RESEARCH ARTICLE

Editorial Process: Submission:05/01/2025 Acceptance:09/30/2025 Published:10/19/2025

Association of *IFNL4* and *IFITM3* Genetic Variants with Susceptibility to HCV-Related Liver Cirrhosis and Hepatocellular Carcinoma

Enas Ahmed Osman[1]*, Marwa Ahmed Mohamed[2], Rania M. Abbas[2], Ethar Mokhtar Badran[2], Heba I Aly[2], Medhat M Madbouly[1], Farida M. Khanany[1]

Abstract

Objective: To ascertain the possible association of *IFITM3* rs12252 and *IFNL4* rs12979860 genetic polymorphisms with cirrhosis and HCC in chronic HCV-infected patients. **Methods:** Genetic variations were assessed in this case-control study using TaqMan real-time polymerase chain reaction (RT-PCR) in 1350 subjects: 450 healthy controls, 450 cirrhosis patients, and 450 patients with HCC. **Results:** The genotyping of the *IFNL4* rs12979860 revealed a significantly higher frequency of the TT genotype in the cirrhotic group compared to healthy controls. Additionally, the proportions of the CT genotype and TT genotypes were significantly higher in the HCC patients than in the healthy controls. Regarding the *IFITM3* rs12252, the frequency of the CC genotype was significantly higher in the cirrhotic group compared to healthy controls. The proportion of the CT and CC genotypes in the HCC group was significantly higher than in healthy controls. **Conclusion:** Our results indicate that the *IFITM3* rs12252 and *IFNL4* rs12979860 genetic polymorphisms are linked to the development of cirrhosis and HCC in patients chronically infected with HCV.

Keywords: Hepatitis C virus (HCV)- genetic polymorphisms- *IFITM3* rs12252- *IFNL4* rs12979860- liver disorders

Asian Pac J Cancer Prev, **26 (10)**, 3805-3813

Introduction

In a global context, chronic hepatitis C (CHC) is a main community health problem, impacting a substantial population [1-3]. A substantial proportion of those with chronic HCV infection progress gradual progress to severe liver diseases, including liver cirrhosis and hepatocellular carcinoma. Approximately 58 million people worldwide suffer from CHC virus infection, with 1.5 million new cases reported annually [4].

Compared to other areas, the Mid East and North Africa (MENA) region is the most adversely impacted by HCV infection [5, 6]. In 2019, the MENA region reported approximately 470,000 new HCV infections, representing 30% of all new cases globally. In that year, HCV resulted in 13,705 deaths from liver tumors and 57,994 deaths from cirrhosis and different chronic liver conditions in this region [7]. These statistics highlight the need to clarify the molecular mechanisms underlying liver disease progression to enable early diagnoses and improve therapy accessibility for this viral infection.

Genes implicated in the regulatory functions of the immunological system, such as *IFNL4*, play a direct role in regulating clearance of viral infections. These genes can activate antiviral responses in specific cells and interrupt the host cell viral replication process [8]. The IFNL binding to its receptor stimulates a phosphorylation-dependent signaling cascade that involves the signal transducer and transcription activator. This cascade stimulates the *JAK/STAT* pathway, enhancing the expression of specific IFN-stimulated genes (ISGs) and mediating various immunological activities [9, 10].

On human chromosome 19, the *IFNL4* locus consists of four duplicated genes: *IFNL1–4* [11]. The most recently discovered gene in this group, *IFNL4*, was identified in 2013 through follow-up studies on HCV [11]. In the interferon-λ3/4 (*IFNL3–IFNL4*) region, the rs12979860 genetic polymorphism significantly influences hepatic inflammatory and fibrotic development in chronic hepatitis patients [12]. Notably, while rs12979860 is located in the *IFNL3* (IL28B) region, it is in strong linkage disequilibrium with *IFNL4* and is frequently used as a proxy for *IFNL4* activity.

The *IFITM3* gene on chromosome 11 is part of the *IFITM* gene family and is identified as an antiviral gene [13, 14]. The *IFITM3* has two transmembrane regions and a molecular weight of 10–15 kDa [15, 16]. It inhibits several viral infections stimulated by enveloped viruses requiring membrane fusion for cellular entry [17]. Additionally, *IFITM3* contributes to cellular

[1]*Clinical Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.* [2]*Ain Shams University, Cairo, Egypt.* **For Correspondence: inasosman@ymail.com*

differentiation, death, cell adhesion, and the regulation of immune cells [18]. Multiple studies have demonstrated an increasing prevalence of abnormal *IFITM3* expression in various human malignancies, suggesting its potential role in promoting tumor growth [18]. In the *IFITM3* gene, several single nucleotide polymorphisms (SNPs) can affect the clinical outcome of HCV infection [19]. SNPs serve as a compelling genetic marker to distinguish an individual's vulnerability to illness. The identification of SNPs is straightforward, uncomplicated, and conducted singularly; these characteristics make them attractive biomarkers in molecular genetics for assessing disease state [20]. Several researchers have explored the link between *IFITM3* rs12252 and *IFNL4* rs12979860 polymorphisms and HCV infection; however, findings have been equivocal and variable.

Therefore, we investigated the potential influence of *IFITM3* rs12252 and *IFNL4* rs12979860 genetic polymorphisms on the development of liver cirrhosis and HCC in chronic HCV infection patients.

Materials and Methods

Study design and subjects

This case-control study was performed from June 2023 to September 2024, involving 1350 subjects. Participants were recruited from the Tropical and Gastroenterology Units and classified into three distinct groups: Group A comprised 450 healthy individuals who underwent health screenings and had no history of chronic liver diseases. Group B included 450 cirrhosis patients, and Group C encompassed 450 HCC patients.

