BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2019, VOL. 76, NO. 1, 11–16
https://doi.org/10.1080/09674845.2018.1518299

Association of genetic polymorphisms of chemokines and their receptors with clearance or persistence of hepatitis C virus infection

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ABSTRACT

Background: Polymorphisms of certain genes may have an effect on either persistence of infection or spontaneous clearance of hepatitis C virus (HCV). We hypothesized that one or more variants of chemokines (CCL2 and CCL5) and chemokine receptors (CC chemokine receptor type 2 [CCR2]) genes are associated with the susceptibility to HCV infection.

Methods: We recruited 1460 patients with chronic HCV (CHC), 108 subjects with spontaneous virus clearance (SVC) and 1446 individuals as a healthy control group. All were genotyped for single nucleotide polymorphisms: rs13900 C/T of *CCL2*, rs3817655 T/A of *CCL5* and rs743660 G/A and rs1799864 G/A of *CCR2* using allelic discrimination real-time PCR technique.

Results: The carriage of the A allele of *CCR2* rs743660 was significantly higher in CHC compared to SVC (odds ratio [OR] 4.03) and to controls (1.42) and in controls compared to SVC (2.85) (all $P < 0.01$). Similarly, the A allele of *CCR2* rs1799864 was significantly higher in the CHC group when compared with both SVC (1.97) and controls (2.13) (both $P < 0.01$), but the OR between controls and SVC was not significant (1.08, $P = 0.723$). Carriage of C allele of *CCL2* rs13900 and the T allele of *CCL5* rs3817655 were significantly higher in SVC group when compared with both CHC (OR = 0.19 and OR = 0.24, respectively) and control groups (OR = 0.65 and OR = 0.45, respectively [all $P < 0.01$]).

Conclusions: Susceptibility to HCV infection is associated with A alleles of both (rs743660 and rs1799864 G/A) of *CCR2* while spontaneous clearance of HCV is associated with the C allele of rs13900 of CCL2 and T allele of rs3817655 of *CCL5*.

ARTICLE HISTORY
Received 2 August 2018
Accepted 21 August 2018

KEYWORDS
Genetic polymorphism; chemokines; HCV susceptibility; viral clearance; intrafamilial

Introduction

Infection with hepatitis C virus (HCV) is a major health problem affecting 170 million people worldwide. Approximately 20% of infected cases are able to clear the virus, the remainder individuals developing chronicity accompanied with significant morbidity and mortality, mainly as a result of disease progression towards cirrhosis and hepatocellular carcinoma (HCC) [1]. Although HCV is often transmitted through direct percutaneous exposure to infected blood, the role of intrafamilial transmission has not been defined with regards to nonsexual household exposures. Among these contacts of index cases, the reappearance of anti-HCV seropositivity was found to be 13.7% in Egypt [2].

Chemokines belong to a family of small chemotactic glycoproteins measuring 8–12 kDa that control cell recruitment [3]. Based on the circumstances under which their expression is dependent, chemokines are usually referred to as either homeostatic or pro-inflammatory [4]. The latter initiate the signalling pathways by which leukocytes undergo migration and extrusion from blood into tissues through ligation with their cognate receptors [5]. Hence, chemokines and chemokine receptors are the primary factors involved in leukocyte aggregation at immune response sites. Four main subfamilies comprise the broader family of chemokines: CXC (α), CC (β), XC (γ) and CX3C (δ) [6,7]. Expression of CC chemokine receptor type 2 (CCR2) occurs predominantly on macrophages and monocytes, as well as dendritic cells (DCs) and T cells. Ligands for CCR2 include CCL2, also known as monocyte chemotactic protein-1, CCL7, CCL8 and CCL13. While all these ligands are widely expressed in a hepatic setting, livers of HCV-infected patients have significantly increased transcription levels of *CCR2* and *CCL2* mRNA [8].

In the absence of animal models, understanding the pathogenesis of the disease is determined mainly by observational studies of HCV-infected patients and *in vitro* experiments. Examination of liver tissues from HCV patients using expression studies has established a definitive role of chemokines in the pathogenesis of HCV infection, a finding confirmed by studies demonstrating a connection between various polymorphisms in

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chemokine and chemokine receptor genes and altered susceptibility to HCV infection, as well as disease progression and outcome [9]. We hypothesized that one or more variants of chemokines (*CCL2* and *CCL5*) and chemokine receptors (*CCR2*) single nucleotide polymorphism (SNPs) are associated with previous or present HCV infection.

