RESEARCH ARTICLE

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Suppressor of Cytokine Signaling-3 Gene Polymorphisms and the Risk of Hepatocellular Carcinoma in Chronic Hepatitis C Patients in Egypt

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Abstract

Background: Hepatocellular carcinoma (HCC) contributes significantly to cancer mortalities worldwide. The association between a specific single nucleotide polymorphism (SNP) located within the *SOCS3* gene as well as the likelihood of hepatocellular carcinoma (HCC) progression in individuals with chronic hepatitis C virus (CHC) was found to be significant. We aimed to study *SOCS3* gene polymorphisms at rs4969168 and rs4969170and HCC susceptibility in individuals with CHC. **Methods:** The current prospective study involved 111 subjects divided in to three groups (HCC, HCV with and with no cirrhosis, and apparently healthy individuals). Tumor staging was done using BCLC staging system. *SOCS3* (rs4969168 and rs4969170) gene polymorphisms' analysis was done utilizing real-time polymerase chain reaction (RT-PCR) (via DNA extracted from all subjects). All subjects underwent a complete history, medical examination, and laboratory and radiological data collection. **Results:** Compared to healthy controls, homozygous AA genotypes and heterozygous GA genotypes were substantially overrepresented in HCC patients as well as those with CHCaccompanied by cirrhosis.AFP, smoking, glucose level, and AA genotype of rs4969170 might be critical significant parameters for HCC development. **Conclusion:** *SOCS3* gene polymorphisms at rs4969168 and rs4969170 are associated with HCC and liver fibrosis progression in the Egyptian population with CHC infection.

Keywords: Cytokine- Real-time polymerase chain reaction- *SOCS3* Gene polymorphisms- hepatocellular carcinoma

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Introduction

HCC is a malignancy that manifests in individuals with liver cirrhosis as well as CHC. HCC demonstrates elevated mortality rates and represents the primary cause of cancer-related mortality on a global scale (Panvichian et al., 2015).CHC is widely considered a significant risk factor for liver cirrhosis development, and it carries a substantial likelihood of progressing to HCC. In addition, insulin resistance, which is induced by HCV, has been proven to have an integral function in liver disease progression (Jadid et al., 2018).Suppressor of cytokine signaling-3 (*SOCS3*) has been identified as a crucial negative regulator of cytokine signaling. It acts by inhibiting Janus kinases (JAKs) activity as well as signal transducer and activator of transcription proteins (STATs), thereby impeding the transmission of signals (through the pathway of JAK/STAT) (Jiang et al., 2015).

The arrangement of the interaction network between HCV proteins and *SOCS3* may potentially serve as a crucial mechanism in the dissemination of viral infection and the development of pathogenesis, which can include metabolic dysfunctions (Panvichian et al., 2015). A number of functionally significant SNPs located within *SOCS3* have been proven to be correlated with susceptibility to insulin resistance as well as type II diabetes mellitus in both HCV-infected and non-infected individuals (Zheng et al., 2013; Zhang et al., 2014). Furthermore, a specific genetic variant known as rs4969170, in the *SOCS3* gene's promoter region, has recently been demonstrated to impact HCV therapeutic response (Aslam et al., 2016). This study aimed to identify the association between specific genetic *SOCS3* variations, namely rs4969168 & rs4969170, and HCC susceptibility in Egyptian individuals infected with HCV.

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Materials and Methods

A total of 111 individuals were included in the present case-control study. They were randomly selected from the Hepatology and Gastroenterology Department Outpatients' Clinic of the National Liver Institute Hospital (Menoufia University-Shebin El-Kom) and Al-Hussein University Hospital (Cairo-Egypt). The enrollment of all subjects in our study was performed following the guidelines of Great Clinical Practice & declaration of Helsinki. Our study protocol was approved by Menoufia University's Ethics Committee and the Institutional Review Board National Liver Institute (IRB No:00333), Menoufia University. All enrolled persons signed informed permission to participate in the study in routine clinical practice.

The studied individuals were classified (into three groups): Group I: 30 HCCcases; Group Π A: 26 subjects with cirrhosis (with no HCC radiological evidence); Group Π B: 25CHC-infectedpatients without cirrhosis; and Group Ш: 30 apparently healthy subjects (with matched age & sex) as controls.

