Associations of Chemokine System Polymorphisms With Clinical Outcomes and Treatment Responses of Chronic Hepatitis C

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Background & Aims: **CCR5Δ32, a 32–base pair deletion of the CC chemokine receptor (CCR) 5 gene, is associated with slowed human immunodeficiency virus disease progression in heterozygotes and protection against infection in homozygotes. A recent study found a higher than expected frequency of CCR5Δ32/Δ32 in patients with hepatitis C virus infection. The roles of other disease-associated chemokine system polymorphisms have not been evaluated in hepatitis C virus infection.** ***Methods:*** **Six chemokine system polymorphisms (CCR5Δ32, CCR5 promoter *59029–G/A*, *CCR2 -64I*, RANTES [regulated upon activation, normal T cells expressed and secreted] *-403 -G/A*, and *-28 -C/G* and stromal derived factor 1 *-3′A*) were studied in 417 patients with liver diseases (339 with hepatitis C) and 2380 blood donors. The clinical parameters of hepatitis C virus infection were compared between carriers and noncarriers of each genetic variant.** ***Results:*** **The frequency of CCR5Δ32 homozygosity was 0.8% in whites with hepatitis C virus and 1.1% in controls ($P = 0.75$). The CCR5Δ32 allele was not associated with any of the clinical parameters of hepatitis C virus infection. Hepatitis C virus–seropositive whites with the RANTES *-403 -A* allele were less likely to have severe hepatic inflammation compared with those without (odds ratio, 0.34; $P = 0.03$). In multivariate analysis, the CCR5 promoter *59029 -A* allele was marginally associated with a sustained response to interferon therapy (odds ratio, 3.07; $P = 0.048$).** ***Conclusions:*** **In this cohort, the frequency of CCR5Δ32 homozygosity in patients with hepatitis C was similar to controls. The high prevalence of CCR5Δ32 homozygosity in the hepatitis C virus patients of the earlier study likely reflects resistance to human immunodeficiency virus infection in hemophiliacs rather than a susceptibility to hepatitis C virus infection. Expression of CCR5 and RANTES may be important in the modulation of hepatic inflammation and response to interferon therapy in chronic hepatitis C.**

Hepatitis C virus (HCV) infection causes a wide spectrum of liver disease ranging from acute and chronic hepatitis to cirrhosis and hepatocellular carcinoma. The precise viral and host factors responsible for these outcomes have not yet been delineated but are expected to include specific immunoregulatory molecules, such as chemokines and chemokine receptors.[1] Chemokines, a large family of leukocyte chemoattractants that act by binding to G protein–coupled receptors,[2] have become increasingly recognized as important mediators of hepatic inflammation and injury.[3,4] In particular, lymphocytes infiltrating HCV-infected liver express high levels of the chemokine receptors CC chemokine receptor (CCR) 5 and CXC chemokine receptor (CXCR) 3.[5] Moreover, intrahepatic T helper type 1 cytokines drive the increased expression of the CXCR3 ligands CXCL10 (interferon-inducible protein-10) and CXCL9 (monokine induced by interferon γ), thereby promoting continued recruitment of T helper type 1 cells into the hepatic lobule.[5] Other chemokines, such as dendritic cell–chemokine 1 and the CCR5 ligand RANTES (regulated upon activation, normal T cells expressed and secreted), may attract naïve and activated T cells to the portal and periportal areas.[6] The chemokine CXCL8 (interleukin 8) can be directly induced by the HCV nonstructural protein 5A and may lead to a partial inhibition of the interferon-induced antiviral response.[7]

Abbreviations used in this paper: AIDS, acquired immune deficiency syndrome; CCR, CC chemokine receptor; CCR5Δ32, a 32–base pair deletion of the CCR5 gene; CI, confidence interval; CXCR, CXC chemokine receptor; HAI, histological activity index; HIV, human immunodeficiency virus; OR, odds ratio; PCR, polymerase chain reaction; RANTES, regulated upon activation, normal T cells expressed and secreted.

