INTRODUCTION
Since its discovery in 1989, hepatitis C virus (HCV) has been recognized as the leading cause of chronic liver disease in the world. Host genetic factors have been implicated in the persistence of HCV infection. Single nucleotide polymorphisms at positions -607 C/A (rs1946518) and -137 G/C (rs187238) in the IL-18 gene promoter have been suggested to be associated with delayed hepatitis C virus clearance and persistence of the disease. Objective - Identify these polymorphisms in a population infected with hepatitis C virus from the Brazilian Amazon region. Methods - In a cross-sectional analytical study conducted in Belém, Pará, Brazil, 304 patients infected with hepatitis C virus were divided into two groups: group A, patients with persistent infection; group B, patients with spontaneous clearance. The control group consisted of 376 volunteers not infected with hepatitis C virus. Samples were analyzed by RT-PCR for the detection of viral RNA and by RFLP-PCR to evaluate the presence of the -137 G/C and -607 C/A IL-18 gene promoter polymorphisms. Results - Comparison of polymorphism allele frequencies between the patient and control groups showed a higher frequency of allele C at position -607 among patients (P=0.02). When the association between the polymorphisms and viral infection was analyzed, patients carrying genotype C/A at position -607 were found to be at higher risk of persistent hepatitis C virus infection (P=0.03). Conclusion - The present results suggest a possible role of the -607 IL-18 gene promoter polymorphism in the pathogenesis of hepatitis C virus infection.
investigated the association between the -607 C/A (rs1946518) and -137 G/C (rs187238) SNPs in the IL-18 gene promoter and the outcomes of HCV infection. The objective of the present study was to identify these polymorphisms in a population infected with HCV from the Brazilian Amazon region.

METHODS
Sample characterization
A total of 304 patients of Tropical Medicine Center of the viral hepatitis program, were selected and divided into 2 groups: Group A - 174 patients with persistent infection, with anti-HCV and HCV polymerase chain reaction (PCR) positive for more than 6 months. Group B - 130 patients with spontaneous clearance, characterized by positive anti-HCV and HCV PCR negative. Viral clearance was defined when anti-HCV serology was positive and HCV RNA was undetectable in serum over a period of 6 months or less in the absence of specific HCV treatment (Figure 1). For the control group, 376 anti-HCV-negative volunteers with negative HCV PCR were selected at random.

None of the patients was co-infected with hepatitis B virus (HBV surface antigen negative) or Human Immunodeficiency Virus (HIV), none of the patients consumed alcoholic beverages, and all were treatment naive. All patients received detailed information about the study and signed a consent form.

All results of serology confirmed by at least these paired serological tests within consecutive 6 months during the follow-up. All subjects were diagnosed by experienced physicians on the basis of clinical and laboratory findings and internationally accepted criteria(18).

All subjects included (patients and control) were of the same socioeconomic status and had similar cultural habits. In addition, all subjects were born in Pará state and had the same ethnic origin, approximately 50% Portuguese, 40% Amerindian, and 10% African(20).

The study was approved by the Ethics Committee of the Tropical Medicine Center, Federal University of Para (Núcleo de Medicina Tropical, Universidade Federal do Pará - NMT/UFPA), Belém, PA, Brazil (Permit No. 042/2011). All patients gave their informed consent to participate in the study.

Serological testing and HCV genotyping
Peripheral venous blood (5 mL) was collected from each participant into EDTA tubes. Plasma was isolated by centrifugation and stored at -80°C until assayed. Serological markers of HCV infection (anti-HCV antibodies) were investigated using the ETI-ABHCVK-4 kit from Diasorin (Saluggia, Italy).

RNA was extracted from all samples using the QIAamp Viral RNA kit (Hilden, Germany). HCV RNA was investigated by nested PCR using primers that target the 5'-UTR region. The first reaction consisted of the synthesis and amplification of cDNA in a single step using 1 mL of the k10

FIGURE 1. Flowchart of inclusion and exclusion criteria of the patients hepatitis C virus (HCV) carriers participating in the study.
(5'-GGC GAC ACT CCA CCA TRR-3') and k11 (5'-GGT GCA CGG TCT ACG AGA CC-3') primers, 5 mL DNase-free ultrapure water, 5 mL RNase, and 1 mL One-Step Taq DNA Polymerase (Invitrogen, São Paulo, Brazil).