In the diseased groups, all patients were positive for HCV antibodies (HCV Ab). Cirrhotic patients were identified using the fibrosis-4 (FIB-4) equation with a cutoff value > 2.67. The FIB-4 score, calculated for all non-HCC patients to evaluate the likelihood of cirrhosis, was determined using the formula:

$$\text{FIB-4} = \frac{age \times aspartate\ aminotransferase\ (AST)}{platelet\ count \times \sqrt{yalanine\ aminotransferase\ (ALT)}}$$

For confirmation, all patients underwent FibroScan and had a score of F4. A comprehensive assessment was conducted for each patient, encompassing a detailed medical history, clinical examination, laboratory tests, and abdominal ultrasonography. For patients with focal lesions detected on ultrasound, triphasic abdominal computed tomography (CT) and/or dynamic magnetic resonance imaging (MRI) were conducted.

The study excluded patients who were seropositive for HBV or HIV, had inflammatory disorders such as bowel disease or systemic sepsis, or had malignancies other than HCC.

Laboratory investigations

Typically, 2 mL of venous blood were taken from all subjects into an EDTA vacutainer for platelet count and into a citrate tube for an international normalized ratio (INR). Additionally, 4 mL of venous blood was withdrawn in a sterile plain vacutainer for basic laboratory tests, including liver function tests (AST, ALT, total and direct serum bilirubin, and albumin) utilizing Beckman Coulter AU 480 chemistry autoanalyzer. Serum alpha-fetoprotein (AFP) levels were measured using chemiluminescence on the Advia Centaur CP device with a kit from Siemens Healthcare Diagnostics. Hepatitis virus markers, including HBsAg, anti-HCV antibodies, and HBcAb, were detected using ELISA to confirm HCV infection status and exclude co-infection with HBV.

SNPs Analysis

Serum preparation and DNA extraction

A sterile EDTA vacutainer was used to collect 2 mL of whole blood samples, and afterward, it was stored at -20 °C. Genomic DNA was obtained from peripheral blood leukocytes of EDTA-anticoagulated blood using ThermoFisher Sci GeneJET Whole Blood Genomic DNA Purification Kit (lot number: 01198940, supplied by Clinilab, Egypt).

Genotyping assay

Amplification and RT-PCR using sequence-specific primers were employed to identify *IFNL4* rs12979860 and *IFITM3* rs12252 SNPs in all investigated participants. RT-PCR allelic discrimination tests created from TaqMan® SNP Genotyping assays (Lot number: 2738630, supplied by Clinilab, Egypt) were assessed using the Applied Biosystems StepOne PCR equipment.

Statistical methods

The pre-existing data were imported into (SPSS v26) software for organization, purification, and statistical analysis. The data were summarized by calculating the mean and standard deviations for quantitative data and determining the number and percentage for qualitative data. The chi-square test was utilized to compare qualitative data, while the nonparametric Kruskal-Wallis test was deployed to quantitative data that lacked a normal distribution. Binary logistic regression was employed to test predictors. A P-value < 0.05 was considered statistically significant.

Results

Demographic, clinical, and laboratory data of the study groups

The present study included a total of 1350 subjects: 450 healthy controls, 450 cirrhosis patients, and 450 HCC patients. There was no significant difference between the groups concerning age (p=0.563) or gender (p=0.765).

Regarding clinical examination, ascites, encephalopathy, and jaundice were significantly more prevalent in the cirrhosis group as compared to the HCC group; however, lower limb edema did not show a statistically significant difference. The HCC group exhibited a significant increase as regards platelet count, AST, ALT, and AFP compared to the cirrhosis group. In the cirrhosis group, albumin was significantly decreased, while total and direct bilirubin were significantly higher compared to the HCC group. INR did not show any significant difference across the study groups. The cirrhosis patients exhibited a higher Child-Pugh score compared to the HCC group. Class A was found only in the HCC group; in contrast, there was an increased incidence of classes B and C in the cirrhosis group compared to the HCC group. The radiological examinations revealed a significant increase in liver size and a higher incidence of portal vein thrombosis in the HCC group. The cirrhosis group had a significant increase in spleen size compared to the HCC group (Table 1).

Genotypic frequency distribution of the studied SNPs among the study population

The *IFNL4* rs12979860 frequency distribution differs significantly among the three studied groups. There was a significantly higher proportion of TT genotype in cirrhotic cases compared to healthy controls. Additionally, the proportion of the CT genotype and TT genotype was significantly higher in the HCC patients compared to the healthy controls (Table 2) (Figure 1).

