 does not influence plasma levels of *CCL2*. Nevertheless, prediction of potential transcription factors using the online software, JASPAR (http://jaspar.genereg.net/), revealed that when the A allele was converted to T, the number of potential binding transcription factors increased from VENTX alone to VENTX and PAX3. Thus, we conjectured that the difference in luciferase activity may be more significant if HEK293 cells were stimulated by some ligands; however, further research is required to confirm this hypothesis.

Our study also has some limitations. There is a lack of a reference group for spontaneous viral clearance. This is a common mistake in studying the genetic susceptibility of HCV (de Brito et al. 2020; Mosaad et al. 2019; Real et al. 2019; Yang et al. 2020). To avoid introducing bias, the most appropriate control set should include patients who could spontaneously clear the virus (El-Bendary et al. 2018, 2019, 2016; Fakhir et al. 2018; Neamatallah et al. 2020). Moreover, we did not evaluate the dynamics of serological indices, and the sample size was relatively small. Therefore, further studies recruiting a spontaneous viral clearance group and enrolling a larger sample size in various populations are warranted.

This is the first study to demonstrate an association between *CCL2* rs1024610 and the risk of chronic HCV infection in the Chinese Han population. Moreover, rs1024610 and the ATGC haplotype in *CCL2* are implicated as candidate biomarkers of liver abnormalities. rs2280788 and the TGCT haplotype in *CCL5* are likely to play a significant role in liver fibrosis during chronic HCV infection.

Conflicts of interest

The authors declare no competing interests regarding the publication of this article.

Funding

This study was supported by The Dalian Municipal Youth Science and Technology Star Grant [grant number 2017RQ170].

References

Alegret JM, Aragones G, Elosua R, Beltran-Debon R, Hernandez-Aguilera A, Romero-Menor C, Camps J, Joven J. 2013. The relevance of the association between inflammation and atrial fibrillation. Eur J Clin Invest. 43:324–31.

Arzumanyan A, Reis HM, Feitelson MA. 2013. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nat Rev Cancer. 13:123–35.

Asti M, Martinetti M, Zavaglia C, Cuccia MC, Gusberti L, Tinelli C, Cividini A, Bruno S, Salvaneschi L, Ideo G, et al. 1999. Human leukocyte antigen class II and III alleles and severity of hepatitis C virus-related chronic liver disease. Hepatology. 29:1272–79.

Baltus TH, Kallaur AP, Lozovoy MA, Morimoto HK, Delongui F, Alfieri DF, Iriyoda TM, Dichi I, Simao AN, Reiche EM. 2016. CCR5Delta32 (rs333) polymorphism is associated with the susceptibility to systemic lupus erythematosus in female Brazilian patients. Rheumatol Int. 36:7–15.

Castera L. 2015. Noninvasive assessment of liver fibrosis. Dig Dis. 33:498–503.

Chen YS, Li L, Cui FQ, Xing WG, Wang L, Jia ZY, Zhou MG, Gong XH, Wang FZ, Zheng H, et al. 2011. [A sero-epidemiological study on hepatitis C in China]. Zhonghua Liu Xing Bing Xue Za Zhi. 32:888–91.

Chiraunyanann T, Changsri K, Sretapunya W, Yuenyongchaiwat K, Akekawatchai C. 2019. CXCL12 G801A polymorphism is associated with significant liver fibrosis in HIV-infected Thais: a cross-sectional study. Asian Pac J Allergy Immunol. 37:162–70.

Chisari FV. 2005. Unscrambling hepatitis C virus-host interactions. Nature. 436:930–32.

Colobran R, Pujol-Borrell R, Armengol MP, Juan M. 2007. The chemokine network. II. On how polymorphisms and alternative splicing increase the number of molecular species and configure intricate patterns of disease susceptibility. Clin Exp Immunol. 150:1–12.

de Brito WB, Queiroz MAF, da Silva Graça Amoras E, Lima SS, da Silva Conde SRS, Dos Santos EJM, Cayres-Vallinoto IMV, Ishak R, Vallinoto ACR. 2020. The TGFB1-509C/T polymorphism and elevated TGF-β1 levels are associated with chronic hepatitis C and cirrhosis. Immunobiology. 225:152002.

