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Original Article

Chemokine and Chemokine Receptor Gene Polymorphism in Tunisian Hemodialysis Patients with HCV Infection

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Abstract

Introduction: Our aim was to investigate the possibility of a significant relationship between chemokines and chemokine receptor genes polymorphisms and the spontaneous clearance or the persistence of HCV infection.

Methods: A total of 96 hemodialysis (HD) patients infected with HCV were classified into two groups: G1 included 73 patients with persistently positive HCV-RNA and G2 included 23 HD patients who have spontaneously eliminated the virus. The control group consisted of 170 healthy blood donors. All subjects were genotyped for CCR5 Δ32, CCR5 (-59029) A/G, CCR2 (64Ile) and MCP-1(-2518) A/G gene polymorphisms.

Results: Our results showed statistically significant increased frequencies of the CCR2 (64Ile) and the (-59029) CCR5 A alleles in patients infected with HCV (22.1% and 35.9%) compared to G1 (24.3% and 40.6%) and compared to controls (14.4% and 20%). We also observed a lower frequency of the MCP-1 G allele and a greater frequency of the CCR5Δ32 variant in G2 (15.2% and 6.5%) compared to G1 (22.6% and 1.4%) that was not statistically significant. However, adjustment for known covariates (age, gender and HCV genotypes) didn't confirm the results of univariate analysis.

Conclusion: In conclusion, our study suggests a possible role for some of the studied chemokines polymorphisms in the spontaneous clearance or persistence of HCV infection in Tunisian population. These results should be further investigated by a prospective cohort studies and large population-based studies.

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Introduction

Hepatitis C virus (HCV) is the major cause of post transfusion hepatitis. HCV infection becomes chronic in about 80% of infected individuals and often leads to serious consequences, including liver cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Only 20% of infected subjects clear the virus spontaneously [3]. Studies of early HCV infection suggest that a vigorous CD4+ T cells response is associated with viral clearance [4-6]. In contrast, patients developing a chronic infection show a predominant Th2 response [7-9]. Interplay between chemokines and their receptors is considered crucial for transmigration of lymphocytes and monocytes from the circulation to the liver portal area of inflammation during viral hepatitis, including HCV induced hepatitis. Differences in chemokine receptor expression between Th1 and Th2 cells might explain the regulating T helper cell polarization and their selective recruitment to liver tissue [10, 11]. Among these inflammatory molecules, monocyte chemoattractant protein-1 (MCP-1) with the corresponding chemokine receptors CCR2 and CCR5, specific receptor for pro-inflammatory chemokines: Rantes, MIP-1α and MIP-1β, are likely to be responsible for mononuclear cell accumulation in progressive of liver injury. Several allelic variants of these proteins have been shown to be important in the pathogenesis viral infection, either by modulating virological response or by influencing the severity of liver injury [12-14].

A bi-allelic G/A polymorphism at position -2518 of the MPC-1 gene, relative to the major transcriptional start site, has been described and was shown to influence the level of MCP-1 production in response to inflammatory stimuli [15].

CCR5 and CCR2 are two of a cluster of six chemokine receptor genes mapped to 3p21. A substitution mutation resulting in a valine (Val) to isoleucine (Ile) designed CCR2 (64Ile), a 32-bp deletion in the CCR5 gene leading to a non-functional protein and CCR5 promoter single nucleotide polymorphisms (SNP) were identified. Both CCR mutations have been implicated in the pathogenesis of human immunodeficiency virus (HIV) infection and the presence of the muted allele confers varying degree of anti-HIV protection that is reflected in slower disease progression [16]. Several studies have examined these polymorphisms in association with HCV and HIV infections [17-19].

The aim of this study was to investigate the distribution of the wild type and mutant alleles of MCP-1 -2518, CCR5-Δ32, CCR5-59029 and CCR2 (64Ile) in HCV infected hemodialysis Tunisian patients in comparison with healthy controls. Furthermore, we have analyzed the association of particular genotypes with outcomes of HCV infection, in terms of viral clearance or viral persistence.

