Case Control Study

Association of interferon lambda-4 rs12979860 polymorphism with hepatocellular carcinoma in patients with chronic hepatitis C infection

Jóice Teixeira de Bitencorte, Tássia Flores Rech, Vagner Ricardo Lunge, Deivid Cruz dos Santos, Mário Reis Álvares-da-Silva, Daniel Simon

ORCID number: Jóice Teixeira de Bitencorte 0000-0002-0156-4225; Tássia Flores Rech 0000-0002-9530-7042; Vagner Ricardo Lunge 0000-0003-4012-8650; Deivid Cruz dos Santos 0000-0001-7300-422X; Mário Reis Álvares-da-Silva 0000-0002-5001-246X; Daniel Simon 0000-0003-1122-8468.

Author contributions: de Bitencorte JT, Álvares-da-Silva MR, and Simon D were involved with conception and design of the study; de Bitencorte JT, Rech TF, and dos Santos DC were involved with acquisition of the samples and data; de Bitencorte JT performed the molecular analysis; de Bitencorte JT, Rech TF, Lunge VR, and Simon D performed the statistical analysis and interpretation of data; de Bitencorte JT, Rech TF, and Simon D drafted the manuscript; All authors read and approved the final version of the manuscript.

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Abstract

BACKGROUND

Hepatitis C virus (HCV) infection is a public health concern worldwide. Several factors, including genetic polymorphisms, may be evolved in the progression of HCV infection to liver diseases. Interferon lamdas (IFNLs) modulate the immune response during viral infections. IFNLs induce antiviral activity, interfering in the viral replication by promoting the expression of several genes that regulate immunological functions. The interferon lambda-4 (IFNL4) rs12979860 polymorphism, which is characterized by a C to T transition in intron 1, is associated with spontaneous and treatment-induced clearance of HCV infection and may play a role in the development of HCV-associated liver diseases, including hepatocellular carcinoma (HCC).

AIM

To investigate the association of IFNL4 rs12979860 polymorphism with fibrosis, cirrhosis, and HCC in patients with chronic HCV infection.

METHODS

This study was comprised of 305 chronic HCV-infected patients (53 fibrosis, 154 cirrhosis, and 98 HCC cases). The control group was comprised of 260 HCV-negative healthy individuals. The IFNL4 rs12979860 polymorphism was genotyped using the TaqMan assay. Fibrosis was diagnosed based on liver biopsy findings, while cirrhosis was diagnosed through clinical, laboratory,
Hepatitis C virus (HCV) infection is a public health concern worldwide as it is associated with increased morbidity and mortality\(^1\). HCV, a hepatotrophic virus, is the etiological factor for chronic hepatitis C. Patients with HCV infection can develop cirrhosis and hepatocellular carcinoma (HCC) and may need liver transplantation\(^1\). According to the World Health Organization report on viral hepatitis, 71 million people were infected with hepatitis C in 2015\(^2\). Generally, acute HCV infections are clinically silent infections. Among the patients with HCV infection, 15%-45% can eliminate the virus spontaneously, with the highest recovery rates observed in children and young women\(^3\). However, a vast majority of infected patients develop chronic hepatitis C, which is characterized by the persistence of HCV in the serum for more than 6 mo. Chronic HCV infection is associated with anatomopathological, and/or imaging data. HCC was diagnosed through imaging tests, tumor, and/or anatomopathological markers.

**RESULTS**

The T allele was observed in the three groups of patients (fibrosis, cirrhosis, and HCC) at a significantly higher frequency when compared with the control group (\(P = 0.047, P < 0.001, \) and \(P = 0.01\), respectively). Also, genotype frequencies presented significant differences between the control group and cirrhosis patients (\(P < 0.001\)) as well as HCC patients (\(P = 0.002\)). The risk analysis was performed using the codominant and dominant T allele models. In the codominant model, it was observed that the CT genotype showed an increased risk of developing cirrhosis in comparison with the CC genotype [odds ratio (OR) = 2.53; 95% confidence interval (CI): 1.55-4.15; \(P < 0.001\)] as well as with HCC (OR = 2.54; 95%CI: 1.44-4.56; \(P = 0.001\)]. A similar result was observed in the comparison of the TT vs CC genotype between the control group and cirrhosis group (OR = 2.88; 95%CI: 1.44-5.77; \(P = 0.001\)) but not for HCC patients. In the dominant T allele model, the CT \& TT genotypes were associated with an increased risk for progression to cirrhosis (OR = 2.60; 95%CI: 1.63-4.19; \(P < 0.001\)) and HCC (OR = 2.45; 95%CI: 1.42-4.31; \(P = 0.001\)).