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Tests of the importance of these and other chemokines and chemokine receptors in HCV pathogenesis are now possible, in part because of the discovery of numerous allelic variants. Several of these have already been associated with human immunodeficiency virus (HIV) pathogenesis, asthma, rheumatoid arthritis, multiple sclerosis, and several other autoimmune diseases.[8–10] The strongest association to date is between HIV/acquired immune deficiency syndrome (AIDS) and CCR5Δ32, a defective allele of the chemokine receptor/HIV co-receptor CCR5 caused by a 32–base pair (BP) deletion in the open reading frame that is found mainly in whites. Accordingly, individuals who are homozygous for CCR5Δ32 are highly resistant to HIV-1 infection, and HIV-positive heterozygotes have delayed progression to AIDS.[11,12] CCR5Δ32 occurs with an allele frequency of approximately 10% in North American whites. Approximately 1% are homozygous, and these individuals appear healthy, suggesting that CCR5 may not be essential.[13] Other polymorphisms associated with HIV disease have also been described in the CCR5 promoter (*59029 -G/A* and *59356 -C/T*),[14,15] the promoter for the CCR5 ligand RANTES (*-28-C/G* and *-403 -G/A*),[16,17] the open reading frame of the minor HIV co-receptor CCR2 (*CCR2 -64I*),[18,19] and the 3′-untranslated region of the gene for CXCL12 (stromal derived factor 1 *-3′A*),[20] the ligand for the major HIV co-receptor CXCR4.

Recently, Woitas et al.[21] reported a highly statistically significant 3-fold increase in the expected frequency of CCR5Δ32 homozygosity in patients infected with HCV. CCR5Δ32 homozygotes also had significantly higher HCV viral loads than patients with other genotypes. Consequently, the authors suggested that this mutation may be a risk factor for HCV infection and disease. However, the great majority of HCV-infected subjects tested in that study were HIV-seronegative hemophiliacs at high risk of HIV infection because of transfusion of factor VIII before HIV screening in the early 1980s. Because the CCR5Δ32 homozygous genotype should be overrepresented among HIV-seronegative individuals, conclusions regarding the role of this mutation as a risk factor specific for HCV must be interpreted with caution.

Here we report a separate analysis of this issue, expanded to incorporate the other known disease-associated chemokine and chemokine receptor polymorphisms, by using a cohort of HIV-seronegative, HCV-infected nonhemophiliacs who are more representative of the HCV-infected population in the United States.

Materials and Methods

Subjects

Patients were recruited from 2 sources: (1) those with various forms of liver disease who had been followed up at the Liver Diseases Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, and (2) blood donors positive for antibodies to HCV who had been followed up by the Department of Transfusion Medicine, Clinical Center, National Institutes of Health. Overall, 417 patients agreed to participate in the study: 339 patients were anti-HCV positive, and 78 patients had other forms of liver disease (chronic hepatitis B, primary biliary cirrhosis, nonalcoholic steatohepatitis, and so on). Among the 339 patients with HCV infection, 14 patients presented with acute hepatitis C. Their clinical presentations were well documented and were consistent with acute infection after a clearly defined exposure. Details of this subgroup with acute hepatitis C will be presented in a separate article. Normal controls were 2380 unrelated white blood donors. Liver biopsy samples were read and scored by a hepatopathologist using a modification of the histology activity index (HAI) for the grading of inflammation and necrosis (0–18)[22] and the Ishak fibrosis score to stage fibrosis (0–6).[23] HCV RNA was quantified with either the Superquant assay (National Genetic Institute, Los Angeles, CA) or the Cobas Amplicor Monitor assay version 2.0 (Roche Diagnostics, Branchburg, NJ). HCV viral titer was standardized to copies per milliliter by using a formula described by Pawlotsky et al.[24] Genotyping of HCV was performed with a line-probe hybridization assay (INNO-LiPA HCV II; Innogenetics, Ghent, Belgium).

The outcome of interferon therapy was assessed in all patients who ever received interferon. Sustained response was defined as an absence of serum HCV RNA by reverse-transcription polymerase chain reaction (PCR) 6 months after the completion of treatment. Nonresponse was defined as the presence of HCV RNA 6 months after the end of treatment, including both relapsers (reappearance of HCV RNA after stopping the treatment) and nonresponders (detectable HCV RNA during treatment).

Written, informed consent was obtained from all participants after detailed explanation of the protocol. The study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Genotyping Methods

Peripheral blood mononuclear cells were isolated from 10 mL of whole blood by Ficoll centrifugation. Genomic DNA was isolated from lymphocytes by standard methods. The CCR5Δ32 genotype was determined by sizing PCR amplicons that included the entire region of the deletion, a modification of methods we had used previously.[13] PCR was conducted in a 15-μL reaction containing 50 ng of genomic DNA, 5 pmol of each primer, 175 μmol/L of deoxynucleotide triphosphates, 1.5 mmol/L of magnesium chloride, 1× PCR buffer, and 0.5

U of Platinum *Taq* polymerase (Invitrogen, La Jolla, CA). The thermocycling procedure (PTC 100; MJ Research, Watertown, MA) consisted of initial denaturation at 94°C for 4 minutes followed by 35 cycles of 94°C for 30 seconds, 52°C for 45 seconds, and 72°C for 1 minute and final extension at 72°C for 7 minutes. The sense primer was 5′-TGTTTGCGTCTCTC-CCAG-3′, and the antisense primer was 5′-CACAGCCCTGT-GCCTCTT-3′, which resulted in a 233-bp product for the wild-type amplicon and 201 bp for the deletion product.

