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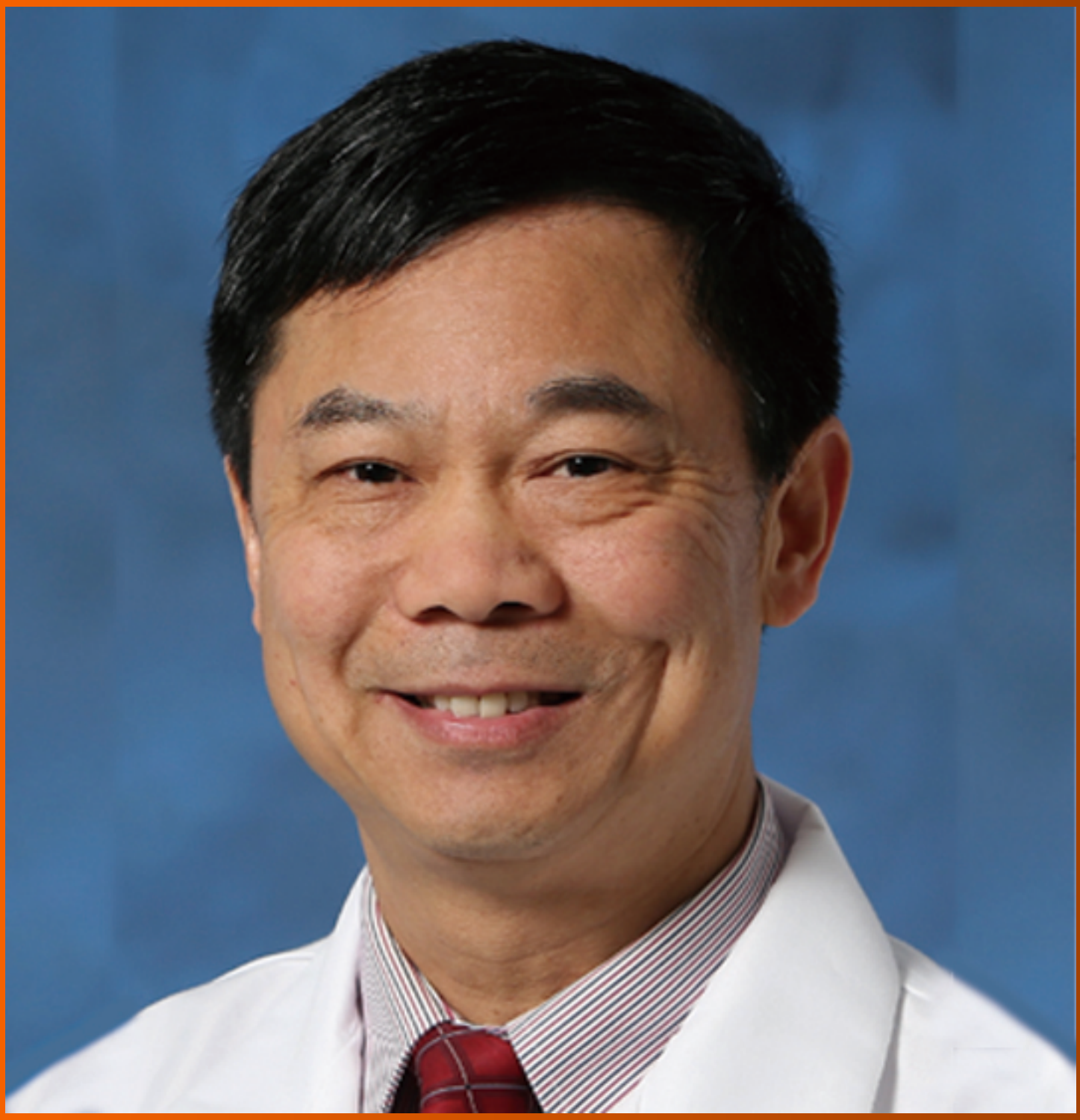

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ABOUT COVER

Editor-in-Chief of *World Journal of Hepatology*, Dr. Ke-Qin Hu is Director of Hepatology Services and Professor of Medicine in the Division of Gastroenterology and Hepatology, University of California, Irvine School of Medicine (United States). Dr. Hu's career efforts emphasize bridging research advances to bedside patient care. His clinical research has focused on the natural history and outcomes of various liver diseases and healthcare disparity. His basic science research has focused on molecular virology and diagnosis of hepatitis B and C virus infection, and chemoprevention of liver cancer. Dr. Hu has coauthored more than 150 research papers, book chapters, and review articles. He is Deputy Editor-in-Chief for Frontiers of Medicine. He is dedicated to community outreach, public health education, and reduction of healthcare disparity. (L-Editor: Filipodia)

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ORIGINAL ARTICLE

Case Control Study

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

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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 lambdas (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,

Committee of Hospital de Clínicas de Porto Alegre under the protocol 15-0126.