**CONCLUSION**

These findings suggest that the T allele of IFNL4 rs12979860 polymorphism is associated with the development of cirrhosis and HCC in chronic HCV-infected patients.

**Key Words:** Hepatitis C; Hepatitis C virus; Cirrhosis; Hepatocellular carcinoma; Genetic polymorphism; Interferon-lambda

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**Core Tip:** Hepatitis C virus (HCV) infection is a major public health problem worldwide as the infection progresses to severe chronic liver diseases in many patients. Interferon lamdas modulate the immune responses against infections, including the antiviral activity by promoting the expression of several genes related to immunological functions. The interferon lambda-4 rs12979860 (C/T) polymorphism, which is associated with spontaneous and treatment-induced clearance of HCV, plays a pivotal role in the host response to HCV-associated liver diseases. In this case-control study, the rs12979860 T allele was found to be associated with the development of cirrhosis and hepatocellular carcinoma in chronic HCV-infected patients.

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**INTRODUCTION**

Hepatitis C virus (HCV) infection is a public health concern worldwide as it is associated with increased morbidity and mortality\(^1\). HCV, a hepatotrophic virus, is the etiological factor for chronic hepatitis C. Patients with HCV infection can develop cirrhosis and hepatocellular carcinoma (HCC) and may need liver transplantation\(^1\). According to the World Health Organization report on viral hepatitis, 71 million people were infected with hepatitis C in 2015\(^2\). Generally, acute HCV infections are clinically silent infections. Among the patients with HCV infection, 15%-45% can eliminate the virus spontaneously, with the highest recovery rates observed in children and young women\(^3\). However, a vast majority of infected patients develop chronic hepatitis C, which is characterized by the persistence of HCV in the serum for more than 6 mo. Chronic HCV infection is associated with...
slow progression, and the patients may remain asymptomatic for several decades. Thus, the persistence of HCV in the organism can cause continuous damage to the liver and can progress to fibrosis, cirrhosis, and HCC\(^{11}\).

HCC, which accounts for 80% of all primary liver cancers, is associated with high mortality rates. Globally, HCC is the third leading cause of cancer-related deaths. HCC is a complex disease with a variety of etiologies and may be associated with different risk factors, such as chronic hepatitis B virus (HBV) and HCV infections, alcoholic liver disease, and nonalcoholic steatohepatitis\(^{6,9}\). HCV infection, which is the second most common risk factor for HCC, accounts for 10%-25% of all HCC cases. Additionally, 80%-90% of HCC cases are reported in patients with cirrhosis\(^{11}\).

The pathogenesis of HCV infection and its progression to chronic liver disease vary among individuals. Several factors, including viral, environmental, and host characteristics, such as age, sex, ethnicity, and genetic factors, contribute to the pathogenesis of HCV\(^{11}\). The immune system-related genes, such as interferon lambda2 (IFN-λ2s), are directly related to modulate viral infections with the ability to induce antiviral activity in target cells and interfere with HCV replication within the host cells. The binding of IFN-λ to its receptor activates the signal transducer and activator of transcription phosphorylation-dependent signaling cascade, inducing hundreds of IFN-stimulated genes and consequently regulating various immune functions\(^{10,11}\).

The interferon lambda-3 gene \((IFNL3)\), which is located on chromosome 19q13.13, encodes IFN-λ3 protein, a cytokine with antiviral properties. Genome-wide association studies have demonstrated the association of single nucleotide polymorphisms, such as rs12979860 and rs8099917, near the \(IFNL3\) gene (formerly known as interleukin-28B \((IL28B)\) gene), both with spontaneous virus elimination after acute infection and with sustained virological response in patients with chronic hepatitis C treated with pegylated interferon plus ribavirin combination therapy\(^{12-14}\). Prokunina-Olsson et al\(^{15}\) demonstrated that the rs12979860 polymorphism, commonly referred as an \(IL28B\) or \(IFNL3\) variant, is in an independent loci and should be called an interferon lambda-4 \((IFNL4)\) variant. The \(IFNL4\) gene is controlled by rs368234815 AG-TT polymorphism, in which the AG allele creates an open reading frame for \(IFNL4\), while the TT allele does not. Furthermore, the AG allele (rs368234815) is reported to be in linkage disequilibrium with the T allele of rs12979860 polymorphism\(^{16,17}\).