Materials and methods

We tested our hypothesis in 3014 people from 845 families in a multicentre case-control study conducted at Molecular Genetic Unit in Endemic Hepatogastroenterology and Infectious Diseases in the Faculty of Medicine of Mansoura University in Egypt during the period between January 2013 and May 2017. All subjects gave written informed consent, and the approval of University Institutional Review Board was obtained. Subjects were categorized into three different groups similar to a paper on Toll-like receptors recently published in this journal [10]. The 1460 patients with chronic hepatitis C infection (CHC) included index patients or household contacts who were anti-HCV antibody positive with detectable HCV-RNA. The second group of 108 patients, the spontaneous virus clearance (SVC) group, had anti-HCV antibodies in absence of detectable HCV-RNA when measured successively in two samples taken at least 6 months apart, never having undergone prior antiviral treatment. The third group was 1446 healthy household contacts as a negative control group. Inclusion and exclusion criteria of both the index and contacts subjects were enrolled as described previously [10,11]. Criteria for inclusion of index cases were adults of both sexes aged above 18, PCR detection of HCV-RNA positivity for >6 months and presence of HCV-related liver disease regardless of stage. Exclusion criteria were co-infecting with hepatitis B (presence of HBV core antibodies) or HIV, as well as patients with anti-HCV positivity in absence of detectable serum HCV-RNA, patients with HCC, autoimmune hepatitis and any metabolic liver disease. Inclusion criteria for healthy household contacts used as control subjects admitted individuals of both sexes aged above 18 years who were related to the index case by no more than two degrees and, hence, shared common family activities with exposure to HCV infection for at least 15 years. These individuals presented with no serological evidence of HCV or HBV, and no history of HCC or any other liver disease.

Three millilitre blood samples were taken. For DNA analysis, 1 ml was taken into K_2EDTA, aliquoted, and stored at −50 °C. The remainder was placed in plain tubes, centrifuged at 4000 rpm for 10 min, the serum being aliquoted and stored at −50 °C. Antibodies to HCV were determined by ELISA (Abbott Laboratories, Abbott Park, IL, USA); quantitative PCR was used to measure HCV in seropositive patients as previously described [10]. The commercial Qiagen DNA isolation kit (Qiamp® DNA Minikit, Qiagen, Germany) was used in accordance to the manufacturer's guidelines to extract and purify genomic DNA from leucocytes. Four SNPs of chemokine receptor and ligand 2 and 5 genes were analysed. Each DNA sample underwent allelic genotyping with real-time PCR (Model 7500, Applied Biosystems, Foster City, CA, USA) employing fluorescein-amidite-labelled SNP primers and probes for these SNPs (Figure 1). These probes and primers were ready-made and also purchased from Applied Biosystems, and comprised one primer and probe for each gene, namely *CCR2* rs743660, *CCR2* rs1799864, *CCL2* rs13900 and *CCL5* rs3817655. TaqMan® Universal Master Mix II (2×) was also supplied from Applied Biosystems, as were the DNA templates, RNase free water, and optical plate (MicroAmp® Optical 96-Well Reaction Plate, Applied Biosystems).

Qualitative variables, presented as frequencies and percentages, were compared using Chi-square (χ^2) and Fisher's exact test (SPSS v16). Quantitative variables were tested for normality distribution by the Shapiro test. Normally distributed variables are presented as mean (SD). One-way ANOVA was used to test for significant differences between the three groups with Tukey's *post-hoc* multiple comparisons. Non-parametric variables are presented as median (1st quartile–3rd quartile) and Kruskall–Wallis was used to test for the significance between the three groups. Mann–Whitney test was used for *post-hoc* multiple comparison. χ^2 tests were also used to calculate Hardy–Weinberg equilibrium (HWE) in each group separately. The number of persons carrying at least one copy of a certain allele is defined as allele carriage, whereas allele frequency is characterized as the number of appearances of the test allele when divided by the total allele number in the group. MedCalc software was used to calculate odds ratio and 95% confidence interval (CI) for a specific allele carriage in comparison to no carriage of that target allele. A *P*-value of ≤0.05 was regarded as significant. Modification of *P* value was made using Bonferroni-corrected *P* value when several statistical tests, both dependent and independent, were simultaneously performed on a solitary data set [12].

