Lack of Higher Frequency of the Chemokine Receptor 5-Δ32/Δ32 Genotype in Hepatitis C

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Purpose: An elevated frequency of the CCR5-Δ32 mutation in German patients with hepatitis C with viremia has been reported. The aim of the present study was to verify whether this mutation occurs in an Italian population with hepatitis C and whether it is an adverse host factor indicative of severity of liver disease and response to antiviral therapy.

Study: The authors amplified 189-bp (wild-type) and 157-bp (Δ32 deletion) fragments of the CCR5 gene by polymerase chain reaction in 130 patients with chronic hepatitis C. Comparisons were drawn with 110 blood donors and 135 patients with primary biliary cirrhosis.

Results: Four (3.1%) patients with chronic hepatitis C and 1 blood donor (0.9%) were CCR5-Δ32 homozygous, whereas there was no CCR5-Δ32 homozygosity among primary biliary cirrhosis patients; the wild-type gene was present in a similar percentage in the 3 groups of patients without any significant difference (83.1% vs 90.4% vs 83.6%, respectively). Among the patients with chronic hepatitis C, no significant correlation was found between CCR5-Δ32 homozygosity and the following parameters: histologic grade/stage, hepatitis C virus genotype, viral load, serum aspartate aminotransferase, serum alanine aminotransferase, and serum gamma-glutamyltransferase. Ninety-five patients received a standard antiviral protocol with pegylated interferon (PEG Intron) + ribavirin; a sustained response was achieved in 59 patients (62.1%), and the remainder did not respond or experienced a relapse. Response to treatment was not influenced by CCR5-Δ32 deletion.

Conclusion: No greater frequency of CCR5-Δ32 homozygosity was seen in an Italian population of patients with chronic hepatitis C. This mutation does not seem to influence either the overall severity of liver disease or the response to viral therapy.

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Chemokine receptor 5 (CCR5) plays a pivotal part in regulating immune response, mainly in the recruitment of inflammatory cells. Woitas et al[1] recently found a greater frequency of CCR5-Δ32 mutation in German patients with hepatitis C, showing a significant association with viral load. This mutation is of scientific interest because it confers resistance to human immunodeficiency virus (HIV)-1 infection. In fact, CCR5 has been identified as a coreceptor for cell entry of macrophage-tropic HIV-1 isolates.[2] This observation provided the rationale for studying the potential role of CCR5 in other viral infections. Although the hepatitis C virus (HCV) does not require CCR5 expression for cell entry, the initial observation of the more frequent CCR5-Δ32 mutation deserves further study in other populations with hepatitis C.

In a subsequent article by Promrat et al,[3] no such higher frequency of CCR5-Δ32 homozygosity was found in patients with hepatitis C than in control individuals, but in a multivariate analysis the authors found that the CCR5 promoter 59029-A allele was marginally associated with a sustained response to interferon therapy. Whether interactions between HCV and CCR5-mediated processes could affect therapeutic response to interferon-alpha and ribavirin is another matter. In fact, these antiviral agents may modulate cytokine production and reduce fibrotic processes without altering viral load.[4] To the best of our knowledge, no one knows whether CCR5-Δ32 mutation is involved in the severity of fibrosis, and only preliminary results are available on the CCR5-Δ32 mutation and response to antiviral therapy.

Moreover, in a recent study performed in two independent cohorts of patients from Germany, no significant association was found between CCR5 genotypes and chronic HCV infection.[5] Furthermore, if such mutations do play a role in hepatic injury, it is important to analyze whether this is specific for virally induced injury.

The aims of the present study were to verify whether this mutation occurs in an Italian population with HCV infection and whether it is an adverse or favorable host

factor correlating to the severity of liver disease or response to antiviral therapy.

PATIENTS AND METHODS

Patients

The study involved 130 consecutive Caucasian patients with chronic HCV infection who gave informed consent to the study. The study protocol was approved by the local ethical committee. Of the 130 patients, 104 were male and 26 were female; the mean age was 46.6 ± 15.0 years. The diagnosis of HCV infection was based on clinical, virologic, and histologic grounds. All patients were HCV-RNA positive according to qualitative polymerase chain reaction and had undergone liver biopsy in the 6 to 12 months before the beginning of the study. None of them were coinfected with hepatitis B surface antigen or HIV. Other causes of liver damage (drugs, alcohol, autoimmunity) were ruled out on the basis of the patients' histories and conventional test results.

Histologic scores for grade/stage were obtained according to the classification of Ishak et al.[6]

HCV load was determined quantitatively by use of a branched DNA technology (Chiron, Emeryville, CA) with a detection limit of 200,000 copies/mL serum. HCV genotypes were determined by the Innolipa II line probe assay (Innogenetics, Zwijndrecht, Belgium).

The frequency of CCR5-Δ32 mutation was compared with 2 control populations: (1) 110 blood donors (108 female, 2 male; mean age 53.2 + 9.4 years) from the same geographic area, and (2) 135 patients with primary biliary cirrhosis (PBC) (125 female, 10 male; mean age 59.9 + years) from the same geographic area; the diagnosis of PBC was based on clinical, immunologic, (positivity for antimitochondrial antibodies at a titer > 40) and histologic grounds.