CCR5 -59029 genomic variants were detected by using PCR followed by restriction enzyme fragment analysis, a slight modification of our previously published procedure.[15] The sense primer, 5′-CCCGTGAGCCCATAGTTAAAACTC-3′, and antisense primer, 5′-TCACAGGGCTTTTCAACAGTA-AGG-3′, pair were used with PCR conditions identical to those for CCR5Δ32 except for an annealing temperature of 65°C. The reaction yields a 268-bp amplicon. A total of 10 μL of PCR product was digested with 10 U of *Bsp*1286I (New England BioLabs, Beverly, MA). The presence of the G nucleotide at position 59029 of the CCR5 gene creates a recognition site for the *Bsp*1286I enzyme. Digested amplicons from homozygotes for 52909 -G appear as a single 130-bp band on agarose gel electrophoresis, homozygotes for 59029 -A appear as a 258-bp band, and heterozygotes have both bands.

Genotyping of CCR2-V64I was performed as originally described by Smith et al.,[18] with modifications. PCR was performed as previously described except for the use of an annealing temperature of 65°C. The sense primer was 5′-TTGGTTTTGTGGGCAACATGATGG-3′, and the antisense primer was 5′-CATTGCATTCCCAAAGACCCACTC-3′. The 173-bp amplicon was then digested with *Bsa*BI (New England BioLabs). An alanine at nucleotide position 190 encodes isoleucine at amino acid position 64 and yields restriction fragments of 149 and 24 bp after *Bsa*BI digestion. In contrast, the 173-bp amplicon remains uncut if a glycine encoding a valine is present. Genotyping procedures for RANTES -403, -28 and stromal derived factor 1 -3′A were performed as previously described.[17,20]

Statistical Analysis

In the analysis of hepatic inflammation and fibrosis, only the liver biopsies that were performed before therapy were included in the calculations. For the purpose of statistical analysis, the degree of hepatic inflammation was classified into 3 groups according to the HAI score[25]—mild (HAI 0–5), moderate (HAI 6–10), and severe (HAI 11–18)—and the degree of fibrosis was classified into mild (Ishak fibrosis score of 0–2) and advanced (Ishak fibrosis score of 3–6).

Statistical analysis was primarily performed with StatView software (SAS Institute Inc., Cary, NC), except as noted. Categorical variables were evaluated with χ^2 or Fisher exact tests (StatXact 4; CYTEL Software Corporation, Cambridge, MA). Two-sample *t* tests or analysis of variance were used to compare means for continuous variables, and when normality was questioned, the Mann–Whitney *U* or Kruskal–Wallis tests were used for comparison of median values. In all cases that similar *P* values were obtained, the nonparametric *P* values were reported. Viral titers were transformed to log base 10. Logistic regression was used to model viral titer categories ($>2 \times 10^6$ vs. 2×10^6 copies per milliliter), treatment response vs. nonresponse, and liver biopsy HAI and fibrosis categorical scores as dependent variables, with genetic markers and other factors as covariates. For the logistic regressions, the logistic likelihood ratio test was used to determine significance, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. In multivariate models, Wald χ^2 *P* values were reported. To test for Hardy–Weinberg equilibrium, gene frequencies were calculated from the numbers of observed heterozygotes and homozygotes; the expected genotype frequencies were calculated according to the Hardy–Weinberg equation and then compared with the observed frequencies by using χ^2 statistics. For all tests, a 2-tailed *P* value of < 0.05 was considered significant.

Results

Six chemokine and chemokine receptor polymorphisms were genotyped in 417 patients with chronic liver diseases. Results for each of these genetic polymorphisms were available in all cases. Demographic characteristics of the entire cohort are summarized in Tables 1 and 2. All patients were HIV seronegative. Three hundred thirty-nine patients were HCV seropositive. The most common source of HCV infection was injection drug use (39.5%), followed by a history of blood transfusion (22.4%). Most patients were white (75.8%).