The rs12979860 polymorphism has a relevant and well-known role in the spontaneous and treatment-induced clearance of HCV infection\(^{18}\). However, the importance of this polymorphism in the progression of HCV-associated liver diseases is still unclear. Therefore, the objective of our study was to investigate the potential role of the variants from \(IFNL4\) rs12979860 polymorphism in the progression to hepatic fibrosis, cirrhosis, and HCC in chronic HCV-infected patients.

**MATERIALS AND METHODS**

**Study population**

This case-control study was conducted using a convenience sampling strategy. The case group was comprised of 305 patients who visited the outpatient clinic of the Gastroenterology-Hepatology Service of the Hospital de Clínicas de Porto Alegre in Brazil. HCV-positive patients diagnosed with fibrosis, cirrhosis, or HCC were included in the case group. Fibrosis (METAVIR F1-F3) was diagnosed based on liver biopsy findings, while cirrhosis was diagnosed based on liver biopsy or clinical evidence, such as liver imaging (abdominal ultrasonography, computed tomography, and magnetic resonance) abnormalities or endoscopic findings as well as current or past clinical evidence of decompensation, including Child-Pugh B or C classification (score of \(>6\)) ascites on physical examination, hepatic encephalopathy, or variceal bleeding. HCC was diagnosed through liver biopsy (64/98; 65.3%) or in cirrhotic patients through dynamic computed tomography or magnetic resonance by the presence of a nodule of at least 1 cm featuring arterial phase enhancement with decreased enhancement during the portal venous phase as recommended by international guidelines. Patients with HCV/human immunodeficiency virus and/or HCV/HBV coinfection were excluded as well as patients with other causes of liver diseases such as HBV, metabolic associated fatty liver disease, alcohol abuse (more than 20 or 30 g daily consumption of ethanol for females and males, respectively), and/or hemochromatosis. The control group was comprised of 260 samples obtained from the donors at the Hospital de Clínicas de Porto Alegre blood bank. As Brazilian laws for blood donation requires, all have been tested negative for HBV, HCV, human
immunodeficiency virus, syphilis, and Chagas disease. This study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (protocol number: 15-0126). All participants provided their written informed consent to participate in the study.

Molecular analysis
DNA was extracted from the blood samples using the salting-out method as described previously[13]. The polymorphism was genotyped using the validated pre-designed real-time PCR TaqMan® Assays (Applied Biosystems Inc., Foster City, CA, United States; catalog 4351376, assay ID: C__7820464_10) in the StepOnePlusTM Real-Time PCR Systems (Applied Biosystems Inc.). PCR was performed in an 18 μL reaction volume containing 10 mmol/L Tris-HCl (pH 8.5), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.0625 mmol/L dNTPs, 0.25 μM of each primer, 0.045 μM of each probe, 1 U Taq DNA polymerase (Cenbiot Enzimas, Porto Alegre, Brazil), and 1 μL extracted DNA (10-200 ng). The PCR conditions were as follows: 95 °C for 10 min (initial DNA denaturation), followed by 40 cycles of 95 °C for 15 s (denaturation) and 60 °C for 1 min (annealing and extension).

Statistical analyses
All statistical analyses were performed using SPSS® software (Statistical Package for the Social Sciences 17.0 version, Chicago, IL, United States). The normal distribution of the quantitative variables was examined using the Kolmogorov-Smirnov test with Lilliefors correction. The quantitative variables, which were expressed as mean ± SD, were analyzed using analysis of variance, followed by Tukey post-hoc test. For the categorical variables, the frequencies were calculated and expressed as percentages. Gene frequencies were determined by direct allele counting. Hardy-Weinberg equilibrium (HWE) deviation and the gene frequencies between groups were compared using the Chi-square test. Yates’ correction for continuity was used to analyze the 2 × 2 contingency table. Odds ratio (OR) was estimated with 95% confidence interval (CI). The differences were considered significant at P < 0.05 (two-tailed). Potential confounding factors were entered in the logistic regression models based on statistical criteria (only if the variable was associated with the study factor and with the outcome at P < 0.20). The statistical methods used in this study were reviewed by Dr. D. Simon from the Human Molecular Genetics Laboratory, Universidade Luterana do Brasil (Canoas, Brazil).

