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Gender Susceptibility to Chronic Hepatitis C Virus Infection Associated with Interleukin 10 Promoter Polymorphism

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Elevated levels of interleukin 10 (IL-10) were previously described for chronically hepatitis C virus (HCV)-infected patients. We determined by a sequence-specific oligonucleotide probing technique the IL-10 promoter genotypes in 286 Argentinean HCV patients grouped according to disease outcome. The GG genotype (position −1082) is known to be associated with high IL-10 production, GA is considered an intermediate producer, and AA is associated with low IL-10 production. We found an increase in frequency of the GG genotype in female patients who do not eliminate the virus (RNA^+). In these patients, the GG frequency was 0.19, versus 0.10 in controls ($P = 0.03$). This association became more significant in those RNA^+ female patients with elevated hepatic transaminases (GG frequency of 0.25; $P = 0.0013$). Additionally, this genotype frequency was higher in noncirrhotic female patients than in controls (GG frequency for noncirrhotic female patients was 0.31; $P = 0.009$). In RNA^- patients, the GA frequency was elevated compared with that in controls (GA frequency of 0.76 in RNA^- patients versus 0.48 in controls; $P = 0.01$), that in all HCV patients (GA frequency of 0.43; $P = 0.001$), and that in RNA^+ patients (GA frequency of 0.40; $P = 0.0005$). We conclude that a gender effect is observed with women carrying the GG high IL-10 producer genotype. The higher levels of IL-10 present in those individuals are associated with a higher risk of an inefficient clearance of the HCV and the development of a chronic HCV infection together with a lower risk of progression to cirrhosis in female patients.

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease worldwide. About 60 to 80% of patients develop chronic infection, which may progress to cirrhosis and hepatocellular carcinoma (2, 26). The mechanisms whereby HCV causes acute liver injury and initiates the cascade of events leading to the establishment of persistent infection and development of chronic liver disease are not clearly established. Many factors, including age, gender, alcohol consumption, body mass index, steatosis, and human immunodeficiency virus (HIV) or hepatitis B virus (HBV) coinfection, affect disease outcome but are insufficient to explain it. Immunologic and genetic factors may play an important role (2).

A strong natural killer (NK) cell- and T helper 1 (Th1) cell-mediated immune response seems to be a key factor in the protection against HCV infection (9, 11). In addition, viral persistence and a deficient response to antiviral therapy have been associated with the production of inappropriate levels of cytokines (2). Interleukin 10 (IL-10) is a Th2 cytokine which down-regulates the Th1 effector mechanisms. Elevated serum levels of this cytokine have been observed to occur in patients with untreated chronic HCV infection (3). Moreover, in vitro production of IL-10 by peripheral blood mononuclear cells of chronically infected patients is higher than that observed with individuals showing a self-limited HCV infection (12, 31). This increase in IL-10 levels was also observed in a prospective study of patients with acute infection who developed a chronic disease (29).

Functional polymorphism was described for the IL-10 gene promoter. The single-nucleotide polymorphisms (SNP) at positions −1082 (G/A), −819 (C/T), and −592 (C/A) from the transcriptional start site are in linkage disequilibrium, and they are responsible for three different haplotypes: GCC, ACC, and ATA. There is a correlation between IL-10 genotype and cytokine production, i.e., ACC/ACC, ACC/ATA, and ATA/ATA (designated the AA genotype) are associated with low IL-10 production, GCC/ACC and GCC/ATA (GA genotype) are considered intermediate producers, and GCC/GCC (GG genotype) is considered a high producer (5). The −1082G allele, by having a lower binding affinity to the transcription factor PU.1, shows an increased transcriptional activity of the IL-10 promoter (27).

The present study was designed to retrospectively analyze the frequencies of IL-10 haplotypes and genotypes in anti-HCV-positive patients, taking into account the different outcomes of HCV infection.

MATERIALS AND METHODS

Patients. This retrospective study included 209 healthy controls (HC) and 286 anti-HCV-positive individuals (HCV patients) derived from the hepatology units of the Infectious Diseases Hospital F. J. Muñiz, Gastroenterology Hospital Dr. C. Bonorino Udaondo, and Buenos Aires Italian Hospital, Buenos Aires, Argentina. The ethnicity of this population is known as Latin American Caucasoid.