El-Bendary M, Neamatallah M, Elalfy H, Besheer T, Elkholi A, El-Diasty M, Elsareef M, Zahran M, El-Aarag B, Gomaa A, et al. 2018. The association of single nucleotide polymorphisms of Toll-like receptor 3, Toll-like receptor 7 and Toll-like receptor 8 genes with the susceptibility to HCV infection. Br J Biomed Sci. 75:175–81.

El-Bendary M, Neamatallah M, Elalfy H, Besheer T, El-Setouhy M, Youssef MM, Zein M, Elhammady D, Hegazy A, Esmat G. 2019a. Association of genetic polymorphisms of chemokines and their receptors with clearance or persistence of hepatitis C virus infection. Br J Biomed Sci. 76:11–16.

El-Bendary M, Neamatallah M, Elalfy H, Besheer T, Kamel E, Mousa H, Eladl AH, El-Setouhy M, El-Gilany AH, El-Waseef A, et al. 2019b. HLA class II-DRB1 alleles with hepatitis C virus infection outcome in Egypt: a multicentre family-based study. Ann Hepatol. 18:68–77.

El-Bendary M, Neamatallah M, Esmat G, Kamel E, Elalfy H, Besheer T, Eldeib D, Eladl AH, El-Setouhy M, El-Gilany AH, et al. 2016. Associations of human leucocyte antigen class II-DQB1 alleles with hepatitis C virus infection in Egyptian population: a multicentre family-based study. J Viral Hepat. 23:961–70.

Fahey S, Dempsey E, Long A. 2014. The role of chemokines in acute and chronic hepatitis C infection. Cell Mol Immunol. 11:25–40.

Fakhir FZ, Lkhider M, Badre W, Alaoui R, Meurs EF, Pineau P, Ezzikouri S, Benjelloun S. 2018. Genetic variations in toll-like receptors 7 and 8 modulate natural hepatitis C outcomes and liver disease progression. Liver Int. 38:432–42.

Guergnon J, Combadiere C. 2012. Role of chemokines polymorphisms in diseases. Immunol Lett. 145:15–22.

He J, Chen Y, Lin Y, Zhang W, Cai Y, Chen F, Liao Q, Yin Z, Wang Y, Tao S, et al. 2017. Association study of MCP-1 promoter polymorphisms with the susceptibility and progression of sepsis. PLoS One. 12:e0176781.

Hellier S, Frodsham AJ, Hennig BJ, Klenerman P, Knapp S, Ramaley P, Satsangi J, Wright M, Zhang L, Thomas HC, et al. 2003. Association of genetic variants of the chemokine receptor CCR5 and its ligands, RANTES and MCP-2, with outcome of HCV infection. Hepatology. 38:1468–76.

Joven J, Coll B, Tous M, Ferre N, Alonso-Villaverde C, Parra S, Camps J. 2006. The influence of HIV infection on the correlation between plasma concentrations of monocyte chemoattractant protein-1 and carotid atherosclerosis. Clin Chim Acta. 368:114–19.

Kim BK, Kim HS, Yoo EJ, Oh EJ, Park JY, Kim DY, Ahn SH, Han KH, Kim SU, Park YN. 2014. Risk assessment of clinical outcomes in Asian patients with chronic hepatitis B using enhanced liver fibrosis test. Hepatology. 60:1911–19.

Ksiaa Cheikh Rouhou L, Gorgi YL, Skhiri HA, Aouadi H, Ayed SJ, Sfar I, Ayed K, Ben Abdallah T. 2011. Chemokine and chemokine receptor gene polymorphism in Tunisian hemodialysis patients with HCV infection. Arab J Nephrol Transplant. 4:117–24.

Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L, Shi Y. 2009. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 19:519–23.

Micallef JM, Kaldor JM, Dore GJ. 2006. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 13:34–41.

Mohs A, Kuttkat N, Reissing J, Zimmermann HW, Sonntag R, Proudfoot A, Youssef SA, De Bruin A, Cubero FJ, Trautwein C. 2017. Functional role of CCL5/RANTES for HCC progression during chronic liver disease. J Hepatol. 66:743–53.

Mosaad YM, Metwally SS, Farag RE, Lotfy ZF, AbdelTwab HE. 2019. Association between Toll-Like Receptor 3 (TLR3) rs3775290, TLR7 rs179008, TLR9 rs352140 and Chronic HCV. Immunol Invest. 48:321–32.

