Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJCBR

International Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg

Investigation of the role of *SOCS3* and *SLC29A1 rs760370* single gene polymorphisms in chronic hepatitis B in a group of Egyptian patients

Alshimaa M. Elmalawany, Wesam E. Hamed, Hind S. AboShabaan, Reham H. Ashour and Eman L. Shehata

PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

EGYPTIAN JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Investigation of the role of *SOCS3* and *SLC29A1 rs760370* single gene polymorphisms in chronic hepatitis B in a group of Egyptian patients

Alshimaa M. Elmalawany[1], Wesam E. Hamed[1], Hind S. AboShabaan[1], Reham H. Ashour[2] and Eman L. Shehata[1]

[1]*Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin Elkom, Egypt*
[2]*Department of Hepatology and Gastroenterology, National Liver Institute, Menoufia University, Shebin Elkom, Egypt*

ABSTRACT

Background: Chronic hepatitis B (CHB) virus is a major global health concern, often progressing to liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Genetic polymorphisms in *SOCS3* and *SLC29A1* have been implicated in disease susceptibility. However, their roles in the Egyptian population remain underexplored. **Aim:** This study aims to investigate the association of *SOCS3* (rs4969168, rs4969170) and *SLC29A1* (rs760370) polymorphisms with the progression of hepatitis B virus (HBV)-related liver diseases in a group of Egyptian patients**. Subjects and Methods:** The study was conducted on 50 healthy controls, 50 CHB patients, and 50 LC patients. Genomic DNA was extracted and analyzed for polymorphisms using quantitative real-time PCR. Routine laboratory tests and clinical assessments were performed. Statistical analysis was used to identify any associations between genetic polymorphisms, immune response, and clinical outcomes**. Results:** The *SLC29A1* rs760370 AA genotype was more prevalent in CHB (70%) and LC (86%) patients than in controls (24%) ($P < 0.001$). Carriers of the A allele had higher odds for CHB (OR = 20.417) and LC (OR = 25.083). The *SOCS3* rs4969168 GG genotype was associated with CHB (50%) and LC (82%) compared to controls (14%) ($P < 0.001$), with increased susceptibility (OR = 6.296 and 22.034). The *SOCS3* rs4969170 GG genotype had the highest odds for CHB (OR = 279.0) and LC (OR = 1302.0). **Conclusion:** This study identifies *SOCS3* and *SLC29A1* gene polymorphisms as major genetic determinants in HBV-related liver disease progression in Egyptian patients, highlighting their use as potential biomarkers for HBV-related risk assessment.

Keywords: Hepatitis, HBV, Cirrhosis, Egyptian, Patients, *SLC29A1, SOCS3,* Virus

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/jcbr.2025.356950.1383

ARTICLE INFO

Article history
Received: January 31, 2025
Revised: February 12, 2025
Accepted: March 07, 2025

Correspondence to
Alshimaa M. Elmalawany
Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin Elkom, Egypt
Email: smalawany@liver.menofia.edu.eg

INTRODUCTION

Hepatitis B virus (HBV) is a major global health concern, responsible for a wide range of liver disorders (Albuquerque-Souza and Sahingur, 2022). It is estimated that over 350 million people worldwide suffer from chronic HBV infection, posing a significant burden on healthcare systems, particularly in developing countries where the prevalence of HBV is higher (Sheena et al., 2022). HBV infection can lead to various clinical outcomes, including acute hepatitis, spontaneous recovery, chronic hepatitis, cirrhosis, hepatocellular carcinoma (HCC), and liver failure. In Egypt, the national prevalence of HBV infection is estimated at approximately 1.7% among individuals aged 15 to 59 years (Azzam et al., 2023). The clinical manifestations of HBV infection vary from asymptomatic carriers to severe complications such as chronic hepatitis B (CHB) (Pisano et al., 2021).

HBV infection is a complex and progressive course that significantly increases the risk of liver cirrhosis and HCC (Trépo et al., 2014; Yuan et al., 2019). Approximately 20-30% of individuals with chronic HBV infection progress to liver cirrhosis, while an additional 5% develop HCC. Notably, HCC leads to end-stage liver disease in 15-40% of patients globally (Khullar, 2015).

Multiple factors, including the virus, the environment, and the host influence the progression and clinical outcome of HBV infection. Among these, immune response and genetic predisposition play critical roles (Jose-Abrego et al., 2023). Although HBV does not directly damage hepatocytes, the immune response against viral antigens in infected hepatocytes triggers liver injury (Thomas and Baumert, 2019)**.** Genetic variations modulating the immune response are identified as key determinants in HBV infection outcomes (Tan et al., 2015).

During HBV infection, liver injury is primarily mediated by the host's immune response. Innate immune responses, particularly the secretion of interferons (IFNs) and cytokines, play a crucial role in controlling viral replication and inflammation during early infection stages. The Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway lies central to these immune processes, and its role in HBV infection has been extensively documented (Khanam et al., 2021)**.**

The *Suppressors of Cytokine Signaling (SOCS)* protein family, which include *SOCS1* to *SOCS7*, act as a classical negative feedback mechanism that regulates cytokine signaling via the JAK/STAT pathway (Larsen et al., 2002, Tamiya et al., 2011). *SOCS3*, located on chromosome 17q25.3, is a key regulator of interleukin-6 (IL-6) and IL-10 responses, which are activated through Toll-like receptor pathways. *SOCS3* inhibits cell proliferation and survival by suppressing signal transducer and activator of transcription 3 *(STAT3)* activation (Yoshimura et al., 2012). *STAT3*, known for its oncogenic potential, is associated with NF-kβ activation, inflammation, and carcinogenesis. *SOCS3* plays a pivotal role in controlling STAT3 activation, thereby regulating inflammation and tumor progression (Hoan et al., 2017).

SOCS3 expression correlates with the severity of liver inflammation and injury in HBV infection (Li et al., 2014). Overexpression of *SOCS3* has been observed in liver tissue of CHB patients and is linked to increased inflammation, suggesting dysregulation of the JAK/STAT pathway in HBV-infected hepatocytes (Hu et al., 2021). Polymorphisms in *SOCS3*, such as rs4969168 and rs4969170, can alter gene expression and function, potentially influencing the progression of CHB to more severe liver conditions, including cirrhosis and HCC (Hoan et al., 2017). Furthermore, hypermethylation of CpG (5′-Cytosine-phosphate-Guanine-3′) islands in the *SOCS3* promoter region has been implicated as a prognostic marker in cancer development, particularly in HBV-related hepatocellular carcinoma (Locke et al., 2019).

The *Solute Carrier Family 29 Member 1 (SLC29A1)* gene encodes the equilibrate nucleoside transporter 1 (ENT1), facilitating nucleoside transport across cell membranes. Located on chromosome 6p21.1, ENT1 plays a crucial role in the metabolism of antiviral drugs used for HBV treatment, impacting drug efficacy (Rehermann, 2024). Additionally, ENT1 is involved in the cellular uptake of ribavirin (RBV), a commonly used antiviral therapy for chronic hepatitis C (Holmes et al., 2014). Polymorphisms in *SLC29A1*, such as rs760370, may influence transporter activity, potentially affecting HBV infection progression and liver cirrhosis development by altering drug metabolism and the antiviral response (Rehermann, 2024). Some studies suggest that *SLC29A1* rs760370 polymorphism is associated with higher plasma RBV levels, enhancing antiviral activity but also increasing the risk of anemia in patients receiving RBV for chronic HCV (Pineda-Tenor et al., 2014). While most research focuses on *SLC29A1*'s role in HCV, its involvement in HBV-related liver diseases remains underexplored.

This study aims to investigate the relationship between single nucleotide polymorphisms in *SOCS3* and *SLC29A1* (rs760370) and HBV-related liver diseases in Egyptian patients.

SUBJECTS AND METHODS

Subjects

A total of 150 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). The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the National Liver Institute (NLI), Menoufia University, Egypt (NIL IRB 00014014/FWA00034015), by the Declaration of Helsinki and clinical practice guidelines ("World Medical Association Declaration of Helsinki," 2013). Before the study began, all participants provided informed written consent after receiving a complete explanation of the research.