For all enrolled subjects, the following was performed: relevant clinical data collection, basic laboratory tests such as prothrombin time (Coagulometer CA-1500-Siemens-Germany), Alpha-fetoprotein (AFP) (cobas-e411-immunoassay analyser-Roche diagnostics-Germany), liver function tests (cobas-6000 autoanalyser-Roche diagnostics-Germany), and hepatitis serology (HCV Ab & HBs Ag) (cobas-e411-immunoassay analyser-Roche Diagnostics-Germany). The confirmation of HCC was based on performing abdominal Triphasic computed tomography (CT) or dynamic magnetic resonance (MRI), which implies diagnostic criteria of hepatic focal imaging lesion, presence of satellites, and vascular invasion (EASL, 2012).

The score of child-Turcotte-Pugh was calculated for the patients (Bilirubin, albumin, international normalized ratio (INR), ascites, encephalopathy) and graded as A, B, or C (Prakash, 2020). The FIB-4 score was also determined as Age (years) × aspartate aminotransferase (AST) (U/L) ÷ platelet count (10^9 /L)×√ alanine transaminase (ALT) (IU/l) (Vallet-Pichard et al., 2007).

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST (U/L)}}{\text{Platelet Count } (10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}}$$

Genomic DNA was extracted from the EDTA blood sample utilizing Pure Link® Genomic DNA Mini Kit as permanufacturer's information (Invitrogen™, Carlsbad, USA) according to manufacturers' suggestions. The extracted DNA concentration and purity were detected utilizing a spectrophotometer (Implen Nano Photometer™-N60 UV/VIS spectrophotometer-Germany). Aliquot of the extracted DNA was done and stored at - 80ºC. Genotyping of SNPsrs4969168 and rs4969170 was identified by real-time PCR fluorescence using a rotor-gene Q real-time PCR system (Rotor-Gene Q MDX- Qiagen-Germany). Two allele-specific fluorescent-labeled probes (VIC/VAM) AGGAGACCAGCTGACCAGCCCATCC(G/A) TCCCCTCCAAATGTTGCTTCCCCCT for rs4969168 and CTTTCCATTGTTTTTAGAGACCACA(G/A) CCTGCTTTCTTCTAGAGTACTTTTT for rs4969170 were used to perform the assay utilizingthe ABI Prism® 7500 Sequence Detection Systemas perthe guidelines of the manufacturer's (Rotor-Gene Q MDX-Qiagen-Germany).

Procedure

PCR reaction mix was prepared by including 5 µL of Genomic DNA, 10 µL of Master mix, 0.5 µL of SNP Genotyping test probe, and 4.5 µL of DNase-free water, so the total volume was 20µL.The tubes were capped and mixed thoroughly to avoid air bubbles. Furthermore, they were subjected to thermal cycling condition for *SOC3* SNP detection (rs4969168 and rs4969170) as follow (AmpliTaq Gold enzyme activation was at 95°C for 10 min, 15 sec of denaturation at a temperature of 95°C then annealing/extension at 60°C for 1 min{PCR (40 Cycles}). The reaction tubes were then loaded onto the rotor gene Q real-time PCR system, and the run was started. Measurement of each well fluorescence profile was done utilizing the Rotor gene Q. Each well's fluorescence signals were detected at the conclusion of each cycle and generated a graphical representation of the fluorescence intensity plotted against the cycle number, as depicted in Figure 1.

Statistical analysis

The 20.0 V of the IBM software (Armonk, NY: IBM Corp) was utilized. We used tests such as Chi-square, ANOVA, Monte Carlo, Student t-test, and Mann-Whitney tests. Any differences in the mean with a P-value of (>0·05) were judged a significant value. Standard errors of all means (+/- deviations) were also calculated. Univariate logistic regression analysis was done to distinguish all baseline factors correlated with HCC. Information from regression models was described as odds ratios (OR) (with confidence intervals (CI) of 95%).