Results

Clinical and laboratory characteristics of studied groups are displayed in Table 1 showing differences in age, sex and (as expected) almost all laboratory indices. All samples were successfully genotyped for rs13900 while 100 samples from CHC group failed to be genotyped for the other three SNPs. Table 2 shows the distribution of allele carriage and allele frequency, HWE and heterozygosity of different SNPs among the different groups. The heterozygosity of all the SNPs in the different groups ranged from 0.19 to 0.51, attesting to the suitability of these SNPs for genetic analysis testing in the study groups.

Table 3 and Figure 1 demonstrate that the carriage of C allele of *CCL2* rs13900 C/T polymorphism was

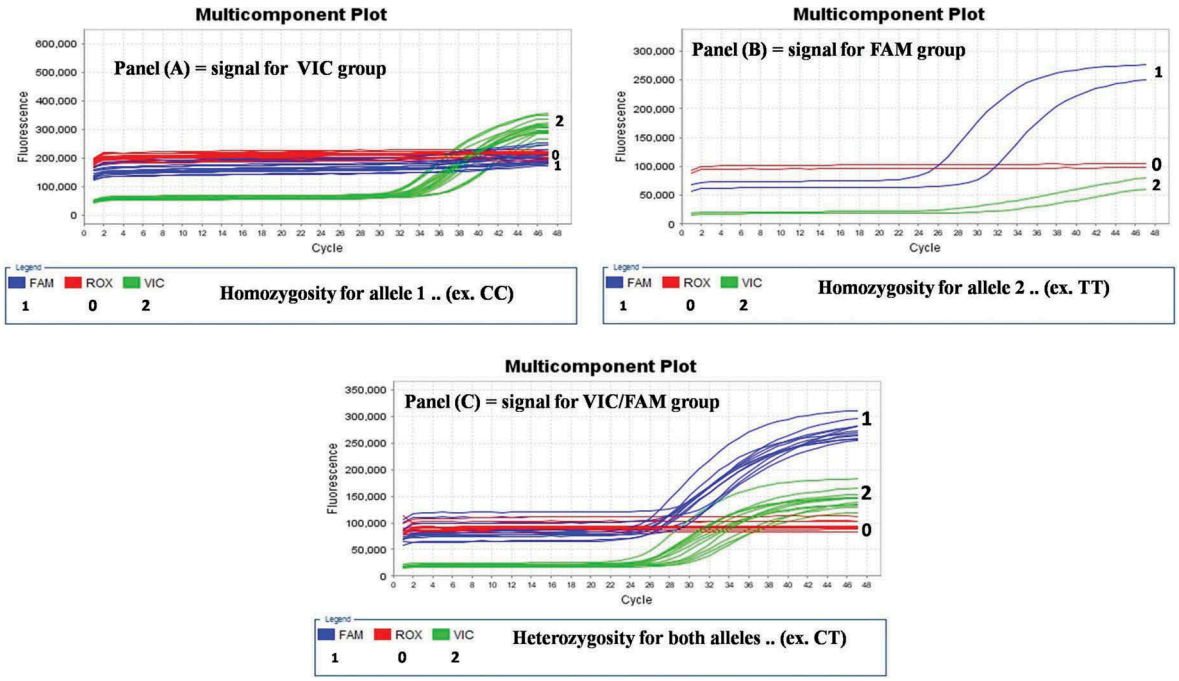

Figure 1. The output file of allele discrimination software plot and multicomponent plot of rs13900 C/T SNP of CCL2 gene: (a) the CT genotyping subjects (carrying the C and T allele) (heterozygote), (b) the CC genotyping subjects (carrying the C allele only) (homozygote) and (c) the TT genotyping subjects (carrying the T allele only) (homozygote). FAM dye: blue colour [1], VIC dye: green colour [2], ROX: red colour [0].

Table 1. Clinical and laboratory characteristics of studied groups.