CCR5-Δ32 Polymorphism Genotyping

We obtained 2 mL of blood samples from each study participant and treated them with ethylenediaminetetra-acetic acid. Genomic DNA was extracted with the QIAmp Blood Midi Kit (Quiagen S.p.A., Milan, Italy) according to the manufacturer's protocol.

Primers flanking the CCR5-Δ32 mutation (sense, 5′–CAA AAA GAA GGT CTT CAT TAC ACC–3′; antisense 5′-CCT GTG CCT CTT CTT CTC ATT TCG-3′)[7] were chosen to amplify 189-bp (wild-type) and 157-bp (Δ32-deletion) fragments of the CCR5 gene, respectively.

Amplification was done on a GeneAmp polymerase chain reaction system 2400 thermocycler (Perkin Elmer Inc; Boston, Massachusetts); 35 cycles at 94°C, 1 minute; 55°C, 1 minute; 72°C, 1 minute. The amplified fragments were visualized on 2% agarose gel (Promega; Madison, Wisconsin).

Statistical Analysis

Data analyses were performed by use of the χ^2 test (Mantel-Haenszel and Fisher exact test) and the Student *t* test, as appropriate. $P < 0.05$ was considered significant. Analyses were carried out with the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois. Deviations of CCR5 wild-type and CCR5Δ32 gene frequencies from Hardy-Weinberg equilibrium were tested with an exact test.[8]

RESULTS

Four (3.1%) patients with chronic hepatitis C and 1 blood donor (0.9%) were CCR5-Δ32 homozygous, whereas no CCR5-Δ32 homozygosity was found in PBC patients; the wild-type gene (wt/wt) was present in a similar percentage in the 2 groups of patients (with chronic hepatitis C and with PBC) and in control individuals without any significant difference (83.1% vs 90.4% vs 83.6%, respectively) (Table 1).

Heterozygosity for CCR5-Δ32 was found in 18 (13.8%) HCV+ patients, 17 blood donors (15.5%), and 13 (9.6%) PBC patients. The difference between the 3 groups revealed no significant difference.

Among chronic hepatitis C patients, there was no significant correlation between CCR5-Δ32 homozygosity and the following parameters: histology (grading and staging), HCV genotype, viral load, and serum levels of aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyltransferase. The presence of any mutation (wt/Δ32 and Δ32/Δ32) was unrelated to the following parameters: sex, age, body mass index, risk factors for acquiring the infection, extrahepatic diseases, histologic grading/staging, or viral load (Table 2). Genotype 1 seemed to be associated more to the presence of any mutation with respect to the other genotypes, but the difference did not reach any statistical significance (Table 2).

Ninety-five chronic hepatitis C patients received a standard antiviral protocol with pegylated interferon (PEG-Intron, Schering-Plough, Schering Corporation, Kenilworth, New Jersey) + ribavirin (Rebetol, Schering-Plough). The duration and dosing of the antiviral treatment were according to the 2002 guidelines of the National Institutes of Health.[9] Sustained response was achieved in 59 patients (62.1%); the remainder either did not respond or experienced relapse. Response to the treatment was uninfluenced by CCR5-Δ32 deletion.

TABLE 1. Distribution of CCR5 Polymorphisms Among HCV+ Individuals, PBC Patients, and Blood Donors

	wt/wt		wt/Δ32		Δ32/Δ32	
Group	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
HCV+ (n = 130)						
Observed	108	(83.1)	18	(13.8)	4	(3.1)
Expected	105.3	(81.0)	23	(18.0)	1	(1.0)
PBC (n = 135)						
Observed	122	(90.4)	13	(9.6)	0	(0.0)
Expected	122.3	(90.4)	12	(9.2)	0	(0.0)
Blood donors (n = 110)						
Observed	92	(83.6)	17	(15.5)	1	(0.9)
Expected	91	(83.5)	18	(16.3)	1	(0.7)

TABLE 2. Frequency of Distribution of CCR5 Polymorphisms in HCV+ Patients According to Virologic, Histologic, and Biochemical Parameters

	wt/wt (n = 108)		Δ32 Deletion (n = 22)		
Parameters	**n**	**(%)**	**n**	**(%)**	***P***
Sex					
F	23	(13.6)	3	(21.3)	
M	85	(86.4)	19	(78.7)	0.563
Age (yrs)	46.6 ± 15.0		41.7 ± 16.4		0.174
BMI (mean ± SD)	25.0 ± 2.3		24.5 ± 2.8		0.325
Risk factors					
None	9	(8.3)	2	(9.1)	
community-acquired	46	(42.6)	7	(31.8)	0.485
blood transfusions	6	(5.6)	2	(9.1)	0.574
drug addiction	47	(43.5)	11	(50.0)	0.659
Extrahepatic conditions					
Absence	85	(78.7)	19	(86.4)	
Presence	23	(21.2)	3	(13.6)	0.310
Grading (mean ± SD)	7.2 ± 4.4		7.4 ± 2.9		0.839
Staging (mean ± SD)	1.8 ± 2.4		2.3 ± 1.9		0.334
Genotype					
1	51	(47.2)	13	(59.1)	
2	24	(22.2)	3	(13.6)	
3	29	(26.9)	3	(13.6)	
4	4	(3.7)	3	(13.6)	0.121
Viral load (copies$X10^6$/l ± SD)	1.16 ± 1.80		1.41 ± 1.25		0.973
Response to antiviral therapy*					
Yes	51	(63.8)	8	(53.3)	
No	29	(36.2)	7	(46.7)	0.447
ALT		146.1 ± 87.8	132.5 ± 53.1		0.354

BMI, body mass index; ALT, serum alanine aminotransferase.
*35 untreated.