The clinical features of HCV patients included in this study are described in Table 1. All individuals showed the presence of anti-HCV antibodies detected by a third-generation enzyme-linked immunosorbent assay (version 4.0; Abbott-

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TABLE 1. Clinical features of anti-HCV-positive patients

Patient group[a]	No. of patients	Mean age (range) (yr)	No. of males:no. of females[b]	Mean HCV RNA (range) (IU/ml)[c]	% Virus of genotype 1[c]
All	286	50 (22–77)	161:125		
HCV RNA$^-$	25	40 (27–61)	8:17		
HCV RNA$^+$	261	51 (22–77)	154:107	1,768,700 (1,589–20,000,000)	73
HCV RNA$^+$ normalALT	61	54 (22–76)	25:36	1,747,607 (126,191–6,234,555)	NA[d]
HCV RNA$^+$ elevatedALT	200	50 (25–77)	126:74	1,771,336 (1,589–20,000,000)	72
HCV RNA$^+$ noncirrhotic	138	49 (25–77)	84:54	1,960,393 (1,589–20,000,000)	63
HCV RNA$^+$ cirrhotic	123	57 (27–76)	69:54	1,837,171 (10,000–10,416,512)	85

[a] All individuals showed the presence of anti-HCV antibodies detected by a third-generation enzyme-linked immunosorbent assay. Circulating HCV RNA was detected by qualitative PCR analysis.
[b] For HCV RNA$^-$ versus HCV RNA$^+$ results, $P = 0.01$ and OR = 3 (CI = 1.3 to 7.3); for HCV RNA$^+$ normalALT versus HCV RNA$^+$ elevatedALT results, $P = 0.003$ and OR = 2.5 (CI = 1.4 to 4.4).
[c] Results were obtained from data available (for HCV RNA results, $n = 45$; for genotype results, $n = 82$).
[d] NA, not available.

Murex). Patients were grouped according to the outcome of HCV infection as follows: (i) 261 HCV RNA$^+$ patients, having circulating HCV RNA detected by qualitative PCR (AMPLICOR hepatitis C virus test, version 2.0), (ii) 25 HCV RNA$^-$ patients, with a self-limited infection identified as being negative for circulating HCV RNA and positive for the confirmatory test for the presence of anti-HCV antibodies by use of a LIA 3 supplementary test (INNO-LIA HCV-AbIII; Inogenetics), (iii) 200 HCV RNA$^+$ elevatedALT patients (from the group of HCV RNA$^+$ patients), who showed serum levels of alanine aminotransferase (ALT) persistently elevated between 1.2 and 7 times over the upper normal limit, and (iv) 61 HCV RNA$^+$ normalALT patients (from the group of HCV RNA$^+$ patients), who had normal levels of transaminases. Liver biopsy samples obtained by intrahepatic punction were available for only 188 HCV RNA$^+$ patients. The METAVIR system was used to assess the degree of liver fibrosis (F0 to F4) (1). For the other 73 patients, the diagnosis of cirrhosis was performed by clinic and ultrasonographic studies.

Within the different groups of HCV patients, we found no significant differences between age at which patients became infected, viral genotype, serum HCV RNA level, or alcohol consumption. Many of these patients are at present under antiviral treatment. However, patient data included in this study were compiled prior to patient enrollment in any treatment. Patients were negative for HIV markers and other causes of liver disease, like HBV infection, metabolic disease, drug-induced hepatitis, or autoimmune hepatitis.

All healthy donors were tested and found to be negative for the presence of infectious diseases (HIV, HBV, HCV, cytomegalovirus, and Chagas' disease).

Informed consent was obtained from each patient and control. The study protocol conforms to the ethical guidelines of each author's institution.

DNA extraction and IL-10 PCR. Blood samples from patients and controls were collected in EDTA sterile tubes. Genomic DNA was obtained by proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation.