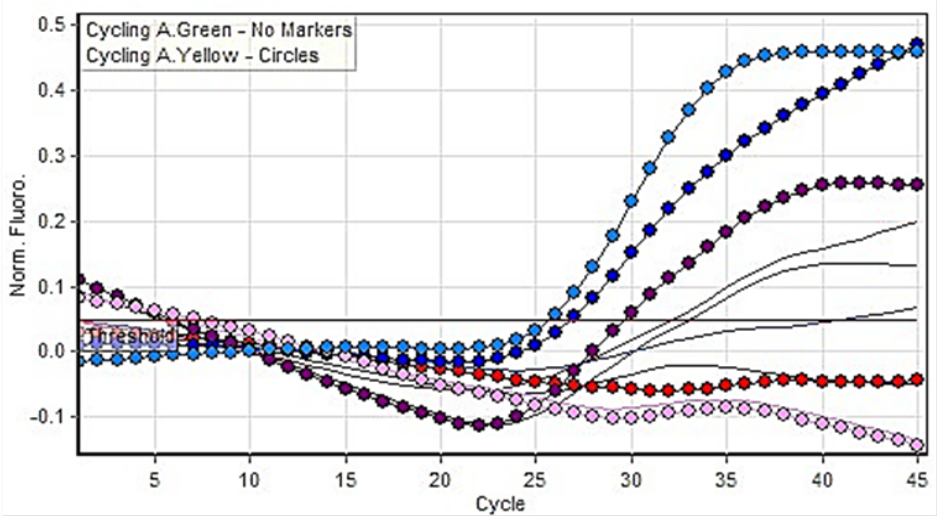
Results

Study subjects' baseline characteristics

There were no substantial differences between the studied groups concerning sex as well as age (P≥0.05). Nevertheless, there were highly substantial differences between the groups regarding the presence of PVT, ascites, smoking, and FIB-4. Moreover, there were statistical differences between the studied groups as regards the laboratory data (ALT, GGT, total bilirubin, ALP, direct bilirubin, INR, AFP, and AST) (Table 1). The noticed distribution regarding genotype frequencies of rs4969168, rs4969170, and Hardy-Weinberg was steady with the expected distribution in Hardy-Weinberg equilibrium (Table 2).

SOCS3 (rs4969168 and rs4969170) gene polymorphisms, alleles distribution, and genotypes among study subjects

When analyzing the studied groups, we noticed a statistically significant increase in genotype frequencies with increased AA & GA genotypes in HCC patients and HCV group with cirrhosis when compared to HCV cases



No.	Color	Name	Genotype	Cycling A.Green	Cycling A.Yellow
1		sNP 168 S 1	Mutant	Reaction	No Reaction
3		3	Wild Type	No Reaction	Reaction
4		sNP170 S1	Heterozygous	Reaction	Reaction
5		2	Heterozygous	Reaction	Reaction
6		3	Heterozygous	Reaction	Reaction

Figure 1. A Graphical Representation of the Fluorescence Intensity Plotted Against the Cycle Number

without cirrhosis and healthy control groups as regards rs4969168, rs4969170. Moreover, there was a marked elevation in the A allele when compared to the G allele in HCC and HCV with cirrhosis groups compared to HCV patients (without cirrhosis) and healthy control group. Furthermore, a substantial elevation was observed in the A-A haplotype in HCC and HCV (with cirrhosis) groups when compared to HCV patients (without cirrhosis) as well as controls (Table 3). According to genotype distribution, there were statistically considerable variations in relation to laboratory investigations among patients (HCC, HCV with cirrhosis, HCV without cirrhosis) regarding GGT, INR, AFP, and fasting serum glucose in rs4969168 and regarding GGT, INR, fasting serum glucose, cholesterol and LDL in rs4969170 when we compare GA+AA genotypes to GG genotype (Table 4). Univariate Logistic regression analysis (for parameters impacting HCC) in Group IIA+IIB (HCV with cirrhosis and HCV without cirrhosis) showed that AFP, smoking, glucose level, and AA genotype of rs4969170 could be significant parameters for HCC development (Table 5).

Discussion

HCC ranks as the sixth most prevalent cancer type globally, and it stands as the third leading cause of reduced survival rates as well as cancer-associated deaths. This is primarily attributed to the high recurrence rate of HCC and its tendency to metastasize (Peng et al., 2019). Additionally, HCC ranks as the fifth most prevalent form of cancer among both males and females in Egypt. Unfortunately, the prognosis for individuals diagnosed with HCC is generally unfavorable, as indicated by 5-year overall rates of survival below 20% (Azim et al., 2018).

HCC is a multifaceted neoplasm, with its pathogenesis likely influenced by a multitude of genetic as well as environmental variables. The hepatitis (C&B) viruses have become known as the primary causative agent linked to HCC development. This association is primarily attributable to their ability to induce persistent cirrhosis, fibrosis, as well as chronic inflammation over an extended period of time (Budha and Wang, 2006).

Options for therapy are exclusively accessible to patients who are in the early stages of their condition. Consequently, the implementation of screening and surveillance approaches for HCC holds significant importance in facilitating early detection and the advancement of targeted curative interventions. However, the efficacy of these strategies remains limited, primarily attributed to the absence of dependable predictive biomarkers (Bruix and Sherman, 2011). Multiple candidate-gene studies have documented substantial correlations between SNPs and HCC occurrence (Nahon and Zucman-Rossi, 2012). *SOCS3* is a member of the suppressor (of the cytokine signaling family) characterized by the potential to impede the activity of multiple cytokines, including IL-11, IL-6, and LIF. The literature has documented the correlation between polymorphisms

