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Original paper

The TM6SF2 variant as a risk factor for hepatocellular carcinoma development in chronic liver disease patients

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Abstract

Introduction: Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide. A non-synonymous single nucleotide polymorphism (SNP) of the transmembrane 6 superfamily member 2 (*TM6SF2*) gene is associated with non-alcoholic fatty liver disease. SNPs of the *TM6SF2* gene play an important role in the pathogenesis of HCC in alcoholic cirrhosis, but there are limited data regarding other possible etiologies. We aimed to evaluate the role of the rs58542926 polymorphism in the development of HCC in Egyptian chronic liver disease (CLD) patients.

Material and methods: A total of 120 participants, including 40 HCC patients, 40 CLD patients, and 40 healthy controls, were selected. Real-time polymerase chain reaction (RT-PCR) was used to detect the *TM6SF2* rs58542926 polymorphism.

Results: There were no significant differences among the three studied groups regarding age ($p = 0.06$) and gender ($p = 0.75$). Frequencies of the CT, TT, CT + TT genotypes and the T allele were significantly higher in HCC patients than in the CLD and control groups ($p < 0.001$, $p = 0.005$, and $p < 0.001$, respectively). CLD patients with the CT genotype had a significantly increased risk of HCC development (OR = 4.67, 95% CI: 1.67-12.90). Patients with the TT genotype had a significantly increased risk of HCC (OR = 9.33, 95% CI: 1.72-50.61). Moreover, the T allele was correlated with an increased risk of HCC (OR = 5.44, 95% CI: 2.09-14.17) compared to the C allele.

Conclusions: The *TM6SF2* rs58542926 genotype is associated with an increased risk of HCC in the Egyptian population.

Key words: hepatocellular carcinoma, CLD, *TM6SF2*, polymorphism.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and is highly prevalent in Egypt. Unfortunately, most HCC patients in Egypt are first diagnosed in the intermediate or advanced stages [1]. For early diagnosis and to reduce HCC-related mortality, new HCC diagnostic and prognostic biomarkers are crucial [2]. Multiple risk factors are correlated with HCC, including alcohol abuse, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, aflatoxin exposure, nonalcoholic steatohepatitis, and metabolic diseases [2].

The transmembrane 6 superfamily member 2 (*TM6SF2*) gene is located on chromosome 19 and encodes a protein composed of 351 amino acids [3]. Expression pattern analysis has shown that *TM6SF2* is mainly expressed in the kidney, small intestine and liver, all of which are tightly associated with lipid metabolism; the expression levels of *TM6SF2* are lower in most other tissues [3]. *TM6SF2* gene E167K variant (rs58542926) was identified in an exome-wide associa-

tion study as a non-synonymous single nucleotide polymorphism (SNP) associated with non-alcoholic fatty liver disease. SNPs can influence gene expression, protein function and disease susceptibility [5]. The E167K variant helps regulate liver fat metabolism, influencing triglyceride secretion and hepatic lipid droplet content, and has been associated with increased hepatocytic triglyceride content, dyslipidemia, and cardiovascular risk, advanced hepatic fibrosis, and cirrhosis [4]. We determined the association between the *TM6SF2* gene SNP rs58542926 and HCC occurrence and progression in Egyptian CLD patients to evaluate its utility as a diagnostic and prognostic biomarker of HCC.

Material and methods

The study was conducted in the Clinical Pathology and Hepatology and Gastroenterology Departments at the National Liver Institute, Menoufia University, Egypt, between January 2019 and March 2020. A total of 120 subjects were enrolled in this case-control study, including 40 patients with HCC and 40 patients with CLD with no radiological evidence of HCC. Infections associated with hepatitis types C or B were the cause of chronic liver disease in this group in addition to 40 healthy age- and sex-matched individuals as a control group, with no previous history of liver or malignant diseases and negative for hepatitis viral markers. Patients with HCC were diagnosed according to definitive criteria detected by triphasic computed tomography with contrast (which showed arterial enhancement and delayed venous washout). Patients were excluded if they had been diagnosed with an inflammatory disease, hematological malignancy, and cancer of any organ other than the liver. The study protocol was approved by the local ethics committee of the National Liver Institute, Menoufia University, Egypt. Informed consent was collected from all participants after receiving information about the study.