Muhlbauer M, Bosserhoff AK, Hartmann A, Thasler WE, Weiss TS, Herfarth H, Lock G, Scholmerich J, Hellerbrand C. 2003. A novel MCP-1 gene polymorphism is associated with hepatic MCP-1 expression and severity of HCV-related liver disease. Gastroenterology. 125:1085–93.

Neamatallah M, El-Bendary M, Elalfy H, Besheer T, El-Maksoud MA, Elhammady D, Abed S, Elegezy M, Kandeel L, Eldeib D, et al. 2020. Impact of Toll-like Receptors 2(TLR2) and TLR 4 gene variations on HCV susceptibility, response to treatment and development of hepatocellular carcinoma in cirrhotic HCV patients. Immunol Invest. 49:462–76.

Nielsen SU, Bassendine MF, Martin C, Lowther D, Purcell PJ, King BJ, Neely D, Toms GL. 2008. Characterization of hepatitis C RNA-containing particles from human liver by density and size. J Gen Virol. 89:2507–17.

Promrat K, McDermott DH, Gonzalez CM, Kleiner DE, Koziol DE, Lessie M, Merrell M, Soza A, Heller T, Ghany M, et al. 2003. Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. Gastroenterology. 124:352–60.

Qidwai T. 2016. Chemokine genetic polymorphism in human health and disease. Immunol Lett. 176:128–38.

Real LM, Macías J, Rivero-Juárez A, Téllez F, Merino D, Moreno-Grau S, Orellana A, Gómez-Salgado J, Sáez ME, Frías M, et al. 2019. Genetic markers of lipid metabolism genes associated with low susceptibility to HCV infection. Sci Rep. 9:9054.

Rebbani K, Ezzikouri S, Marchio A, Ababou M, Kitab B, Dejean A, Kandil M, Pineau P, Benjelloun S. 2014. Common polymorphic effectors of immunity against hepatitis B and C modulate susceptibility to infection and spontaneous clearance in a Moroccan population. Infect Genet Evol. 26:1–7.

Renou C, Halfon P, Pol S, Cacoub P, Jouve E, Bronowicki JP, Arpurt JP, Rifflet H, Picon M, Causse X, et al. 2002. Histological features and HLA class II alleles in hepatitis C virus chronically infected patients with persistently normal alanine aminotransferase levels. Gut. 51:585–90.

Seki E, De Minicis S, Gwak GY, Kluwe J, Inokuchi S, Bursill CA, Llovet JM, Brenner DA, Schwabe RF. 2009. CCR1 and CCR5 promote hepatic fibrosis in mice. J Clin Invest. 119:1858–70.

Sun D, Sun L, Xu Q, Wang H, Yang J, Yuan Y. 2017. Promoter polymorphism of toll-like receptor 4 is associated with a decreased risk of coronary artery disease: a case-control study in the Chinese han population. Med Sci Monit. 23:276–84.

Toson B, dos Santos EJ, Adelino JE, Sandrin-Garcia P, Crovella S, Louzada-Junior P, Oliveira RD, Pedroza LS, de Fatima Lobato Cunha Sauma M, de Lima CP, et al. 2017. CCR5Delta32 and the genetic susceptibility to rheumatoid arthritis in admixed populations: a multicentre study. Rheumatology (Oxford). 56:495–97.

van Beek JH, de Moor MH, de Geus EJ, Lubke GH, Vink JM, Willemsen G, Boomsma DI. 2013. The genetic architecture of liver enzyme levels: GGT, ALT and AST. Behav Genet. 43:329–39.

Viola A, Luster AD. 2008. Chemokines and their receptors: drug targets in immunity and inflammation. Annu Rev Pharmacol Toxicol. 48:171–97.

Wei X, Tian Y, Lu W, Li W, Zhang M, Lu X, Liu Y. 2015. Functional polymorphisms in monocyte chemoattractant protein-1 are associated with increased susceptibility to ovarian cancer. DNA Cell Biol. 34:37–42.

Yang XJ, Wang XO, Chen Y, Ye SD. 2020. Associations of content and gene polymorphism of macrophage inhibitory factor-1 and chronic hepatitis C virus infection. World J Gastroenterol. 26:6378–90.

Yu RB, Hong X, Ding WL, Tan YF, Zhang YX, Sun NX, Wu GL, Zhan SW, Ge DF. 2008. The association between the genetic polymorphism of HLA-DQA1, DQB1, and DRB1 and serum alanine aminotransferase levels in chronic hepatitis C in the Chinese population. J Gastroenterol Hepatol. 23:1394–402.