CCR5Δ32 and Hepatitis C Virus

CCR5Δ32 homozygosity was found in 2 of 257 (0.8%) whites with hepatitis C and 26 of 2380 (1.1%) controls (Table 3). Among anti-HCV–positive patients who were not white (n = 82), none was homozygous for CCR5Δ32, and 6 (7.3%) were heterozygous. Fourteen patients (7 whites, 5 blacks, 1 Hispanic, and 1 Native American) who presented with acute hepatitis C were all negative for the CCR5Δ32 allele. The frequencies of CCR5Δ32 homozygotes and heterozygotes were not sta-

Table 1. Distribution of the Overall Cohort

Characteristic	*n* = 417
HCV	339
At least 1 liver biopsy	252 (74.3%)
Received therapy	174 (51.3%)
Persistently normal ALT	16 (4.7%)
Spontaneous recovery	12 (3.5%)
Acute HCV	14 (4.1%)
Others	78
HBV	46 (59.0%)
PBC	16 (20.5%)
Others	16 (20.5%)

HBV, hepatitis B virus; PBC, primary biliary cirrhosis.

Table 2. Demographic Characteristics of Participants With Hepatitis C and Controls

Characteristic	HCV (n = 339)	Blood donors (n = 2380)
Mean age, *yr* (range)	44.8 (21–76)	44.6 (22–71)
Male (%)	201 (59)	1357 (57)
Race (%)		
White	257 (75.8)	2380 (100)
Black	58 (17.1)	—
Asian	16 (4.7)	—
Other	8 (2.3)	—
Source of infection (%)		
Injection drug use	134 (39.5)	—
Transfusion	76 (22.4)	—
Nasal cocaine	18 (5.3)	—
Unknown	31 (9.1)	—
Others[a]	80 (23.6)	—
Genotype (%)		
1	243 (71.7)	—
2	34 (10.0)	—
3	16 (4.7)	—
4	3 (0.9)	—
5	1 (0.3)	—
Not done	42 (12.4)	—

[a]Other sources of infection include occupational exposure, sexual transmission, and origin from an endemic area.

tistically different between the HCV-seropositive whites and controls ($P = 0.75$; Fisher exact test). The observed frequency of CCR5Δ32/Δ32 was consistent with published reports in the general population and was not significantly different from the frequency expected under Hardy–Weinberg equilibrium conditions (expected frequency, 1.1%; observed frequency, 0.8%; $P = 0.91$). Patients with other forms of liver disease had a similar CCR5Δ32 distribution, although the group was small and heterogeneous. Patients who were carriers of CCR5Δ32 (CCR5Δ32 heterozygotes and homozygotes) had no significant differences in serum alanine aminotransferase (ALT), HCV viral titer, degree of hepatic inflammation, fibrosis, and response to interferon therapy compared with those with wild-type CCR5. We were unable to directly analyze the association between CCR5Δ32 homozygosity and HCV disease outcomes because the number of patients in this group was too small (n = 2).

Serum Alanine Aminotransferase

There was no significant difference in mean serum ALT between carriers and noncarriers of each genetic marker. The distribution of patients who had persistently normal ALT and persistently high ALT (>4 times the upper limit of normal) was similar among carriers and noncarriers of each genetic marker (data not shown).

Hepatitis C Virus Viral Titers

Table 4 shows the frequencies of carrier (heterozygous and homozygous) of each genetic polymorphism in relationship to HCV titers. The distribution of patients who had low ($\leq 2 \times 10^6$ copies per milliliter) and high ($>2 \times 10^6$ copies per milliliter) HCV titers was not statistically different between carriers and noncarriers of each genetic marker. Similarly, mean HCV titers were not statistically different between carriers and noncarriers of each genetic polymorphism (Figure 1).

Hepatic Inflammation

In HCV-seropositive whites (Table 5), the proportion of RANTES promoter -403A carriers who had severe hepatic inflammation (HAI score >10) was significantly less than those lacking this allele (OR, 0.34;

Table 3. Frequencies of Distribution of Chemokine Polymorphisms Among Whites With Hepatitis C and Controls