Regarding the *IFITM3* rs12252 frequency distribution,

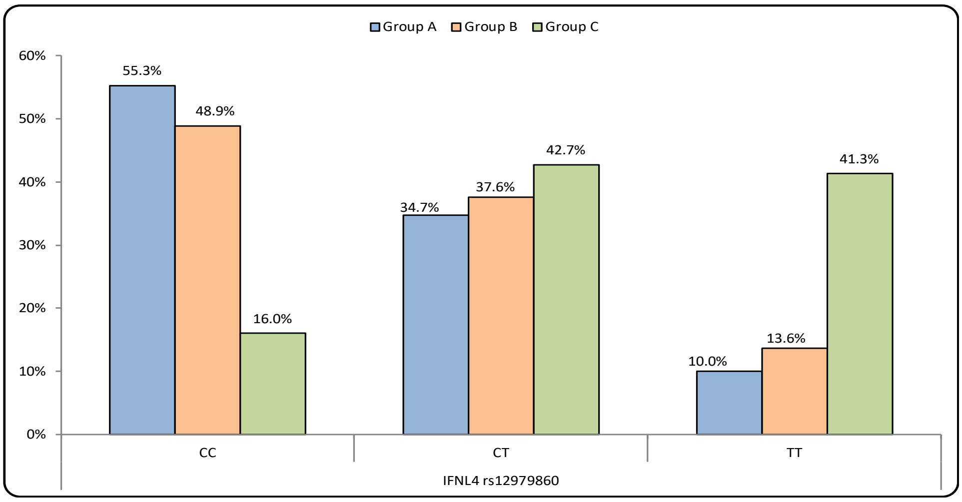

Figure 1. Frequency Distribution of *IFNL4* rs12979860 Genotypes among the Study Groups. Group A: Healthy controls; Group B: Cirrhosis patients; Group C: HCC patients.

Table 1. Basic Clinical, Lab and Radiological Data among Cirrhosis and HCC Groups

		Cirrhosis (n = 450)	HCC (n = 450)	P-value
PLT	Mean ± SD	140.54 ± 51.32	168.71 ± 74.82	<0.001
AST U/L	Median (IQR)	33 (25 - 50)	76 (37 - 95)	<0.001
ALT U/L	Median (IQR)	22 (16 - 31)	47 (31 - 7)	<0.001
Total bilirubin mg/dl	Mean ± SD	1.78 ± 0.96	1.51 ± 1.04	<0.001
Direct bilirubin mg/dl	Mean ± SD	0.95 ± 0.64	0.85 ± 0.74	0.045
Albumin g/dl	Mean ± SD	2.87 ± 0.55	3.36 ± 0.55	<0.001
INR	Mean ± SD	1.26 ± 0.24	1.27 ± 0.28	0.725
Child class	A	0 (0%)	282 (62.7%)	<0.001
	B	382 (84.9%)	120 (26.7%)	
	C	68 (15.1%)	48 (10.7%)	
Child score	Median (IQR)	8 (7 -9)	6 (5 - 7)	<0.001
AFP ng/ml	Median (IQR)	2 (1.3 - 3)	730 (79 - 3113)	<0.001
Liver size cm	Mean ± SD	12.46 ± 2.45	13.87 ± 2.27	<0.001
Splenic size cm	Mean ± SD	16.25 ± 3.46	15.4 ± 2.4	<0.001
Portal vein thrombosis	No	337 (74.9%)	162 (36%)	<0.001
	Yes	113 (25.1%)	288 (64%)	
LL edema	No	272 (60.4%)	276 (61.3%)	0.785
	Yes	178 (39.6%)	174 (38.7%)	
Ascites	No	141 (31.3%)	270 (60%)	<0.001
	Yes	309 (68.7%)	180 (40%)	
Encephalopathy	No	260 (57.8%)	396 (88%)	<0.001
	Yes	190 (42.2%)	54 (12%)	
Jaundice	No	318 (70.7%)	348 (77.3%)	0.023
	Yes	132 (29.3%)	102 (22.7%)	

HCC, hepatocellular carcinoma; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; AFP, Alpha fetoprotein; LL edema, lower limb edema.

Table 2. Frequency Distribution of *IFNL4* rs12979860 Genotypes among the Study Groups

	Control (n = 450)	Cirrhosis (n = 450)	HCC (n = 450)	P-value
CC	249 (55.3%)	220 (48.9%)	72 (16%)	
CT	156 (34.7%)	169 (37.6%)	192 (42.7%)	<0.001
TT	45 (10%)	61 (13.6%)	186 (41.3%)	
	Control	Cirrhosis	OR (95% CI)	P-value
CC	249 (55.3%)	220 (48.9%)	Reference	
CT	156 (34.7%)	169 (37.6%)	1.2 (0.92- 1.6)	0.158
TT	45 (10%)	61 (13.6%)	1.5 (1- 2.3)	0.048
	Control	HCC	OR (95% CI)	P-value
CC	249 (55.3%)	72 (16%)	Reference	
CT	156 (34.7%)	192 (42.7%)	4.3 (3 -5.9)	<0.001
TT	45 (10%)	186 (41.3%)	14.3 (9.4-21.7)	<0.001

HCC, hepatocellular carcinoma.

Table 3. Frequency Distribution of *IFITM 3* rs12252 Genotypes among the Study Groups

	Control (n = 450)	Cirrhosis (n = 450)	HCC (n = 450)	P-value
TT	217 (48.2%)	183 (40.7%)	96 (21.3%)	
CT	170 (37.8%)	175 (38.9%)	198 (44%)	<0.001
CC	63 (14%)	92 (20.4%)	156 (34.7%)	
	Control	Cirrhosis	OR (95% CI)	P-value
TT	217 (48.2%)	183 (40.7%)	Reference	
CT	170 (37.8%)	175 (38.9%)	1.2 (0.9 -1.6)	0.175
CC	63 (14%)	92 (20.4%)	1.7 (1.2 -2.5)	0.004
	Control	HCC	OR (95% CI)	P-value
TT	217 (48.2%)	96 (21.3%)	Reference	
CT	170 (37.8%)	198 (44%)	2.6 (1.9 -3.6)	<0.001
CC	63 (14%)	156 (34.7%)	5.6 (3.8 -8.2)	<0.001

HCC, hepatocellular carcinoma.