DNA was amplified using a PTC-100 MJ Research thermocycler (MJ Research Inc., Watertown, CT). Each 25 μl of reaction mixture contained 100 to 300 ng of DNA sample, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, pH 9, 250 μM each deoxynucleoside triphosphate, 3 mM $MgCl_2$, 600 μM each primer (sense, 5′-ATCCAAGACAACACTACTAA-3′; antisense, 5′-TAAATA TCCTCAAAGTTCC-3′ [22]), and 1 U *Taq* polymerase (Inbio-Highway, Buenos Aires, Argentina). After an initial melting time of 3 min at 94°C, samples were subjected to 39 cycles of 30 s at 94°C, 30 s at 51°C, and 1 min at 72°C, with a final extension time of 5 min at 72°C. The obtained 587-bp fragment (amplified region, −1115 to −528) was monitored by electrophoresis on a 2% agarose gel with ethidium bromide (0.5 μg/ml).

Sequence-specific oligonucleotide probing. Two 5′-digoxigenin-labeled probes were used to positively identify each SNP by a dot blot technique. The probe sequences are as follows: for −1082G, 5′TTCTTTGGGAGGGGGAAG3′, and for 1082A, 5′ACTTCCCCTTCCCAAAGAA3′; for −819C, 5′GAGGTGATGT AACATCTCTGTGC3′, and for −819T, 5′GCACAGAGATATTACATCACC TGT3′; for −592C, 5′CCGCCTGTCCTGTAGGAA3′, and for −592A, 5′TTC CTACAGTACAGGCGGG3′ (22). A total of 2 μl of PCR product was blotted onto each Hybond N+ nylon transfer membrane (Amersham International, Slough, United Kingdom). The double-stranded DNA was separated by incubation of the membranes in 0.4 M NaOH for 5 min and then neutralized with SSPE 2× solution (0.3 M NaCl, 20 mM PO_4H_2Na, 2 mM EDTA, pH 7.4) for 5 min. After DNA was immobilized by cross-linking in a UV Stratalinker (Stratagen Ltd., Cambridge, United Kingdom) at 1,200 mJ/cm^2, membranes were incubated in 5 ml of prehybridization solution (5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 1% casein, 0.1% *N*-lauryl sarcosine, and 0.02% sodium dodecyl sulfate [SDS]) for 30 min at 42°C and hybridized overnight by addition of 800 pmol of the specific probe to each tube. Membranes were washed in SSPE 2× containing 0.1% SDS for 10 min at room temperature, in 5× SSC with 0.5% SDS for 20 min at probe-specific temperatures (51°C for −1082G, 58°C for −1082A, −592C, and −592A, 62°C for −819T, and 66°C for −819C), and in

TABLE 2. IL-10 promoter haplotype and genotype frequencies in Argentinean and other populations

Population	Reference(s)	Haplotype frequency			Genotype frequency[a]		
		GCC	ACC	ATA	GG	GA	AA
Argentinean		0.35	0.40	0.26	0.10	0.48	0.42
Brazilian	23				0.11	0.45	0.44
Australian white	5	0.51	0.28	0.21			
English white	13	0.49	0.29	0.22			
Norwegian	19				0.26	0.49	0.24
Sicilian	14				0.25	0.64	0.11
North American	30				0.17	0.39	0.44
Italian	15				0.16	0.46	0.39
Japanese	7, 17	0.04	0.27	0.69	0.00	0.08	0.92

[a] GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies; AA, ACC/ACC plus ACC/ATA plus ATA/ATA frequencies.

TABLE 3. IL-10 promoter haplotype and genotype frequencies in anti-HCV-positive patients according to gender

Study group	No. of controls or patients	Haplotype frequency			Genotype frequency[c]		
		GCC	ACC	ATA	GG	GA	AA
HC	209	0.35	0.39	0.26	0.10	0.48	0.42
Female	88	0.34	0.40	0.26	0.10	0.50	0.40
Male	121	0.34	0.39	0.27	0.09	0.50	0.41
HCV patients	286	0.36	0.35	0.29	0.15	0.43	0.42
Female[a]	125	0.35	0.41	0.24	0.17	0.36*	0.47
Male	161	0.38	0.30	0.32	0.13	0.49	0.38
CPD[b] patients	55	0.30	0.36	0.34	0.07	0.46	0.47

[a] $P < 0.039$ (comparing GG/GA/AA frequencies with those of HC).
[b] CPD, chronic pulmonary disease.
[c] GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies; AA, ACC/ACC plus ACC/ATA plus ATA/ATA frequencies. *, $P < 0.03$ (comparing GA versus non-GA frequencies with those of HC).