Relevant clinical data were collected from all participants. Laboratory blood analyses included those for liver and renal function. The Cobas 6000 autoanalyser (Roche Diagnostics, Germany) was used to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, serum albumin, creatinine and urea. Alpha-fetoprotein (AFP, Cobas e411 immunoassay analyzer, Roche Diagnostics, Germany), prothrombin time (Coagulometer CA-1500, Siemens, Germany), and hepatitis serology (HBsAg and HCVAb) (Cobas e411 immunoassay analyzer, Roche Diagnostics, Germany) were also assessed.

SNP genotyping for the *TM6SF2* rs58542926 polymorphism was performed using a real-time PCR assay (Applied Biosystems, USA). Total DNA was extracted from EDTA-treated blood samples using the QIAamp DNA Mini Kit (Thermo Fisher Scientific, Lithuania).

After ethanol precipitation, the DNA was purified and dissolved in double-distilled water and stored at –20°C until analysis [6]. The *TM6SF2* gene polymorphism (rs58542926) was genotyped by real-time PCR fluorescence detection on an ABI 7500 Real-Time PCR system using fluorescence-labeled probes. Context Sequence [VIC/FAM]: The PCR primers (Thermo Fisher Scientific) were as follows: forward 5'GTGAGGAAGAAGGCAGGCCTGATCT3' and reverse 5'GGAGCTGTATTTGCCTTCCATGGTG3'.

Preparation of solution reaction (Total volume 20 µl): [Master Mix (2X) 10 µl, TaqMan assay 20 k 0.5 µl, Template DNA 5 µl, Water and nuclease-free 4.5 µl]. PCR cycling conditions were 95°C for 10 min, then 40 cycles of amplification of 95°C for 10 s, 60°C for 30 s, and 68°C for 15 s.

Automatic or manual allele calls were marked. The software analyzed the before and after fluorescence levels and calculated normalized dye fluorescence (ΔRn) as a function of cycle number for Allele1 (wild-type) or Allele2 (mutant). Based on this number (the relative number of the possible outcomes), the software makes an automatic call of Allele1 (homozygous 1/1), Allele2 (homozygous 2/2), or heterozygous (1/2).

Statistical analysis

Statistical analysis of the data was conducted using SPSS version 22 (SPSS Inc., USA). Data were analyzed descriptively and quantitatively using a χ^2 test, one-way ANOVA, Kruskal-Wallis test, Fisher's exact test, odds ratio (OR), and confidence interval (CI) test. *P*-values < 0.05 were considered statistically significant.

Results

There were no significant differences between the groups in terms of age ($p = 0.06$) or gender ($p = 0.750$) (Table 1). There were significant differences between the three groups regarding liver cirrhosis, ascites, portal vein thrombosis and Child-Pugh score ($p \leq 0.001$), but no significant differences regarding smoking habits ($p = 0.39$) and diagnosed diabetes mellitus ($p = 0.55$) (Table 2). Blood concentrations of AST, ALT, GGT, ALP, total bilirubin, direct bilirubin, serum albumin, creatinine, urea, AFP and international normalized ratio (INR) were significantly different between the groups ($p \leq 0.001$) (Table 3).