Table 4. Relation between *IFNL4* rs12979860 Genotypes and the Parameters Studied among Cirrhosis Group

Cirrhosis group		*IFNL4* rs12979860			P-value
		CC	CT	TT	
		No.= 220	No.= 169	No.= 61	
PLT	Mean ± SD	141.8 ± 54.8	138.73 ± 47.49	141.07 ± 49.17	0.841
AST U/L	Median (IQR)	31 (23 - 44)	34 (28 - 54)	33 (28 - 44)	<0.001
ALT U/L	Median (IQR)	21 (16 - 28)	25 (16 - 33)	28 (21 - 39)	0.069
Total bilirubin mg/dl	Mean ± SD	1.76 ± 0.8	1.72 ± 1.22	2.01 ± 0.61	0.122
Direct bilirubin mg/dl	Mean ± SD	0.87 ± 0.49	0.95 ± 0.78	1.22 ± 0.64	<0.001
Albumin g/dl	Mean ± SD	2.82 ± 0.56	3.02 ± 0.56	2.63 ± 0.33	<0.001
INR	Mean ± SD	1.28 ± 0.25	1.22 ± 0.16	1.3 ± 0.36	0.011
AFP ng/ml	Median (IQR)	2 (1.5 - 2.3)	2 (1 - 3)	2 (1.5 - 3.1)	0.94
Liver size cm	Mean ± SD	12.07 ± 2.42	12.82 ± 2.51	12.88 ± 2.16	0.004
Splenic size cm	Mean ± SD	17.08 ± 3.33	15.62 ± 3.23	15.02 ± 3.82	<0.001
Post Hoc Analysis					
Parameters		CC vs CT	CC vs TT	CT vs TT	
AST		<0.001	0.01	0.963	
Direct bilirubin		0.178	<0.001	0.004	
Albumin		<0.001	0.012	<0.001	
INR		0.008	0.609	0.02	
Liver size cm		0.003	0.023	0.871	
Splenic size cm		<0.001	<0.001	0.24	

HCC, hepatocellular carcinoma; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; AFP, Alpha fetoprotein; LL edema, lower limb edema.

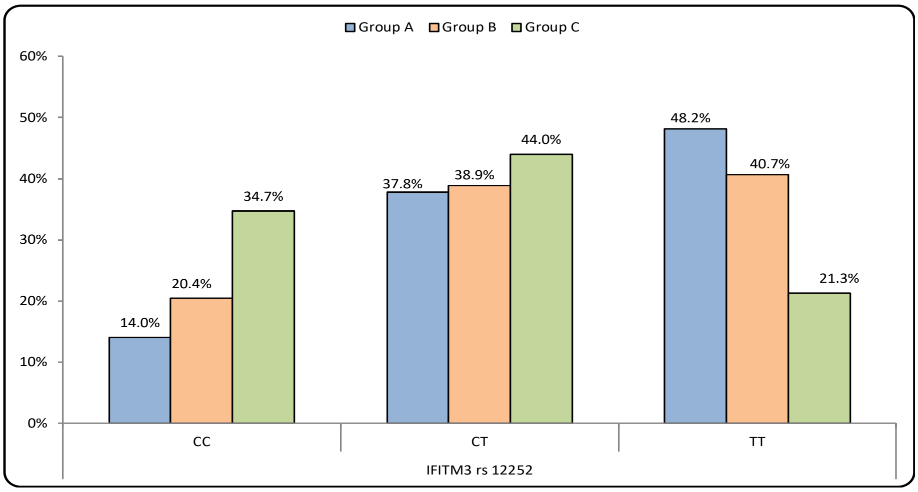

Figure 2. Frequency Distribution of *IFITM3* rs12252 Genotypes among the Study Groups . Group A: Healthy controls; Group B: Cirrhosis patients; Group C: HCC patients.

a statistically significant difference was observed between the studied groups. The Frequency of the CC genotype was significantly higher in cirrhotic cases compared to healthy controls. The proportion of the CT and CC genotypes in HCC patients was significantly higher than that in normal controls (Table 3) (Figure 2).

The relation between IFNL4 rs12979860 and IFITM3

Table 5. Relation between *IFNL4* rs12979860 Genotype and the Parameters Studied among HCC group

HCC Group		IFNL4 rs12979860			P-value
		CC No.= 72	CT No.= 192	TT No.= 186	
PLT	Mean ± SD	149.75 ± 66.6	180.69 ± 60.1	163.68 ± 88.55	0.005
AST U/L	Median (IQR)	54.5 (31 - 79.5)	84.5 (40.5 - 95.5)	70 (44 - 95)	<0.001
ALT U/L	Median (IQR)	33.5 (26 - 47)	48 (37.5 - 77.5)	59 (30 - 81)	<0.001
Total bilirubin mg/dl	Mean ± SD	1.59 ± 1.28	1.42 ± 1.08	1.57 ± 0.9	0.289
Direct bilirubin mg/dl	Mean ± SD	0.87 ± 0.74	0.78 ± 0.79	0.93 ± 0.69	0.142
Albumin g/dl	Mean ± SD	3.19 ± 0.4	3.49 ± 0.57	3.28 ± 0.54	<0.001
INR	Mean ± SD	1.21 ± 0.16	1.2 ± 0.24	1.35 ± 0.34	<0.001
Child score	Median (IQR)	6 (5.5 - 7.5)	6 (5 - 7)	6 (5 - 8)	0.011
AFP ng/ml	Median (IQR)	53 (5.2 - 2475.5)	207.5 (41.5 - 764.5)	2327 (760 - 3497)	0.047
Liver size cm	Mean ± SD	13.83 ± 1.83	13.86 ± 2.38	13.9 ± 2.33	0.97
Splenic size cm	Mean ± SD	15.42 ± 2.45	15.5 ± 2.43	15.29 ± 2.36	0.697
Number of HFL	Mean ± SD	2.83 ± 1.69	2.25 ± 1.44	2.23 ± 1.29	0.005
	Range	1 – 5	1 – 6	1 – 5	
Size of HFLs if multiple the largest	Mean ± SD	8.28 ± 6.27	8.15 ± 8.57	6.51 ± 3.06	0.025
	Range	2.2 – 20	2 – 53	2.5 – 17	
Post Hoc Analysis					
Parameters		CC vs CT	CC vs TT	CT vs TT	
PLT		0.003	0.176	0.026	
AST		<0.001	<0.001	0.722	
ALT		<0.001	<0.001	0.611	
Albumin		<0.001	0.248	<0.001	
INR		0.875	<0.001	<0.001	
Child score		0.011	0.471	<0.001	
AFP		0.047	<0.001	<0.001	
Number of HFL		0.003	0.002	0.869	
Size of HFLs if multiple the largest		0.893	0.049	0.013	