	Frequency (%)		
CCR5	+/+	+/Δ32	Δ32/Δ32
Hepatitis C (n = 257)	207 (80.5)	48 (18.7)	2 (0.8)
Other liver diseases (n = 54)	41 (75.9)	12 (22.2)	1 (1.9)
Blood donors (n = 2380)	1950 (81.9)	404 (17.0)	26 (1.1)
CCR5 promoter 59029	G/G	G/A	A/A
Hepatitis C (n = 257)	68 (26.5)	130 (50.6)	59 (22.9)
Blood donors (n = 2212)	417 (18.8)	1110 (50.2)	685 (31.0)
CCR2 -641	+/+	+/641	641/641
Hepatitis C (n = 257)	209 (81.3)	47 (18.3)	1 (0.4)
Blood donors (n = 1985)	1631 (82.2)	331 (16.7)	23 (1.1)
RANTES -403	G/G	G/A	A/A
Hepatitis C (n = 257)	174 (67.7)	76 (29.6)	7 (2.7)
Blood donors (n = 2177)	1456 (66.9)	654 (30.0)	67 (3.1)
RANTES -28	C/C	C/G	G/G
Hepatitis C (n = 257)	245 (95.3)	12 (4.7)	0 (0.0)
Blood donors (n = 2177)	2062 (94.7)	112 (5.1)	3 (0.1)
SDF1 - 3′A	+/+	+/3′A	3′A/3′A
Hepatitis C (n = 257)	152 (59.1)	100 (38.9)	5 (1.9)
Blood donors (n = 1976)	1241 (62.8)	647 (32.7)	88 (4.5)

NOTE. CCR5: $P = 0.73$ (χ^2, overall cohorts); $P = 0.75$ (exact test, hepatitis C vs. blood donors).

Table 4. Relationship Between HCV Viral Titer and Chemokine Polymorphisms

Genetic marker	Frequency of carriers (%) High viral titer (>2 × 10^6 copies/mL)	Frequency of carriers (%) Low viral titer (≤2 × 10^6 copies/mL)	OR (95% CI)	*P* value[a]
All participants				
CCR5Δ32	23/123 (18.7)	29/180 (16.1)	1.20 (0.66–2.19)	0.64
CCR2 -64I	25/123 (20.3)	34/180 (18.9)	1.10 (0.62–1.95)	0.77
CCR5 promoter				
59029A	87/123 (70.7)	138/180 (76.7)	0.74 (0.44–1.24)	0.28
RANTES -403A	43/123 (35.0)	69/180 (38.3)	0.87 (0.54–1.39)	0.63
RANTES -28G	6/123 (4.9)	5/180 (2.8)	1.80 (0.54–6.02)	0.36
SDF1 -3′A	46/123 (37.4)	64/180 (35.6)	1.08 (0.67–1.74)	0.81
Whites				
CCR5Δ32	20/89 (22.5)	27/140 (19.3)	1.21 (0.63–2.33)	0.62
CCR2 -64I	14/89 (15.7)	28/140 (20.0)	0.75 (0.37–1.51)	0.49
CCR5 promoter				
59029A	65/89 (73.0)	111/140 (79.3)	0.71 (0.38–1.32)	0.34
RANTES -403A	28/89 (31.5)	43/140 (30.7)	1.04 (0.58–1.84)	0.99
RANTES -28G	5/89 (5.6)	4/140 (2.9)	2.02 (0.53–7.75)	0.32
SDF1 -3′ A	37/89 (41.6)	58/140 (41.4)	1.01 (0.59–1.73)	0.99

[a]Fisher exact test.

95% CI, 0.13–0.90; $P = 0.03$). However, this was not statistically significant in the overall HCV cohort (OR, 0.47; 95% CI, 0.20–1.08; $P = 0.07$). Mean HAI scores among Caucasians who were carriers of the -403A polymorphism were 7.90 ± 2.81 as compared with 8.60 ± 2.82 among noncarriers ($P = 0.13$). Because the 2 single nucleotide polymorphisms in the RANTES gene promoter region, -403G/A and -28C/G, are in complete linkage disequilibrium, we then performed an analysis of compound genotype and hepatic inflammation. The 2 most common compound genotypes were -403G/G, -28C/C (67%) and -403G/A, -28C/C (25%). HCV-infected whites who had the -403G/A, -28C/C compound genotype were less likely to have severe hepatic inflammation in comparison with the -403G/G, -28C/C genotype (OR, 0.35; 95% CI, 0.12–1.01; $P = 0.05$).