This study was conducted on 150 Egyptian individuals divided into three groups. Group 1 included 50 healthy individuals who were recruited from the Blood Donation Unit of the National Liver Institute at Menoufia University. They have no history of liver illness, exhibited normal liver function tests, and demonstrated negative serological results for HBV and HCV. They served as the control group. Group 2 included 50 patients diagnosed with chronic hepatitis B (CHB), while Group 3 included 50 patients with HBV who had developed cirrhosis. Patients with alternative causes of liver cirrhosis, such as HCV, metabolic liver disorders, alcoholic liver disease, autoimmune liver disorders, and fatty liver disease, as well as those with hepatocellular carcinoma or other hepatic tumors, were excluded.

Sample collection

Peripheral blood samples (3 ml each) were collected from patients diagnosed with HBV for serological and PCR techniques. All samples underwent comprehensive clinical and laboratory testing, including the detection of HBV antibodies using the ELISA technique and routine liver function tests.

A comprehensive medical history was obtained for all participants, followed by thorough clinical examinations. Baseline laboratory evaluations included complete blood counts and liver function tests, performed using enzyme-linked immunosorbent assay (ELISA) kits (Human ELISA kits, Adaltis, Freiburg, Germany). The tests measured serum alanine aminotransferase (U/L), serum aspartate aminotransferase (U/L), serum albumin (g/dL), total and direct bilirubin (mg/dL), and prothrombin concentration.

Additional analyses encompassed serum creatinine (mg/dL), HBV surface antigen, HBV envelope antigen, total HBV core antibodies, HBV envelope antibodies, and antibodies to the hepatitis C virus. Serum alpha-fetoprotein (AFP) levels were quantified using Human AFP ELISA kits (Glory Science, USA). Liver stiffness measurement was performed by transient elastography (fibro scan) in kilopascals (kPa) (Eddowes et al., 2019). The Child-Turcotte-Pugh (CTP) score was assessed for the patients based on bilirubin, albumin, international normalized ratio (INR), ascites, and encephalopathy and categorized into grades A, B, or C (Prakash and B., 2019).

Quantitative Real-Time PCR Analysis

A total of 150 samples were subjected to molecular analysis using quantitative real-time PCR.

DNA extraction

Genomic DNA was isolated from whole blood utilizing the QIAamp DNA Blood Mini Kit (Catalogue number 51104; Qiagen, Hilden, Germany). The concentration and purity of the extracted DNA were assessed using a Nanophotometer™ N60 UV/VIS spectrophotometer (Implen, Germany). Aliquots of the DNA were prepared and stored at -80°C until further analysis.

DNA Amplification for *SOCS3* and *SLC29A1* rs760370 single nucleotide polymorphisms using PCR

DNA amplification for *SOCS3* and *SLC29A1* single nucleotide polymorphisms was performed using specific primers and detected using real-time PCR analysis without agarose gel electrophoresis (Bustin et al., 2009).

Genomic DNA Extraction and SNP Genotyping

Genotyping of *the SOCS3* (rs4969168 and rs4969170) (Thermo Fisher Scientific Baltics UAB, catalog number #4351379) and *SLC29A1* (rs760370) (Thermo Fisher Scientific Baltics UAB, catalog number #4351379) single-nucleotide polymorphisms was carried out by real-time PCR using the Rotor-Gene Q real-time PCR system (Rotor-Gene Q MDX, Qiagen, Germany) and run on Software Version Rotor-Gene Q Software 2.3.1.49 (Figure 1). Allele-specific fluorescent-labeled probes were employed to detect the SNP variants. The probes for *SOCS3* were:

For rs4969168:
AGGAGACCAGCTGACCAGCCCATCC(G/A)TCCCCTCCA AATGTTGCTTCCCCCT
For rs4969170:
CTTTCCATTGTTTTTAGAGACCACA(G/A)CCTGCTTTCT TCTAGAGTACTTTTT

The *SLC29A1* (rs760370) SNP genotyping was performed using the following probe:
For rs760370:
TGGGTGGAGGTGGAGACAGGTTTGC(A/G)GGAAGGA GTGAAAGACAACCCCACC

All assays were conducted according to the manufacturer's guidelines for the Rotor-Gene Q real-time PCR system (QIAGEN, Germany) (Sample & Assay Technologies Rotor-Gene ® Q MDx User Manual (US), 2018). The PCR products were loaded into the Rotor-Gene Q real-time PCR system, and fluorescence was measured at the end of each cycle. The results were analyzed using the Rotor-Gene Q software, which plotted fluorescence intensity against cycle numbers to determine genotyping results.

Statistical analysis

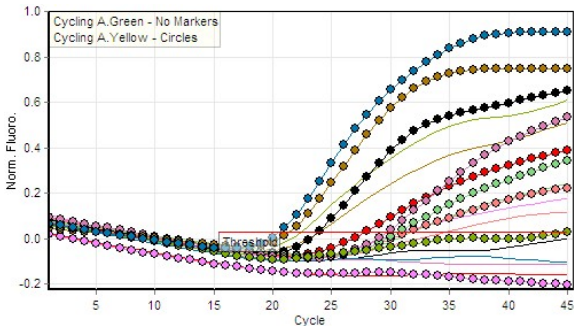
The statistical package of social sciences (SPSS 22.0, IBM/SPSS Inc., Chicago, IL) was employed to analyze the results. Mean ± Standard deviations (SD) were used to summarize continuous data that passed the normal distribution, while median and interquartile range (IQR) were used to summarize non-normal data. Categorical data was represented as a percentage. Statistical comparisons were conducted using the student t-test or ANOVA test for normally distributed data, while Mann-Whitney or Kruskal-Wallis was used for non-normal data. Depending on the appropriateness, categorical variables were compared using either the Pearson Chi-square ($\chi 2$) test or Fisher's exact test.

To validate genetic association findings, Hardy-Weinberg equilibrium (HWE) was assessed in the control group using the Chi-square goodness-of-fit test. The p-values for HWE are reported in the respective tables to ensure unbiased genotype distribution. Effect sizes were calculated using Cohen's d for continuous variables and Odds Ratios (OR) with 95% confidence intervals (CI) for categorical genetic associations. A significance level of 0.05 and a required statistical power of 80% were used to determine the appropriate sample size. Power analysis was conducted using SPSS software to ensure that the selected sample size was sufficient to detect statistically significant differences between the groups.

RESULTS

Evaluation of Laboratory Parameters

Several laboratory parameters were measured to assess liver disease progression and its association with *SOCS3* and *SLC29A1* polymorphisms. Liver function markers (ALT, AST, bilirubin, albumin) were analyzed to evaluate hepatic injury and synthetic capacity. Kidney function (creatinine) was assessed



No.	Color	Name	Genotype	Cycling A. Green	Cycling A. Yellow
1		snp4969168 s 41	Wild Type	No Reaction	Reaction
5		50	Mutant	Reaction	No Reaction
8		149	Heterozygous	Reaction	Reaction
11		snp4949170 s41	Wild Type	No Reaction	Reaction
14		46	Heterozygous	Reaction	Reaction
19		159	Wild Type	No Reaction	Reaction
21		snp760370 s41	Heterozygous	Reaction	Reaction
22		43	Mutant	Reaction	No Reaction
24		46	Wild Type	No Reaction	Reaction

Figure 1. A graphical display of the fluorescence intensity correlation with the cycle number. (resolution is poor)

to detect potential complications, while coagulation markers (prothrombin time and INR) reflected hepatic synthetic impairment. Additionally, hematological parameters (TLC, HB, PLTs) were examined to detect blood abnormalities often associated with liver disease. AFP was measured as a potential marker for hepatocellular carcinoma (HCC), and fibrosis severity was assessed using Fibro Scan values and Child-Pugh scores. Lastly, HBV DNA viral load was quantified to evaluate viral replication and disease progression.

Routine Laboratory Parameters Across the Studied Groups

There were statistically significant differences between the three studied groups concerning ALT, AST, total bilirubin, albumin, creatinine, prothrombin time, INR, TLC, hemoglobin (HB), platelet count (PLTs), AFP, Fibro Scan values, Child-Pugh scores, and HBV PCR viral load (Table 1).

Liver Function Parameters

The ALT levels were markedly elevated in the CHB and LC groups compared to the control group ($P < 0.001$). The median ALT level in CHB patients was 94.32U/L, significantly higher than in controls (17.5 U/L). LC patients exhibited the highest ALT levels (153.13 U/L), indicating a progressive increase in disease severity. Similarly, AST levels showed a significant rise among CHB and LC patients compared to controls ($P < 0.001$), further supporting the hepatic injury associated with HBV progression. Total bilirubin levels demonstrated a substantial increase among cirrhotic patients (0.90 mg/dL) compared to CHB (0.63 mg/dL) and controls (0.40 mg/dL) ($P < 0.001$). This trend was accompanied by a significant decline in serum albumin, with cirrhotic patients showing the lowest levels (3.9 ± 0.7 g/dL) compared to CHB (4.2 ± 0.39 g/dL) and controls (4.6 ± 0.2 g/dL) ($P < 0.001$).