RESULTS
The sociodemographic and clinical characteristics of patients are described in Table 1. Patients were stratified into the following three groups: Fibrosis (n = 53), cirrhosis (n = 154), and HCC (n = 98). The mean age of the patients was 59.85 ± 8.83 years, with a statistically significant difference among the groups studied (P = 0.019). A significant statistical difference (P = 0.024) was also observed in the frequency of males in the HCC group (58.2%) when compared to the fibrosis (37.7%) and cirrhosis groups (43.5%). The mean value of body mass index presented a statistically significant difference between the groups with cirrhosis and HCC (27.80 ± 5.39 and 26.34 ± 4.15 kg/m², respectively; P = 0.038). Blood transfusion was the most frequent possible infection source among patients (41.0%). The frequencies of HCV 1 and 3 genotypes, which were the most common, were 40.7% and 36.7%, respectively.

Table 2 shows the allele and genotype frequencies of the IFNL4 rs12979680 polymorphism in the patient and control groups. The success rate for genotyping IFNL4 rs12979680 polymorphism was 100% in all studied groups. Statistically significant differences were observed regarding the allele frequencies, in which the frequency of the T allele was significantly higher in the three groups of patients analyzed when compared to the controls: [fibrosis group vs control group (OR = 1.57; 95%CI: 1.03-1.68; P = 0.047), cirrhosis group vs control group (OR = 1.75; 95%CI: 1.30-2.36; P < 0.001), and HCC group vs control group (OR = 1.57, 95%CI: 1.11-2.23; P = 0.01)].

Compared with those in the control group, the IFNL4 genotype frequencies were significantly higher in the cirrhotic and (P < 0.001) HCC groups (P = 0.002). The genotype distribution in the control and fibrosis groups was in agreement with those expected from HWE (P = 0.81 and P = 0.88, respectively). In contrast, the genotype frequencies in the cirrhosis and HCC groups deviated from those expected from HWE (P = 0.02 and P = 0.01, respectively). When the genotype distribution was analyzed in
Table 1  Sociodemographic and clinical features of chronic hepatitis C virus positive patients