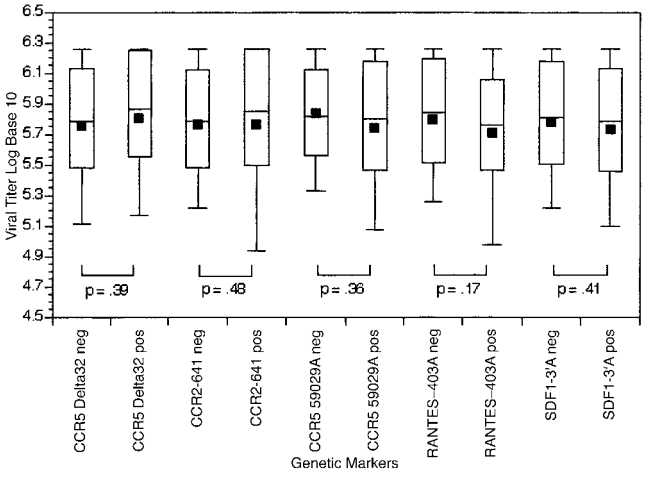

Figure 1. Comparison of HCV viral titers among carriers and noncarriers of various genetic markers. Note: RANTES -28G was not included because of a small number of -28G carriers.

Hepatic Fibrosis

Forty-two percent of patients in this study had advanced fibrosis (Ishak score 3–6) on their initial liver biopsy; 13% had cirrhosis. There was no significant association between the degree of hepatic fibrosis and each genetic marker. Distributions of patients who had advanced-stage fibrosis were similar among carriers and noncarriers of each genetic marker (Table 6).

Progression of Hepatic Fibrosis

Fifty-nine patients in this study were part of a natural history study of HCV infection.[26] These patients had 2 sequential liver biopsies without receiving therapy, to evaluate the natural progression of liver fibrosis. The mean duration between the liver biopsies was 6.4 years (range, 0.6–19.2 years). Among these 59 patients, 19 had at least a 1-point increase in their Ishak fibrosis score (progressors), and 40 patients had either a stable or improved score on subsequent biopsy (nonprogressors). Mean changes of the Ishak fibrosis scores were not statistically different between carriers and noncarriers of each genetic marker (data not shown).

Response to Interferon-Based Therapy

Response to interferon therapy was strongly associated with HCV genotype and, to a lesser extent, with

Table 5. Relationship Between Hepatic Inflammation and Chemokine Polymorphisms

Genetic marker	Frequency of carriers (%) Mild inflammation (HAI ≤5)	Severe inflammation (HAI >10)	OR (95% CI)	*P* value[a]
All participants				
CCR5Δ32	7/46 (15.2)	8/53 (15.1)	0.99 (0.33–2.98)	0.99
CCR2 -64I	10/46 (21.7)	9/53 (17.0)	0.74 (0.27–2.01)	0.55
CCR5 promoter				
59029A	33/46 (71.7)	42/53 (79.2)	1.50 (0.60–3.79)	0.39
RANTES -403A	21/46 (45.7)	15/53 (28.3)	0.47 (0.20–1.08)	0.07
RANTES -28G	2/46 (4.3)	2/53 (3.8)	0.86 (0.12–6.38)	0.89
SDF1 -3′A	14/46 (30.4)	20/53 (37.7)	1.38 (0.60–3.20)	0.45
Whites				
CCR5Δ32	7/39 (17.9)	6/43 (13.9)	0.74 (0.23–2.43)	0.62
CCR2 -64I	7/39 (17.9)	7/43 (16.3)	0.89 (0.28–2.81)	0.84
CCR5 promoter				
59029A	29/39 (74.3)	37/43 (86.0)	2.13 (0.69–6.54)	0.19
RANTES -403A	17/39 (43.6)	9/43 (20.9)	0.34 (0.13–0.90)	**0.03**
RANTES -28G	2/39 (5.1)	2/43 (4.7)	0.90 (0.12–6.74)	0.92
SDF1 -3′A	10/39 (25.6)	17/43 (39.5)	1.90 (0.74–4.87)	0.18

[a]Fisher exact test.

viral titer, in agreement with the published literature.[27] In this cohort, we observed no significant association between each genetic polymorphism and treatment response in univariate analyses (Table 7). However, in multivariate logistic regression analysis controlling for factors known to affect treatment response (sex, race, viral genotype, and titer), there was a marginally positive association between 59029 -A allele carriers and sustained treatment response among patients with HCV (OR, 3.07; 95% CI, 0.93–10.13; $P = 0.048$).