Coagulation Parameters

There was a significant prolongation of prothrombin time and INR in LC patients compared to both CHB and control groups ($P < 0.001$), reflecting impaired liver synthetic function. The median INR values were 1.20 in LC patients, 1.04 in CHB, and 1.03 in controls.

Hematological Parameters

Total leukocyte count (TLC) was notably lower in the LC group (4.15 $\times 10^3/\mu L$) compared to CHB (6.20 $\times 10^3/\mu L$) and controls (6.9 $\times 10^3/\mu L$) ($P < 0.001$). A similar trend was observed in platelet count (PLTs), where LC patients had a significantly reduced median value (101.5$\times 10^3/\mu L$) compared to CHB (22 $\times 10^3/\mu L$) and controls (278.5 $\times 10^3/\mu L$) ($P < 0.001$).

Alpha-fetoprotein (AFP) and Fibrosis Markers

The AFP levels were significantly elevated in LC patients (5.02 ng/mL) compared to CHB (2.18 ng/mL) and controls (2.4 ng/mL) ($P < 0.001$). Fibrosis assessment using Fibro Scan values revealed a progressive increase from CHB (10.95 kPa) to LC (19.1 kPa) ($P < 0.001$). Additionally, the Child-Pugh score, used to assess liver disease severity, was significantly elevated in cirrhotic patients (5.0) ($P < 0.001$).

HBV DNA Viral Load

The HBV PCR viral load was significantly higher in LC patients (5609.0 IU/mL) than in CHB patients (8934.5 IU/mL) ($P = 0.001$), highlighting the increased viral replication associated with advanced liver disease.

Genotypic and Allelic Distribution

The Hardy-Weinberg equilibrium (HWE) test confirmed that the control group followed expected allele distribution patterns (*P*-values provided in Tables 2-7). This validation ensures that genetic variation within the study is representative and free from selection bias.

Genotypic and Allelic Distribution of *SLC29A1 rs760370* Polymorphisms

The genotypic distribution of rs760370 demonstrated a significant association with chronic hepatitis B (CHB) and liver cirrhosis (LC) in Egyptian patients. The AA genotype was notably more prevalent in CHB (70%) and LC (86%) patients compared to controls (24%), while the GG genotype was rare in CHB and LC groups (2%) but present in

Table 1. Comparison of routine laboratory parameters across the three studied groups

Parameters	Control (n = 50)	CHB (n = 50)	LC (n = 50)	*Significance test*	Pairwise comparisons*
ALT Min – Max Median (IQR)	 10.0 – 29.0 17.50(13.0 – 22.0)	 52.0 – 134.0 94.32(89.84 – 99.66)	 74.67 – 200.79 153.13(125.0–181.24)	H=119.632* P-value <0.001*	P1<0.001* P2<0.001* P3<0.001*
AST Min – Max Median (IQR)	 13.0 – 29.0 17.50(15.0 – 19.0)	 30.0 – 96.0 71.04(59.0 – 82.0)	 84.95 – 187.0 109.87(96.0 – 129.0)	H=129.421 P-value <0.001*	P1<0.001* P2<0.001* P3<0.001*
TBIL Min – Max Median (IQR)	 0.23 – 0.70 0.40(0.40 – 0.56)	 0.30 – 1.70 0.63(0.50 – 0.80)	 0.30 – 3.20 0.90(0.70 – 1.90)	H=54.257 P-value <0.001*	P1<0.001* P2<0.001* P3=0.002*
ALB Min – Max Mean ± SD	 4.0 – 4.90 4.62 ± 0.23	 2.70 – 4.80 4.27 ± 0.40	 2.25 – 4.90 3.90 ± 0.74	F= 25.758 P-value <0.001 *	P1=0.002* P2<0.001* P3=0.001*
CREA Min – Max Median (IQR)	 0.61 – 0.90 0.69(0.66 – 0.82)	 0.60 – 1.48 0.80(0.69 – 0.90)	 0.47 – 1.36 0.88(0.70 – .97)	H=10.314 P-value= 0.009*	P1=0.066 P2=0.001* P3=0.174
Prothrombin Min – Max Mean ± SD	 78.30 – 97.0 92.37 ± 4.98	 50.0 – 120.0 91.59 ± 14.53	 47.80 – 109.90 71.69 ± 14.18	F= 47.184 P-value <0.001*	P1=0.944 P2<0.001* P3<0.001*
INR Min – Max Median (IQR)	 1.0 – 1.19 1.03(1.02 – 1.06)	 0.90 – 1.48 1.04(1.02 – 1.19)	 0.95 – 1.54 1.20(1.14 – 1.29)	H=42.577 P-value <0.001*	P1=0.025* P2<0.001* P3<0.001*
TLC Min – Max Median (IQR)	 5.70 – 9.60 6.90(6.10 – 7.70)	 2.80 – 14.80 6.20(4.60 – 7.80)	 2.0 – 9.20 4.15(3.40 – 6.20)	H=27.746* P-value <0.001*	P1=0.094 P2<0.001* P3<0.001*
HB Min – Max Mean ± SD	 11.20 – 15.60 13.12 ± 1.14	 7.90 – 17.20 13.81 ± 2.02	 8.40 – 12.80 10.47 ± 0.96	F= 73.979 P-value <0.001*	P1<0.092 P2<0.503 P3<0.004
PLTs Min – Max Median (IQR)	 187.0 – 412.0 278.50(213.0 – 378.0)	 135.0 – 374.0 226.0(176.0 – 274.0)	 38.0 – 225.0 101.50(77.0 – 101.50)	H=82.545 P-value <0.001*	P1=0.048* P2<0.001* P3<0.001*
AFP Min – Max Median (IQR)	 1.70 – 5.0 2.40 (2.0 – 3.0)	 0.50 – 5.0 2.18(1.60 – 3.0)	 2.76 – 6.80 5.02(4.72 – 5.93)	H=87.279* P-value <0.001*	P1=0.015* P2<0.001* P3<0.001*
Fibroscan value Min – Max Median (IQR)	–	 7.0 – 16.80 10.95(7.50 – 11.80)	 7.0 – 31.0 19.10(18.50 – 19.60)	U=48.50 P-value <0.001*	–
CHILD pugh Value Min – Max Median (IQR)	–	–	 0.0 – 9.0 5.0(5.0 – 6.0)	–	–
PCR HBV Min – Max Median (IQR)	–	 2456.0 – 5988000.0 8934.50(6500–14000)	 2056.0 – 3532741.0 5609.0(3567–7653)	U=751.0 P-value= 0.001*	–

F: F for One way ANOVA test, pairwise comparison was done using Post Hoc Test (Tukey), H: H for Kruskal-Wallis test, pairwise comparison was done using Post Hoc Test (Dunn test), U: Mann-Whitney test was used for non-normal data (Two groups), p1: p-value for the difference between healthy control and CHB (G1 vs. G2). p2: p-value for the difference between healthy control and LC (G1 vs. G3), p3: p-value for the difference between CHB and LC (G2 vs. G3). *The significance level was set at a P-value of 0.05.

14% of controls (*p < 0.001*). The allelic analysis further revealed a higher frequency of the A allele in CHB (84%) and LC (92%) patients compared to controls (55%), highlighting a strong association (*p < 0.001*) (Table 2). Odds ratio analysis confirmed that the AA genotype significantly increased the risk of CHB (OR = 20.4, *p = 0.007*) and LC (OR = 25.08, *p = 0.004*) compared to the GG genotype. Additionally, carriers of the A allele showed a markedly higher risk of CHB (OR = 4.2) and LC (OR = 9.4, *p < 0.001*). These findings underline the potential role of rs760370 polymorphisms in the progression of chronic hepatitis B and liver cirrhosis (Table 3).