TABLE 4. Frequencies of IL-10 promoter genotypes according to outcome of HCV infection

Study group	No. of controls or patients	Genotype frequency[a]			Groups compared[b]	*P* value
		GG	GA	AA		
HC	209	0.10	0.48	0.42		
HCV patients	286	0.15	0.43	0.42		
HCV RNA^-	25	0.04	0.76	0.20	RNA^- vs HC	0.03
					RNA^- vs total HCV	0.005
					RNA^- vs RNA^+	0.002
HCV RNA^+	261	0.16	0.40	0.44		
Female	107	0.19	0.31	0.50	Female RNA^+ vs HC	0.004
					Female RNA^+ vs male RNA^+	0.04
Male	154	0.14	0.46	0.40		
HCV RNA^+ normalALT	61	0.13	0.32	0.55		
HCV RNA^+ elevatedALT	200	0.19	0.42	0.39	RNA^+ elevatedALT vs HC	0.02
Female	74	0.25	0.30	0.45	Female elevatedALT vs HC	0.0006
					Female elevatedALT vs male elevatedALT	0.028
Male	126	0.16	0.49	0.36		

[a] GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies; AA, ACC/ACC plus ACC/ATA plus ATA/ATA frequencies.
[b] GG versus GA versus AA genotypes were compared by the chi-square test.

SSPE 2× for 10 min at room temperature. After 5 min of washing in buffer 1 (0.1 M Tris, 0.15 M NaCl, pH 7.5), the membranes were blocked to reduce nonspecific binding in buffer 1 containing 1% nonfat milk (buffer 2) and incubated with 1/10,000 alkaline phosphatase-conjugated antidigoxigenin antibody (Roche Diagnostics, Indianapolis, Ind.) in buffer 2 for 30 min at room temperature. The membranes were washed twice in buffer 1 (10 min each wash) and once in buffer 3 (0.1 M Tris, 0.05 M $MgCl_2$, 0.1 M NaCl, pH 9.5; 5 min), incubated with 25 mM chemiluminescent substrate CSPD (Roche Diagnostics, Indianapolis, Ind.) in buffer 3 for 20 s at room temperature, and stored for 15 min at 37°C. X-ray films were exposed for 30 min before being developed. Allele-specific probes were used to determine each SNP genotype, and the IL-10 promoter haplotypes were inferred after compound analysis of the three SNP results.

Statistical analysis. We compared haplotype and genotype frequencies between the groups of patients and controls with the chi-square test for independence for 3 by 2 contingency tables (GG versus GA versus AA) or the two-sided Fisher exact test for 2 by 2 tables (GG versus non-GG and GA versus non-GA). *P* values of <0.05 were considered statistically significant, *P* values of <0.01 very significant, and *P* values of <0.001 extremely significant. Bonferroni's correction (p_c) was applied to *P* values obtained by the Fisher test (three groups). The odds ratio (OR) with a 95% confidence interval (CI) was calculated to evaluate the relative risk in each patient group.

RESULTS

Haplotype and genotype frequencies of IL-10 promoter in different populations. Ethnic differences in the haplotype and genotype frequencies within the IL-10 promoter have been reported previously (8, 18, 25). The genotype frequencies of GG, GA, and AA observed in the Argentinean population were quite similar to the frequencies found in the Brazilian Caucasian population (23). However, as depicted in Table 2, these frequencies differed from those previously reported for other populations. For example, in England and Australia the GCC haplotype is present in approximately 50% of white individuals (5, 13). With Norwegian, North American, and Italian individuals, 16 to 26% of the population is homozygous for this haplotype (14, 15, 19, 30). On the other hand, in Japan, the GCC haplotype is less frequent (4 to 6%) and the GG homozygous genotype was not found in any control individual (7, 8, 17).