HCC, hepatocellular carcinoma; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; AFP, Alpha fetoprotein; LL edema, lower limb edema.

Table 6. Relation between *IFITM* rs12252 and the Parameters Studied among the Cirrhosis Group

Cirrhosis group			*IFITM3* rs12252		P-value
		CC	CT	TT	
		No.= 92	No.= 175	No.= 183	
PLT	Mean ± SD	92.45 43.32	151.03 55.03	154.7 34.46	<0.001
AST U/L	Median (IQR)	29 (25 - 59)	33 (28 - 50)	33 (23 - 44)	0.765
ALT U/L	Median (IQR)	21 (17 - 33)	25 (16 - 40)	25 (20 - 30)	0.157
Total bilirubin mg/dl	Mean ± SD	2.37 ± 1.59	1.8 ± 0.73	1.47 ± 0.48	<0.001
Direct bilirubin mg/dl	Mean ± SD	1.19 ± 0.93	0.92 ± 0.53	0.85 ± 0.53	<0.001
Albumin g/dl	Mean ± SD	2.83 ± 0.59	2.77 ± 0.42	2.99 ± 0.61	<0.001
INR	Mean ± SD	1.3 ± 0.3	1.31 ± 0.24	1.19 ± 0.19	<0.001
Child score	Median (IQR)	9 (8 - 11)	8 (7 - 8)	7 (7 - 8)	<0.001
AFP ng/ml	Median (IQR)	2.2 (2 - 4)	2 (1.5 - 3)	1.6 (1 - 2)	0.003
Liver size cm	Mean ± SD	10.17 ± 1.2	12.91 ± 2.61	13.18 ± 2.04	<0.001
Splenic size cm	Mean ± SD	20.02 ± 2.41	15.27 ± 3.3	15.29 ± 2.68	<0.001
Post Hoc Analysis					
Parameters		CC vs CT	CC vs TT	CT vs TT	
PLT		<0.001	<0.001	0.443	
Total bilirubin		<0.001	<0.001	<0.001	
Direct bilirubin		<0.001	<0.001	0.327	
Albumin		0.352	0.022	<0.001	
INR		0.695	<0.001	<0.001	
Child score		<0.001	<0.001	0.01	
AFP		0.003	<0.001	<0.001	
Liver size cm		<0.001	<0.001	0.233	
Splenic size cm		<0.001	<0.001	0.947	

HCC, hepatocellular carcinoma; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; AFP, Alpha fetoprotein; LL edema, lower limb edema.

rs12252 genotypes and the examined variables

The relation between *IFNL4* rs12979860 genotypes and the parameters studied in the cirrhosis group is shown in Table 4, while its relation with the HCC group is presented in Table 5. The relation between *IFITM3* rs12252 genotypes and the examined parameters in the cirrhosis group is shown in Table 6, while its relation with the HCC group is presented in Table 7.

Discussion

Cirrhosis and hepatocellular carcinoma are severe liver disorders that may develop in a subset of individuals infected with HCV [20, 21]. Interferons prevent viral replication in host cells by stimulating innate immune reactions via the transcriptional activation of several genes [22, 23]. The severity of HCV-related liver disorders and variations in individuals' susceptibility to these conditions may result from a combination of hereditary and environmental dynamics. Polymorphisms may serve as important genetic markers for identifying individuals at increased risk of disease development [24].

This research evaluated the association of *IFNL4* rs12979860 and *IFITM3* rs12252 genetic polymorphisms with the development of cirrhosis and hepatocellular carcinoma in individuals chronically infected with HCV. Genotyping of the *IFNL4* rs12979860 revealed a significant difference among the studied groups; cases with liver cirrhosis showed a significantly higher proportion of the TT genotype compared to normal controls. The CT and TT genotypes were significantly more frequent in the HCC group than the control group. These findings suggest that allelic variations in the *IFNL4* gene may influence immunomodulatory pathways, thereby contributing to the progression of cirrhosis and hepatocellular carcinoma in patients with chronic HCV infection.

Our findings partially align with a prior Brazilian study [25] investigating the association between the *IFNL4* rs12979860 polymorphism and the progression of fibrosis, cirrhosis, and HCC in HCV-infected patients. They stated that the CT and TT genotypes were linked to a heightened risk of cirrhosis in HCV patients compared to the control group. Also observed that the CT genotype conferred an increased risk for HCC.