Genotypic and allelic distribution of *SOCS3* (rs4969168) polymorphism

The genotypic distribution of rs4969168 revealed significant differences between the studied groups. The AA genotype was most frequent in controls (56%) but markedly reduced in CHB (6%) and LC patients (2%). In comparison, the GG genotype was

significantly more prevalent in CHB (50%) and LC (82%) patients compared to controls (14%) (p < 0.001, Table 4). Allelic analysis showed a higher frequency of the G allele in CHB (72%) and LC (90%) patients compared to controls (29%), highlighting its potential association with disease progression (p < 0.001). Odds ratio analysis confirmed that the GG genotype significantly increased the risk of CHB (OR = 33.3, p < 0.001) and LC (OR = 164, p < 0.001) compared to the AA genotype. The dominant model analysis showed that carriers of the AG+GG genotypes had a markedly higher risk of CHB (OR = 19.9, p < 0.001) and LC (OR = 62.364, p < 0.001). Similarly, the G allele was associated with significantly elevated odds for CHB (OR = 6.296) and LC (OR = 22.0, p < 0.001, Table 5).

Table 2. Comparison between the two studied groups according to rs760370

	Control (n = 50)	CHB (n = 50)	LC (n = 50)	Test of sig.	p
rs760370					
GG	7 (14.0%)	1(2.0%)	1(2.0%)	χ^2= 44.443*	<0.001*
GA	31 (62.0%)	14(28.0%)	6(12.0%)		
AA	12 (24.0%)	35(70.0%)	43(86.0%)		
^{HW}p	0.074	0.768	0.191		
Dominant					
GG	7 (14.0%)	1(2.0%)	1(2.0%)	FET= 6.961*	0.020*
GA + AA	43(86.0%)	49(98.0%)	49(98.0%)		
Recessive					
GG + GA	38(75.0%)	15(30.0%)	7(14.0%)	χ^2= 44.163*	<0.001*
AA	12 (24.0%)	35(70.0%)	43(86.0%)		
Co-dominant–1					
GG	7 (14.0%)	1(2.0%)	1(2.0%)	FET= 1.070	0.755
GA	31 (62.0%)	14(28.0%)	6(12.0%)		
Co-dominant–2					
GG	7 (14.0%)	1(2.0%)	1(2.0%)	FET= 15.563	<0.001*
AA	12 (24.0%)	35(70.0%)	43(86.0%)		
Allele					
G	45 (45.0%)	16(16.0%)	8(8.0%)	42.801*	<0.001*
A	55 (55.0%)	84(84.0%)	92(92.0%)		

χ^2: Chi-square test, FET: Fisher Exact test. $^{HW}p_0$: p-value for Chi-square for the goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE). p: p value for comparing between the studied groups. *: Statistically significant at p < 0.05

Table 3. Association of rs760370 genetic risk factors with susceptibility to CHB and LC groups

	Control (n = 50)	CHB (n = 50)	p_1	OR_1 (CI. 95%)	LC (n = 50)	p_2	OR_2 (CI. 95%)	p_3	OR_3 (CI. 95%)
rs760370									
GG®	7 (14%)	1(2%)			1(2%)				
GA	31 (62%)	14(28%)	0.303	3.161 (0.354–28.198)	6(12%)	0.793	1.355 (0.140 – 13.118)	0.571	0.429 (0.023 – 8.043)
AA	12 (24%)	35(70%)	0.007*	20.417 (2.272 – 183.429)	43(86%)	0.004*	25.083 (2.805 – 224.309)	0.886	1.229 (0.074 – 20.355)
Dominant									
GG	7 (14%)	1(2%)			1(2%)				
GA + AA	43(86%)	49(98%)	0.057	7.977 (0.943 – 67.456)	49(98%)	0.057	7.977 (0.943 – 67.456)	1.000	1.000 (0.061 – 16.444)
Recessive									
GG + GA	38(75%)	15(30%)			7(14%)				
AA	12 (24%)	35(70%)	<0.001*	7.389 (3.043 – 17.942)	43(86%)	<0.001*	19.452 (6.950 – 54.446)	0.058	2.633 (0.967 – 7.170)
Co-dominant–1									
GG	7 (14%)	1(2%)			1(2%)				
GA	31 (62%)	14(28%)	0.303	3.161 (0.354–28.198)	6(12%)	0.793	1.355 (0.140 – 13.118)	0.571	0.429 (0.023 – 8.043)
Co-dominant–2									
GG	7 (14%)	1(2%)			1(2%)				
AA	12 (24%)	35(70%)	0.007*	20.417 (2.272 – 183.429)	43(86%)	0.004*	25.083 (2.805 – 224.309)	0.886	1.229 (0.074 – 20.355)
Allele									
G	45 (45%)	16(16%)			8(8%)				
A	55 (55%)	84(84%)	<0.001*	4.296 (2.211 – 8.345)	92(92%)	<0.001*	9.409 (4.132 – 21.426)	0.087	2.191 (0.892 – 5.381)

OR_1: Odds ratio for Control and CHB, OR_2: Odds ratio for Control and LC, OR_3: Odds ratio for CHB and LC, CI: Confidence interval, LL: Lower limit, UL: Upper Limit, ®: reference group, *: Statistically significant at p < 0.05

Table 4. Comparison between the two studied groups according to rs4969168

	Control (n = 50)	CHB (n = 50)	LC (n = 50)	χ^2	p
rs4969168					
AA	28 (56.0%)	3 (6.0%)	1(2.0%)	72.752*	<0.001*
AG	15 (30.0%)	22 (44.0%)	8(16.0%)		
GG	7 (14.0%)	25 (50.0%)	41(82.0%)		
^{HW}p	0.055	0.519	0.432		
Dominant					
AA	28 (56.0%)	3 (6.0%)	1(2.0%)	53.946*	<0.001*
AG + GG	22(44.0%)	47(94.0%)	49(98.0%)		
Recessive					
AA + AG	43(86.0%)	25 (50.0%)	9(18.0%)	46.326*	<0.001*
GG	7 (14.0%)	25 (50.0%)	41(82.0%)		
Co-dominant–1					
AA	28 (56.0%)	3 (6.0%)	1(2.0%)	22.254*	<0.001*
AG	15 (30.0%)	22 (44.0%)	8(16.0%)		
Co-dominant–2					
AA	28 (56.0%)	3 (6.0%)	1(2.0%)	61.321*	<0.001*
GG	7 (14.0%)	25 (50.0%)	41(82.0%)		
Allele					
A	71 (71.0%)	28 (28.0%)	10(10.0%)	84.932*	<0.001*
G	29 (29%)	72 (72.0%)	90(90.0%)		

χ^2: Chi-square test, $^{HW}p_0$: p-value for Chi-square for the goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE), p: p-value for comparing between the studied groups, *: Statistically significant at p < 0.05.

Table 5. Association of rs4969168 genetic risk factors with susceptibility to CHB and LC groups

	Control (n = 50)	CHB (n = 50)	p_1	OR_1 (CI.95%)	LC (n = 50)	p_2	OR_2 (CI.95%)	p_3	OR_3 (CI.95%)
rs4969168									
AA®	28 (56%)	3(6%)			1(2%)				
AG	15(30%)	22(44%)	<0.001*	13.689 (3.515 –53.311)	8(16%)	0.015*	14.933 (1.703 – 130.972)	0.943	1.091 (0.099 – 12.067)
GG	7 (14%)	25(50%)	<0.001*	33.333 (7.772 – 142.968)	41(82%)	<0.001*	164.0 (19.11 – 1407.47)	0.178	4.920 (0.485 – 49.923)
Dominant									
AA	28 (56%)	3 (6%)			1(2%)				
AG + GG	22(44%)	47(94%)	<0.001*	19.939 (5.468 – 72.714)	49(98%)	<0.001*	62.364 (7.972 – 487.887)	0.331	3.128 (0.314 – 31.142)
Recessive									
AA + AG	43(86%)	25 (50%)			9(18%)				
GG	7 (14%)	25 (50%)	<0.001*	6.143 (2.323 – 16.242)	41(82%)	<0.001*	27.984 (9.538 – 82.108)	0.001*	4.556 (1.834 – 11.316)
Co-dominant–1									
AA	28 (56%)	3 (6%)			1(2%)				
AG	15 (30%)	22 (44%)	<0.001*	13.689 (3.515 – 53.311)	8(16%)	0.015*	14.933 (1.703 – 130.972)	0.943	1.091 (0.099 – 12.067)
Co-dominant–2									
AA	28 (56%)	3 (6%)			1(2%)				
GG	7 (14%)	25 (50%)	<0.001*	33.333 (7.772 – 142.968)	41(82%)	<0.001*	164.0 (19.11 – 1407.47)	0.178	4.920 (0.485 – 49.923)
Allele									
A	71 (71%)	28 (28%)			10(10%)				
G	29 (29%)	72 (72%)	<0.001*	6.296 (3.407 – 11.634)	90(90%)	<0.001*	22.034 (10.068 – 48.222)	0.002*	3.500 (1.595 – 7.679)

OR_1: Odds ratio for Control and CHB, OR_2: Odds ratio for Control and LC, OR_3: Odds ratio for CHB and LC, CI: Confidence interval, LL: Lower limit, UL: Upper Limit, ®: reference group, *: Statistically significant at p ≤ 0.05.