Table 1. Sociodemographic data of the studied groups

Parameter	Group I (*n* = 40)		Group II (*n* = 40)		Group III (*n* = 40)		*p*
	n	%	*n*	%	*n*	%	
Gender							
Male	30	75.0	29	72.5	27	67.5	0.750
Female	10	25.0	11	27.5	13	32.5	
Age (years)							
Min.-Max.	44.0-68.0		40.0-68.0		42.0-60.0		0.06
Mean ±SD	55.23 ±7.13		52.40 ±7.10		51.92 ±5.31		

χ^2 – chi-square test, F – F for ANOVA test
p – p value for comparing between the studied groups
Group I: Patients with HCC, Group II: Patients with chronic hepatic disease, Group III: Control

Table 2. Comparison between the two studied groups according to risk factors

Parameter	Group I (*n* = 40)		Group II (*n* = 40)		χ^2	*p*
	n	%	*n*	%		
Smoking						
No	22	55.0	26	65.0	0.833	0.361
Yes	18	45.0	14	35.0		
Diabetes mellitus						
No	22	55.0	22	55.0	0.0	1.000
Yes	18	45.0	18	45.5		
Splenomegaly						
No	8	20.0	26	65.0	16.573*	< 0.001*
Yes	32	80.0	14	35.0		
Cirrhotic						
No	0	0.0	30	75.0	48.0*	< 0.001*
Yes	40	100.0	10	25.0		
PVT						
No	24	60.0	40	100.0	20.0*	< 0.001*
Yes	16	40.0	0	0.0		

χ^2 – chi-square test
p – p value for comparing between the studied groups
**Statistically significant at p ⩽ 0.05*
Group I: Patients with HCC, Group II: Patients with chronic hepatic disease

The frequency distributions of the different genotypes for the *TM6SF2* polymorphism were significantly different between HCC patients and the other groups (Table 4). HCC patients had a higher incidence of TT and TC genotypes than CLD patients and healthy controls ($p = 0.001$ and $p = 0.008$, respectively). A statistically higher incidence of the T allele was observed in HCC than in the CLD and healthy control groups ($p < 0.001$) (Table 4, Fig. 1).

In evaluating the risk of HCC according to *TM6SF2* genotype, we identified significantly higher TC and TT genotype frequencies in the HCC group (50% and 20%, respectively) than in the other groups (Table 4, Fig. 1). In contrast, the CC genotype frequency was significantly higher in the control group (95%) than in the other groups ($p < 0.001$).

In the additive genetic model, CLD patients with the CT genotype had a significantly higher risk of HCC development (OR = 4.67, 95% CI: 1.67-12.90). Individuals carrying the TT genotype had a significantly increased risk of HCC (OR = 9.33, 95% CI: 1.72-50.61) (Table 4, Fig. 1). Furthermore, HCC patients

Table 3. Comparison between the different studied groups according to laboratory data