| Characteristics                        | Total, n = 305 | Fibrosis, n = 53 | Cirrhosis, n = 154 | HCC, n = 98 | P value |
|----------------------------------------|----------------|-----------------|--------------------|-------------|---------|
| Age in yr                              | 59.85 ± 8.83   | 57.89 ± 10.43   | 59.29 ± 8.43       | 61.78 ± 8.22| 0.019   |
| Male                                   | 144 (47.2)     | 20 (37.7)       | 67 (43.5)          | 57 (58.2)   | 0.024   |
| Ethnicity, Caucasian                   | 218 (71.5)     | 35 (66.1)       | 110 (71.4)         | 73 (74.5)   | 0.547   |
| BMI in kg/m²                           | 27.08 ± 4.85   | 26.39 ± 4.14    | 27.80 ± 5.39       | 26.34 ± 4.15| 0.038   |
| Level of education                     |                |                 |                    |             | 0.366   |
| Completed primary education or less    | 196 (62.0)     | 31 (56.6)       | 100 (62.3)         | 65 (64.3)   |         |
| Secondary or higher education          | 102 (24.9)     | 20 (34.0)       | 51 (25.3)          | 31 (19.4)   |         |
| Smoker                                 | 59 (19.3)      | 16 (30.2)       | 31 (20.1)          | 12 (12.2)   | 0.001   |
| Alcohol consumption                    |                |                 |                    |             | 0.004   |
| No                                     | 260 (85.2)     | 49 (92.5)       | 137 (89.0)         | 74 (75.5)   |         |
| Former                                 | 45 (14.8)      | 4 (7.5)         | 17 (11.0)          | 24 (24.5)   |         |
| Illicit drug use                       |                |                 |                    |             | 0.164   |
| No                                     | 243 (79.7)     | 43 (81.1)       | 122 (79.2)         | 78 (79.6)   |         |
| Yes                                    | 9 (3.0)        | 4 (7.5)         | 4 (2.6)            | 1 (1.0)     |         |
| Former user                            | 53 (17.4)      | 6 (1.1)         | 28 (18.2)          | 19 (19.4)   |         |
| Coffee drinker                         | 213 (69.8)     | 39 (73.6)       | 112 (72.7)         | 62 (63.3)   | 0.226   |
| Age at infection of HCV in yr          | 27.43 ± 9.75   | 28.47 ± 9.12    | 27.48 ± 9.77       | 26.64 ± 10.26| 0.735   |
| Age at diagnosis of HCV in yr          | 49.11 ± 11.11  | 46.88 ± 12.99   | 49.17 ± 10.97      | 50.24 ± 10.11| 0.223   |
| HCV infection via blood transfusion    | 125 (41.0)     | 24 (45.3)       | 64 (41.6)          | 37 (37.8)   | 0.706   |
| HCV-RNA as log_{10}UI/mL              | 6.05 ± 0.86    | -               | 6.11 ± 0.87        | 5.86 ± 0.78 | 0.141   |
| HCV genotypes                          |                |                 |                    |             | 0.060   |
| 1                                      | 124 (40.7)     | -               | 86 (55.8)          | 38 (38.8)   |         |
| 2                                      | 7 (2.3)        | -               | 4 (2.6)            | 3 (3.1)     |         |
| 3                                      | 112 (36.7)     | -               | 61 (39.6)          | 51 (52.0)   |         |
| Antiviral treatment                    | 178 (58.4)     | -               | 115 (74.7)         | 63 (64.3)   | 0.077   |
| Diabetes                               | 85 (27.9)      | -               | 50 (32.5)          | 35 (35.7)   | 0.595   |
| Steatosis                              | 24 (7.9)       | -               | 13 (8.4)           | 11 (11.2)   | 0.431   |
| Ascites                                | 66 (21.6)      | -               | 31 (20.1)          | 35 (35.7)   | 0.005   |
| Portal hypertension                    | 146 (47.9)     | -               | 72 (46.8)          | 74 (75.5)   | < 0.001 |
| Esophageal varices                     | 156 (51.1)     | -               | 91 (59.0)          | 65 (66.3)   | 0.231   |
| Upper gastrointestinal bleeding        | 49 (16.0)      | -               | 26 (16.9)          | 23 (23.5)   | 0.184   |
| Spontaneous bacterial peritonitis      | 13 (4.3)       | -               | 7 (4.5)            | 6 (6.1)     | 0.568   |
| Hepatic encephalopathy                 | 24 (7.9)       | -               | 13 (8.4)           | 11 (11.2)   | 0.431   |
| Child-Pugh                             |                |                 |                    |             | 0.083   |
| A                                      | 137 (44.9)     | -               | 95 (61.7)          | 42 (42.9)   |         |
| B                                      | 45 (14.1)      | -               | 28 (18.2)          | 15 (15.3)   |         |
| C                                      | 9 (3.0)        | -               | 3 (1.9)            | 6 (6.1)     |         |
| Number of tumors                       |                |                 |                    |             |         |
| 1                                      | -              | -               | 62 (63.37)         |             |         |
| 2                                      | -              | -               | 17 (17.35)         |             |         |
| ≥3                                     | -              | -               | 18 (18.37)         |             |         |
The risk of developing fibrosis, cirrhosis, and HCC was calculated using the following two genetic models: Codominant and dominant T allele models (Table 3). In the codominant model, it was observed that the CT vs CC genotype conferred an increased risk of developing cirrhosis in HCV patients when compared with the control group (OR = 2.53; 95%CI: 1.55-4.15; P < 0.001). Additionally, the CT vs CC genotype conferred an increased risk for HCC (OR = 2.54; 95%CI: 1.44-4.56; P = 0.001). A similar result was observed in the comparison of the TT vs CC genotype between cirrhosis patients and controls (OR = 2.88; 95%CI: 1.44-5.77; P = 0.001) but not for HCC. In the dominant T allele model, the CT + TT genotypes conferred an increased risk of developing cirrhosis (OR = 2.60; 95%CI: 1.63-4.19; P < 0.001) and HCC (OR = 2.45; 95%CI: 1.42-4.31; P = 0.001) when compared with the CC genotype. The observed associations remained significant when logistic regression models were analyzed controlling for potential confounding factors (data not shown).