These results highlight the role of rs4969168 polymorphisms in chronic hepatitis B and liver cirrhosis susceptibility.

Genotypic and Allelic Distribution of *SOCS3* (rs4969170) Polymorphisms

The distribution of rs4969170 genotypes showed significant differences among the studied groups. The AA genotype was predominant in controls (62%) but significantly reduced in CHB (6%) and LC patients (2%). In contrast, the GG genotype was rare in controls (2%) but highly frequent in CHB (54%) and LC patients (84%) (p < 0.001, Table 6). Allelic analysis revealed a higher frequency of the G allele in CHB (74%) and LC (91%) patients compared to controls (20%), indicating a strong association with disease progression (p < 0.001, Table 6). Odds ratio analysis highlighted that the GG genotype significantly increased the risk of CHB (OR = 279, p < 0.001) and LC (OR = 1302, p < 0.001) compared to the AA genotype. The dominant model further demonstrated that carriers of the AG+GG genotypes had markedly elevated odds for CHB (OR = 25.5, p < 0.001) and LC (OR = 79.9, p < 0.001). Additionally, the G allele was strongly associated with a higher risk of CHB (OR = 11.38) and LC (OR = 40.4, p < 0.001, Table 7). These results underscore the significant role of rs4969170 polymorphisms in susceptibility to chronic hepatitis B and liver cirrhosis.

The relationship between the polymorphisms and ALT, AST, fibro scan values, and CHILD-Pugh scores

The findings in Table 8 indicate that ALT and AST levels were higher in patients carrying the AA genotype, particularly in the LC group (ALT: 145.7 U/L, AST: 118.7 U/L). However, statistical analysis using the student's t-test revealed no significant differences among genotypes in ALT (P = 0.0.4) or AST (P = 0.4). Fibrosis severity, as assessed by Fibro Scan values, was highest in LC patients with the AA genotype (19.1 kPa), yet the Kruskal-Wallis test showed no statistically significant difference (P = 0.44). Likewise, Child-Pugh scores remained comparable among genotypes (P = 0.541), suggesting no significant impact of rs760370 on liver disease progression.

As presented in Table 9, the mean ALT levels were slightly higher in LC patients with the AA genotype (121 U/L) compared to AG (167. U/L), but this difference was not statistically significant (*P* = 0.386). Similarly, AST levels did not vary significantly among genotypes in either CHB or LC patients (P > 0.05). Fibrosis assessment using Fibro Scan values showed a tendency for higher readings in LC patients carrying the AG genotype (19.2 kPa), but the Mann-Whitney test indicated no significant difference (*P*=0.822). Additionally, Child-Pugh scores remained consistent across genotypes (*P*=0.401), suggesting that rs4969168 does not have a significant influence on liver function or fibrosis severity. The results in Table 10 highlight a significant association between rs4969170 and ALT levels in CHB patients, with the AA genotype showing the highest median ALT (103 U/L, P < 0.01). However, in LC patients, ALT levels did not significantly differ among genotypes (P = 0.208). AST levels followed a comparable pattern, with no statistically significant differences in either CHB (P=0.06) or LC (P=0.8) groups. The Fibro Scan values were slightly elevated in LC patients with the AA genotype (19.0kPa), but the Kruskal-Wallis test

Table 6. Comparison between the two studied groups according to rs4969170

	Control (n = 50)	CHB (n = 50)	LC (n = 50)	Test of sig.	p
rs4969170					
AA	31 (62.0%)	3 (6.0%)	1 (2.0%)	χ^2= 91.648*	<0.001*
AG	18(36.0%)	20 (40.0%)	7 (14.0%)		
GG	1(2.0%)	27 (54.0%)	42 (84.0%)		
^{HW}p	0.377	0.780	0.304		
Dominant					
AA	31 (62.0%)	3 (6.0%)	1 (2.0%)	χ^2= 62.907*	<0.001*
AG + GG	19(38.0%)	47(94.0%)	49(98.0%)		
Recessive					
AA + AG	49(98.0%)	23(46.0%)	8(16.0%)	χ^2= 69.161*	<0.001*
GG	1(2.0%)	27 (54.0%)	42 (84.0%)		
Co-dominant–1					
AA	31 (62.0%)	3 (6.0%)	1 (2.0%)	FET= 19.956*	<0.001*
AG	18(36.0%)	20 (40.0%)	7 (14.0%)		
Co-dominant–2					
AA	31 (62.0%)	3 (6.0%)	1 (2.0%)	χ^2= 84.095*	<0.001*
GG	1(2.0%)	27 (54.0%)	42 (84.0%)		
Allele					
A	80 (80.0%)	26 (26.0%)	9 (9.0%)	116.277*	<0.001*
G	20 (20.0%)	74 (74.0%)	91 (91.0%)		

χ^2: Chi-square test. $^{HW}p_0$: p-value for Chi-square for the goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE).p: p-value for comparing between the studied groups. *: Statistically significant at p ≤ 0.05

Table 7. Association of rs4969170 genetic risk factors with susceptibility to CHB and LC groups

	Control (n = 50)	CHB (n = 50)	p_1	OR_1 (CI. 95%)	LC (n = 50)	p_2	OR_2 (CI. 95%)	p_3	OR_3 (CI. 95%)
rs4969170									
AA®	31 (62%)	3(6%)		1.000	1 (2%)		1.000		1.000
AG	18(36%)	20 (40%)	<0.001*	11.481 (2.990 – 44.082)	7 (14%)	0.025*	12.056 (1.371–106.041)	0.968	1.050 (0.093 – 11.824)
GG	1(2%)	27 (54%)	<0.001*	279.0 (27.38– 2842.53)	42 (84%)	<0.001*	1302.0 (78.36– 21634.57)	0.192	4.667 (0.461 – 47.214)
Dominant									
AA	31 (62%)	3 (6%)			1 (2%)				
AG + GG	19(38%)	47(94%)	<0.001*	25.561 (6.971 – 93.732)	49(98%)	<0.001*	79.947 (10.184–627.623)	0.331	3.128 (0.314 – 31.142)
Recessive									
AA + AG	49(98%)	23(46%)			8(16%)				
GG	1(2%)	27 (54%)	<0.001*	57.522 (7.357 – 449.733)	42 (84%)	<0.001*	257.250 (30.899– 2141.720)	0.002*	4.472 (1.749 – 11.433)
Co-dominant–1									
AA	31 (62%)	3 (6%)			1 (2%)				
AG	18(36%)	20 (40%)	<0.001*	11.481 (2.990 – 44.082)	7 (14%)	0.025*	12.056 (1.371–106.041)	0.968	1.050 (0.093 – 11.824)
Co-dominant–2									
AA	31 (62%)	3 (6%)			1 (2%)				
GG	1(2%)	27 (54%)	<0.001*	279.0 (27.38– 2842.53)	42 (84%)	<0.001*	1302.0 (78.36– 21634.57)	0.192	4.667 (0.461 – 47.214)
Allele									
A	80(80%)	26 (26%)		1.000	9 (9%)		1.000		1.000
G	20(20%)	74 (74%)	<0.001*	11.385 (5.866 – 22.096)	91 (91%)	<0.001*	40.444 (17.423– 93.883)	0.002*	3.553 (1.568 – 8.05)

OR_1: Odds ratio for Control and CHB, OR_2: Odds ratio for Control and LC, OR_3: Odds ratio for CHB and LC, CI: Confidence interval, LL: Lower limit, UL: Upper Limit, ®: reference group, *: Statistically significant at p < 0.05