IL-10 promoter haplotype and genotype frequencies in HCV patients according to gender. The comparison of the haplotype and genotype frequencies between our healthy controls and HCV patients (including all anti-HCV-positive patients) showed no significant differences. However, we found a significant difference in the genotype frequencies (GG, GA, and AA) between female HCV patients and controls ($P = 0.039$) (Table 3).

Additionally, we evaluated GA versus non-GA and GG versus non-GG frequencies. This comparison showed a significant

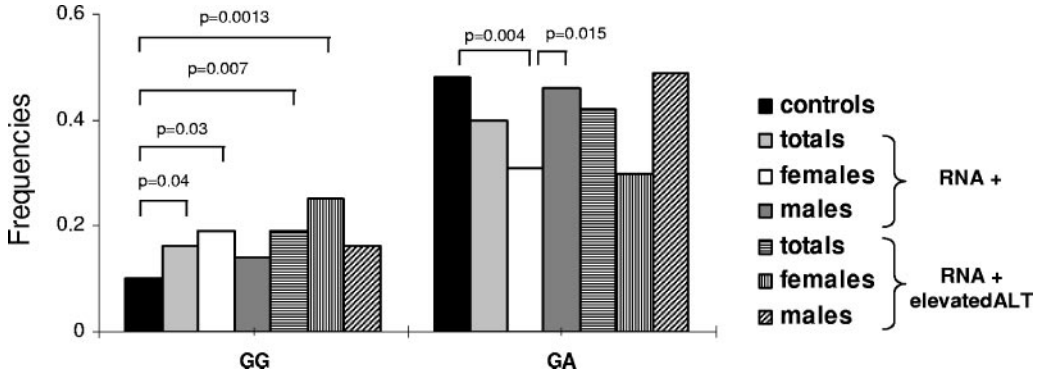

FIG. 1. Genotype frequencies of the IL-10 promoter in RNA^+ HCV patients according to gender. GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies. *P* values were obtained by the Fisher test (GG versus non-GG or GA versus non-GA).

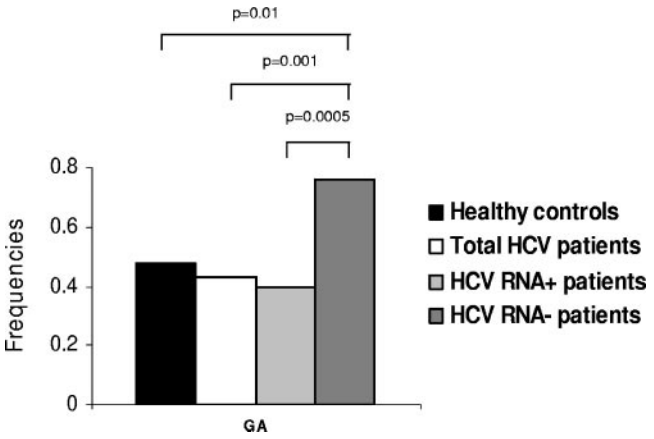

FIG. 2. Increase of GA frequency in self-limited (RNA^-) HCV patients. GA, GCC/ACC plus GCC/ATA frequencies. *P* values were obtained by the Fisher test (GA versus non-GA).

decrease of GA frequencies in the same group of female patients ($P = 0.03$, $p_c = 0.09$, OR = 0.9, CI = 0.4 to 0.95) and a tendency to an increase of the GG frequency (0.17 in female HCV patients versus 0.10 in controls).

We found no differences in the IL-10 promoter haplotype and genotype frequencies according to viral genotype or age of HCV patients (data not shown).

In our control population, the IL-10 promoter haplotype and genotype frequencies showed no differences between males and females (Table 3). Therefore, all subsequent comparisons involving female or male patients were made with the total number of control individuals (males plus females).

Gender effect on IL-10 promoter frequency for those HCV patients who do not eliminate the virus (RNA^+). The genotype frequencies (GG/GA/AA) showed significant differences when we compared RNA^+ female patients either with controls ($P = 0.004$) or with male RNA^+ patients ($P = 0.04$) (Table 4). These frequencies showed an even stronger significance in those RNA^+ female patients with elevated levels of ALT in comparison with controls ($P = 0.0006$) or with males who also had elevated levels of ALT ($P = 0.028$) (Table 4).