Methods

Subjects: This is a retrospective study which involved 96 HCV infected individuals with confirmed antibody positivity to HCV. They were negative for hepatitis B surface antigen (HBsAg) and HIV infection. All patients were dialyzed during 2004 at different hemodialysis (HD) centers (private and public) in various regions of Tunisia. They were divided into two groups. Group I (G1) included 73 patients with persistent HCV infection as assessed by two positive PCR tests for HCV-RNA separated by more than one year. Group II (G2) consisted of 23 subjects considered to have spontaneously recovered from HCV infection on the basis of two negative PCR tests for HCV-RNA one year apart. None of the patients had received treatment for HCV infection before entering the study. Data obtained from each patient at the HD center included age at diagnosis, gender and possible risk factors for HCV (such as blood transfusion, invasive procedures or intravenous drug abuse). These two groups of patients were comparable in viral characteristics and average duration on HD (Table-1). In addition, blood samples were obtained from 170 ethnical and geographically matched healthy individuals who tested negative for HBsAg, HIV-Ab and HCV-Ab. These 170 subjects served as controls. The study was approved by the ethics committee of Charles Nicolle Hospital (Tunis, Tunisia) and all participants gave informed consent to participate in the study.

HCV RNA detection: HCV-RNA in serum was detected by reverse transcriptase PCR (Inno-Lipa HCV II, Innogenetics Belgium) according to the manufacturer's instructions. Patients who were HCV-PCR positive on the initial assessment and became consistently HCV-PCR negative were classified in G2.

Typing HCV: HCV genotypes were determined by means of inverse hybridization using specific oligonucleotide probes assay (HCV Genotype Assay Lipa, Innogenetics Belgium) according to the manufacturer's instructions.

DNA Extraction: Blood samples were collected on EDTA, and DNA was isolated by the Salting-Out method reported by Miller *et al* [20].

MCP-1 promoter genotyping: The identification of polymorphism was carried out using PCR, followed by a restriction fragment length polymorphism (RFLP) assay, using PvuII site which is introduced by the presence of the G nucleotide. The regulatory region of the MCP1 gene (-1817 to -2746) was amplified by PCR resulting in 930 bp fragment. Primers used were 5'CCGAGATGTTCCCAGCCAG-3' (forward) and 5'-CTGCTTTGCTTGTGCCTCTT-3' (reverse). 1µl of genomic DNA (~100ng) were added to 19µl of amplification buffer containing 11µl of H2O, 4µl of 5x PCR buffer, 1.6µl of MgCl2 (2mM), 0.4µl of dNTPs (0.2mM), 0.1 µl of Taq DNA polymerase (0.5U) and 5µM of each forward and reverse primers. PCR was run for 40 cycles using the following temperature profile: denaturation at 94°C for 60s, annealing at 55°C for 60s, extension at 72°C for 1min and 30s, followed by a single final extension step at 72°C for 10min. 4µl of the PCR products were digested with 5U of PvuIIin 10x buffer and H2O up to a final volume of 20µl at 37°C for 2h and 30min. The resulting products were separated by gel-electrophoresis in 1.5% agarose gel. Samples showing only a 930bp were assigned as AA, samples showing two bands of 708bp and 222bp were considered GG and samples showing three bands 930, 708 and 222bp were typed AG.

CCR5-59029genotyping: CCR5-59029genomic variants were detected by using RFLP-PCR. The presence of the G nucleotide at position -59029 of the CCR5 gene creates a recognition site for the BspI286I enzyme. Primers used were 5'-CCCGTGAGCCCATAGTTAAAACTC-3' (forward) and 5'-TCACAGGGCTTTTCAACAGTAAGG-3' (reverse). 2µl of genomic DNA (~100ng) were added to 18µl of amplification buffer containing 11.8µl of H2O, 4µl of 5x PCR buffer, 1.2µl of MgCl2 (1.5mM), 0.35µl

of dNTPs (0.175mM), 0.1 µl of Taq DNA polymerase (0.5U), 10µM of each forward and reverse primers. PCR was run for 35 cycles using the following temperature profile: a single initial denaturation at 94°C for 4min, denaturation at 94°C for 30s, annealing at 55°C for 45s, extension at 72°C for 1min, followed by a single final extension step at 72°C for 7min. 5µl of the PCR products were digested with 3U of BspI286I in 10x buffer and H2O up to a final volume of 20µl at 37°C for 1 night. The resulting products were separated by gel-electrophoresis in 2% agarose gel. Samples showing only a 130bp were assigned as GG, samples showing only 258bp band were considered AA and heterozygote have both bands.