Table 8. Relation between rs760370 with ALT, AST, fibro scan value, and CHILD Pugh in each group

	rs760370					
	CHB			LC		
	GG (n= 1#)	GA (n= 14)	AA (n= 35)	GG (n= 1#)	GA (n= 6)	AA (n= 43)
ALT						
Min. – Max.	89.0	52.0 – 103.5	59.0 – 134.0	90.0	79.0– 191.0	74.67 – 200.8
Median (IQR)		94.96 (89.84–100.52)	94.54 (90.13 – 99.24)		145.79 (88.0 – 176.35)	154.36 (131.0 – 182.46)
U(p)		230.0(0.740)			105.50(0.483)	
AST						
Min. – Max.	85.0	57.0 – 96.0	30.0 – 92.50	140.0	90.67 – 160.0	84.95 – 187.0
Median (IQR)		72.87 (61.0 – 85.0)	68.0 (58.50 – 79.50)		118.73 (100.0 – 126.0)	107.83 (96.0 – 126.50)
U(p)		197.50(0.293)			102.50(0.429)	
Fibro scan value						
Min. – Max.	16.0	7.0 – 16.0	7.0 – 16.80	18.50	17.45 – 30.0	7.0 – 31.0
Median (IQR)		11.10(7.70– 12.80)	10.10(7.40– 11.42)		19.45(19.0–19.60)	19.10(18.55–19.55)
U(p)		204.50(0.370)			103.50(0.446)	
CHILD pugh						
Min. – Max.	–	–	–	5.0	5.0 – 9.0	0.0 – 9.0
Median (IQR)		–	–		5.0(5.0 – 5.06)	5.03(5.0 – 6.0)
U(p)		–			108.0(0.541)	

U: Mann Whitney test, p: p-value for comparison between the categories studied

Table 9. Relation between rs4969168 with ALT, AST, fibro scan value, and CHILD Pugh in each group

	rs4969168					
	CHB			LC		
	AA (n= 3)	AG (n= 22)	GG (n= 25)	AA (n= 1[#])	AG (n= 8)	GG (n= 41)
ALT						
Min. – Max.	91.97 – 96.25	52.0 – 134.0	59.0 – 103.5	121.0	99.0 – 200.8	74.67 – 198.3
Median (IQR)	92.40 (92.19 – 94.32)	94.11 (89.84 – 97.53)	94.54 (89.0 – 100.09)		167.80 (132.29–188.57)	151.91 (125.0–178.80)
Test of sig. (p)		H(p)=0.303(0.859)			U(p)=131.0(0.386)	
AST						
Min. – Max.	46.0 – 82.0	42.0 – 88.0	30.0 – 96.0	133.0	88.76 – 187.0	84.95 – 160.0
Median (IQR)	59.0 (52.50 – 70.50)	69.54 (60.0 – 79.0)	72.30 (59.0 – 85.0)		111.65 (95.0 – 130.14)	109.74 (96.0 – 126.0)
Test of sig. (P)		H(p)=0.896(0.639)			U(p)=154.50(0.801)	
Fibro scan value						
Min. – Max.	7.0 – 16.0	7.0 – 16.80	7.0 – 16.0	19.70	17.33 – 19.80	7.0 – 31.0
Median (IQR)	7.10(7.05–11.55)	9.49(7.6–12.8)	11.0(7.5–11.27)		19.20(19.0–19.5)	19.10(18.5–19.6)
Test of sig.(p)		H(p)=0.835(0.659)			U(p)=155.50(0.822)	
CHILD Pugh						
Min. – Max.	–	–	–	5.0	5.0 – 6.0	0.0 – 9.0
Median (IQR)	–	–	–		5.0(5.0–5.1)	5.06(5.0 – 6.0)
Test of sig.(p)		–			U(p)=132.50(0.401)	

U: Mann-Whitney test, H: H for Kruskal-Wallis test, p: p-value for comparison between the categories studied

Table 10. Relation between rs4969170 with ALT, AST, fibro scan value, and CHILD Pugh in each group

	rs4969170					
	CHB			LC		
	AA (n= 3)	AG (n= 20)	GG (n= 27)	AA (n= 1[#])	AG (n= 7)	GG (n= 42)
ALT						
Min. – Max.	97.53 – 134.0	52.0 – 103.9	79.0 – 103.5	144.60	95.0 – 200.8	74.67 – 198.3
Median (IQR)	103.08 (100.30–118.54)	91.76 (68.50 – 96.03)	95.39 (90.90 – 99.83)		166.58 (155.58–188.57)	150.69 (121.0–178.80)
Test of sig. (p)		H(p)=9.185*(0.010*)			U(p)=102.0(0.208)	
AST						
Min. – Max.	62.0 – 88.0	42.0 – 96.0	30.0 – 94.0	100.0	87.0 – 187.0	84.95 – 160.0
Median (IQR)	82.0 (72.0 – 85.0)	59.50 (56.0 – 75.87)	75.96 (61.50 – 85.0)		115.46 (101.92–120.64)	109.87 (96.0–130.0)
Test of sig. (p)		H(p)=5.529(0.063)			U(p)=139.50(0.834)	
Fibro scan value						
Min. – Max.	7.80 – 11.27	7.0 – 16.80	7.10 – 16.0	19.0	19.0 – 29.60	7.0 – 31.0
Median (IQR)	7.80(7.80–9.53)	11.08(7.3–14.0)	10.90(7.5–11.27)		19.30(19.1–19.5)	19.10(18.4–19.6)
Test of sig. (p)		H(p)=0.324(0.851)			U(p)=113.0(0.346)	
CHILD pugh						
Min. – Max.	–	–	–	6.0	5.0 – 8.0	0.0 – 9.0
Median (IQR)		–	–		5.0(5.0 – 5.5)	5.01(5.0 – 6.0)
Test of sig. (p)		–			U(p)=125.50(0.547)	

U: Mann-Whitney test, H: H for Kruskal-Wallis test, p: p-value for comparison between the studied categories, *: Statistically significant at $p \leq 0.05$.

showed no statistical significance (P=0.346). Similarly, Child-Pugh scores remained comparable among genotypes (P=0.54), indicating that rs4969170 may be associated with ALT elevation in CHB but does not significantly impact liver fibrosis or disease severity. These findings suggest that while genetic variations may influence liver function, their impact on fibrosis and disease severity remains uncertain and warrants further investigation.

DISCUSSION

This study provides significant insights into the role of genetic polymorphisms in the progression of chronic hepatitis B (CHB) and liver cirrhosis (LC) among Egyptian patients. The investigation focused on the SNPs of *SOCS3 rs4969168, rs4969170*, and *SLC29A1 rs760370*, which have been implicated in immune response regulation and liver disease pathology. The study revealed significant alterations in liver function tests, hematological parameters, and fibro scan values across the studied groups, alongside notable associations between specific polymorphisms and disease susceptibility.

SLC29A1, encoding the *equilibrate nucleoside transporter 1* (*ENT1*), plays a crucial role in cellular nucleoside transport and nucleotide metabolism (Mikdar et al., 2021). In the process of HBV infection, *ENT1* has been implicated in modulating the intracellular environment to favor viral replication (Tian et al., 2021).

Regarding the *SLC29A1* rs760370 Polymorphism, the AA genotype of rs760370 is strongly associated with increased susceptibility to CHB and LC compared to the GG genotype. The frequency of the A allele was significantly higher in CHB (84%) and LC (92%) patients compared to controls (55%), and the AA genotype presented markedly higher odds of CHB (OR = 20.417) and LC (OR = 25.083). These findings suggest a critical role for *SLC29A1* in disease progression.

This study significantly contributes to understanding the role of the *SLC29A1* rs760370 polymorphism in liver disease progression, particularly in Egyptian patients with CHB and LC. Prior research by Doehring et al. (2011); Fouad et al. (2017), and Milazzo et al., (2015) highlighted associations between *SLC29A1* polymorphisms and liver diseases. The current study is the first to explore the gene's role, specifically in HBV-infected Egyptian patients. The marked

association of the AA genotype and the A allele with increased disease susceptibility underscores its potential as a population-specific genetic marker. This, in turn, may provide new insights into disease mechanisms and lay the groundwork for future therapeutic and diagnostic advancements.

SOCS3 (*Suppressor of Cytokine Signaling 3*) is a key negative regulator of cytokine signaling pathways, particularly the *JAK/STAT* pathway, which is central to antiviral immunity (Carow and Rottenberg, 2014). Dysregulation of *SOCS3* has been implicated in the pathogenesis of chronic viral infections, including HBV (Xie et al., 2021).

The association of *SOCS3 rs4969168* polymorphism with disease progression has been highlighted by the significant increase in the frequency of the GG genotype from controls (14%) to CHB (50%) and LC (82%). Carriers of the G allele demonstrated notably higher odds for CHB (OR = 6.2) and LC (OR = 22). These results underscore the potential role of this polymorphism in modulating inflammatory responses and contributing to the pathogenesis of chronic liver diseases, particularly within the context of HBV infection.