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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

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 lambdas 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[1,2]. 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[2-4]. 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[5]. 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

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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[5,6].

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[7,8]. 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[9,10].

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 lambdas (IFN-λs), 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[12-14].

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 gene; *IL28B*), 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[15-18].

Prokunina-Olsson *et al*[19] 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 ΔG-TT polymorphism, in which the ΔG allele creates an open reading frame for *IFNL4*, while the TT allele does not. Furthermore, the ΔG allele (rs368234815) is reported to be in linkage disequilibrium with the T allele of rs12979860 polymorphism[13,19].

The rs12979860 polymorphism has a relevant and well-known role in the spontaneous and treatment-induced clearance of HCV infection[20]. 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[21]. 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 $MgCl_2$, 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 tables. 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/m^2, 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	43 (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)	

Tumor size in cm		-	-	2.8 ± 1.81	
Portal vein thrombosis		-	-	10 (10.20)	
Extrahepatic metastases		-	-	7 (7.14)	
Liver transplantation		-	-	47 (47.96)	
Deaths	14 (4.59)	-	8 (5.19)	6 (6.12)	0.754

Characteristics expressed as number and percentage or mean ± SD. BMI: Body mass index; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

Table 2 Allele and genotype frequencies of interferon lambda-4 rs12979860 polymorphism in patients with hepatitis C virus-associated liver diseases and healthy control subjects

rs12979860	Control, *n* = 260	Total patients, *n* = 305	Fibrosis, *n* = 53	Cirrhosis, *n* = 154	HCC, *n* = 98	*P* value				
						Fibrosis *vs* Control	Cirrhosis *vs* Control	HCC *vs* Control	Fibrosis *vs* Cirrhosis	Cirrhosis *vs* HCC
Allele						0.047	< 0.001	0.010	0.708	0.618
C	345 (66.3)	331 (54.3)	59 (55.7)	163 (52.9)	109 (55.6)					
T	175 (33.7)	279 (45.7)	47 (44.3)	145 (47.1)	87 (44.4)					
Genotype						0.113	< 0.001	0.002	0.541	0.665
CC	115 (44.2)	76 (24.9)	16 (30.2)	36 (23.4)	24 (24.5)					
CT	115 (44.2)	179 (58.7)	27 (50.9)	91 (59.1)	61 (62.2)					
TT	30 (11.6)	50 (16.4)	10 (18.9)	27 (17.5)	13 (13.3)					

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

the total sample of patients (n = 305), deviations from HWE were maintained (P = 0.001).

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)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Codominant model										
CC	1.00 (Ref.)	-	1.00 (Ref.)	-	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	1.50 (0.67-3.28)	0.277	1.01 (0.52-1.95)	0.986
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	1.20 (0.43-3.45)	0.702	0.72 (0.28-1.80)	0.447
T allele dominant model										
CC	1.00 (Ref.)	-	1.00 (Ref.)	-	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	1.42 (0.66-2.97)	0.325	0.94 (0.50-1.79)	0.840

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[22]. 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*[23] 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*[24] 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[25].

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[26].

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

		Genotypes		Codominant model	T allele dominant model
Variable	**CC, *n* = 24**	**CT, *n* = 61**	**TT, *n* = 13**	***P* value**	***P* value**
HCV genotypes				0.052	0.017
1	3 (14.3)	27 (46.6)	8 (61.5)		0.004
2	1(4.8)	2 (3.4)	-		
3	17 (81.0)	29 (50.0)	5 (38.5)		0.007
Diabetes	10 (41.7)	19 (31.1)	6 (46.2)	0.463	0.484
Steatosis	1(4.2)	8 (13.3)	2 (16.7)	0.409	0.195
Ascites	10 (41.7)	20 (32.8)	5 (41.7)	0.679	0.511
Portal hypertension	17 (70.8)	48 (78.7)	9 (75.0)	0.741	0.469
Esophageal varices	17 (70.8)	39 (63.9)	9 (75.0)	0.682	0.646
Upper gastrointestinal bleeding	8 (33.3)	10 (16.4)	5 (41.7)	0.075	0.201
Spontaneous bacterial peritonitis	1 (4.2)	5 (8.2)	-	0.500	0.636
Hepatic encephalopathy	3 (12.5)	2 (3.3)	3 (25.0)	**0.030**	0.383
Child-Pugh				0.209	0.156
A	8 (61.5)	26 (63.4)	8 (88.9)		
B	2 (15.4)	12 (29.3)	1 (11.1)		
C	3 (23,1)	3 (7.3)	-		
Number of tumors				0.325	0.684
1	17 (70.8)	39 (65.0)	6 (46.2)		
2	3 (12.5)	12 (20.0)	2 (15.4)		
≥ 3	4 (16.7)	9 (15.0)	5 (38.5)		
Portal vein thrombosis	4 (16.7)	4 (6.6)	2 (16.7)	0.286	0.238
Extrahepatic metastases	1 (4.2)	5 (8.6)	1 (7.7)	0.780	0.487