Discussion

In this cohort, the proportion of patients with chronic hepatitis C who had the CCR5Δ32 mutation was similar to controls and was not significantly different from expectations predicted by Hardy–Weinberg equilibrium conditions. Thus, we were unable to confirm the report of Woitas et al.,[21] which showed an increased frequency of CCR5Δ32 homozygosity among HCV-seropositive patients. Woitas et al.[21] found a 7.8%

Table 6. Relationship Between Hepatic Fibrosis and Chemokine Polymorphisms

Genetic marker	Frequency of carriers (%) Mild fibrosis (Ishak score 0–2)	Advanced fibrosis (Ishak score 3–6)	OR (95% CI)	*P* value[a]
All participants				
CCR5Δ32	25/139 (18.0)	17/105 (16.2)	0.88 (0.45–1.73)	0.74
CCR2 -64I	28/139 (20.1)	21/105 (20.0)	0.99 (0.53–1.87)	0.99
CCR5 promoter				
59029A	106/139 (76.3)	76/105 (72.4)	0.82 (0.46–1.46)	0.55
RANTES -403A	50/139 (36.0)	41/105 (39.0)	1.14 (0.68–1.92)	0.69
RANTES -28G	6/139 (4.3)	4/105 (3.8)	0.89 (0.24–3.19)	0.99
SDF1 -3′A	53/139 (38.1)	37/105 (35.2)	0.88 (0.52–1.50)	0.69
Whites				
CCR5Δ32	24/115 (20.9)	15/71 (21.1)	1.02 (0.49–2.10)	0.99
CCR2 -64I	19/115 (16.5)	14/71 (19.7)	1.24 (0.58–2.67)	0.69
CCR5 promoter				
59029A	89/115 (77.4)	54/71 (76.1)	0.93 (0.46–1.87)	0.86
RANTES -403A	40/115 (34.8)	19/71 (26.8)	0.69 (0.36–1.31)	0.33
RANTES -28G	5/115 (4.3)	4/71 (5.6)	1.31 (0.34–5.06)	0.73
SDF1 -3′A	47/115 (40.9)	28/71 (39.4)	0.94 (0.52–1.72)	0.88

[a]Fisher exact test.

Table 7. Relationship Between Treatment Response and Chemokine Polymorphisms

Genetic marker	Frequency of carriers (%)		OR (95% CI)	*P* value[a]
	Responder	Nonresponder		
All participants				
CCR5Δ32	11/56 (19.6)	18/115 (15.7)	1.32 (0.58–3.02)	0.52
CCR2 -64I	10/56 (17.9)	25/115 (21.7)	0.78 (0.35–1.77)	0.69
CCR5 promoter				
59029A	46/56 (82.1)	83/115 (72.2)	1.77 (0.80–3.93)	0.19
RANTES -403A	20/56 (35.7)	39/115 (33.9)	1.08 (0.55–2.11)	0.86
RANTES -28G	2/56 (3.6)	5/115 (4.3)	0.81 (0.15–4.34)	0.99
SDF1 -3′A	21/56 (31.5)	39/115 (33.9)	1.17 (0.60–2.27)	0.73
Whites				
CCR5Δ32	11/47 (23.4)	14/74 (18.9)	1.31 (0.54–3.19)	0.65
CCR2 -64I	9/47 (19.1)	14/74 (18.9)	1.02 (0.40–2.58)	0.99
CCR5 promoter				
59029A	40/47 (85.1)	55/74 (74.3)	1.97 (0.76–5.14)	0.18
RANTES -403A	13/47 (27.7)	19/74 (25.7)	1.11 (0.49–2.53)	0.83
RANTES -28G	1/47 (2.1)	5/74 (6.8)	0.30 (0.03–2.65)	0.40
SDF1 -3′A	20/47 (42.6)	31/74 (41.9)	1.03 (0.49–2.15)	0.99

[a]Fisher exact test.

CCR5Δ32 homozygosity rate among HCV-seropositive/HIV-seronegative patients, in comparison to 0.8% in this study. The frequency of CCR5Δ32/Δ32 in our study is in agreement with the reported frequency in the general population.[11–13] The underlying reason for this discrepancy is most likely caused by the different levels of HIV exposure of the 2 study populations.