CCR2 (64Ile) genotyping: For characterization of the CCR2 polymorphism, sequence specific primer PCR was used (SSP-PCR). The first primer was specific for the wild type allele sequence. The second primer was specific for the sequence of the mutant allele. In the case of homozygous wild type individual (+/+), the product was observed only in the first reaction. In the case of a homozygous mutant individual (64Ile/64Ile), the product was detected only in the second reaction. When the typed proband was heterozygous (+/64Ile), the product were detected in both the wild and the mutant specific reaction. Primers used were CCR2 440 5'-GTGGGCAACATGCTGGTCA-3', CCR2441 5'-CCCAAAGACCCACTCATTTG-3', and CCR2 442 5'-GTGGGCAACATGCTGGTCG-3'. 1µl of genomic DNA (~100ng) was added to 14µl of amplification buffer containing 7.5µl of H2O, 3µl of 5x PCR buffer, 1.2µl of MgCl2 (2mM), 0.3µl of dNTPs (0.2mM), 0.05 µl of Taq DNA polymerase (0.25U) and 5µM of each primer. PCR was run for 5 cycles using a single initial denaturation at 94°C for 1min, 5 cycles of denaturation at 96°C for 25s, annealing at 65°C for 50s, extension at 72°C for 45s, 21 cycles of denaturation at 96°C for 25s, annealing at 70°C for 45s, extension at 72°C for 45s, and 4 cycles of denaturation at 96°C for 25s, annealing at 55°C for 60s, extension at 72°C for 2min.

CCR5Δ32 genotyping: CCR5Δ32 genotype was determined by sizing PCR amplicons that include the entire region of the deletion. PCR was conducted in 15µl reaction containing 0.5µl of genomic DNA (50ng), 5µM of each primer, 0.25µl of dNTPs (0.175Mm), 0.9µl of MgCl2 (1.5mM), 3µl of 5xPCR buffer and 0.1 µl of Taq DNA polymerase (0.5U). Thermocycling procedure consisted of initial denaturation at 94°C for 4min, followed by 35 cycles of 94°C for 30s, 52°C for 45s, 72°C for 7min; amplicons were visualized in 2% agarose gel. The sense primer was 5'-TGTTTGCGTCTCTCCCAG-3', and antisense was 5'-CACAGCCCTGTGCCTCTT-3', which result in a 233bp product for the wild type amplicon (+/+) and 201 bp for the deletion product (Δ32/Δ32).

Statistical methods: Genotypes and alleles frequencies were calculated using Epi-stat program (version 6; center for disease control and prevention [CDC], Atlanta, GA). Statistical comparisons between different groups of patients and controls was conducted by the χ^2 test calculated on 2x2 contingency tables. Fisher exact test was used when an expected cell value was less than 5. P value < 0.05 was considered statistically significant. The strength of the association between genotypes or alleles in each group was estimated by calculating of the odds ratios (OR) and 95% confidence intervals (CI) using the same software. Logistic regression models were built with clearance of HCV infection (RNA-HCV negative) as the response variable to estimate adjusted ORs.

Results

Epidemiological characteristics

Clinical and virological characteristics are summarized in Table-1. Our results showed that 1b genotype was the predominant genotype among G1 (64.4%) and G2 (70%). The prevalence of risk factors was similar in both groups. None of the patients was an intravenous drug user. Ninety patients (93.7%) had history indicating risk of exposure to HCV due to blood transfusion (56.3%) or due to surgical or medical invasive procedures (37.5%). No significant differences were found in the distribution according to regions, private versus public nature of the HD centers or the duration of HD between the two groups of patients.

Genetic polymorphisms

All analyzed allele frequencies and genotype distributions were in Hardy-Weinberg equilibrium both in patients and controls. All polymorphism frequencies are described below and summarized in Table-2.

MCP-1 gene polymorphism: Comparison of allele frequencies in patients infected with HCV and controls did not reveal significant difference for the MCP-1 gene polymorphism. Nevertheless, the frequency of genotype GG was lower in patients (5.2%) than in controls (17%). Regarding the two groups of HD patients, there was an increased frequency of the G allele in G1 (22.6%) compared with G2 (15.2%) but the difference did not reach statistical significance.