	Group I (*n* = 40)		Group II (*n* = 40)		Group III (*n* = 40)		*p*	Sig. bet. groups		
	n	%	*n*	%	*n*	%		p_1	p_2	p_3
Albumin (g/dl)										
Min.-Max.	2.0-4.60		3.80-4.80		4.30-4.90		< 0.001*	< 0.001*	< 0.001*	< 0.001*
Mean ±SD	3.34 ±0.59		4.17 ±0.29		4.58 ±0.16					
TP (g/dl)										
Min.-Max.	5.0-7.10		6.0-7.10		5.80-7.40		< 0.001*	< 0.001*	< 0.001*	0.029*
Mean ±SD	5.73 ±0.53		6.54 ±0.36		6.79 ±0.39					
ALP (µ/l)										
Min.-Max.	81.0-165.0		61.0-111.0		35.0-61.0		< 0.001*	< 0.001*	< 0.001*	< 0.001*
Mean ±SD	114.20 ±21.57		87.55 ±16.16		45.20 ±7.77					
γ-GT (mg/dl)										
Min.-Max.	78.0-160.0		10.0-41.0		13.0-35.0		< 0.001*	< 0.001*	< 0.001*	0.018*
Median (IQR)	95.0 (90.50-113.0)		30.50 (23.5-36.5)		23.50 (22.0-25.0)					
AST (µ/l)										
Min.-Max.	25.0-238.0		18.0-135.0		18.0-30.0		< 0.001*	< 0.001*	< 0.001*	0.003*
Median (IQR)	74.0 (42.5-102.5)		37.0 (29.0-67.0)		21.50 (20.0-24.0)					
ALT (µ/l)										
Min.-Max.	11.0-170.0		20.0-173.0		20.0-34.0		< 0.001*	< 0.001*	< 0.001*	0.015*
Median (IQR)	78.0 (46.5-115.0)		43.0 (27.5-85.0)		26.0 (24.0-29.5)					
Total bilirubin (mg/dl)										
Min.-Max.	0.50-3.0		0.30-1.20		0.30-0.75		< 0.001*	< 0.001*	< 0.001*	0.010*
Median (IQR)	1.04 (0.75-1.40)		0.60 (0.40-0.85)		0.50 (0.40-0.60)					
Direct bilirubin (mg/dl)										
Min.-Max.	0.10-2.0		0.10-1.10		0.10-0.30		< 0.001*	< 0.001*	< 0.001*	0.001*
Median (IQR)	0.50 (030-0.80)		0.30 (0.20-0.40)		0.15 (0.10-0.19)					
PT										
Min.-Max.	9.0-14.50		11.0-15.50		11.0-14.0		< 0.001*	< 0.001*	0.146	0.002*
Median (IQR)	11.75 (10.1-13.35)		14.0 (12.0-14.5)		12.0 (12.0-12.60)					
INR										
Min.-Max.	1.0-1.18		1.0-1.30		1.0-1.12		< 0.001*	0.003*	0.068	< 0.001*
Median (IQR)	1.0 (1.0-1.05)		1.12 (1.0-1.18)		1.0 (1.0-1.0)					
AFP (ng/ml)										
Min.-Max.	16.10-2650.0		2.0-18.0		2.0-5.0		< 0.001*	< 0.001*	< 0.001*	0.075
Median (IQR)	510.0 (75.5-769.5)		4.0 (3.0-8.15)		3.0 (3.0-4.0)					
Urea (mg/dl)										
Min.-Max.	15.0-80.0		15.0-41.0		17.0-37.0		< 0.001*	< 0.001*	< 0.001*	0.336
Median (IQR)	38.0 (29.5-50.5)		26.0 (21.0-35.0)		24.0 (21.5-30.5)					
Creatinine (mg/dl)										
Min.-Max.	0.40-1.30		0.40-1.02		0.50-1.0		0.002*	0.002*	0.013*	0.848
Mean ±SD	0.87 ±0.27		0.72 ±0.16		0.75 ±0.13					

AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, γ-GT – γ-glutamyltransferase, TP – total protein, INR – international normalized ratio
χ^2 – chi-square test, F – F for ANOVA test, Pairwise comparison between each 2 groups was done using a post hoc test (Tukey), H – H for Kruskal-Wallis test, pairwise comparison between each 2 groups was done using a post hoc test (Dunn's test for multiple comparisons)
p – p value for comparing between the studied groups, p_1 – value for comparing between HCC and liver cirrhosis, p_2 – value for comparing between HCC and control, p_3 – value for comparing between liver cirrhosis and control
**Statistically significant at p < 0.05*

Table 4. Comparison of genotype and allele frequencies of rs58542926 between HCC patients, CLD and control group