	Control subject (1446)	SVC (108)	CHC (1460)	Significance (*P* value)
Sex: M/F	621/825	71/37	843/617	<0.001
Age (years)	33.2 (23.9)^{A,B}	43.0 (10.5)^{A}	39.5 (16.0)^{B}	<0.001
Bilirubin (μmol/L)	14 (3)^{B}	15 (3)^{C}	24 (12)^{B,C}	<0.001
Albumin (μmol/L)	69 (7.5)^{B}	65 (7)^{C}	57 (9)^{B,C}	<0.001
AST (IU/L)	19 (6)^{B}	22 (5)^{C}	45 (11)^{B,C}	<0.001
ALT (IU/L)	20 (8)^{B}	19 (5)^{C}	52 (11)^{B,C}	<0.001
ALP (U/L)	48 (18)^{A,B}	62 (18)^{A,C}	78 (16)^{B,C}	<0.001
AFP (ng/mL)	1 (1,2)^{A}	7 (5–8)^{A}	12 (5–24)^{A,B}	<0.001
ANA (U)	0.4 (0.4–0.5)	0.4 (0.4–0.6)	0.4 (0.4–0.6)	0.8
Creatinine (μmol/L)	71 (62–80)^{A,B}	80 (71–88)^{A,C}	88 (71–89)^{B,C}	<0.001

Data mean (SD) or median (IQR). AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; AFP: alpha-fetoprotein; ANA: antinuclear antibodies; SVC: spontaneous virus clearance; CHC: chronic hepatitis C infection. ^{A,B,C}Significant difference at $P < 0.05$ between corresponding groups by Tukey's test or Mann–Whitney test *post-hoc* multiple comparison as appropriate.

significantly lower in the CHC group when compared to that of both SVC group and the control group and that it was lower in controls compared to the SVC group. This signifies that the C allele of rs13900 C/T polymorphism is a protective allele against development of CHC. Similarly, the allele carriage of T allele of *CCL5* rs3817655 T/A polymorphism was significantly lower in the CHC group in comparison to that of both SVC group and control group, and the control group compared to the SVC group, again indicative of the T allele of *CCL5* rs3817655T/A polymorphism being protective against development of CHC. Conversely, the carriage of allele A of *CCR2* rs743660 G/A polymorphism was shown to be significantly higher in the CHC group when compared to that of both SVC group and control group, and in the controls versus the SVC group, suggesting that the allele A of *CCR2* rs743660 G/A polymorphism is a risk allele for development of CHC. In addition, the allele carriage of at least one copy of A allele of *CCR2* rs1799864 G/A polymorphism (also known as CCR2-V64I) was found to be significantly higher in the CHC group when compared to that of both SVC group and control group, respectively, suggesting once again that the A allele of rs1799864 G/A polymorphism proposes higher risk for development of CHC.

Discussion

Of all individuals infected with HCV, at least 70% develop of chronic disease, with 20–50% of patients advancing to cirrhosis and 1–2% to HCC within a period of 10–20 years [13]. Chemokines play a crucial role in regulation of host immunity and modulation of

Table 2. Allele carriages and allele frequencies of candidate SNPs.

	CCL2 rs13900 C/T			CCl5 rs3817655 A/G			CCR2 rs743660 A/G			CCR2 rs743660 A/G		
	SVC (108)	Control (1446)	CHC (1460)	SVC (108)	Control (1446)	CHC (1360)	SVC (108)	Control(1446)	CHC (1360)	SVC (108)	Control (1446)	CHC (1360)
Wild allele	85 (78.7%)	771 (53.3%)	610 (41.78%)	70 (64.8%)	725 (50.1%)	421 (31%)	88 (81.5%)	878 (60.7%)	710 (52.2%)	79 (73.2%)	1080 (74.7%)	790 (58.1%)
Heterozygous	21 (19.4%)	589 (40.7%)	645 (44.18%)	33 (30.6%)	603 (41.7%)	694 (51%)	19 (17.6%)	483 (33.4%)	525 (38.6%)	25 (23.1%)	336 (23.3%)	476 (35%)
Rare allele	2 (1.85%)	86 (5.95%)	205 (14.04%)	5 (4.6%)	118 (8.2%)	245 (18%)	1 (0.9%)	85 (5.9%)	125 (9.2%)	4 (3.7%)	30 (2%)	94 (6.9%)
MAF	0.15	0.26	0.36	0.2	0.29	0.44	0.10	0.23	0.29	0.15	0.14	0.24
PIC	0.20	0.33	0.35	0.25	0.36	0.40	0.17	0.30	0.30	0.21	0.24	0.32
Heterozygosity	0.19	0.41	0.44	0.31	0.42	0.51	0.18	0.33	0.39	0.23	0.23	0.35
No of alleles	216	2892	2920	216	2892	2720	216	2892	2720	216	2892	2720
Hardy Weinberg *P*	0.60	0.06	0.10	0.66	0.64	0.16	0.98	0.09	0.05	0.27	0.52	0.06