Table 1. Comparison between the Four Studied Groups According to Liver Function

	Group I (n= 30)	Group IIA (n= 26)	Group IIB (n= 25)	Group III (n= 30)	Test of sig.	p
Albumin (gm/dl)						
Min. – Max.	1.90 – 4.90	1.70 – 4.80	1.40 – 5.0	4.40 – 4.90	F=23.299*	<0.001*
Mean ± SD.	3.30 ± 0.69	3.45 ± 0.87	4.24 ± 0.92	4.64 ± 0.15		
p_1	<0.001*	<0.001*	0.172			
Sig.bet.Grps			p_2=0.876, p<0.001*, p_4=0.001*			
AST (U/L)						
Min. – Max.	27.0 – 290.0	18.0 – 82.0	12.0 – 68.0	19.0 – 30.0	H=61.814*	<0.001*
Median (IQR)	80.50 (55.0 – 140.0)	44.50 (34.0 – 59.0)	35.0 (20.0 – 37.0)	23.0 (21.0 – 26.0)		
p_1	<0.001*	<0.001*	0.021*			
Sig.bet.Grps			p_2=0.004*, p<0.001*, p_4=0.045*			
ALT (U/L)						
Min. – Max.	11.0 – 167.0	11.0 – 112.0	14.0 – 48.0	20.0 – 33.0	H=32.606*	<0.001*
Median (IQR)	75.0 (45.0 – 105.0)	29.50 (15.0 – 69.0)	37.0 (18.0 – 45.0)	27.0 (24.0 – 30.0)		
p_1	<0.001*	0.282	0.084			
Sig.bet.Grps			p_2=0.019*, p<0.001*, p_4=0.659			
Total bilirubin (mg/dL)						
Min. – Max.	0.60 – 17.60	0.37 – 8.50	0.40 – 1.40	0.30 – 0.80	H=36.751*	<0.001*
Median (IQR)	1.20 (0.90 – 1.70)	0.80 (0.50 – 1.20)	0.69 (0.60 – 1.20)	0.50 (0.30 – 0.70)		
p_1	<0.001*	0.001*	0.006*			
Sig.bet.Grps			p_2=0.012*, p3=0.003*, p_4=0.613			
Direct bilirubin (mg/dL)						
Min. – Max.	0.20 – 9.51	0.08 – 4.24	0.20 – 0.90	0.10 – 0.40	H=31.389*	<0.001*
Median (IQR)	0.56 (0.40 – 0.90)	0.25 (0.20 – 0.30)	0.30 (0.20 – 0.80)	0.20 (0.10 – 0.30)		
p_1	<0.001*	0.15	0.002*			
Sig.bet.Grps			p_2<0.001*, p3=0.041*, p_4=0.114			
ALK Ph						
Min. – Max.	49.0 – 597.0	68.0 – 219.0	61.0 – 109.0	35.0 – 71.0	H=69.921*	<0.001*
Median (IQR)	114.50 (101.0– 153.0)	106.0 (86.0 – 126.0)	91.0 (66.0 – 92.0)	51.0 (42.0 – 60.0)		
p_1	<0.001*	<0.001*	<0.001*			
Sig.bet.Grps			p_2=0.133, p3=0.001*, p_4=0.071			
GGT						
Min. – Max.	32.0 – 689.0	11.0 – 97.0	12.0 – 32.0	10.0 – 35.0	H=68.616*	<0.001*
Median (IQR)	100.0 (88.0 – 129.0)	33.50 (21.0 – 42.0)	26.0 (14.0 – 31.0)	22.0 (19.0 – 25.0)		
p_1	<0.001*	0.004*	0.299			
Sig.bet.Grps			p_2<0.001*, p3<0.001*, p_4=0.078			
INR						
Min. – Max.	1.0 – 1.58	1.0 – 2.54	1.0 – 1.70	1.0 – 1.18	H=33.870*	<0.001*
Median (IQR)	1.07 (1.0 – 1.18)	1.15 (1.05 – 1.32)	1.18 (1.10 – 1.20)	1.0 (1.0 – 1.0)		
p_1	0.002*	<0.001*	<0.001*			
Sig.bet.Grps			p_2=0.056, p3=0.047*, p_4=0.015*			

IQR, Inter quartile range; SD, Standard deviation; H, H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p, p value for comparing between the studied groups; p_1, p value for comparing between Group III and other groups; p_2, p value for comparing between Group I and Group IIA; p_3, p value for comparing between Group I and Group IIB; p_4, p value for comparing between Group IIA and Group IIB; *, Statistically significant at $p \leq 0.05$

in the *SOCS3* gene as well as a range of human diseases (Babon and Nicola, 2012).