A prior meta-analysis [26] was undertaken to explore the connection of the *IFNL4* rs12979860 with the development of hepatitis virus-related HCC; the TT genotype indicated a substantial association with higher HCC risk, which fits hand in hand with our results. Interestingly, a study [27] conducted in a Taiwanese population treated for HCV with peg-IFNα/RBV indicated that the *IFNL4* rs12979860 CC genotype was associated with a lower risk of cirrhosis, suggesting a protective effect in this specific context.

Table 7. Relation between *IFITM* rs12252 and the Parameters Studied among HCC Group

HCC Group		*IFITM3* rs 12252			P-value
		CC No.= 156	CT No.= 198	TT No.= 96	
PLT	Mean ± SD	149.54 ± 80.86	163.18 ± 61.65	211.25 ± 73.56	<0.001
AST U/L	Median (IQR)	76.5 (47 - 85)	82 (37 - 95)	56 (33.5 - 101)	0.25
ALT U/L	Median (IQR)	47 (30 - 69)	47 (34 - 80)	64.5 (42.5 - 78)	0.118
Total bilirubin mg/dl	Mean ± SD	1.77 ± 1.3	1.5 ± 0.95	1.13 ± 0.53	<0.001
Direct bilirubin mg/dl	Mean ± SD	0.99 ± 0.88	0.89 ± 0.71	0.57 ± 0.44	<0.001
Albumin g/dl	Mean ± SD	3.12 ± 0.52	3.41 ± 0.55	3.63 ± 0.41	<0.001
INR	Mean ± SD	1.29 ± 0.25	1.3 ± 0.32	1.15 ± 0.22	<0.001
Child score	Median (IQR)	7 (6 - 8)	6 (5 - 7)	5 (5 - 6)	<0.001
AFP ng/ml	Median (IQR)	382 (79 - 2567)	769 (169 - 2861)	191 (23 -3409)	0.029
Liver size cm	Mean ± SD	13.92 ± 2.19	13.82 ± 2.31	13.91 ± 2.36	0.9
Splenic size cm	Mean ± SD	16.35 ± 2.44	15.09 ± 2.24	14.5 ± 2.16	<0.001
Number of HFL	Mean ± SD	2.31 ± 1.44	2.12 ± 1.32	2.81 ± 1.56	<0.001
Size of HFLs if multiple the largest	Mean ± SD	10.04 ± 9.45	6.35 ± 3.56	5.71 ± 3.14	<0.001
Post Hoc Analysis					
Parameters		CC vs CT	CC vs TT	CT vs TT	
PLT		0.075	<0.001	<0.001	
Total bilirubin		0.013	<0.001	0.004	
Direct bilirubin		0.189	<0.001	<0.001	
Albumin		<0.001	<0.001	<0.001	
INR		0.673	<0.001	<0.001	
Child score		<0.001	<0.001	<0.001	
AFP		0.029	0.724	0.114	
Splenic size cm		<0.001	<0.001	0.039	
Number of HFL		0.219	0.006	<0.001	
Size of HFLs if multiple the largest		<0.001	<0.001	0.411	

HCC, hepatocellular carcinoma; PLT, platelet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; AFP, Alpha fetoprotein; LL edema, lower limb edema.

Conversely, a previous study conducted in Pakistan [28] demonstrated a significant association between the CC genotype and liver cirrhosis. Furthermore, this study examined the relationship between the *IFNL4* rs12979860 genotypes and laboratory variables, reporting no significant association. This discrepancy may be attributed to the fact that the research was conducted on a distinct ethnic group.

Regarding *IFITM3*, accumulating data suggests that its expression is significantly associated with the progression of many cancers and their prognosis [29, 30]. Chinese research demonstrated markedly elevated *IFITM3* expression in colon cancer, suggesting a significant association with disease progression and metastasis [31]. Similarly, recent research has shown that *IFITM3* expression can be upregulated in hepatocellular carcinoma [32]. Moreover, in liver cancer patients with heightened *IFITM3* levels experience comparatively worse prognoses, identifying *IFITM3* as a crucial factor in carcinogenesis [33, 34].

Our findings align with previous Chinese research [35] that found a potential association between the CC genotype of the *IFITM3* rs12252 and the development of HCC. Also, in an Egyptian study on a cohort of HCC patients and a control group infected with HCV, they established a significant association between CC genotype and HCC development. They also observed that patients with HCC and the *IFITM3* rs12252-CC genotype had higher serum *IFITM3* protein levels compared to those with CT or TT genotypes. This suggests a potential link between the CC genotype and increased *IFITM3* protein production in the context of HCC [18, 35]. Earlier studies reported a significant correlation between the CC genotype in the *IFITM3* rs12252 and worse prognosis [18].

In the current study, the CC genotype in the *IFITM*3 rs12252 in the HCC group was substantially correlated with increased size of HFLs, showing a connection between the CC genotype and the severity of HCC. This comes in agreement with prior research [36] on HCC patients compared to those with persistent HBV infection or liver cirrhosis, which observed that cases with the CC genotype exhibited significant correlation with larger tumor sizes compared to CT/TT patients. Authors also performed immunohistochemistry staining and reported association of the CC genotype with low tumor differentiation, which indicates worse prognosis [36].

This study has several limitations. It was conducted at a single center and lacked patient follow-up. Additionally, this study did not assess whether patients with *IFITM3* rs12252 and *IFNL4* rs12979860 genetic polymorphisms remain at higher risk of developing HCC after treatment with direct-acting antivirals.

In conclusion, the results of this study suggest that in *IFNL4* rs12979860, the TT genotype may be associated with susceptibility to cirrhosis, and that the CT and TT genotypes may impact the risk of HCC among individuals with chronic HCV infection. In *IFITM3* rs12252, the CC genotype may be connected with cirrhosis development, whereas the CT and CC genotypes may be associated with HCC risk.