Table 4 presents the distribution of the IFNL4 rs12979680 polymorphism genotypes regarding clinical features of HCC patients. A significantly higher frequency of the T allele in the dominant T allele model was observed among patients with HCV genotypes 1 and 3 with a frequency of 92% and 67%, respectively (P = 0.017). In addition, a higher frequency of the TT genotype was observed among patients with hepatic encephalopathy (P = 0.03).
**Table 3 Genetic models of association between interferon lambda-4 rs12979860 polymorphism and hepatitis C virus-associated liver diseases**

| rs12979860 | Fibrosis vs Control | Cirrhosis vs Control | HCC vs Control | Fibrosis vs Cirrhosis | Cirrhosis vs HCC |
|------------|---------------------|----------------------|----------------|-----------------------|------------------|
|            | OR (95%CI)          | P value              | OR (95%CI)     | P value              | OR (95%CI)     |
| **Codominant model** |                     |                      |                |                      |                 |
| CC         | 1.00 (Ref.)         | -                    | 1.00 (Ref.)    | -                    | 1.00 (Ref.)    |
| CT         | 1.69 (0.82-3.54)    | 0.126                | 2.53 (1.55-4.15)| < 0.001              | 2.54 (1.44-4.56)|0.001            |
| TT         | 2.40 (0.87-6.27)    | 0.053                | 2.88 (1.44-5.77)| 0.001                | 2.08 (0.86-4.83)| 0.068            |
| **T allele dominant model** |                     |                      |                |                      |                 |
| CC         | 1.00 (Ref.)         | -                    | 1.00 (Ref.)    | -                    | 1.00 (Ref.)    |
| CT + TT    | 1.83 (0.94-3.71)    | 0.061                | 2.60 (1.63-4.19)| < 0.001              | 2.45 (1.42-4.31)| 0.001            |

CI: Confidence interval; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; OR: Odds ratio; Ref.: Reference.

**DISCUSSION**

This study investigated the association of the IFNL4 rs12979860 polymorphism with the development of fibrosis, cirrhosis, and HCC among patients with chronic HCV infection. The frequency of the T allele in the case group was higher than that in the control group. Additionally, the risk analyses indicated that patients with HCV infection harboring the T allele were more susceptible to develop cirrhosis and HCC.

The studies on the role of IFNL4 rs12979860 polymorphism in HCV-related liver diseases have yielded controversial results. A recent meta-analysis of 18 studies involving different ethnicities attempted to elucidate the global association of this polymorphism with HCV and HBV\(^2\)\(^2\). The meta-analysis revealed that the IFNL4 rs12979860 polymorphism is a risk factor for both HCV- and HBV-related HCC. Although the meta-analysis enhanced our understanding of the role of IFNL4 rs12979860 polymorphism in the outcomes of liver diseases with viral etiologies, the results must be carefully analyzed. Some limiting factors, such as ethnic differences, discrepancies in clinical characteristics among different studies, genotyping methods, HCV genotypes, nonuniform controls in case-control studies, and the influence of confounding factors should be considered.

Various studies have evaluated the role of IFNL4 rs12979860 polymorphism in the development of HCC. De la Fuente et al.\(^2\)\(^3\) examined the association of rs12979860 polymorphism with the development of HCC in both chronic HCV infection and nonviral cirrhosis. The authors reported that the TT genotype is highly prevalent in cirrhotic patients infected with HCV genotype 1 who were subjected to liver transplantation. However, there was no significant association between polymorphism variants and hepatocarcinogenesis.

The risk of developing HCC in patients responding to pegylated interferon plus ribavirin treatment is lower than that in nonresponders. Chang et al.\(^2\)\(^4\) evaluated 800 patients who received pegylated interferon plus ribavirin combination therapy but did not respond to treatment to evaluate the risk factors for HCC. The CT + TT genotypes of rs12979860 polymorphism were an independent risk factor for the development of HCC in these patients, which further indicated the importance of this polymorphism in the progression to HCC. Similarly, a study on 200 patients with advanced fibrosis revealed that the IFNL4 rs12979860 TT genotype was significantly associated with HCC development after direct-acting antiviral therapy for chronic hepatitis C\(^2\)\(^5\).