SOCS3 plays a pivotal role in regulating cytokine signaling and inflammation. Studies by Koeberlein et al. (2010) and Wang et al. (2020) demonstrated that altered expression of *SOCS3* can exacerbate liver damage by modulating the *JAK/STAT* signaling pathway, a mechanism also implicated in hepatitis B pathogenesis. The findings of this study align with those observations, highlighting the genetic predisposition conferred by *SOCS3* polymorphisms in CHB and LC patients.

The findings regarding the *SOCS3* rs4969170 polymorphism reveal its strong association with disease progression, particularly in CHB and LC patients. The GG genotype showed a significantly higher frequency in CHB (54%) and LC (84%) patients compared to controls (2%), with odds ratios of 279.0 for CHB and 1302.0 for LC. These results indicate that *SOCS3* rs4969170 acts as a major genetic determinant of risk (Hoan et al., 2017).

This polymorphism has been linked to the dysregulation of cytokine-mediated immune responses. Jiang et al. (2015) identified the GG genotype as contributing to fibrosis and cirrhosis through immune modulation, supporting its role in disease severity. Conversely, Zhang et al., (2015) and Zheng et al. (2021) reported inconsistent associations, potentially due to genetic variability or environmental factors, highlighting the need for further investigation.

Although significant genotypic associations were observed, the relationship between these polymorphisms and clinical parameters such as ALT, AST, Fibro Scan values, and Child-Pugh scores were limited. Among CHB patients, ALT levels were slightly higher in rs760370 AA carriers, rs4969168 and rs4969170 GG genotypes were associated with higher ALT and AST levels in LC patients, but these trends lacked statistical significance. This suggests that while these polymorphisms influence susceptibility, their impact on clinical severity may be moderated by other factors.

The findings underscore the importance of genetic screening for *SLC29A1* rs760370 and *SOCS3* rs4969168 and rs4969170 in assessing the risk of CHB and LC. These polymorphisms could serve as potential biomarkers for early detection and risk stratification. Further functional studies are warranted to elucidate the mechanistic role of these genetic variations in the pathogenesis of chronic liver diseases. Additionally, large-scale multicentric studies must validate these findings and explore their translational potential in therapeutic strategies.

LIMITATIONS

One limitation of this study is the relatively small sample size, which may affect the generalizability of the findings. Future studies with larger cohorts are needed to confirm these associations. Additionally, the study focused on a specific population, and the results may not apply to other ethnic groups.

CONCLUSION

This study highlights the significant role of *SLC29A1* rs760370 and *SOCS3* rs4969168 and rs4969170 polymorphisms in Egyptian patients' susceptibility to CHB and LC. The findings contribute to understanding genetic determinants in chronic liver diseases and provide a basis for future research into personalized medicine approaches.

ABBREVIATIONS

AFP	Alpha-Fetoprotein
ALB	Albumin
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CHB	Chronic Active Hepatitis
CI	Confidence Intervals
CpG	5’-Cytosine-phosphate-Guanine-3
H	Kruskal Wallis test
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma
HCC	Hepatocellular Carcinoma
HW	Hardy-Weinberg
HWE	Hardy-Weinberg Equilibrium

IQR	Interquartile Range
JAK/STAT	*The Janus kinase/Signal Transducer and Activator of Transcription*
LC	Liver Cirrhosis
OR	Odds Ratios
PLT	Platelet Count
SD	Standard Deviations
SLC29A1	*Solute Carrier Family 29 Member 1 (Augustine Blood Group)*
SOCS	*Suppressors of Cytokine Signalling*
SOCS3	*Suppressors of Cytokine Signalling-3*
STAT3	*Signal transducer and activator of transcription 3*
TBIL	Total Bilirubin
TLC	Total Leukocyte Count

ETHICS APPROVAL

This study was approved by the Research Ethical Committee at the National Liver Institute (NLI), Menoufia University; NLI IRB Protocol number: 00615/ 2024; IRB: 00014014/FWA00034015; Date: June 2024 National Liver Institute, Menoufia University.

CLINICAL TRIAL

Clinical trial number: not applicable. This study is not part of a randomized controlled clinical trial; the board approval was attached as a supplementary.

CONSENT FOR PUBLICATION

All authors have read and approved the final manuscript for publication.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

No potential conflicts of interest were disclosed.

FUNDING

This study received no funds.

AUTHORS' CONTRIBUTIONS

Conceptualization; AME, WEH, HSA, RHA, ELS. Methodology; AME, WEH, HSA, RHA, ELS. Resources; AME, WEH, ELS, HSA, RHA. Data curation; AME. Writing-original draft; AME, WEH, ELS, HSA, RHA. Writing—review and editing: AME, WEH, ELS, HSA, RHA. Visualization; AME, WEH. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Albuquerque-Souza E, Sahingur SE (2022). Periodontitis, chronic liver diseases, and the emerging oral-gut-liver axis. Periodontology 2000, 89(1): 125–141. https://doi.org/10.1111/prd.12427

Azzam A, Khaled H, Elbohy OA, Mohamed SA, Mohamed SMH, Abdelkader AH, Ezzat AA, Elmowafy AOI, El-Emam OA, Awadalla M, Refaey N, Rizk SMA. (2023). Seroprevalence of hepatitis B virus surface antigen (HBsAg) in Egypt (2000–2022): a systematic review with meta-analysis. BMC Infectious Diseases, 23(1): 151. https://doi.org/10.1186/s12879-023-08110-5

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer C T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry, 55(4): 611–622. https://doi.org/10.1373/clinchem.2008.112797

Carow B, Rottenberg ME. (2014). *SOCS3*, a Major Regulator of Infection and Inflammation. Frontiers in Immunology, 5. https://doi.org/10.3389/fimmu.2014.00058

Doehring A, Hofmann WP, Schlecker C, Zeuzem S, Sarrazin C, Berg T, Müller T, Herrmann E, Geisslinger G, Lötsch J. (2011). Role of nucleoside transporters SLC28A2/3 and SLC29A1/2 genetics in ribavirin therapy. Pharmacogenetics and Genomics, 21(5): 289–296. https://doi.org/10.1097/FPC.0b013e32834412e7

Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Guha IN, Cobbold JF, Deeks JJ, Paradis V, Bedossa P, Newsome PN. (2019). Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients with Nonalcoholic Fatty Liver Disease. Gastroenterology, 156(6): 1717–1730. https://doi.org/10.1053/j.gastro.2019.01.042

Fouad R, Zachariah K, Khairy M, Khorshied M, Ezzat W, Sheta MM, Heiba A. (2017). Single Nucleotide rs760370 Polymorphism at the Main Ribavirin Transporter Gene Detection by PCR-RFLP Assay Compared with the TaqMan Assay and Its Relation to Sustained Virological Response in Chronic HCV Patients Treated with Pegylated Interferon–Ribavirin Therapy. Journal of Interferon & Cytokine Research, 37(2): 90–96. https://doi.org/10.1089/jir.2016.0099

Hoan NX, Van Tong H, Giang DP, Cuong BK, Toan NL, Wedemeyer H, Bock CT, Kremsner PG, Song LH, Velavan TP. (2017). SOCS3 genetic variants and promoter hypermethylation in patients with chronic hepatitis B. Oncotarget, 8(10): 17127–17139. https://doi.org/10.18632/oncotarget.15083

Holmes JA, Roberts SK, Ali RJ, Dore GJ, Sievert W, McCaughan GW, Crawford DH, Cheng WS, Weltman MD, Bonanzinga S, Visvanathan K, Sundararajan V, Desmond PV, Bowden D S, Matthews GV, Thompson AJ. (2014). ITPA genotype protects against anemia during peginterferon and ribavirin therapy but does not influence virological response. Hepatology, 59(6): 2152–2160. https://doi.org/10.1002/hep.27022

Hu X, li J, Fu M, Zhao X, Wang W. (2021). The JAK/STAT signaling pathway: from bench to clinic. Signal Transduction and Targeted Therapy, 6(1): 402. https://doi.org/10.1038/s41392-021-00791-1

Jiang B, Yang Y, Liu H, Gu F, Yang Y, Zhao LH, Yuan S, Wang R, Zhang J, Zhou W. (2015). SOCS3 Genetic

Polymorphism Is Associated with Clinical Features and Prognosis of Hepatocellular Carcinoma Patients Receiving Hepatectomy. Medicine, 94(40): e1344. https://doi.org/10.1097/MD.0000000000001344