MAF: Minor allele frequency; PIC: polymorphic information content >0.1; CHC: chronic hepatitis C; SVC: spontaneous virus clearance. Rare alleles = Minor allele; the minor allele for CCL2 rs13900 is T, the major is C; the minor allele for CCl5 rs3817655 is G allele while the major is A; the minor allele for CCR2rs743660 is G allele, the major is A; the minor allele for CCR2 rs743660 is G allele, the major is A.

inflammation during every stage of HCV infection through activation of cytokine cascades that determine the immune response of the host to the virus [14], including expression of high levels of CCR5 receptor by many of the cells infiltrating the liver. Furthermore, an HCV-infected liver demonstrates increased levels of the chemokines CCL3, CCL4 and CCL5 [15]. However, continuous expression of chemokines in the setting of chronic infection supplies the driving force for chronic inflammation which, when associated with lack of effective host antiviral immunity, leads to consequent liver injury culminating in cirrhosis and HCC [9].

Several genetic polymorphism studies showed that the association of SNPs of several genes with HCV susceptibility, response to treatment, progression to end stage liver diseases and development of HCC [16,17]. Genetic analysis of subjects taking part in the current study demonstrates the contribution of *CCL2, CCR2* and *CCL5* in the pathogenesis of HCV infection. The allele frequency of the current study is consistent with the published global frequency, indicating that the allele A of *CCR2* rs743660 and the allele A of *CCR2* rs1799864 can be considered risk alleles, while the allele C of *CCL2* rs13900 and the allele T of *CCL5* rs3817655 may be regarded as protective alleles.

A previous study of haemodialysis patients with HCV reported that the frequency of the *CCR2* rs1799864 genotype was found to be significantly increased in the HCV-infected patients group when compared to control group, but no significant difference was detected when compared to the clearance group [18]. Another study reported that the *CCR2* rs1799864 was linked to the inability to eliminate the virus in individuals infected with HCV [19]. Conversely, others [20] reported that *CCR2* rs1799864 was no more frequent in HCV patients than in a control group, and that *CCR2* rs1799864 G/A polymorphism showed no significant association with HCV. Analysis of the haplotype of *CCR2* and *CCL5* genotypes additionally failed to detect any relationship with HCV clearance [21,22]. The expression of chemokine receptor CCR2 occurs predominantly by memory T lymphocytes, DCs, monocytes, B cells, basophils and occasionally neutrophils under certain conditions. The affiliation of receptor to ligand is demonstrated by the apparent inclination of CCR2 for ligands CCL2, CCL7, CCL8 and CCL13 ligands [23].

CCL2 is the member of the CC chemokine subfamily that is produced by various immune and non-immune cells including macrophages, neutrophils, lymphocytes, vascular endothelial cells, fibroblasts, keratinocytes and various cancer cell lines, in response to stimulation by a large group of mediators that include cytokines, lipopolysaccharides and growth factors [23]. Transcription of *CCL2* and *CCR2* mRNA is markedly increased in the livers of HCV-infected patients, while elevated serum CCL2 was also associated with escalating liver inflammation

Table 3. Association of SNPs of minor alleles in chemokines and their receptors.

Candidate alleles	CHC vs. SVC	CHC vs. control	Control vs. SVC
CCL2 rs13900 (C)			
Odds ratio (95% CI)	0.19 (0.12–0.31)	0.65 (0.56–0.75)	0.30 (0.19–0.48)
P	0.0003	0.0003	0.0001
CCL5 rs3817655 (T)			
Odds ratio (95% CI)	0.24 (0.16–0.37)	0.45 (0.16–0.37)	0.55 (0.36–0.82)
P	0.0001	0.001	0.0108
CCR2 rs743660 (A)			
Odds ratio (95% CI)	4.03 (2.45–6.62)	1.42 (1.22–1.64)	2.85 (1.73–4.68)
P	0.0001	0.001	0.001
CCR2 rs1799864 (A)			
Odds ratio (95% CI)	1.97 (1.27–3.05)	2.13 (1.81–2.50)	1.08 (0.70–1.69)
P	0.0078	0.0001	0.7229