The analysis of GG versus non-GG genotypes showed a significant increase of GG frequency in RNA^+ female patients compared with that in controls ($P = 0.03$, $p_c = 0.09$, OR = 2.2, CI = 1.1 to 4.2). This comparison became even more significant in RNA^+ female patients with elevatedALT versus controls ($P = 0.0013$, $p_c = 0.039$, OR = 3.3, CI = 1.6 to 6.5) (Fig. 1). This increase in GG frequency of RNA^+ female patients was also accompanied by a decrease in the GA frequency compared with that for controls ($P = 0.004$, $p_c = 0.012$, OR = 0.5, CI = 0.3 to 0.8) or with that for RNA^+ male patients ($P = 0.015$, $p_c = 0.045$, OR = 0.5, CI = 0.3 to 0.9) (Fig. 1).

IL-10 promoter frequencies in self-limited HCV infection (RNA^-). In patients with self-limited HCV infection, the genotype distributions were significantly different when patients who had cleared the virus were compared with controls ($P = 0.03$), with all HCV patients ($P = 0.005$), or with RNA^+ HCV patients ($P = 0.002$) (Table 4). As depicted in Fig. 2, this difference was caused by an increase in the frequency of GA (RNA^- versus HC, $P = 0.01$, $p_c = 0.03$, OR = 0.3, CI = 0.1 to 0.76; RNA^- versus total HCV patients, $P = 0.001$, $p_c = 0.003$, OR = 0.23, CI = 0.09 to 0.26; RNA^- versus RNA^+, $P = 0.0005$, $p_c = 0.0015$, OR = 0.2, CI = 0.08 to 0.5).

In females, the high IL-10 producer genotype (GG) seems to be associated with an anticirrhotic effect. We next addressed the association of IL-10 promoter genotypes and the development of cirrhosis. As depicted in Table 5, the genotype frequencies (GG/GA/AA) in cirrhotic patients are quite similar to those in controls. Again, a significant difference was present only when we evaluated these frequencies in noncirrhotic female patients in comparison with frequencies either in controls ($P = 0.003$) or in male noncirrhotic patients ($P = 0.009$).

Further analysis of genotype combinations confirmed the increase of the GG frequency in female noncirrhotic patients ($P = 0.009$, $p_c = 0.027$, OR = 0.3, CI = 0.15 to 0.7), indicative of the antifibrotic role of the high IL-10 producer genotype (Fig. 3). In this group of patients, we found that the increase in GG frequency was also accompanied by a decrease in GA frequency, in comparison with results for controls ($P = 0.009$, $p_c = 0.027$, OR = 2.4, CI = 1.3 to 4.7) and for male noncirrhotic patients ($P = 0.003$, $p_c = 0.009$, OR = 3, CI = 1.4 to 6.2) (Fig. 3).

It is known that the age at which a patient became infected influences the outcome of HCV infection. Thus, we analyzed the IL-10 promoter genotypes according to age/gender and development of fibrosis (Table 6). Differences in the genotype frequencies were observed for noncirrhotic female patients whose age was above 40 years old either versus controls ($P =$

TABLE 5. IL-10 promoter genotype frequencies and development of cirrhosis in HCV-infected patients

Study group (score)	No. of controls or patients	Genotype frequency[b]			Groups compared[c]	*P* value
		GG	GA	AA		
HC	209	0.10	0.48	0.42		
HCV cirrhotic (F4)[a]	123	0.16	0.40	0.44		
Female	54	0.16	0.35	0.49		
Male	69	0.17	0.44	0.40		
HCV noncirrhotic (F0 to F3)[a]	138	0.16	0.44	0.39		
Female	54	0.24	0.27	0.49	Female noncirrhotic vs HC	0.003
					Female noncirrhotic vs male noncirrhotic	0.009
Male	84	0.12	0.53	0.34		

[a] Patients were from the HCV RNA^+ group. METAVIR scores for fibrosis stages (F0 to F4) were used.
[b] GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies; AA, ACC/ACC plus ACC/ATA plus ATA/ATA frequencies.
[c] GG versus GA versus AA genotypes were compared by the chi-square test.