A large international study involving 2916 patients, mostly the European Caucasian population, revealed that the increased number of the T allele was significantly associated with the prevalence of cirrhosis/transition to cirrhosis in patients infected with HCV genotype 1. This association was evident in Caucasian European patients but not in Asian, Latin American, or Middle Eastern patients infected with HCV genotype 1\(^2\)\(^6\).

The genetic background of populations can contribute to variable results among...
Table 4 Distribution of the interferon lambda-4 rs12979860 genotypes based on the clinical features of patients with hepatocellular carcinoma, n = 98

| Variable                          | Genotypes | Codominant model | T allele dominant model |
|-----------------------------------|-----------|------------------|------------------------|
|                                   | CC, n = 24|                  |                        |
|                                   | CT, n = 61|                  |                        |
|                                   | TT, n = 13|                  |                        |
| P value                           |           |                  |                        |
| P value                           |           |                  |                        |
| HCV genotypes                     | 0.052     | 0.017            |                        |
| 1                                 |           |                  |                        |
| 2                                 |           |                  |                        |
| 3                                 |           |                  |                        |
| Diabetes                          | 0.004     | 0.484            |                        |
| Steatosis                         | 0.195     | 0.469            |                        |
| Ascites                           | 0.511     | 0.646            |                        |
| Portal hypertension               | 0.201     | 0.636            |                        |
| Esophageal varices                | 0.030     | 0.383            |                        |
| Child-Pugh                        | 0.209     | 0.156            |                        |
| A                                 | 0.325     | 0.684            |                        |
| B                                 |           |                  |                        |
| C                                 |           |                  |                        |
| Number of tumors                  |           |                  |                        |
| 1                                 |           |                  |                        |
| 2                                 |           |                  |                        |
| ≥ 3                               |           |                  |                        |
| Portal vein thrombosis            | 0.238     | 0.286            |                        |
| Extrahepatic metastases           | 0.487     | 0.780            |                        |

Variables expressed as number (percentage). HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

different studies as the allele frequencies of IFNL4 rs12979860 polymorphism vary among populations. In this study, the minor allele frequencies of the IFNL4 rs12979860 polymorphism, represented by the T allele, in the case and control groups were 0.46 and 0.34, respectively. The minor allele frequencies reported for European, Japanese, and Chinese populations in the 1000 Genomes database were 0.28, 0.10, and 0.06, respectively.

The role of IFN-λ4 in the pathophysiology of chronic HCV infection-mediated liver diseases is still under investigation. IFN-λ4 activates interferon-stimulated genes, induces cell death, and inhibits cell proliferation\(^{[27]}\). In the IFN-λ4-expressing cells, enhanced cell death may cause tissue inflammation, while the antiproliferative effect of IFN-λ4 could decrease the capacity of tissue remodeling\(^{[27,28]}\). In this sense, our study may provide significant information about the association of the genetic variants of the IFNL4 rs12979860 polymorphism with disease progression and clinical features of hepatitis C, demonstrating that this polymorphism has relevance in the HCV spontaneous and treatment-induced clearance of HCV infection. Also, the present study can stimulate the clarification of this issue by the analyses of large samples as well as the correlation of genetic variants with gene expression and protein interactions.

This study has some limitations. The sample size of this study is relatively small. A more representative sample could enhance the statistical power to detect genetic differences. In this study, the fibrosis group, which had the lowest sample number, exhibited a trend of association with the TT genotype and the T allele when compared with the control group. A larger sample size could clarify the role of this
polymorphism in the development of fibrosis. In addition, some data are missing in
the liver fibrosis group (such as HCV RNA, HCV genotype, number of patients on
antiviral treatment, diabetes, and steatosis), which precluded a more detailed
comparison with the other groups. Besides, the analysis of a nonfibrotic (F0) HCV-
infected group would be important because it makes the study more comprehensive.
The analysis of a single polymorphism is insufficient to fully explain the genetic basis
of HCC. In the cirrhosis and HCC groups, the genotype frequencies of the IFNL4
rs12979860 polymorphism did not concur with those expected from HWE. The
deviations from HWE can be due to the population stratification and selection or may
indicate disease association[29,30]. As population stratification may have caused
disequilibrium among the cirrhosis and HCC groups, HWE analysis was performed
on the case group. However, the genotype frequency in the case group deviated from
that expected from HWE. Thus, the observed imbalance could be explained by the
effective role of this polymorphism in the sample of patients with HCV-related liver
diseases.