Most of the patients (83.7%) in the study by Woitas et al.[21] were hemophiliacs who probably received factor VIII or IX concentrate before the HIV screening era began, whereas none of the patients in our cohort were hemophiliacs and instead acquired HCV through a variety of exposures that carry a much lower risk of HIV infection. The 2 most common HIV risk factors in our cohort were injection drug use and blood transfusion for incidental reasons; these are also the most common risk factors for HCV in the general US population.[28] It is well known that CCR5 is a co-receptor for cellular entry of macrophage-tropic HIV and that decreased expression of CCR5 protects against HIV infection.[11–13] Before 1986, patients with hemophilia were at a particularly high risk of acquiring HIV infection because of repeated parenteral exposure to HIV-contaminated factor VIII or IX concentrate. The risk was shown to be directly dependent on both the nature and intensity of treatment. One study showed that only 6% of hemophiliacs heavily treated with factor VIII or IX concentrate between 1978 and 1985 remained HIV-1 seronegative.[29] Hemophiliacs who remain uninfected with HIV are expected to have, and have been shown in the Multicenter Hemophilia Cohort Study to have, a disproportionately higher frequency of CCR5Δ32.[30] In the Multicenter Hemophilia Cohort Study, 12 of 219 (5.5%) overall[30] and 7 of 43 (16.3%) high-risk (>20,000 U/yr or >100,000 U of non–heat-treated clotting factor between 1978 and 1985) HIV-1 seronegative hemophiliacs[31] were noted to be CCR5Δ32 homozygotes. In another group of highly exposed HIV-seronegative homosexual men from the Multicenter AIDS Cohort Study of homosexual men, CCR5Δ32 homozygosity was detected in 4.5%.[13] Therefore, the high prevalence of CCR5Δ32 homozygosity in the HCV-seropositive patients of the Woitas study likely reflects resistance to HIV infection rather than a susceptibility to HCV infection. In addition, among the 14 patients with well-documented acute HCV infection in this study, none were CCR5Δ32 homozygotes or heterozygotes, suggesting that the CCR5Δ32 allele is not more commonly seen in patients who are exposed to and become infected with HCV, although the sample size is relatively small. CCR5Δ32 was also not associated with a higher ALT or HCV viral titer in our cohort, in contrast to previous reports.[21,30] Overall, with respect to the association between the CCR5Δ32 mutation and HCV, we found no evidence of an increased rate of CCR5Δ32 homozygosity in our HCV cohort, and those who were CCR5Δ32 carriers did not seem to have altered clinical manifestations of chronic HCV infection or response to interferon treatment.

In this study, a genetic polymorphism of a CC chemokine (RANTES -403A), which can activate several chemokine receptors, including CCR1, CCR3, and CCR5, was found to be associated with less hepatic inflammation in whites with hepatitis C. This is surprising because increased RANTES expression is often asso-

ciated with hepatic inflammation and transient transfection of the human mast cell line HMC-1 and the T-cell Jurkat line with reporter vectors driven by the -403A variant of the RANTES promoter result in an 8-fold higher constitutive transcriptional activity as compared with that of the -403G promoter.[32] However, it is not known how this promoter variant behaves under conditions in which RANTES production is induced. It is therefore possible that increased basal expression of RANTES could somehow lead to less hepatic inflammation.

Viral factors, in particular the HCV genotype, are strong predictors of interferon treatment response.[27] The host factors of young age, female sex, and lesser degrees of fibrosis on liver biopsy correlate with a greater likelihood of a sustained response. To date, few host genetic factors have been associated with treatment response to interferon. Examples include polymorphisms of the promoter of interleukin 10[34] and MxA genes.[33] In this study, we showed that carriers of the 59029 -A allele were more likely to achieve a sustained virological response to interferon than those without this allele. This was independent of HCV viral genotype, titer, sex, and ethnicity. Therefore, expression of CCR5 may be involved in the indirect antiviral effects of interferon, which result from the host immune response against HCV-infected hepatocytes. However, we did not find any association between other variants that may affect CCR5 functions (CCR5Δ32, RANTES -403 and -28) and response to interferon therapy, and we were unable to detect other significant associations between 59029 -A or RANTES -403A and other clinical manifestations of HCV infection, namely, serum ALT, HCV viral titer, fibrosis, and progression of fibrosis. Thus, these associations need to be interpreted with caution and validated in future studies.

In conclusion, our study did not show that individuals homozygous for the defective CCR5 allele CCR5Δ32 are more susceptible to infection with HCV. However, we did observe an association between hepatic inflammation and the RANTES promoter -403 -G/A . In addition, 59029 -G/A was associated with the treatment response to interferon in HCV patients, independently of race, sex, viral genotype, and titer. Although these results are consistent with earlier studies showing the presence of RANTES and CCR5 in HCV liver disease, follow-up studies in independent cohorts will be needed to further assess the role of these polymorphisms as genetic risk factors in this disease and to explore the complex interactions between the chemokine system and HCV infection.

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