Author Contribution Statement

The study was planned by F.M.K. M.M. carried out the radiological studies. E.A.O. wrote the paper and R.M.A. performed the measurements. While E.M.B. helped with data analysis and manuscript preparation. H.A. and M.A.M. collected the samples. Each author discussed the results and offered feedback on the writing.

Acknowledgements

The authors extend their appreciation to all study participants for their invaluable contribution.

Ethical approval

This study is a case control study, but not a clinical trial, thus the clinical trial number is not applicable. Before conducting this study, we obtained clearance from the Institutional Research Board (IRB) of Ain Shams University following research ethics. The study protocol was assigned to the serial number: R152. Each participant in the study gave informed consent, following the ethical rules set by our hospital's ethics committee, as well as the 1975 Helsinki Declaration and its subsequent amendments.

Data availability statement

The data is available upon reasonable request from the corresponding author.

Conflict of interest

None.

References

1. Brunner N, Bruggmann P. Trends of the Global Hepatitis C Disease Burden: Strategies to Achieve Elimination. J Prev Med Public Health. 2021;54(4):251-8. https://doi.org/10.3961/jpmph.21.151
2. Yang J, Qi JL, Wang XX, Li XH, Jin R, Liu BY, et al. The burden of hepatitis C virus in the world, China, India, and the United States from 1990 to 2019. Front Public Health. 2023;11:1041201. https://doi.org/10.3389/fpubh.2023.1041201
3. Stasi C, Milli C, Voller F, Silvestri C. The Epidemiology of Chronic Hepatitis C: Where We Are Now. Livers. 2024;4(2):172-81. https://doi.org/10.3390/livers4020013
4. World Health Organization. Hepatitis C [Internet]. Geneva: WHO; 2024 [cited 2025 Apr 17]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c.
5. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet. 2016;388(10049):1081-8. https://doi.org/10.1016/s0140-6736(16)30579-7
6. World Health Organization. Global hepatitis report 2017 [Internet]. Geneva: WHO; 2017 [cited 2025 Apr17]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c
7. Lakkis NA, Mokalled NM, Osman MH, Musharrafieh UM, Eljammal M. Liver cancer and risk factors in the mena region: Epidemiology and temporal trends based on the 2019 global burden of disease data. Cancer Control. 2024;31:10732748241297346. https://doi.org/10.1177/10732748241297346
8. Hemann EA, Gale M, Jr., Savan R. Interferon Lambda Genetics and Biology in Regulation of Viral Control. Front Immunol. 2017;8:1707. https://doi.org/10.3389/fimmu.2017.01707
9. 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(11):829-38. https://doi.org/10.1089/jir.2013.0136
10. Syedbasha M, Egli A. Interferon Lambda: Modulating Immunity in Infectious Diseases. Front Immunol. 2017;8:119. https://doi.org/10.3389/fimmu.2017.00119
11. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. 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(2):164-71. https://doi.org/10.1038/ng.2521
12. Thabit K, Bkeet A, Abdelhalem E, Sharaf A, Eltahir H, Abouzied MM, et al. Single nucleotide polymorphisms in INFL3-INFL4 region are associated with fibrosis and cirrhosis in Egyptian patients with chronic hepatitis C. J Adv Biomedical Pharm Sci. 2023;6(4):228-33. https://doi.org/10.21608/jabps.2023.233765.1199
13. Zhao B, Wang H, Zong G, Li P. The role of *IFITM3* in the growth and migration of human glioma cells. BMC Neurol. 2013;13:210. https://doi.org/10.1186/1471-2377-13-210
14. Appourchaux R, Delpeuch M, Zhong L, Burlaud-Gaillard J, Tartour K, Savidis G, et al. Functional Mapping of Regions Involved in the Negative Imprinting of Virion Particle Infectivity and in Target Cell Protection by Interferon-Induced Transmembrane Protein 3 against HIV-1. J Virol. 2019;93(2). https://doi.org/10.1128/jvi.01716-18
15. Desai TM, Marin M, Chin CR, Savidis G, Brass AL, Melikyan GB. *IFITM3* restricts influenza A virus entry by blocking the formation of fusion pores following virus-endosome hemifusion. PLoS Pathog. 2014;10(4):e1004048. https://doi.org/10.1371/journal.ppat.1004048
16. Feeley EM, Sims JS, John SP, Chin CR, Pertel T, Chen LM, et al. *IFITM3* inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog. 2011;7(10):e1002337. https://doi.org/10.1371/journal.ppat.1002337
17. Xie Q, Wang L, Liao X, Huang B, Luo C, Liao G, et al. Research Progress into the Biological Functions of *IFITM3*. Viruses. 2024;16(10). https://doi.org/10.3390/v16101543
18. Bondok RM, Barakat LA, Elsergany AR, Mahsoub N, Ghattas MH. Interferon-induced transmembrane protein 3 in hepatocellular carcinoma patients. BMC Cancer. 2023;23(1):584. https://doi.org/10.1186/s12885-023-11071-2
19. Rajapaksa US, Jin C, Dong T. Malignancy and *IFITM3*:

Friend or Foe? Front Oncol. 2020;10:593245. https://doi.org/10.3389/fonc.2020.593245