In addition, SOCS-3 can influence the progression of cancer (Jiang et al., 2017), regulate immune responses and inflammatory processes (Meng et al., 2020), exert inhibitory effects on insulin and leptin signaling pathways through negative feedback mechanisms (Thon et al., 2016), and function as a signaling molecule for various hormones such as resistin and growth hormone (Lin et al., 2019). The involvement of *SOCS3* in suppressing tumor growth and metastasis of HCC has been observed. The promotion of hepatitis-induced hepatocarcinogenesis is facilitated by *SOCS3* gene deletion in liver parenchymal cells (Jiang et al., 2015). Our study aimed to evaluate

Table 2. Comparison between the Three Studied Groups According to Clinical Data

	Group I (n= 30)		Group IIA (n= 26)		Group IIB (n= 25)		Test of sig.	p
	No.	%	No.	%	No.	%		
PVT								
No	18	60	24	92.3	25	100	χ^2=16.891*	<0.001*
Yes	12	40	2	7.7	0	0		
Sig.bet.Grps	p_1=0.005*, p_2<0.001*, $^{FE}p_3$=0.490							
Ascites								
No	23	76.7	20	76.9	25	100	χ^2=8.221*	0.016*
Yes	7	23.3	6	23.1	0	0		
Sig.bet.Grps	p_1=1.000, $^{FE}p_2$=0.012*, $^{FE}p_3$=0.023*							
Smoking								
No	5	16.7	16	61.5	21	84	χ^2=26.203*	<0.001*
Yes	25	83.3	10	38.5	4	16		
Sig.bet.Grps	p_1=0.001*,p_2<0.001*, p_3=0.072							
Hypertension								
No	14	46.7	12	46.2	18	72	χ^2= 4.556	0.102
Yes	16	53.3	14	53.8	7	28		
DAA								
No	22	73.3	18	69.2	15	60	χ^2= 1.143	0.565
Yes	8	26.7	8	30.8	10	40		
Child-Pugh Score								
A	21	70	20	76.9			χ^2= 3.076	MCp=
B	7	23.3	2	7.7				0.248
C	2	6.7	4	15.4				
FIB 4								
Min. – Max.	1.31 – 22.99		1.17 – 14.87		0.71 – 2.43		H=45.020*	<0.001*
Mean ± SD.	5.65 ± 5.06		4.38 ± 4.28		1.20 ± 0.39			
Sig.bet.Grps	p_1=00.98, p_2<0.001*, p_3<0.001*							

χ^2, Chi square test; MC, Monte Carlo test; H, H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p, p value for comparing between the studied groups; p_1, p value for comparing between Group I and Group IIA; p_2, p value for comparing between Group I and Group IIB; p_3, p value for comparing between Group IIA and Group IIB; *, Statistically significant at $p \leq 0.05$; Group I, patients with HCC complicating chronic HCV infected; Group IIA, patients with chronic hepatitis C infection with cirrhosis (HCV); Group IIB, patients with chronic hepatitis C infection without cirrhosis (HCV) ; PVT, Portal Vein Thrombosis; DAA, Direct Acting Anti-virals

the association between specific genetic variations in the *SOCS3* gene, specifically at the rs4969168 and rs4969170 loci, and the likelihood of developing HCC (in individuals with CHC).

The findings presented in this study demonstrate a statistically significant disparity in genotype distribution between HCC cases and the other groups under investigation. HCC patients and the HCV group (with cirrhosis) had an elevated AA & GA genotypes' incidence compared to controls and HCV cases (without cirrhosis) and healthy control groups. Moreover, regarding the Allele frequencies, there was a substantial rise in the A allele compared to the G allele in HCC patients and cases with CHC infection with cirrhosis when compared to patients with CHC infection without cirrhosis and healthy controls for rs4969168 and rs4969170. Jadid et al., (2018), underline the significance of functional *SOCS3* polymorphisms in the regulation of CHC progression. The authors propose that these polymorphisms contribute to developing HCC by influencing the expression of its mRNA and disrupting crucial metabolic variables. The researcher also discovered a significant overrepresentation of (heterozygous GA &homozygous AA genotypes in advanced liver disease patients (related to HCV), as compared to mild fibrosis cases. This observation was made for both the rs4969168 and rs4969170 polymorphisms (Jadid et al., 2018).