95% CI: Confidence interval. Risk allele: odd ratio >1, protective allele: odd ratio <1. *P* = corrected *P* value.
CHC: Chronic hepatitis C infection; SVC: spontaneous virus clearance.

when infected individuals were compared to healthy control subjects. IFN-inducible CCL2 is released by Kupffer cells early in the infection, resulting in stimulation of infiltrating monocytes including $CCR2^+$ plasmacytoid DCs. During persistent HCV infections, CCR2 expressing $CD8^+$ T cells are also enhanced in the inflamed liver [8].

Interaction of CCL5 with CCR5 is probably most significant during HCV infection, where clearance of the virus from infected hepatocytes is mediated by recruitment of T cells to the liver parenchyma [24]. This is evidenced by considerable increase in levels of CCL5, tumour necrosis factor alpha, malondialdehyde and nitric oxide in HCV-infected patients when compared with a control group, levels which exhibited significant positive correlation with the HCV-RNA viral load [25]. Although there are no data in the literature on the association of *CCL5* rs3817655 with susceptibility to HCV infection, available data report that CCL5-403 G/A SNP is associated with decreased risk of severe inflammation in HCV-seropositive patients who are heterozygous or homozygous for the *CCL5* promoter alleles −403*A [22,26]. Furthermore, current findings suggest that *CCL5* rs3817655 T/A polymorphism has a protective role against HCV infection. In contrast, no association was found between *CCL5* polymorphisms and HCV infection disease outcome or severity [23]. Another study demonstrated that presence of both haplotype D and E of *CCL5* was detected at lower frequencies in patients seropositive for HCV in comparison to their seronegative counterparts, in contrast to the frequency of *CCL5* haplotype C which was higher in HCV-seropositive patients compared to those who were seronegative [27]. There is also compelling *in vitro* and *in vivo* evidence of the important role of *CCL5* as a mediator of experimental liver fibrosis [28]. Another polymorphism possibly associated with degree of inflammation in HCV infection is CCR5Δ32, although results are conflicting. Frequency of the CCR5Δ32-polymorphism decreases from northern to southern Europe and is completely missing in African and Asian cohorts [15].

Some of the contradictory findings from different analytical studies of the effect of chemokine gene mutations may be clarified by the complex role that chemokines play during the distinct stages of infection with HCV, signifying the intricacy of targeting chemokines for therapeutic benefits [29]. This work represents an advance in medical science because it shows that SNPs in genes for chemokines and their receptors may be associated with HCV infection – either resolution of the infection or the development of a chronic persistent infection.

Summary table

What is known about this subject:
- The final outcome of HCV infection is either spontaneous recovery (20%) or development of chronic infection (80%) with progression to end stage.
- Polymorphisms in chemokines genes and their receptors may have an effect on persistence of HCV infection or spontaneous clearance of the virus.
- Studies of chemokines genetic polymorphisms with the susceptibility to HCV infection showed conflicting results in different population.

What this paper adds:
- Susceptibility to HCV infection was associated with A alleles of both (rs743660 and rs1799864 G/A) of *CCR2*.
- Spontaneous clearance of HCV was associated with C allele of rs13900 of CCL2 gene and T allele of rs3817655 of *CCL5*.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by Science and Technology Development Foundation (STDF): [Project No. 1784 (TC/2/Health/2009/hep-1.3)].