The samples were incubated in a thermocycler at 42°C for 45 min. The amplification conditions were initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 45 s. A final extension was performed at 72°C for 7 min and the samples were cooled to 4°C.

The second reaction mixture contained 2.5 mL buffer, 4 mL dNTPs, 1.5 mL MgCl₂, 1 mL k15 (5'-ACC ATR RAT CAC TCC CCT GT-3') and k16 (5'-CAA GCA CCC TAT CAG GCA GT-3') primers, 12.5 mL DNase- and RNase-free ultrapure water, and 0.5 mL Platinum Taq DNA Polymerase (Invitrogen).

The virus was genotyped by the restriction fragment length polymorphism (RFLP) technique using Ava II and Rsa I restriction enzymes. Positive and negative controls were included in all reactions.

Detection of the IL-18 gene polymorphisms (-137 G/C and -607 C/A)

Human DNA was isolated from whole blood samples (patients and controls) using the PureLink™ Genomic DNA kit (Invitrogen).

Specific primers were used for the polymorphisms at position -137 G/C: (forward) 5'-TGCTTCTAATGGACCTAGGAGGATGG-3' and (reverse) 5'-CTCTTTTATGTAATATCACTATTTTCATGAGA-3', and at position -607 C/A: (forward) 5'-TTCGTTGCGAAGTAGTGA-AAAATTTT-3' and (reverse) 5'-AAAGGATAGTGTAGCAAGGCCAT-3'.

The IL-18 gene polymorphisms were identified as described by An et al. For RFLP-PCR, the restriction enzymes Bgl II and Dra I were used to detect the -137 and -607 polymorphisms, respectively. Digestion with Bgl II produces a single band of 141 bp for allele C and two bands of 105 and 36 bp for allele G (Figure 1). Digestion with Dra I produces a single band of 154 bp for allele C and two bands of 125 and 28 bp for allele A.

Histopathology analysis

A liver biopsy was obtained from all patients anti-HCV positive. The biopsies were fixed in 10% buffered formalin, submitted to routine processing, and embedded in paraffin. The paraffin blocks were cut and the sections were stained with hematoxylin-eosin and analyzed by a pathologist.

Fibrosis staging and inflammatory activity grading were used for the classification of chronic hepatitis. Fibrosis was staged as follows: 0: no fibrosis, 1: portal fibrosis without septa, 2: few septa, 3: numerous septa delineating nodules without cirrhosis, and 4: cirrhosis. Inflammatory activity was graded taking into account activities in the portal tract and in the periportal and lobular regions: 0: no histological activity, 1: minimal damage, 2: mild activity, 3: moderate activity and 4: severe activity.

Statistical analysis

The results were analyzed using the BioEstat 5.0 program. The chi-square test was used to compare frequencies between groups. The frequency distribution of the variables is expressed as percentage and odds ratios (OR) were calculated. A level of significance of 95% was adopted.

RESULTS

Table 1 shows the demographic data of each group. No significant difference in mean age or gender distribution was observed between the three groups studied. However, comparison of the frequency of HCV genotypes showed a higher prevalence of genotype 1 in patients with persistent infection (group A) compared to the group with spontaneous clearance (group B).

Histological analysis revealed the presence of chronic hepatitis in all patients. Grade 0 or 1 necroinflammatory activity was observed in 93% (284/304) of these patients and grade 2 or 3 in 7% (20/304). Regarding the stage of fibrosis, 90% (275/304) of the patients had stage 0 or 1 and 10% (29/304) stage 2. Analysis of histopathological alterations between the groups A and B showed no statistically significant difference (P>0.05).