Polymorphism data	Group I (*n* = 40)		Group II (*n* = 40)		Group III (*n* = 40)		*p*	Significance between groups		
	n	%	*n*	%	*n*	%		I vs. II	I vs. III	II vs. III
Additive genetic model										
CC	12	30.0	28	70.0	38	95.0	$^{MC}p < 0.001$*	0.001*	$^{MC}p < 0.001$*	^{MC}p 0.008*
CT	20	50.0	10	25.0	2	5.0				
TT	8	20.0	2	5.0	0	0.0				
OR (95% CI)			*I and II CC reference CT: 4.67 [1.67-12.90] TT: 9.33 [1.72-50.61]		*I and III CC reference CT: 31.68 [6.45-155.6] TT: –					
Recessive genetic model										
CT + CC	32	80.0	38	95.0	40	100.0	$^{MC}p = 0.005$*	0.043*	$^{FE}p = 0.005$*	$^{FE}p = 0.494$
TT	8	20.0	2	5.0	0	0.0				
OR (95%CI)			*I and II 4.75 [0.94-23.98]		*I and III –					
Dominant genetic model										
CC	12	30.0	28	70.0	38	95.0	$^{MC}p < 0.001$*	< 0.001*	< 0.001*	0.003*
CT + TT	28	70.0	12	30.0	2	5.0				
OR (95%CI)		*I and II 5.44 (2.09-14.17)	*I and III 44.33 (9.18-214.06)							
Alleles										
C	44	55.0	66	82.5	78	97.5	< 0.001*	0.001*	< 0.001*	0.006*
T	36	45.0	14	17.5	2	2.5				
OR (95% CI)			*I and II 3.86 (1.87-7.97)		*I and III 31.909 (7.33-138.93)					
HWE *p*					0.87					
HWE χ^2					0.026					

HWE – p value for Hardy-Weinberg, χ^2 – chi-square test, MC – Monte Carlo
p – p value for comparing between the studied groups, FE – Fisher exact
OR – odds ratio, CI – confidence interval, LL – lower limit, UL – upper limit

**Statistically significant at p < 0.05*

had a significantly higher frequency ($p < 0.001$) of the T allele (45.0%) than CLD and control groups (17.5% and 2.5%, respectively).

The dominant genetic model combination of TC and TT genotypes was significantly greater in the HCC group (70.0%) than in the CLD and control groups (30% and 5%, respectively; $p < 0.001$). Also, CLD patients carrying the T allele (TC + TT) had a 5.44-fold increased risk for HCC development (OR = 5.44, 95% CI: 2.09-14.17) (Table 4, Fig. 1).

In the control group, the study results were in accordance with the Hardy-Weinberg law of disequilibrium ($\chi^2 = 0.026$, $p = 0.87$).

Univariate analysis of potential HCC risk factors indicated that age and presence of the T allele (CT + TT)

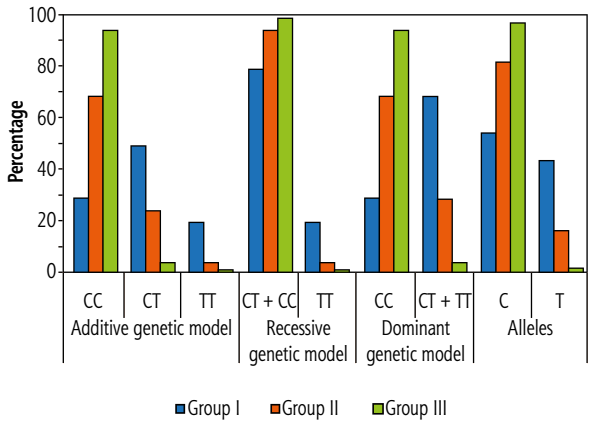

Fig. 1. Comparison between the studied groups according to polymorphism rs58542926

Table 5. Univariate and multivariate logistic regression analysis for the parameters affecting the HCC group compared to the control

Parameter	Univariate		Multivariate#	
	p	OR (95% CI)	*p*	OR (95% CI)
Gender (male)	0.460	0.692 (0.261-1.835)		
Age (years)	0.025*	1.088 (1.011-1.171)	0.154	1.078 (0.972-1.194)
Smoking	–	–		
Diabetes mellitus	–	–		
Splenomegaly	–	–		
Cirrhotic	–	–		
rs58542926 (CT + TT)	< 0.001*	44.333 (9.182-214.062)	< 0.001*	42.359 (8.560-209.598)

OR – odds ratio, CI – confidence interval, LL – lower limit, UL – upper limit
#All variables with p < 0.05 were included in the multivariate analysis
**Statistically significant at p < 0.05*

Table 6. Univariate and multivariate logistic regression analysis for the parameters affecting the HCC group (*n* = 40) compared to the chronic liver disease group (*n* = 40)