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References

[1] Koike K. The oncogenic role of hepatitis C virus. Recent Results Cancer Res. 2014;193:97–111.
[2] El-Bendary M, Esmat G, Neamatallah M, et al. Epidemiological aspects of intrafamilial spread of HCV infection in Egyptian population: a pilot study. Open J Gastroenterol. 2014;4(05):228.
[3] Viola A, Luster AD. Chemokines and their receptors: drug targets in immunity and inflammation. Annu Rev Pharmacol Toxicol. 2008;48:171–197.
[4] Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000;12 (2):121–127.
[5] Kehrl JH. Chemoattractant receptor signaling and the control of lymphocyte migration. Immunol Res. 2006;34(3):211–227.
[6] Mellado M, Rodríguez-Frade JM, Mañes S, et al. Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. Annu Rev Immunol. 2001;19(1):397–421.
[7] Eberlein J, Nguyen TT, Victorino F, et al. Comprehensive assessment of chemokine expression profiles by flow cytometry. J Clin Invest. 2010;120 (3):907.
[8] Fahey S, Dempsey E, Long A. The role of chemokines in acute and chronic hepatitis C infection. Cell Mol Immunol. 2014;11(1):25–40.
[9] Heydtmann M, Adams DH. Chemokines in the immunopathogenesis of hepatitis C infection. Hepatology. 2009;49(2):676–688.
[10] El-Bendary M, Neamatallah M, Elalfy H, et al. 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. 2018;75:175–181.
[11] El-Bendary M, Neamatallah M, Esmat G, et al. 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. 2016;23(12):961–970.
[12] Perneger TV. What's wrong with Bonferroni adjustments. BMJ. 1998;316(7139):1236.
[13] Ruiz-Ferrer M, Barroso N, Antinolo G, et al. Analysis of CCR5-Δ32 and CCR2-V64I polymorphisms in a cohort of Spanish HCV patients using real-time polymerase chain reaction and fluorescence resonance energy transfer technologies. J Viral Hepat. 2004;11(4):319–323.
[14] Kang W, Shin E-C. Clinical implications of chemokines in acute and chronic hepatitis C virus infection. Yonsei Med J. 2011;52(6):871–878.
[15] Coenen M, Nattermann J. The role of CCR5 in HCV infection. Eur J Med Res. 2010;15(3):97.
[16] Micallef J, Kaldor J, Dore G. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 2006;13(1):34–41.
[17] Attallah A, Omran D, Marie M, et al. IL-28B rs12979860 polymorphism affect the course of chronic hepatitis and the development of HCC in Egyptian patients with hepatitis C type 4. B J Biomed Sci. 2018;75:157–162.
[18] Rouhou LKC, Gorgi YL, Skhiri HA, et al. Chemokine and chemokine receptor gene polymorphism in Tunisian hemodialysis patients with HCV infection. Arab J Nephrol Transplant. 2011;4(3):117–124.
[19] Mascheretti S, Hinrichsen H, Ross S, et al. Genetic variants in the CCR gene cluster and spontaneous viral elimination in hepatitis C-infected patients. Clin Exp Immunol. 2004;136(2):328–333.
[20] Promrat K, McDermott DH, Gonzalez CM, et al. Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. Gastroenterol. 2003;124(2):352–360.
[21] Goulding C, Murphy A, MacDonald G, et al. The CCR5-Δ32 mutation: impact on disease outcome in individuals with hepatitis C infection from a single source. Gut. 2005;54(8):1157–1161.
[22] Hellier S, Frodsham AJ, Hennig BJ, et al. Association of genetic variants of the chemokine receptor CCR5 and its ligands, RANTES and MCP-2, with outcome of HCV infection. Hepatology. 2003;38(6):1468–1476.
[23] Navratilova Z. Polymorphisms in CCL2&CCL5 chemokines/chemokine receptors genes and their association with diseases. Biomed Pap. 2006;150(2):191–204.
[24] Heydtmann M, Shields P, McCaughan G, et al. Cytokines and chemokines in the immune response to hepatitis C infection. Curr Opin Infect Dis. 2001;14 (3):279–287.
[25] Tawadrous GA, Aziz AA, Amin DG, et al. RANTES, TNF-α, oxidative stress, and hematological abnormalities in hepatitis C virus infection. J Invest Med. 2012;60 (6):878–882.
[26] Promrat K, Liang TJ. Chemokine systems and hepatitis C virus infection: is truth in the genes of the beholders? Hepatology. 2003;38(6):1359–1362.
[27] Huik K, Avi R, Pauskar M, et al. A CCL5 haplotype is associated with low seropositivity rate of HCV infection in people who inject drugs. PLoS One. 2016;11 (6):e0156850.
[28] Berres M-L, Koenen RR, Rueland A, et al. Antagonism of the chemokine Ccl5 ameliorates experimental liver fibrosis in mice. J Clin Invest. 2010;120(11):4129–4140.
[29] Heydtmann M, Adams DH. chemokines in the immunopathogenesis of hepatitis c infection. Hepatology. 2009;49(2):676–688.