CCR2 (64Ile) gene polymorphism: Significant differences were found between HCV infected patients and controls regarding the CCR2 (64Ile) gene polymorphism. The frequency of the +/+ genotype was significantly higher in controls (73.5%) compared to HCV infected patients as a whole (60%) and to G1 patients (56.9%) [P = 0.03; OR = 1.9; CI 95% = 1.3-2.7, and P = 0.02; OR = 2.1, CI 95% = 1.1-3.9 respectively].

Table 1: Clinical and virological characteristics of the study subjects

Characteristics	G1 (n = 73)	G2 (n = 23)
Gender ratio (Male/Female)	33/40	13/10
Mean age (years)	58 ± 13.08	55.5 ± 11.7
HCV genotype: n (%)*		
1a	2 (2,6)	-
1b	47 (64.4)	7 (70%)
2a/2c	7 (9.2)	1 (10%)
4	14 (18.4)	2 (20%)
1b/4	3 (4)	-
Risk factors: n (%)		
Blood transfusion	40 (64.8)	14 (60.8)
Invasive procedure	29 (39.7)	7 (30.4)
Intravenous drug abuse	0	0
Regional distribution: n (%)		
Tunis (Capital region)	62 (84.9)	23 (100)
North	10 (13.7)	0
Central	0	0
South	1(1.3)	0
HD in private sector: n (%)	64 (87.7)	18 (78.26)
HD in public sector: n (%)	9 (12.3%)	5 (21,7)
Mean duration on HD (months)	158.1 ± 58	164 ± 60.21

* HCV genotype was determined for only 10 patients in G2 who were HCV-RNA positive at baseline and spontaneously cleared HCV infection as suggested by two subsequent negative HCV-PCR detections one year apart.

On the other hand, the frequency of the +/64Ile genotype was significantly increased in the HCV infected patients group as a whole (35.8%) and in G1 (37.5%) compared to controls (24.1%) [P = 0.04; OR = 0.6; CI 95% = 0.3-1.0; P = 0.05; OR = 0.5; CI 95% = 0.3-1.0 respectively]. The distribution of allele frequencies showed a significantly increased frequency of the 64Ile allele in HCV infected patients group (22.1%) and in G1 (24.3%) compared to controls (14.4%) [P = 0.03; OR = 0.6; CI 95% = 0.4-1.0 and P = 0.09; OR = 0.5; CI 95% = 0.3-0.9 respectively]. The comparison of these frequencies according to the evolution of HCV infection also revealed an increased frequency of the 64Ile allele in G1 (24.3%) compared to G2 (15.2%). However, this difference did not attain statistical significance.

CCR5-59029 gene polymorphism: The AA CCR5-59029 genotype occurred more frequently among HCV infected patients and G1 compared to controls (35.9 and 40.6% versus 20%) [P = 0.01; OR = 0.5, CI 95% = 0.3-0.8 and P = 0.002; OR = 0.4; CI 95% = 0.2-0.7 respectively]. The frequency of the AG CCR5-59029 genotype was decreased in G1 compared with controls (36.2% versus 52.4%) [P = 0.03, OR = 1.9, CI 95% = 1.1-3.6]. Comparison of allele frequencies showed an increased frequency of the A allele in HCV infected patients group (56%) and in G1 (58.7%) compared to controls (46.2%) [P = 0.04; OR = 0.7; CI 95% = 0.5-1.0 and P = 0.02; OR = 0.6; CI 95% = 0.4-0.9 respectively]. The comparison of these frequencies between the two groups of patients revealed a non significant higher frequency of the A allele in G1 (58.7%) compared to G2 (47.8%).

CCR5 Δ32 gene polymorphism: No significant differences in the genotype distribution or in allele frequencies were observed between patients infected with HCV and controls. No homozygous mutant CCR5 Δ32 subjects were detected in either the control group or G1.When transmission of these genetic variants was analyzed in the two groups of HD patients, the Δ32 variant was present at a greater frequency in G2 compared to G1 (6.5% versus 1.4%), but this difference did not reach statistical significance.

Haplotype distribution: The allelic distribution of the CC-chemokine G MCP-1-2518 in combination with the corresponding chemokine receptor CCR2 (64Ile) did not differ significantly between patients and controls nor between G1 and G2. The same applies for the other haplotype associations: A(-59029)CCR5/64IleCCR2, A(-59029)CCR5/CCR5Δ32 and CCR5Δ32/64IleCCR2 (Data not shown). After adjustment for age, gender and HCV genotypes as confounders, multivariate analysis did not reveal any statistical association for the alleles and genotype frequencies studied.