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.

REFERENCES

1 **Petruzziello A**, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol* 2016; **22**: 7824-7840 [PMID: 27678366 DOI: 10.3748/wjg.v22.i34.7824]

2 **World Health Organization**. Global hepatitis report, 2017 [Internet]. [cited 6 July 2020]. Available from: https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/

3 **Webster DP**, Klenerman P, Dusheiko GM. Hepatitis C. *Lancet* 2015; **385**: 1124-1135 [PMID: 25687730 DOI: 10.1016/S0140-6736(14)62401-6]

4 **Westbrook RH**, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; **61**: S58-S68 [PMID: 25443346 DOI: 10.1016/j.jhep.2014.07.012]

5 **Lingala S**, Ghany MG. Natural History of Hepatitis C. *Gastroenterol Clin North Am* 2015; **44**: 717-734 [PMID: 26600216 DOI: 10.1016/j.gtc.2015.07.003]

6 **Maasoumy B**, Wedemeyer H. Natural history of acute and chronic hepatitis C. *Best Pract Res Clin Gastroenterol* 2012; **26**: 401-412 [PMID: 23199500 DOI: 10.1016/j.bpg.2012.09.009]

7 **Makarova-Rusher OV**, Altekruse SF, McNeel TS, Ulahannan S, Duffy AG, Graubard BI, Greten TF, McGlynn KA. Population attributable fractions of risk factors for hepatocellular carcinoma in the United States. *Cancer* 2016; **122**: 1757-1765 [PMID: 26998818 DOI: 10.1002/cncr.29971]

8 **Zhu RX**, Seto WK, Lai CL, Yuen MF. Epidemiology of Hepatocellular Carcinoma in the Asia-Pacific Region. *Gut Liver* 2016; **10**: 332-339 [PMID: 27114433 DOI: 10.5009/gnl15257]

9 **Bertuccio P**, Turati F, Carioli G, Rodriguez T, La Vecchia C, Malvezzi M, Negri E. Global trends and predictions in hepatocellular carcinoma mortality. *J Hepatol* 2017; **67**: 302-309 [PMID: 28336466 DOI: 10.1016/j.jhep.2017.03.011]

10 **Bosetti C**, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. *Best Pract Res Clin Gastroenterol* 2014; **28**: 753-770 [PMID: 25260306 DOI: 10.1016/j.bpg.2014.08.007]

11 **Matsuura K**, Tanaka Y. Host genetic variants influencing the clinical course of hepatitis C virus infection. *J Med Virol* 2016; **88**: 185-195 [PMID: 26211651 DOI: 10.1002/jmv.24334]

12 **Hemann EA**, Gale M Jr, Savan R. Interferon Lambda Genetics and Biology in Regulation of Viral Control. *Front Immunol* 2017; **8**: 1707 [PMID: 29270173 DOI: 10.3389/fimmu.2017.01707]

13 **O'Brien TR**, Prokunina-Olsson L, Donnelly RP. IFN-λ4: the paradoxical new member of the interferon lambda family. *J Interferon Cytokine Res* 2014; **34**: 829-838 [PMID: 24786669 DOI: 10.1089/jir.2013.0136]

14 **Syedbasha M**, Egli A. Interferon Lambda: Modulating Immunity in Infectious Diseases. *Front Immunol* 2017; **8**: 119 [PMID: 28293236 DOI: 10.3389/fimmu.2017.00119]

15 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis

C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

16 **Rauch A**, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY; Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; **138**: 1338-1345, 1345.e1-1345. e7 [PMID: 20060832 DOI: 10.1053/j.gastro.2009.12.056]