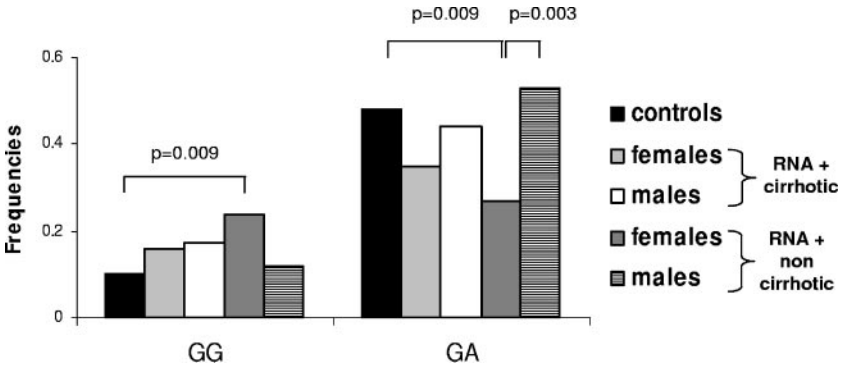

FIG. 3. Genotype frequencies of the IL-10 promoter in cirrhotic and noncirrhotic HCV patients according to gender. GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies. *P* values were obtained by the Fisher test (GG versus non-GG or GA versus non-GA).

0.0026) or versus males with the same features ($P = 0.02$). These differences were caused by an increase of the GG frequency (for noncirrhotic females of >40 years versus HC, $P = 0.007$, $p_c = 0.021$, OR = 0.29, and CI = 0.13 to 0.66; for noncirrhotic females of >40 years versus noncirrhotic males of >40 years, $P = 0.056$) and a decrease of GA frequency in those female patients (for noncirrhotic females of >40 years versus HC, $P = 0.015$, $p_c = 0.045$, OR = 2.5, and CI = 1.2 to 5.4; for noncirrhotic females of >40 years versus noncirrhotic males of >40 years, $P = 0.02$, $p_c = 0.06$, OR = 2.9, and CI = 1.2 to 6.9). Frequencies found in female patients less than 40 years old did not prove to be significantly different from those of the controls, but the number of patients included in the former group was smaller.

We also analyzed the IL-10 promoter polymorphism in 55 patients with chronic pulmonary disease (Table 3) and 102 patients with celiac disease (data not shown). The IL-10 promoter haplotype and genotype frequencies in these samples resulted in no significant differences from frequencies in HC samples.

DISCUSSION

The present study demonstrated an increase in the GG frequency as well as a decrease of the GA frequency in female HCV patients, in particular, in those RNA^+ patients with elevated levels of ALT. Additionally, we found an increased frequency of the GA genotype in self-limited HCV infection.

In spite of ethnic differences, our results are in line with similar findings reported by the Mayo Clinic (30) and by an extensive study performed in the United Kingdom (10). These studies have also found that the GG frequency increased in patients with chronic HCV infection. Also in accordance with our report, the ATA haplotype (15) and ATA/ATA and −1082A/G genotypes (10) were found to be associated with the self-limited infection.

This study addressed for the first time the gender effect in the association between IL-10 promoter polymorphism and the HCV infection. Our understanding is that the gender effect described in the present study might explain many contradictory reports regarding the effect of the IL-10 promoter associ-

TABLE 6. IL-10 promoter genotype frequencies related to age/gender and development of fibrosis

Study group (age [yr])	No. of controls or patients	Genotype frequency[c]			Groups compared	*P* value
		GG	GA	AA		
HC	209	0.10	0.48	0.42		
HCV cirrhotic[a]	123	0.16	0.40	0.44		
Female (<40)	2	0.00	0.00	1.00		
Male (<40)	9	0.17	0.17	0.67		
Female (>40)	52	0.13	0.42	0.45		
Male (>40)	60	0.18	0.47	0.34		
HCV noncirrhotic[a]	138	0.16	0.44	0.39		
Female (<40)	13	0.23	0.23	0.54		
Male (<40)	27	0.11	0.63	0.26		
Female (>40)[b]	41	0.27	0.27	0.46	Female noncirrhotic (>40 yr) vs HC	0.0026
					Female vs male noncirrhotic (>40 yr)	0.02
Male (>40)	57	0.10	0.53	0.38		

[a] Patients were from the HCV RNA^+ group. METAVIR scores for fibrosis stages (F0 to F4) were used: HCV cirrhotic patients scored F4, and HCV noncirrhotic patients scored F0 to F3.