Jose-Abrego A, Roman S, Laguna-Meraz S, Panduro A. (2023). Host and HBV Interactions and Their Potential Impact on Clinical Outcomes. Pathogens, 12(9): 1146. https://doi.org/10.3390/pathogens12091146

Khanam A, Chua JV, Kottilil S. (2021). Immunopathology of Chronic Hepatitis B Infection: Role of Innate and Adaptive Immune Response in Disease Progression. International Journal of Molecular Sciences, 22(11): 5497. https://doi.org/10.3390/ijms22115497

Khullar V. (2015). Hepatitis C cirrhosis: New perspectives for diagnosis and treatment. World Journal of Hepatology, 7(14): 1843. https://doi.org/10.4254/wjh.v7.i14.1843

Koeberlein B, Hausen A, zur Bektas N, Zentgraf H, Chin R, Toan NL, Kandolf R, Torresi J, Bock CT. (2010). Hepatitis B virus overexpresses suppressor of cytokine signaling-3 (SOCS3) thereby contributing to severity of inflammation in the liver. Virus Research, 148(1–2): 51–59. https://doi.org/10.1016/j.virusres.2009.12.003

LARSEN L, RÖPKE C. (2002). Suppressors of cytokine signaling: SOCS. APMIS, 110(12): 833–844. https://doi.org/10.1034/j.1600-0463.2002.1101201.x

Li Y, Han M, Li W, Shi A, Zhang Y, Wang H, Wang F, Li L, Wu T, Ding L, Chen T, Yan W, Luo X, Ning Q. (2014). SOCS3 expression correlates with severity of inflammation in mouse hepatitis virus strain 3-induced acute liver failure and HBV-ACLF. Journal of Huazhong University of Science and Technology [Medical Sciences], 34(3): 348–353. https://doi.org/10.1007/s11596-014-1281-5

Locke WJ, Guanzon D, Ma C, Liew YJ, Duesing KR, Fung KYC, Ross JP. (2019). DNA Methylation Cancer Biomarkers: Translation to the Clinic. Frontiers in Genetics, 10. https://doi.org/10.3389/fgene.2019.01150

Mikdar M, González-Menéndez P, Cai X, Zhang Y, Serra Mt, Dembele AK, Boschat AC, Sanquer S, Chhuon C, Guerrera IC, Sitbon M, Hermine O, Colin Y, Le Van Kim C, Kinet S, Mohandas N, Xia Y, Peyrard T, Taylor N, Azouzi S. (2021). The equilibrative nucleoside transporter ENT1 is critical for nucleotide homeostasis and optimal erythropoiesis. Blood, 137(25): 3548–3562. https://doi.org/10.1182/blood.2020007281

Milazzo L, Peri AM, Mazzali C, Magni C, Calvi E, De Nicolò A, Clementi E, Cheli S, D'Avolio A, Antinori S, Falvella FS. (2015). SLC29A1 polymorphism and prediction of anemia severity in patients with chronic hepatitis C receiving triple therapy with telaprevir. Journal of Antimicrobial Chemotherapy, 70(4): 1155–1160. https://doi.org/10.1093/jac/dku519

Pineda-Tenor D, Micheloud D, Berenguer J, Jiménez-Sousa MA, Fernández-Rodríguez A, García-Broncano P, Guzmán-Fulgencio, M, Diez C, Bellón JM, Carrero A, Aldámiz-Echevarria T, García-Álvarez M, Resino S. (2014). SLC30A8 rs13266634 polymorphism is related to a favorable cardiometabolic lipid profile in HIV/hepatitis C virus-coinfected patients. AIDS, 28(9): 1325–1332. https://doi.org/10.1097/QAD.0000000000000215

Pisano MB, Giadans CG, Flichman DM, Ré VE, Preciado MV, Valva P. (2021). Viral hepatitis update: Progress and perspectives. World Journal of Gastroenterology, 27(26): 4018–4044. https://doi.org/10.3748/wjg.v27.i26.4018

Prakash BC, B AS. (2019). Correlation between APRI Index, MELD Score and Child Pugh Score in cirrhosis of liver. International Journal of Advances in Medicine, 7(1), 46. https://doi.org/10.18203/2349-3933.ijam20195615

Rehermann B. (2024). Toward a better understanding of chronic hepatitis B virus infection. Journal of Clinical Investigation, 134(19). https://doi.org/10.1172/JCI185568

Sample & Assay Technologies Rotor-Gene ® Q MDx User Manual (US). (2018).

Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari, Z., Abbastabar H, Abdoli A, Abubaker Ali H, Adane MM, Adegboye OA, Adnani QES, Advani SM, Afzal MS, Afzal S, Aghaie Meybodi M, Ahadinezhad B, Ahinkorah BO, Ahmad S, … Dirac MA. (2022). Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet Gastroenterology & Hepatology, 7(9): 796–829. https://doi.org/10.1016/S2468-1253(22)00124-8

Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A. (2011). Suppressors of Cytokine Signaling (SOCS) Proteins and JAK/STAT Pathways. Arteriosclerosis, Thrombosis, and Vascular Biology, 31(5): 980–985. https://doi.org/10.1161/ATVBAHA.110.207464

Tan A, Koh S, Bertoletti A. (2015). Immune Response in Hepatitis B Virus Infection. Cold Spring Harbor Perspectives in Medicine, 5(8): a021428. https://doi.org/10.1101/cshperspect.a021428

Thomas E, Baumert TF. (2019). Hepatitis B Virus–Hepatocyte Interactions and Innate Immune Responses: Experimental Models and Molecular Mechanisms. Seminars in Liver Disease, 39(03): 301–314. https://doi.org/10.1055/s-0039-1685518

Tian J, Li C, Li W. (2021). Entry of hepatitis B virus: going beyond NTCP to the nucleus. Current Opinion in Virology, 50, 97–102. https://doi.org/10.1016/j.coviro.2021.08.001

Trépo C, Chan HLY, Lok A. (2014). Hepatitis B virus infection. The Lancet, 384(9959): 2053–2063. https://doi.org/10.1016/S0140-6736(14)60220-8

Wang T, Wang X, Zhuo Y, Si C, Yang L, Meng L, Zhu B. (2020). Antiviral activity of a polysaccharide from Radix Isatidis (Isatis indigotica Fortune) against hepatitis B virus (HBV) in vitro via activation of JAK/STAT signal pathway. Journal of Ethnopharmacology, 257, 112782. https://doi.org/10.1016/j.jep.2020.112782

World Medical Association Declaration of Helsinki. (2013). JAMA, 310(20): 2191. https://doi.org/10.1001/jama.2013.281053

Xie J, Wang, M., Cheng A, Jia R, Zhu D, Liu M, Chen S, Zhao X, Yang Q, Wu Y, Zhang S, Luo Q, Wang Y, Xu Z, Chen Z, Zhu L, Liu Y, Yu Y, Zhang L, Chen X. (2021). The role of SOCS proteins in the development of virus-induced

hepatocellular carcinoma. Virology Journal, 18(1): 74. https://doi.org/10.1186/s12985-021-01544-w

Yoshimura A, Suzuki M, Sakaguchi R, Hanada T, Yasukawa H. (2012). SOCS, Inflammation, and Autoimmunity. Frontiers in Immunology, 3. https://doi.org/10.3389/fimmu.2012.00020

Yuan L, Jiang J, Liu X, Zhang Y, Zhang L, Xin J, Wu K, Li X, Cao J, Guo X, Shi D, Li¬ ¬Jun Jiang L, Sun S, Wang T, Hou W, Zhang T, Zhu H, Zhang J, … Xia N. (2019). HBV infection-induced liver cirrhosis development in dual-humanized mice with human bone mesenchymal stem cell transplantation. Gut, 68(11), 2044–2056. https://doi.org/10.1136/gutjnl-2018-316091.

Zhang X, You Q, Zhang X, Chen X. (2015). SOCS3 Methylation Predicts a Poor Prognosis in HBV Infection-Related Hepatocellular Carcinoma. International Journal of Molecular Sciences, 16(9): 22662–22675. https://doi.org/10.3390/ijms160922662 Zheng H, Yan Y, Cheng J, Yu S, Wang Y. (2021). Association between SOCS3 hypermethylation and HBV-related hepatocellular carcinoma and effect of sex and age. Medicine, 100(43): e27604. https://doi.org/10.1097/MD.0000000000027604.