20. O'Brien TR, Witt DJ, Saxena V, Morrissey KG, Chen S, Baker FS, et al. *IFNL4* genotype and other personal characteristics to predict response to 8-week sofosbuvir-based treatment for chronic hepatitis C. J Infect. 2024;89(5):106258. https://doi.org/10.1016/j.jinf.2024.106258
21. Narayana SK, Helbig KJ, McCartney EM, Eyre NS, Bull RA, Eltahla A, et al. The Interferon-induced Transmembrane Proteins, *IFITM1, IFITM2,* and *IFITM3* Inhibit Hepatitis C Virus Entry. J Biol Chem. 2015;290(43):25946-59. https://doi.org/10.1074/jbc.M115.657346
22. Evans JG, Novotny LA, Meissner EG. Influence of Canonical and Non-Canonical IFNLR1 Isoform Expression on Interferon Lambda Signaling. Viruses. 2023;15(3). https://doi.org/10.3390/v15030632
23. Boeren M, Van Breedam E, Buyle-Huybrecht T, Lebrun M, Meysman P, Sadzot-Delvaux C, et al. Activation of Interferon-Stimulated Genes following Varicella-Zoster Virus Infection in a Human iPSC-Derived Neuronal In Vitro Model Depends on Exogenous Interferon-α. Viruses. 2022;14(11). https://doi.org/10.3390/v14112517
24. Hassan MM, Kaseb A, Etzel CJ, El-Serag H, Spitz MR, Chang P, et al. Genetic variation in the PNPLA3 gene and hepatocellular carcinoma in USA: risk and prognosis prediction. Mol Carcinog. 2013;52 Suppl 1(0):E139-47. https://doi.org/10.1002/mc.22057
25. de Bitencorte JT, Rech TF, Lunge VR, Dos Santos DC, Álvares-da-Silva MR, Simon D. Association of interferon lambda-4 rs12979860 polymorphism with hepatocellular carcinoma in patients with chronic hepatitis C infection. World J Hepatol. 2021;13(1):109-19. https://doi.org/10.4254/wjh.v13.i1.109
26. Zhang Y, Zhu SL, Chen J, Li LQ. Meta-analysis of associations of interleukin-28B polymorphisms rs8099917 and rs12979860 with development of hepatitis virus-related hepatocellular carcinoma. Onco Targets Ther. 2016;9:3249-57. https://doi.org/10.2147/ott.S104904
27. Onabajo OO, Wang F, Lee MH, Florez-Vargas O, Obajemu A, Tanikawa C, et al. Intracellular Accumulation of IFN-λ4 Induces ER Stress and Results in Anti-Cirrhotic but Pro-HCV Effects. Front Immunol. 2021;12:692263. https://doi.org/10.3389/fimmu.2021.692263
28. Wellington D, Laurenson-Schafer H, Abdel-Haq A, Dong T. *IFITM3*: How genetics influence influenza infection demographically. Biomed J. 2019;42(1):19-26. https://doi.org/10.1016/j.bj.2019.01.004
27. Rauff B, Amar A, Chudhary SA, Mahmood S, Tayyab GUN, Hanif R. Interferon-λ rs12979860 genotype association with liver fibrosis in chronic hepatitis C (CHC) patients in the Pakistani population. Arch Virol. 2021;166(4):1047-56. https://doi.org/10.1007/s00705-020-04901-2
30. Manjunath S, Saxena S, Mishra B, Santra L, Sahu AR, Wani SA, et al. Early transcriptome profile of goat peripheral blood mononuclear cells (PBMCs) infected with peste des petits ruminant's vaccine virus (Sungri/96) revealed induction of antiviral response in an interferon independent manner. Res Vet Sci. 2019;124:166-77. https://doi.org/10.1016/j.rvsc.2019.03.014
31. Li D, Peng Z, Tang H, Wei P, Kong X, Yan D, et al. KLF4-mediated negative regulation of *IFITM3* expression plays a critical role in colon cancer pathogenesis. Clin Cancer Res. 2011;17(11):3558-68. https://doi.org/10.1158/1078-0432.Ccr-10-2729
32. Liu W, Feng Q, Liao W, Li E, Wu L. TUG1 promotes the expression of *IFITM3* in hepatocellular carcinoma by competitively binding to miR-29a. J Cancer. 2021;12(22):6905-20. https://doi.org/10.7150/jca.57477
33. Hou Y, Wang S, Gao M, Chang J, Sun J, Qin L, et al. Interferon-Induced Transmembrane Protein 3 Expression Upregulation Is Involved in Progression of Hepatocellular Carcinoma. Biomed Res Int. 2021;5612138. https://doi.org/10.1155/2021/5612138
34. Koyama N, Hakura A, Toritsuka N, Sonoda J, Seki Y, Tohyama O, et al. Wif1 and ifitm3 gene expression preferentially altered in the colon mucosa of benzo[a]pyrene pre-treated mice following exposure to dextran sulfate sodium. Chem Biol Interact. 2015;240:164-70. https://doi.org/10.1016/j.cbi.2015.07.012.
35. Barakat LA, Elsergany AR, Ghattas MH, Mahsoub N, Bondok RM. Relationship between interferon-induced transmembrane protein 3 and matrix metalloproteinase-9 gene polymorphisms in patients with hepatocellular carcinoma. Clin Res Hepatol Gastroenterol. 2023;47(5):102110. https://doi.org/10.1016/j.clinre.2023.102110
36. Hou Y, Zhang Y, Qin L, Zhang C, Wang S, Chen D, et al. Interferon-induced transmembrane protein-3 rs12252-CC is associated with low differentiation and progression of hepatocellular carcinoma. Medicine (Baltimore). 2019;98(2):e13996. https://doi.org/10.1097/md.0000000000013996