TABLE 1. Demographic and clinical characteristics of HCV-infected patients with persistent infection and spontaneous clearance and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>37.5</td>
<td>38</td>
<td>36</td>
<td>0.6251</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>120 (69)</td>
<td>92 (70)</td>
<td>240 (64)</td>
<td>0.1561</td>
</tr>
<tr>
<td>Female</td>
<td>54 (31)</td>
<td>38 (30)</td>
<td>136 (36)</td>
<td></td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>140 (80)</td>
<td>91 (70)</td>
<td>-</td>
<td>0.0481</td>
</tr>
<tr>
<td>Non-1</td>
<td>34 (20)</td>
<td>39 (30)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Group A: patients with persistent infection; Group B: patients with spontaneous clearance; Group C: control group. a: one-way ANOVA; b: Kruskal-Wallis test; c: X² test.

Distribution of the IL-18 gene promoter polymorphisms in patients and controls

Table 2 shows the genotype frequencies of the -137 G/C and -607 C/A polymorphisms in the IL-18 gene in patients and in the control group. A higher frequency of genotypes C/A and C/C of the -607 polymorphism was observed in patients when compared to controls (Table 2).
Analysis of polymorphisms in the interleukin 18 gene promotor (-137 G/C and -607 C/A) in patients infected with hepatitis C virus from the Brazilian Amazon

Santos KN, Almeida MKC, Fecury AA, Costa CA, Martins LC. Analysis of polymorphisms in the interleukin 18 gene promotor (-137 G/C and -607 C/A) in patients infected with hepatitis C virus from the Brazilian Amazon. Arq Gastroenterol 2015;52(3):225

Association of IL-18 gene polymorphisms with the resolution of HCV infection

To evaluate the influence of the IL-18 gene promoter polymorphisms (-137 G/C and -607 C/A) on the course of HCV infection, we compared allele frequencies between the patient groups: group A (persistent infection) and group B (spontaneous clearance). The frequency of allele C at position -607 was higher in patients with persistent infection than in those with spontaneous clearance (Table 3). No significant difference between patients was observed for the allele distribution at position -137.

Table 2. Distribution of the IL-18 gene promoter polymorphisms (-137 G>C, -607 C>A) in patients and controls

<table>
<thead>
<tr>
<th>SNP/ genotype</th>
<th>Patients (n=304)</th>
<th>Controls (n=376)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>-607 C/A A/A</td>
<td>36 (11.8)</td>
<td>68 (18.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C/A</td>
<td>156 (51.3)</td>
<td>192 (51.1)</td>
<td>1.53</td>
<td>0.97-2.42</td>
<td>0.08</td>
</tr>
<tr>
<td>C/C</td>
<td>112 (36.9)</td>
<td>116 (30.8)</td>
<td>1.82</td>
<td>1.12-2.94</td>
<td>0.01</td>
</tr>
<tr>
<td>C/A+C/C</td>
<td>268</td>
<td>308</td>
<td>1.64</td>
<td>1.06-2.54</td>
<td>0.03</td>
</tr>
<tr>
<td>Alleles A</td>
<td>228 (37.5)</td>
<td>328 (43)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>380 (62.5)</td>
<td>424 (57)</td>
<td>1.28</td>
<td>1.03-1.6</td>
<td>0.02</td>
</tr>
<tr>
<td>-137 G/C C/C</td>
<td>100 (32.9)</td>
<td>128 (34)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G/C</td>
<td>120 (39.5)</td>
<td>132 (35.1)</td>
<td>1.16</td>
<td>0.81-1.66</td>
<td>0.46</td>
</tr>
<tr>
<td>G/G</td>
<td>84 (27.6)</td>
<td>116 (30.9)</td>
<td>0.92</td>
<td>0.63-1.36</td>
<td>0.77</td>
</tr>
<tr>
<td>GC + GG</td>
<td>204</td>
<td>248</td>
<td>1.05</td>
<td>0.76-1.45</td>
<td>0.81</td>
</tr>
<tr>
<td>Alleles C</td>
<td>320 (52.6)</td>
<td>388 (51.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td>288 (47.4)</td>
<td>364 (48.5)</td>
<td>0.95</td>
<td>0.77-1.18</td>
<td>0.74</td>
</tr>
</tbody>
</table>

OR: odds ratio; 95% CI: 95% confidence interval.