Parameter	Univariate		Multivariate#	
	p	OR (95% CI)	*p*	OR (95% CI)
Gender (male)	0.799	0.879 (0.324-2.381)		
Age (years)	0.082	1.058 (0.993-1.128)		
Smoking	0.362	1.519 (0.618-3.738)		
Diabetes mellitus	1.000	1.0 (0.414-2.413)		
Splenomegaly	< 0.001*	7.429 (2.703-20.419)	0.003*	5.131 (1.763-14.937)
Cirrhotic	0.998	–		
rs58542926 (CT + TT)	0.001*	5.444 (2.092-14.168)	0.021*	3.409 (1.203-9.660)

OR – odds ratio, CI – confidence interval, LL – lower limit, UL – upper limit
#All variables with p < 0.05 were included in the multivariate analysis
**Statistically significant at p < 0.05*

were associated significantly with a higher risk of HCC than in the control group (OR = 1.088, 95% CI: 1.011-1.171 and $p = 0.025$; OR = 44.333, 95% CI: 9.182- 214.062 and $p < 0.001$, respectively) (Table 5). Univariate analysis of potential HCC risk factors of HCC against CLD indicated that splenomegaly and T allele in the homozygous and heterozygous genotypes (CT + TT) were associated significantly with an increased risk of HCC against CLD (OR = 7.429, 95% CI: 2.703-20.419 and $p < 0.001$; OR = 5.444, 95% CI: 2.092-14.168 and $p = 0.001$, respectively) (Table 6).

Multivariate analysis showed that the T allele in homozygous and heterozygous forms (CT + TT) was associated significantly with a higher risk of HCC than the control group (OR = 42.359, 95% CI: 8.560-209.598 and $p < 0.001$) (Table 5). Multivariate analysis conducted on potential risk factors of HCC against CLD also showed that splenomegaly and the presence of the T allele in homozygous and heterozygous forms (CT + TT) were associated significantly with an increased risk of HCC against CLD (respectively, OR = 5.131, 95% CI: 1.763-14.937 and $p = 0.003$; OR = 3.409, 95% CI: 1.203-9.660 and $p = 0.021$) (Table 6).

Discussion

Hepatocellular carcinoma is the most common primary malignancy of the liver and the third leading cause of cancer mortality worldwide. The incidence of HCC is increasing, presenting a major global health challenge [7]. In Egypt, HCC is the second most common cancer in men and the sixth most common cancer in women [8]. Furthermore, it is associated with a low 5-year survival rate and an increased mortality rate [9].

The mean age (±SD) of HCC patients in our study was 55.23 ±7.13 years. These results are similar to those of Mohammed *et al.* [10], who studied an HCC patient cohort with a mean age of 55.4 ±9.757 years, and Sang-Wook *et al.* [11], who studied an HCC cohort with

a mean age of 53.0 ±9.7 years. However, Fotos *et al.* [12] found that HCC is more frequent in patients ≥ 65 years of age.

The mean age of patients with CLD was 52.40 ±7.10 years. This was consistent with Banait *et al.* [13], who reported that CLD was more frequent in patients ≥ 50 years. In contrast, Abdelmenan *et al.* [14] found that the mean age of CLD patients was between 30 to 40 years.

We found a statistically significant difference between HCC patients and the other two groups in values of AST, ALT, ALP, GGT, AFP, albumin, total and direct bilirubin and INR. These results are consistent with Zekri *et al.* [15], who found a statistically significant difference between the HCC patient group and control group in AST, ALT, albumin, bilirubin and INR values. Similarly, Redwan [16] obtained the same result. In contrast, Sarwar *et al.* [17] found no significant difference in serum albumin and INR between the HCC group and the non-HCC group.

The *TM6SF2* gene E167K variant (rs58542926) is associated with non-alcoholic fatty liver disease, a disease frequently associated with metabolic disorders and fat deposition in liver cells. Some studies have demonstrated that this variant increases the risk of developing liver fibrosis and steatohepatitis [18]. Furthermore, it has emerged as a predictor of fibrosis progression and hepatocellular carcinoma occurrence [18].