Discussion

This study showed an association between chemokines/ chemokine receptors and the outcomes of HCV infection in Tunisian HD patients. Our results suggested a possible role for the -2518 MCP-1 G allele in the persistence of HCV infection. We speculate that individuals bearing G at position -2518 produce more MCP-1 protein than individuals with A allele. In consequence, they could have a stronger inflammatory response with higher liver tissue damage. Our results, which indicated an increased prevalence of -2518 G allele in G1 patients compared to G2, are in agreement with this hypothesis. Indeed, Rovin *et al* reported that in vitro cells obtained from GG or GA subjects produce more MCP-1 molecule than those isolated from AA individuals [15]. On the other hand, HCV infected patients with the -2518 MCP-1 G allele were reported to be more prone to hepatic inflammation and fibrosis [21]. The same higher level variant G allele was suggested to contribute in the chronicity of HBV infection in Korean population [22]. However, the wide CI in many of our findings reflects the relatively small

Table 1: MCP1, CCR2 and CCR5 genotype and allele frequencies in patients and controls

Polymorphisms	Controls		Total Patients		G1		G2	
	N	%	N	%	N	%	N	%
MCP1-2518								
Genotype								
AA	91	53	61	63.5	45	61.6	16	69.6
AG	67	40	30	31.3	23	31.5	7	30.4
GG	12	17	5	5,2	5	6.9	0	0
Allele								
A	146	73.2	152	79.2	113	77.4	39	84.8
G	91	26.8	40	20.8	33	22.6	7	15.2
CCR2 (64Ile)*								
Genotype								
+/+†	125	73.5	57	60	41	56.9	16	69.6
+/64Ile‡	41	24.1	34	35.8	27	37.5	7	30.4
641/64Ile	4	2.4	4	4,2	4	55.6	0	0
Allele								
+	291	85.6	148	77.9	109	75.7	39	84.8
64Ile§	49	14.4	42	22.1	35	24.3	7	15.2
CCR5 Δ32								
Genotype								
+/+	162	95.3	92	95.8	71	97.3	21	91.4
+/ Δ32	8	4.7	3	3.1	2	2.7	1	4.3
Δ32/ Δ32	0	0	1	1.1	0	0	1	4.3
Allele								
+	332	97.6	187	97.4	144	98.6	43	93.5
Δ32	8	2.4	5	2.6	2	1.4	3	6.5
CCR5 59029 ‖								
Genotype								
GG	47	27.6	22	23.9	16	23.2	6	26
AG¶	89	52.4	37	40.2	25	36.2	12	52.2
AA**	34	20	33	35.9	28	40.6	5	21.8
Allele								
G	183	53.8	81	44	57	41.3	24	52.2
A††	157	46.2	103	56	81	58.7	22	47.8

* Only 95 total patients were tested for CCR2 (64Ile) (72 in G1 and 23 inG2).

† The homozygous +/+ CCR2 (64Ile) genotype was significantly higher in controls than in total patients group and in G1 HCV patients [P = 0.0; OR = 1.9; CI 95% = 1.3-2.7 and P = 0.02; OR = 2.1, CI 95% = 1.1-3.9].

‡ The heterozygous +/64Ile CCR2 (64Ile) genotype was significantly increased in patients group and in G1 versus controls [P = 0.043; OR = 0.6; CI 95% = 0.3-1.02; P = 0.049; OR = 0.5; CI 95% = 0.3-1.0].

§ Comparing CCR2 (64Ile) allele frequency in total patients group and G1with controls [P = 0.03; OR = 0.6; CI 95% = 0.4-1.0 and P = 0.09; OR = 0.5; CI 95% = 0.3-0.9].

‖ Only 92 total patients were tested for (-59029)CCR5 A/G (69 in G1 and 23 in G2).

¶ The frequency of (-59029) CCR5AG genotype was significantly lower in G1compared to controls [P = 0.03, OR = 1.9, CI 95% =1.1-3.6].

** The frequency of (-59029) CCR5 AA genotype was significantly higher in total patients group and G1 compared to controls [P = 0.01; OR = 0.5, CI 95% = 0.2-0.8 and P = 0.0017; OR = 0.4; CI 95% = 0.2-0.7].