17 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]

18 **Tanaka Y**, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-1109 [PMID: 19749757 DOI: 10.1038/ng.449]

19 **Prokunina-Olsson L**, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, Hergott D, Porter-Gill P, Mumy A, Kohaar I, Chen S, Brand N, Tarway M, Liu L, Sheikh F, Astemborski J, Bonkovsky HL, Edlin BR, Howell CD, Morgan TR, Thomas DL, Rehermann B, Donnelly RP, O'Brien TR. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet* 2013; **45**: 164-171 [PMID: 23291588 DOI: 10.1038/ng.2521]

20 **Matsuura K**, Watanabe T, Tanaka Y. Role of IL28B for chronic hepatitis C treatment toward personalized medicine. *J Gastroenterol Hepatol* 2014; **29**: 241-249 [PMID: 24325405 DOI: 10.1111/jgh.12475]

21 **Lahiri DK**, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**: 5444 [PMID: 1681511 DOI: 10.1093/nar/19.19.5444]

22 **Qin S**, Wang J, Zhou C, Xu Y, Zhang Y, Wang X, Wang S. The influence of interleukin 28B polymorphisms on the risk of hepatocellular carcinoma among patients with HBV or HCV infection: An updated meta-analysis. *Medicine (Baltimore)* 2019; **98**: e17275 [PMID: 31568008 DOI: 10.1097/MD.0000000000017275]

23 **de la Fuente S**, Citores MJ, Duca A, Cisneros E, Baños I, Vilches C, Cuervas-Mons V. Interleukin-28B TT genotype is frequently found in patients with hepatitis C virus cirrhosis but does not influence hepatocarcinogenesis. *Clin Exp Med* 2017; **17**: 217-223 [PMID: 27083168 DOI: 10.1007/s10238-016-0418-1]

24 **Chang KC**, Ye YH, Wu CK, Lin MT, Tsai MC, Tseng PL, Hu TH. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C without sustained response to combination therapy. *J Formos Med Assoc* 2018; **117**: 1011-1018 [PMID: 29254684 DOI: 10.1016/j.jfma.2017.11.008]

25 **Simili A**, Mazzella G, Ravaioli F, Festi D, Bacchi-Reggiani ML, Porro A, Bazzoli F, Azzaroli F. Interleukin 28 Polymorphisms and Hepatocellular Carcinoma Development after Direct Acting Antiviral Therapy for Chronic Hepatitis C. *J Gastrointestin Liver Dis* 2019; **28**: 449-456 [PMID: 31826071 DOI: 10.15403/jgld-309]

26 **Mangia A**, De Ledinghen V, Bailly F, Brahm J, Keiss J, Valantinas J, Rasmann N, Messinger D, Tatsch F, Bakalos G, Foster GR; Gen-C study group. *IL28B* genotype is associated with cirrhosis or transition to cirrhosis in treatment-naive patients with chronic HCV genotype 1 infection: the international observational Gen-C study. *Springerplus* 2016; **5**: 1990 [PMID: 27917361 DOI: 10.1186/s40064-016-3663-6]

27 **Bantel H**, Schulze-Osthoff K. Apoptosis in hepatitis C virus infection. *Cell Death Differ* 2003; **10** Suppl 1: S48-S58 [PMID: 12655346 DOI: 10.1038/sj.cdd.4401119]

28 **Eslam M**, Hashem AM, Leung R, Romero-Gomez M, Berg T, Dore GJ, Chan HL, Irving WL, Sheridan D, Abate ML, Adams LA, Mangia A, Weltman M, Bugianesi E, Spengler U, Shaker O, Fischer J, Mollison L, Cheng W, Powell E, Nattermann J, Riordan S, McLeod D, Armstrong NJ, Douglas MW, Liddle C, Booth DR, George J, Ahlenstiel G; International Hepatitis C Genetics Consortium (IHCGC). Interferon-λ rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease. *Nat Commun* 2015; **6**: 6422 [PMID: 25740255 DOI: 10.1038/ncomms7422]

29 **Balding DJ**. A tutorial on statistical methods for population association studies. *Nat Rev Genet* 2006; **7**: 781-791 [PMID: 16983374 DOI: 10.1038/nrg1916]

30 **Wittke-Thompson JK**, Pluzhnikov A, Cox NJ. Rational inferences about departures from Hardy-Weinberg equilibrium. *Am J Hum Genet* 2005; **76**: 967-986 [PMID: 15834813 DOI: 10.1086/430507]

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