[b] For GG versus non-GG noncirrhotic females of >40 years versus HC, $P = 0.007$, $p_c = 0.021$, OR = 0.29, and CI = 0.13 to 0.66; for GG versus non-GG noncirrhotic females of >40 years versus noncirrhotic males of >40 years, $P = 0.056$; for GA versus non-GA noncirrhotic females of >40 years versus HC, $P = 0.015$, $p_c = 0.045$, OR = 2.5, and CI = 1.2 to 5.4; and for GA versus non-GA noncirrhotic females of >40 years versus noncirrhotic males of >40 years, $P = 0.02$, $p_c = 0.06$, OR = 2.9, and CI = 1.2 to 6.9.

[c] GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies; AA, ACC/ACC plus ACC/ATA plus ATA/ATA frequencies.

ated with HCV infection. As examples of these contradictory results, Edwards-Smith et al. (5) found no significant differences in genotype frequencies including a gender effect, but the small number of patients analyzed might preclude detection of this effect. Along the same lines, additional reports were unable to confirm differences in the haplotype or genotype frequencies between patients and controls (15, 16, 24). However, those studies failed to analyze a gender effect or also were based on a small number of cases. On the other hand, Lio et al., whose study was based on data from only 60 HCV patients (18 RNA^- and 42 RNA^+ patients), reported that the GG genotype was associated with a self-limited HCV infection (14).

A vigorous $CD4^+$ and $CD8^+$ T-cell response with a predominant Th1 cytokine profile seems to be responsible for recovery from an HCV infection (4, 11). Conversely, patients who develop a chronic infection show a predominant Th2 response that down-regulates the Th1 response and therefore favors persistent HCV infection (6, 26). In this context, it is tempting to speculate that those individuals carrying the high IL-10 producer genotype (GG) will be prone to down-regulate the Th1 response, resulting in a failure of the HCV clearance.

Experimental and clinical data suggest a protective role of IL-10 in hepatic fibrogenesis (20, 28). Accordingly, we found that the increased frequency of GG was observed only with noncirrhotic female patients, mainly in those with stages 1 and 2 of liver fibrosis (not shown), but not in patients with stage 4 (cirrhosis). A study of Japanese chronically HCV-infected patients reported the GCC haplotype to be associated with less hepatic fibrosis. In line with our findings, this Japanese study suggests that high production of IL-10 may cause inhibition of liver fibrosis progression (7).

Chronically HCV-infected patients who received a short treatment with recombinant IL-10 showed a decreased hepatic inflammation and reduced liver fibrosis (20). On the other hand, and in concordance with our findings, a 12-month IL-10 therapy in patients with advanced fibrosis led to increased levels of serum HCV RNA and a reduction in fibrosis score (21), suggesting that high levels of IL-10 not only decrease fibrogenesis but also lead to an increased HCV viral burden. This could be achieved by decreasing the number of HCV-specific $CD4^+$ and $CD8^+$ gamma interferon-secreting T cells and polarizing the immune response towards a Th2-dominant profile.

Similarly, it has been published that the antibody-induced blockage of the IL-10 receptor generates a favorable balance of $CD4^+$ T-cell response to HCV. Also, this anti-IL-10 receptor reverses the inhibitory effect of IL-10 on HCV-specific T-cell proliferation, demonstrating the major role of IL-10 in suppressing antiviral T-cell responses (28). Moreover, clinical evidence suggests that individuals with cellular immune dysfunction, such as that due to HIV infection, show a much faster disease progression (11).

In conclusion, the present study confirms that the host's genetic background plays a significant role in the outcome of HCV infection. In particular, we demonstrate a gender effect associated with the susceptibility to develop a persistent HCV infection and a chronic liver disease together with an inhibition of fibrogenic process in women carrying the GG IL-10 promoter genotype.

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