CONCLUSION
The findings of this study suggest that the T allele of IFNL4 rs12979860 polymorphism
is a potential genetic factor that determines the susceptibility to cirrhosis and HCC
development among patients with chronic HCV.

ARTICLE HIGHLIGHTS

Research background
As a serious public health problem worldwide, hepatitis C virus (HCV) infection has
unfavorable trends in morbidity and mortality. Due to high hepatotrophic potential,
HCV may cause chronic complications, such as fibrosis, cirrhosis, and hepatocellular
carcinoma (HCC). Progression to chronic liver disease usually varies and is influenced
by different factors, including genetic factors. The interferon lambda-4 (IFNL4)
rs12979860 polymorphism, characterized by a C to T transition in the intron 1, has
been associated with spontaneous and treatment-induced clearance of HCV infection
and may play a role in HCV-associated liver diseases, including HCC.

Research motivation
Although the rs12979860 polymorphism has a relevant and well-known role in the
spontaneous and treatment-induced clearance of HCV infection, the importance of
genetic variants of this polymorphism in the progression of HCV-associated liver
diseases is still unclear.

Research objectives
We aimed to investigate the potential role of the variants in the progression to hepatic
fibrosis, cirrhosis, and HCC in chronic HCV-infected patients. In addition, the
distribution of the rs12979860 IFNL4 genetic variants was analyzed in accordance with
clinical features of patients.

Research methods
This case-control study included 305 patients with chronic HCV infection patients (53
with fibrosis, 154 with cirrhosis, and 98 with HCC), and 260 HCV-negative healthy
individuals as controls. Diagnosis of fibrosis (METAVIR F1-F3) was performed by liver
biopsy findings, while the diagnosis of cirrhosis was performed through clinical,
laboratorial, anatomopathological, and/or imaging data. Lastly, diagnosis of HCC was
performed through dynamic imaging tests, and/or anatomopathological markers.
Patients with HCV/human immunodeficiency virus and/or HCV/ hepatitis B virus
coinfection were excluded. Molecular analysis was performed using validated pre-
designed real-time PCR TaqMan® Assays.

Research results
A higher frequency of the T allele was observed among the groups of patients (fibrosis,
cirrhosis, and HCC) as compared to the controls: (P = 0.047; P < 0.001; and P = 0.01,
respectively). Also, significant differences were observed concerning genotype
frequencies between HCC (P = 0.002) and cirrhosis patients (P < 0.001) in comparison with controls. Two genetic models were tested in the risk analysis: Codominant model and dominant T allele model. In the codominant model, it was observed that the CT genotype was related to an increased risk of cirrhosis [odds ratio (OR) = 2.53; 95% confidence interval (CI): 1.55-4.15; P < 0.001] and HCC (OR = 2.54; 95% CI: 1.44-4.56; P = 0.001) as compared to CC genotype. In the comparison of the TT vs CC genotype, a significant difference was observed between the control group and cirrhosis group (OR = 2.88; 95% CI: 1.44-5.77; P = 0.001) but not the HCC group. In the dominant T allele model, the CT + TT genotypes confer an increased risk for the progression to cirrhosis (OR = 2.60; 95% CI: 1.63-4.19; P < 0.001) and HCC (OR = 2.45; 95% CI: 1.42-4.31; P = 0.001). Finally, a significant higher frequency of the T allele among patients with HCV genotypes 1 and 3 (92% and 67%, respectively; P = 0.017) and a higher frequency of TT genotype among patients with hepatic encephalopathy (P = 0.03) was observed.

Research conclusions
This study suggests that the T allele from IFNL4 rs12979860 polymorphism is associated with the development of cirrhosis and HCC in chronic HCV-infected patients.

Research perspectives
As an important factor related to spontaneous and treatment-induced clearance of HCV infection, the analysis of IFNL4 rs12979860 polymorphism in the present study may provide a better understanding of the genetic variants with disease progression and clinical features. In order to clarify this issue, large samples are needed to verify the association of genetic polymorphisms with hepatitis C as well as the correlation of genetic variants with gene expression and protein interactions.

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