Table 3. Association between IL-18 gene polymorphisms and PCR results

<table>
<thead>
<tr>
<th>IL-18 polymorphism</th>
<th>Group A</th>
<th>Group B</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>-607 C/A A/A</td>
<td>14 (8)</td>
<td>22 (17)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C/A</td>
<td>96 (55)</td>
<td>60 (46)</td>
<td>2.51</td>
<td>1.19-5.28</td>
<td>0.02</td>
</tr>
<tr>
<td>C/C</td>
<td>64 (37)</td>
<td>48 (37)</td>
<td>2.09</td>
<td>0.97-4.51</td>
<td>0.06</td>
</tr>
<tr>
<td>C/A+C/C</td>
<td>160</td>
<td>108</td>
<td>2.32</td>
<td>1.14-4.70</td>
<td>0.02</td>
</tr>
<tr>
<td>-137 G/C C/C</td>
<td>62 (35.6)</td>
<td>38 (29.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G/C</td>
<td>64 (36.8)</td>
<td>56 (43.1)</td>
<td>1.42</td>
<td>0.83-2.45</td>
<td>0.24</td>
</tr>
<tr>
<td>G/G</td>
<td>48 (27.6)</td>
<td>36 (27.7)</td>
<td>1.22</td>
<td>0.67-2.21</td>
<td>0.60</td>
</tr>
<tr>
<td>G/G+G/G</td>
<td>112</td>
<td>92</td>
<td>1.34</td>
<td>0.82-2.18</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Group A: patients with persistent infection; Group B: patients with spontaneous clearance.
OR: odds ratio; 95% CI: 95% confidence interval.

DISCUSSION

Progression of hepatitis C varies between patients. There is sufficient evidence suggesting that the cause of progression is multifactorial and includes viral, immunological and genetic factors.

Among viral factors, the infecting HCV genotype seems to play an important role. In the present study, a higher frequency of genotype 1 was observed in the population studied. In addition, the prevalence of genotype 1 was higher among patients with persistent infection compared to those with spontaneous clearance.

Differences in the distribution of HCV genotypes between different geographic areas have been demonstrated. In Brazil, genotype 1 is the most frequent, followed by genotypes 3 and 2. Similar results have been reported for the state of Pará.

Studies conducted in different countries suggest that the genomic heterogeneity of HCV has a significant impact on the severity of liver disease and on the response to treatment. In this respect, genotype 1 has been associated with severe liver damage.

Cytokines play a crucial role in the differentiation, maturation and functional activation of immune cells, as well as in the regulation of the immune response that controls the clearance of persistent HCV  patients. The main genes involved in the progression of disease and persistence of HCV are related to viral replication and to triggering the immune response. Host genetic factors have been implicated in the persistence of HCV infection.

The inflammatory cytokine IL-18 has been indicated as a marker of inflammation and liver damage. Two SNPs have been described in the promoter region of the IL-18 gene at positions -607 C/A (rs1946518) and -137 G/C (rs187238).
These polymorphisms are associated with the transcription activity of the IL-18 promoter and cause a reduction in serum IL-18 levels\(^2,10\).

Some research groups suggested that these polymorphisms influence the clinical evolution of HCV infection. In the present study, comparison of the -607 C/A and -137 G/C polymorphisms between HCV-infected patients and controls showed a higher frequency of allele C at position -607 in the patient group. In addition, genotype C/A at position -607 was associated with a higher risk of persistent infection with HCV (OR=2.51).

Similar studies conducted in America\(^1\), India\(^13\), and Europe\(^11\) also showed that the mutation from C to A at position -607 was associated with viral clearance and protection against severe liver disease in the populations studied.

CONCLUSION

The importance of -607 and -137 polymorphisms in the IL-18 gene promoter for HCV infection should be further studied, but our studies suggest an association between the polymorphism -607 and the persistence of viral infection.

Authors’ contributions

REFERENCES