DISCUSSION

Our study failed to confirm any increased frequency of CCR5-Δ32 homozygosity in an Italian population of chronic hepatitis C patients. Moreover, this mutation seemed to have no adverse host factor effect on severity of liver disease or any negative influence on response to antiviral therapy. This lack of any more frequent CCR5-Δ32 polymorphism in HCV-infected patients is consistent with reports by other authors.[3,5,10] Patient selection may partially explain the difference between these results and the findings reported by Woitas et al[1] because the major risk factor for HCV acquisition in their case was hemophilia (> 80% of cases), whereas the majority of our patients had community-acquired infection (with a positive history of injection with reusable materials before the 1980 s) and drug abuse.

A large control group with a different type of liver disease (PBC) was chosen for comparison. PBC patients form a peculiar control group for two reasons: (1) PBC is a primary liver disease with an autoimmune pathogenesis, in which the target for damage is the biliary epithelium, and although a genetic predisposition has been recognized, the triggering event is unknown; (2) several infectious agents have been hypothesized, including a retroviral agent.[11,12] Because chemokines and chemokine receptors are essential in protecting against infectious pathogens, chemokine receptor gene mutations might modulate the host's response to other viral infections. In particular, CCR5-Δ32 mutation has been found to be associated with protection against rheumatoid arthritis and multiple sclerosis,[13] so CCR5-Δ32 mutation might conceivably be associated with protection against PBC. Our study failed to find any association between CCR5-Δ32 mutation and HCV disease. Moreover, we failed to find a different distribution of CCR5-Δ32 mutation between PBC patients and control individuals. This finding is in agreement with our previous preliminary data, which focused on the CCR5-D32 mutation as a candidate susceptibility locus for PBC in two distinct cohorts of PBC patients from Italy and the United Kingdom.[14] These data provided no evidence for involvement of the CCR5-D32 mutation in the genetic susceptibility/resistance to PBC or in disease severity. Actually, the genetic basis of PBC remains largely unknown, and further studies are necessary to identify candidate genes involved in the genesis and progression of PBC.

Our failure to detect any greater frequency of the mutation in our HCV+ patients than in blood donors might have been due to the relatively small size of our sample, however. The initial study by Woitas et al[1] included 153 HCV+ patients and 102 blood donors. Wasmuth et al[5] included two independent cohorts of German patients: 112 in Aachen and 221 in Berlin. Thus, our sample size, being constituted by consecutive patients (the majority of whom were treated

with antiviral therapy), is adequate also in terms of statistical reasons.

Given the low frequency of CCR5-Δ32 mutation in our study group, it is hardly surprising that there should be no statistically significant associations between CCR5-Δ32 polymorphism and viral, histologic, and biochemical parameters.

It is a matter of speculation whether CCR5-Δ32 mutation can interfere with response to antiviral therapy. There is weak evidence to suggest that the CCR5-Δ32 allele is more frequent in primary nonresponders than in responders to interferon-alpha monotherapy, whereas the difference in relation to sustained response does not reach statistical significance.[15] Our study revealed no such difference in the distribution of CCR5-Δ32 mutation with respect to response to combination therapy. This observation is consistent with the conclusions reached by other authors.[15,16] An attractive hypothesis suggested by Ahlenstiel et al[15] is that ribavirin enhances HCV-specific T helper cell type 1 immune responses, which are insufficiently generated because of the CCR5-Δ32 mutation.

In our opinion, all published contributions on this issue, including our own study, still leave open the potential role of CCR5 in the pathophysiology of hepatitis C infection. We would like to stress, however, that our negative results prompt two practical messages.

First, the relationship between CCR5-Δ32 mutation and HCV genotype may be of interest. We found that genotype 1 was more frequently associated with the presence of any mutation with respect to the other genotypes, but the difference did not reach any statistical significance. The previous articles by Woitas et al[1] and Promrat et al[3] did not focus on this aspect, which deserves further study on larger groups of HCV-infected patients.

Second, extensive proliferative genetic association studies are currently under way. Our standard will evolve as knowledge is gained on complex traits and appropriate strategies for conducting association studies.[17]

In contrast with previously reported data, our results do not confirm any significant role for CCR5 expression in either chronic HCV-related disease severity or response to antiviral treatment.

In conclusion, data on the role of CCR5-Δ32 mutation in HCV infection are still lacking. There is an urgent need for independent replication of an initial study in populations with different genetic backgrounds and with appropriately matched control individuals.

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