†† Comparing (-59029) CCR5 A allele frequency in total patients group and G1 with controls [P = 0.04; OR = 0.7; CI 95% = 0.5-1.0 and P = 0.02; OR = 0.6; CI 95% = 0.4-0.9].

number of patients, particularly in G2, with a consequent loss of statistical power. Another study that includes a greater number of patients who have spontaneously recovered from HCV infection would be required to verify this association. CCR2 signaling promotes Th1 development in infection models. Indeed, Traynor *et al* reported that CCR2 knock out mouse have 46% reduction in lymphocyte recruitment to sites of infection and inflammation and 80% reduction in CD4+T cells locally [23].Our study showed a significant association of the CCR2 (64Ile) allele with susceptibility to HCV infection and persistence of infection This variant was significantly increased in patients infected with HCV and in G1 compared to controls. In addition, this allele was more represented in persistent HCV infection group than in the clearance group. This is in agreement with results reported by Mascheretti *et al*, which showed that the CCR2 (64Ile) variant was under-represented in patients with spontaneous viral elimination compared to patients suffering from chronic infection, and that the frequency of the homozygous variant genotype was higher in patients who cleared the virus compared to both patients with persistent infection and controls in a German study [21]. This genetic variant has also been related to the onset of a variety of autoimmune/inflammatory diseases such as familial multiple sclerosis, pulmonary sarcoidosis, type 1 diabetes mellitus, asthma and inflammatory bowel disease [24]. In the majority of these studies, this genetic CCR2 variant has been found to be associated with a milder course and/or a reduced risk of developing diseases. In our study, the association between HCV persistence and CCR2 (64Ile) allele may reflect the same process with a reduction in the function of CCR2 in the HCV immune response and disease persistence. On the other hand, this polymorphism represents a conservative change in the first transmembrane domain of the protein. The receptor containing the variance has been shown to be expressed efficiently on the cell surface. Its signal transcription in response to MCP-1 binding and protein expression is not altered. This hypothesis could explain the defect of statistical association between haplotype G MCP1(-2518)/ CCR2(64Ile) and the outcome of HCV infection in our studied population. Previous studies have established that A(59029)CCR5 allele had higher promoter activity than G(59029) and could regulate CCR5 gene expression [17, 25]. This observation is in accordance with our findings that reveal a significant association of the CCR5-59029 A allele with susceptibility to infection and progression to chronicity. Chang *et al* corroborate similar association between CCR5-59029 A allele and an increased risk of HBV infection in a Korean population [26].

In the CCR5 gene the most studied polymorphism was the 32 base pair deletion CCR5 Δ32 that creates a truncated protein that fails to reach the cell surface. Patients homozygous for this mutation cannot express CCR5 on the cell surface, whereas the heterozygous state results in decreased expression of the functional CCR5 protein [27]. In an Irish study, the heterozygote CCR5 Δ32 was associated with spontaneous clearance of HCV infection [19]. In agreement with this report, Hellier *et al* found an association between CCR5 Δ32 and reduced portal inflammation and milder fibrosis in a large European cohort [28]. Interestingly, CCR5Δ 32 carriers showed significantly reduced response rates to anti HCV interferon therapy [29, 30].

In accordance with cited studies, we observed a protective effect of this variant in our study. The Δ32 variant was more frequent in the cleared HCV infection group compared to the chronically infected group. The lack of statistical significance observed here might be due to the small size of our cohort, particularly the spontaneous clearance group.

Because the CCR2 and CCR5 genes are closely located on the chromosome 3p21, we suggest that the haplotype associationsCCR5(-59029)A/CCR264Ile,CCR5(-59029) A/CCR5Δ32 and CCR5Δ32/CCR264Ile, combining the different genetic variants, may be important in the persistence of HCV infection.

Because of the small size of our cohort and the retrospective nature of the study, it should be considered an exploratory study and our results must be treated with caution. Future studies are needed to confirm these chemokine/chemokine receptor polymorphism associations with HCV infection progression and to further clarify their mechanism of action.

Conclusion

In conclusion, this study has attempted to elucidate the role of CC chemokines and their receptors in the persistence or clearance of HCV infection. Our data suggests that the CCR5(-59029) A, CCR2 (64Ile) and MCP-1(-2518) G allele profiles predispose to persistent HCV infection. They may be part of the spectrum of immunogenetic factors involved in chronic HCV disease in Tunisian population.

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