The *TM6SF2* E167K variant features a C-to-T substitution at nucleotide 499, encoding a glutamate-to-lysine change at codon 167, resulting in a misfolded protein, accelerated protein degradation and reduced protein levels in the body [19].

The present study was designed to evaluate the role of rs58542926 and the development of HCC in an Egyptian cohort. Multiple studies have focused on the role of a common non-synonymous polymorphism in *TM6SF2* (rs58542926, E167K) in lipid metabolism and chronic liver disease, and multiple studies have focused on the role of the *TM6SF2* rs58542926 variant in chronic liver disease and HCC.

Genotyping will permit more precise HCC risk stratification of patients with chronic liver diseases, and genotype-guided screening algorithms would optimize patient care [20]. Assessing genetic risk factors associated with the development of HCC may allow for earlier diagnosis of malignancy and could potentially lead to decreased disease-specific mortality rates.

Current studies have shown that the (*TM6SF2*) rs5854292 gene polymorphism is associated with non-alcoholic fatty liver disease. Meanwhile, multiple investigators in China and other countries have conducted many studies to assess the relationship between the *TM6SF2* rs5854292 gene polymorphism and liver cancer. Some studies concluded that the *TM6SF2* rs5854292 variant was associated with the risk of developing liver cancer [21]. Other studies, however, demonstrated that the presence of the *TM6SF2* variant did not appear to be associated with a further increased risk of developing HCC [22].

We found in this study that the genotype distribution among the studied groups was significantly different between HCC patients and the other groups. HCC patients had a higher incidence of TT and TC genotypes than CLD patients and healthy controls. The T allele frequency in HCC patients was higher than in CLD patients and healthy controls.

The TC and TT genotype frequencies were significantly higher in the HCC group (50% and 20%, respectively) than in the other groups, while the CC genotype was significantly higher in the control group 95% than in other groups. The dominant genetic model combination of TC and TT genotypes was statistically significantly higher in the HCC group (70.0%) than in the CLD and control groups (30% and 5%, respectively).

These results were similar to those of the metanalysis of Tang *et al.* [21], who found that the pooled risk of liver cancer was higher in the TT + CT genotype than in the CC genotype (CC vs. CT + TT, OR = 1.675, 95% CI: 1.413-1.985, $p = 0.000$).

We found that in the additive genetic model, CLD patients with the CT genotype correlated with an increased risk of HCC development (OR = 4.67, 95% CI: 1.67-12.90). Patients with the TT genotype had a significantly higher risk of HCC (OR = 9.33, 95% CI: 1.72-50.61). This was consistent with the results of Stickel *et al.* [20] (OR = 1.66, 95% CI: 1.30-2.13, $p = 5.13 \times 10^{-5}$), who used an additive model controlling for sex and age.

In our cohort, HCC patients had a significantly higher frequency of the T allele (45.0%) than the CLD and control groups (17.5% and 2.5%, respectively). Also, in the dominant genetic model, CLD patients carrying the T allele (TC + TT) had an increased risk for HCC development (by 5.44-fold). Our results were similar to the results obtained by Tang *et al.* [21] and Raksayot *et al.* [23], who found that patients with the T allele were at increased risk for HCC (OR = 1.621, 95% CI: 1.379-1.905, $p = 0.000$; and OR = 2.22, 95% CI: 1.34-3.65, $p = 0.002$, respectively).

Conclusions

The *TM6SF2* gene E167K variant (rs58542926) polymorphism was found to be associated with an increased risk of HCC in our Egyptian cohort. More

studies are needed that include larger sample sizes and more ethnic groups. Knowledge of the mechanisms involved in HCC carcinogenesis may help to identify targets for the development of chemoprevention or therapeutic strategies.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to confidential and institutional ethical issues.

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Disclosure

The authors declare no conflict of interest.

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