Similarly, in a study by Layden et al., (2014), it was found that the minor A allele frequency for rs4969168 of the *SOC3* gene was associated with an elevated risk of rapid fibrosis progression in individuals with chronic HCV. The OR was determined to be 2.09 (1.12 – 3.89); P = 0.02) (Layden et al., 2014). The results of our univariate analysis indicate a statistically significant overrepresentation of homozygous AA genotypes and heterozygous GA genotypes in HCC patients and those with HCV (and cirrhosis), as compared to controls. Individuals with AA genotypes exhibited a significantly higher risk for cirrhosis and HCC with a 14.25-fold increase in OR (OR= 14.25, 95% confidence interval (CI) = 2.79– 72.72) compared to

Table 3. Comparison between the Three Studied Groups According to Genetic Polymorphisms atrs4969168

	I vs III			IIA vs III			IIB vs III		
	N	p	OR (95% C.I)	N	p	OR (95% C.I)	N	p	OR (95% C.I)
rs4969168									
GG®	6/19		1 (Reference)	6/19		1 (Reference)	15/19		1 (Reference)
GA	14/9	0.012*	4.926 (1.422 – 17.064)	12/9	0.025*	4.222 (1.197 – 14.896)	7/9	0.981	0.985 (0.297 – 3.263)
AA	10/2	0.002*	15.833 (2.686–93.334)	8/2	0.006*	12.667 (2.092–76.700)	3/2	0.511	1.900 (0.281– 12.869)
Dominant									
GG®	6/19		1 (Reference)	6/19		1 (Reference)	15/19		1 (Reference)
GA+AA	24/11	0.001*	6.909 (2.160 – 2.098)	20/11	0.004*	5.758 (1.766–18.668)	10/11	0.8	1.152 (0.387 – 3.430)
Recessive									
GG + GA®	20/28		1 (Reference)	18/28		1 (Reference)	22/28		1 (Reference)
AA	10/2	0.019*	7.0 (1.381 – 35.478)	8/2	0.031*	6.222 (1.185 – 32.684)	3/2	0.499	1.909 (0.293 – 12.440)
Allele									
G®	26/47		1 (Reference)	24/47		1 (Reference)	37/47		1 (Reference)
A	34/13	0.001*	4.728 (2.127 – 10.509)	28/13	0.006*	4.218 (1.855 – 9.589)	13/13	0.595	1.270 (0.526 – 3.066)
	I vs IIA			I vs IIB			IIA vs IIB		
rs4969168									
GG®	6/6		1 (Reference)	6/15		1 (Reference)	6/15		1 (Reference)
GA	14/12	0.825	1.167 (0.297 – 4.588)	14/7	0.016*	5.0 (1.347 – 18.555)	12/7	0.032*	4.286 (1.135– 16.182)
AA	10/8	0.765	1.250 (0.289 – 5.407)	10/3	0.009*	8.333 (1.682 – 41.288)	8/3	0.023*	6.667 (1.306– 34.027)
Dominant									
GG®	6/6		1 (Reference)	6/15		1 (Reference)	6/15		1 (Reference)
GA+AA	24/20	0.78	1.200 (0.334 – 4.306)	24/10	0.003*	6.0 (1.807 – 19.925)	20/10	0.009*	5.0 (1.486 – 16.826)
Recessive									
GG + GA®	20/18		1 (Reference)	20/22		1 (Reference)	18/22		1 (Reference)
AA	10/8	0.838	1.125 (0.365 – 3.472)	10/3	0.074	3.667 (0.882 – 15.249)	8/3	0.114	3.259 (0.753 – 14.116)
Allele									
G®	26/24		1 (Reference)	26/37		1 (Reference)	24/37		1 (Reference)
A	34/28	0.765	1.121 (0.531 – 2.366)	34/13	0.002*	3.722 (1.652 – 8.387)	28/13	0.005*	3.321 (1.441 – 7.650)

OR, Odds ratio; CI, Confidence interval; ®, reference group; *, Statistically significant at $p \leq 0.05$; Group I, patients with HCC complicating chronic HCV infected; Group ΠA, patients with chronic hepatitis C infection with cirrhosis (HCV); Group ΠB, patients with chronic hepatitis C infection without cirrhosis (HCV); Group III, control

other genotypes. Similarly, individuals with GA genotypes had a significantly greater risk for cirrhosis and HCC with a 4.57-fold elevation in odds ratio (OR= 4.57, 95%CI = 1.61 – 13.04).

These findings align with Jadid et al., (2018) who established a substantial SNP correlation with HCC development. AA genotype carriage (at both polymorphisms rs4969168 and rs4969170) was strongly associated with elevated HCC risks (OR=4.1; 95% CI, 1.7–9.7; P=0.04 and OR=2.7; 95% CI, 1.5–4.8; P=0.001).

A study was performed aimed to determiners4969170 polymorphism association in *SOCS3* gene promoter region and RNA expression (with liver fibrosis development) in CHC patients, revealing a substantial disparity in rs4969170 genotypes distribution between advanced and mild fibrosis cases. The prevalence of the AA genotype was found to be substantially greater in advanced liver disease cases caused by HCV compared to mild liver disease caused by HCV. Furthermore, individuals with the AA genotype had a five-fold increased risk of developing advanced liver disease when compared to those with mild CHC. This association was supported by an odds ratio of 5.14 (OR = 5.14; 95% CI, 2.29 - 11.54; P=0.00004) (Chihab et al., 2017).

Contrarily, Jiang et al., (2015) determinedthat, for rs4969170 polymorphism, the HCC patients had an elevated GG prevalence than controls. A significant correlation was observed between the genetic variant *SOCS3* rs4969170 and the clinical features and prognosis of hepatocellular carcinoma (HCC). Based on the rs4969170 A allele as the reference, the odds ratio (OR) for carrying the rs4969170 G allele was found to be 1.40 (P < 0.001). Additionally, it was discovered that the rs4969170 variant exhibited a significant association with both lymph node metastasis as well as clinical stage. In comparison to individuals with the rs4969170 AA genotype, those with the rs4969170 GG genotype exhibited a higher prevalence among HCC patients who displayed more favorable serum (AFP) levels, advanced disease stage, and a greater incidence of cirrhosis (Jiang et al., 2015).

A statistically substantial elevation in the distribution of the rs4969168 genotype was observed when comparing the GA+AA genotypes to the GG genotype. This increase was observed concerning all laboratory criteria among individuals in the HCC group, as well as patients with

Table 4. Comparison between the Three Studied Groups According to Genetic Polymorphisms atrs4969170

	I vs III			IIA vs III			IIB vs III		
	N	P	OR (95% C.I)	N	p	OR (95% C.I)	N	p	OR (95% C.I)
rs4969170									
GG®	5/17		1 (Reference)	4/17		1 (Reference)	14/17		1 (Reference)
GA	13/11	0.033*	4.018 (1.117 – 14.455)	14/11	0.014*	5.409 (1.41 – 20.77)	8/11	0.833	0.883 (0.279 – 2.798)
AA	12/2	0.001*	20.400 (3.38 – 123.24)	8/2	0.003*	17.0 (2.56 – 112.98)	3/2	0.541	1.821 (0.266– 12.473)
Dominant									
GG®	5/17		1 (Reference)	4/17		1 (Reference)	14/17		1 (Reference)
GA + AA	25/13	0.002*	6.538 (1.967–21.739)	22/13	0.003*	7.192 (1.986–26.051)	11/13	0.96	1.027 (0.352 – 7.996)
Recessive									
GG + GA®	18/28		1 (Reference)	18/28		1 (Reference)	22/28		1 (Reference)
AA	12/2	0.007*	9.333 (1.866–46.684)	8/2	0.031*	6.222 (1.185 – 32.684)	3/2	0.499	1.909 (0.293–12.440)
Allele									
G®	23/45		1 (Reference)	22/45		1 (Reference)	36/45		1 (Reference)
A	37/15	0.001*	4.826 (2.21 – 10.55)	30/15	0.001*	4.091 (1.83 – 9.13)	14/15	0.723	1.167 (0.499 – 2.73)
	I vs IIA			I vs IIB			IIA vs IIB		
rs4969170									
GG®	5/4		1 (Reference)	5/14		1 (Reference)	4/14		1 (Reference)
GA	13/14	0.701	0.743 (0.163 – 3.383)	13/8	0.028*	4.550 (1.181 – 17.524)	14/8	0.012*	6.125 (1.50 – 25.10)
AA	12/8	0.822	1.200 (0.245 – 5.886)	12/3	0.004*	11.200 (2.20 – 56.92)	8/3	0.011*	9.333 (1.65 – 52.69)
Dominant									
GG®	5/4		1 (Reference)	5/14		1 (Reference)	4/14		1 (Reference)
GA + AA	25/22	0.896	0.909 (0.217 – 3.815)	25/11	0.004*	6.364 (1.836 – 22.061)	22/11	0.004*	7.0 (1.859–26.365)
Recessive									
GG + GA®	18/18		1 (Reference)	18/22		1 (Reference)	18/22		1 (Reference)
AA	12/8	0.473	1.500 (0.495 – 4.541)	12/3	0.027*	4.889 (1.193 – 20.028)	8/3	0.114	3.259 (0.753–14.116)
Allele									
G®	23/22		1 (Reference)	23/36		1 (Reference)	22/36		1 (Reference)
A	37/30	0.669	1.180 (0.55 – 2.52)	37/14	0.001*	4.137 (1.84 – 9.27)	30/14	0.003*	3.506 (1.53 – 8.02)

OR, Odds ratio; CI, Confidence interval; ®, reference group; *, Statistically significant at $p \leq 0.05$; Group I, patients with HCC complicating chronic HCV infected; Group IIA, patients with chronic hepatitis C infection with cirrhosis (HCV); Group IIB, patients with chronic hepatitis C infection without cirrhosis (HCV); Group III, control

Table 5. Univariate Logistic Regression Analysis for the Parameters Affecting Group I (n = 30) for Group IIA + IIB (n= 51)

	Univariate	
	P	OR (95%C.I)
Gender (Female)	0.179	1.868 (0.750 – 4.652)
Age (years)	0.244	1.029 (0.981 – 1.080)
AFP (>17)	<0.001*	340.750 (36.29–3199.65)
Smoking	<0.001*	13.214 (4.225 – 41.328)
Hypertension	0.29	1.633 (0.658 – 4.050)
Glucose	<0.001*	1.057 (1.031 – 1.084)
rs4969168		
GG®		
GA	0.103	2.579 (0.825 – 8.064)
AA	0.069	3.182 (0.914 – 11.079)
Dominant		
GG®		
GA + AA	0.056	2.800 (0.976 – 8.035)
Recessive		
GG + GA®		
AA	0.246	1.818 (0.662 – 4.995)

Table 5. Continued

	Univariate	
	P	OR (95%C.I)
rs4969170		
GG®		
GA	0.22	2.127 (0.638 – 7.098)
AA	0.037*	3.927 (1.087 – 14.195)
Dominant		
GG®		
GA + AA	0.079	2.727 (0.891 – 8.349)
Recessive		
GG + GA®		
AA	0.079	2.424 (0.901 – 6.520)

OR, Odd`s ratio; C.I, Confidence interval; LL, Lower limit ; UL, Upper Limit; #, All variables with p<0.05 was included in the multivariate; *, Statistically significant at $p \leq 0.05$; Group I, patients with HCC complicating chronic HCV infected; Group IIA, patients with chronic hepatitis C infection with cirrhosis (HCV); Group IIB, patients with chronic hepatitis C infection without cirrhosis (HCV)

CHC disease with cirrhosis and patients with CHC disease without cirrhosis, specifically in relation to serum fasting glucose levels (P=0.002). Additionally, it was observed that individuals carrying the rs4969170-AA genotype exhibited considerably elevated blood glucose levels in comparison to those with the GA genotype, with a statistically marked difference (P=0.003), as well as the GG genotype, with statistically substantial differences (P=0.005).

The aforementioned findings align with the research conducted by Jadid et al., (2018) which demonstrated a significant association between rs4969170 and elevated fasting glucose levels (P = 0.005) in individuals with persistent HCV infection. This study emphasized the significance of functional *SOCS3* polymorphisms in influencing the progression of CHC and their role in the development of HCC through mRNA expression modulation and perturbing key metabolic parameters.

Our univariate investigation revealed that glucose level, smoking, AFP, and AA genotype (of rs4969170) could be potential substantial predictors for HCC development. Jiang and his colleagues illustrated that the rs4969170A>G polymorphism is correlated with HCC patients' clinical features. Individuals(with GG genotype and *SOCS3* rs4969170 G allele) demonstrate elevated HCC risk compared with those with genotype AA after adjusting for alcohol consumption, age, smoking status, as well as sex. Furthermore, these patients displayed larger tumor sizes and elevated AFP levels (Jiang et al., 2015).

In conclusion, *SOCS3* gene polymorphisms at rs4969168 and rs4969170 are related to HCC and liver fibrosis progression in Egyptians with CHC infection.

The association between genetic variations in the *SOCS3* gene at rs4969168 and rs4969170 loci and the development of HCC and liver fibrosis progression in individuals of Egyptian descent with CHC infection has been observed.

Author Contribution Statement

All authors made significant contributions to various aspects of the research project, including resource identification, design, conceptualization, data curation, formal analysis, and resource identification. The technique and validation, along with the revision of novel software utilized in the study, are crucial aspects to consider. The writing of this work was a collaborative effort among all authors. All authors thoroughly examined and evaluated the manuscript.

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Participation consent &ethical approval

All subjects provided formal consent. The ethical committee reviewed and acknowledged the report {Institutional Review Board-National Liver Institute-Menoufia University-IRB: 00333}.

Consent for publication

All authors have confirmed the content of the manuscript.

Availability of data and material

All relevant data can be obtained by contacting the first author (Dr. Amal Elsharnoby).

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