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## TM6SF2 and NCAN polymorphism impact on HCV in North African Egyptian patients

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### Abstract:

**Background:** TM6SF2 and NCAN are genes known to be related to fibrosis and steatosis but are not thoroughly investigated in case of chronic Hepatitis C (HCV) in Egyptians. **Aim:** this study is carried to investigate the role of TM6SF2 and NCAN in chronic HCV Egyptian patients. **Methods:** this retrospective study was carried on 165 patients with chronic HCV who received treatment for it. **Results:** TM6SF2 showed statistical significance with viral load with p value of 0.02 but no statistical significance with fibrosis or activity. NCAN showed statistical significance with activity with p value of 0.011. **Conclusion:** this is the first work recording the prevalence of TM6SF2 (rs58542926) and NCAN (rs2228603) polymorphism in upper African HCV patients. TM6SF2 is not associated with fibrosis or activity in Egyptian patients infected with chronic hepatitis C but associated with high viral load. On the other side, NCAN is associated with severity of activity in the same studied group but no relation with the viral load. These results explain their additive effect exerted during HCV infection which should be further extensively studied.

**Key words:** Transmembrane 6superfamily member 2 (TM6SF2), neurocan (NCAN), chronic HCV (CHC)

## **Introduction:**

Globally, about 130–150 million people are living with chronic HCV infection (1) with about 350,000–500,000 lives lost every year (2,3). The clinical presentation ranges from asymptomatic acute hepatitis to complete clearance in one third of cases or progression to chronic hepatitis in two thirds of cases. Previously, this diverse in progression was thought to be just due to viral or host factors as genotype, age, presence of steatosis or cirrhosis, ethnicity or body mass index. Recently, after the genome wide association studies that investigated the association between single nucleotide polymorphism and the presentation and course of disease, multiple genes were identified to be related to the way of disease progression and response to treatment. Interleukin 28B gene on chromosome 19 was the first one with its effect of spontaneous clearance in acute hepatitis C infection and better response for pegylated interferon with ribavirin in case of chronic hepatitis C (4,5). After that, polymorphism in patatin-like phospholipase domain (PNPLA3) was associated with steatosis (6). This was followed by identifying more variants at 5 loci with multiple genes affecting steatosis, namely NCAN/TM6SF2/CLIP2/PBX4 (7). TM6SF2 rs58542926 C>T variant was established to be related to NAFLD (8, 9, 10) but its role in chronic hepatitis C is debatable (11,12). NCAN has been also assessed to be related to NAFLD. It contains about 20 genes in chromosome 19p13. It was thought that it exists only in neuronal tissue being involved in cell adhesion and migration in the nervous system (13). Later, it was shown to be expressed in the liver (14, 15,16) also and strongly related to plasma low density lipoproteins and triglycerides. This allele also was not studied in case of chronic hepatitis C (17). As it is based now, NAFLD is a prominent feature of HCV (18). Being considered a systemic disease, genetic predisposition to HCV response to treatment and progression of the disease specially when linked to steatosis (19), has to be investigated.

This study aimed to studying the role of TM6SF2 (rs58542926) and NCAN (rs2228603) polymorphism in chronic hepatitis C (CHC) along

its course in Egyptian population. Work was done at National Research Centre

## **Materials and Methods:**

### **Patients:**

This retrospective study was carried on 165 patients with chronic hepatitis C who received treatment either interferon and ribavirin or direct acting antiviral drugs at National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt.

Inclusion criteria were patients above 18 year old, availability of liver biopsy before beginning of the treatment and the presence of blood sample stored at -80 obtained at the same time of treatment. Exclusion criteria were presence of other concomitant infection as HIV or HBV, alcohol abuse, autoimmune hepatitis and liver transplant patients. All patients carried out PCR to detect viral load.

### **Clinical and laboratory evaluation:**

Clinical data and lab investigations were collected at time of liver biopsy. Liver enzymes, CBC, lipid profile, kidney function tests, total bilirubin, albumin, alpha fetoprotein, PCR for HCV were all recorded. BMI was calculated and history of diabetes mellitus and smoking were considered. HCV genotyping was done using the VERSANT HCV genotype 2.0 Assay (LiPA - Bayer).

### **Histological evaluation:**

Histopathology was assessed by the same pathologist who was blinded to this study with detecting degrees of activity and fibrosis according to Metavir scoring system.

### **Association between TM6SF2, NCAN and histopathological changes:**

The association of studied genotypes with the histopathological changes was done by comparing Metavir score of fibrosis (F1,2) and F(3,4), and the degree of activity (A1) versus (A2,3).

### **Statistical analysis:**

Categorical variables were shown as percentage while continuous ones as mean+/- standard

deviation. Uni-variate and multivariate regression analysis were performed after adjustment of clinically relevant variables to evaluate the association of studied genotypes with activity, fibrosis and viral load. A probability value ( $p$  value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

### **Results:**

We retrospectively studied 165 Egyptian patients with chronic HCV, 89 males (53.9%) and 76 females (46.1%). TM6SF2 was detected in 145 patients, CC allele in 74.8%, 23.8% CT and 1.4% TT. NCAN was detected in 151 patients presented as 15.2% with CC allele and 84.8% with CT allele but no patients had TT allele.

#### ***TM6SF2 and studied metabolic and viral data:***

The different metabolic and demographic data were found to have no statistical significance with TM6SF2 (Table 1). This was not the case for the viral load which has  $p$  value of 0.002, with the highest mean viral load detected in patients carrying TT genotype. Evaluating the activity and fibrosis didn't show statistical significance with TM6SF2.

#### ***NCAN and studied metabolic and viral data:***

On examination of the different data with NCAN, no statistical significance was found with blood chemistry, liver markers or viral load but it was found with activity having  $p$  value of 0.011 (Table 2).

#### ***Regression analysis for different confounders:***

Regression analysis was done for studying different related possible confounders to activity, viral load and fibrosis. NCAN was found to be an independent confounder to severe activity with  $p$  value of 0.024 by simple and multiple regression analysis with  $p$  value of 0.035, odd ratio 0.334 and  $p$  value of 0.024 and Odd ratio of 0.274 respectively. Univariate analysis showed  $\alpha$  fetoprotein

protein and age were recorded as independent predictors of viral load with Odd ratio and  $p$  value of 1.190, 0.001 for  $\alpha$  fetoprotein, 1.640 and 0.001 for age. On multiple regression analysis, fibrosis,  $\alpha$  fetoprotein and activity were independent factors associated with the viral load. This was presented by Odd ratio and  $p$  value of 0.397, 0.03 for fibrosis, 1.483, 0.00 for  $\alpha$  fetoprotein and 4.373, 0.24 for activity. The BMI was found to be an independent factor affecting severity of fibrosis with  $p$  value of 0.015 and Odd ratio of 1.110 on univariate analysis. (table 3).

### **Discussion:**

HCV infection represents a global burden with its recorded prevalence of more than 185 million around the world. In Egypt, HCV was estimated to be 7.3 % of the population (20). According to blood bank surveys, it reached 40% in some areas (21).

Before, the viral infection by HCV was one of the factors responsible for developing hepatocellular carcinoma. After the direct acting antiviral drugs and the easier method for viral clearance, investigating the genetic map of the patient became a must to predict the possibility of developing liver injury regardless of the viral cure. The high prevalence in Egypt was a motive for us to investigate the genetic factor in Egyptian population as a cause of disease progression. We investigated 2 genes, transmembrane 6 superfamily 2 and neurocan, as known genes associated with liver fibrosis and steatosis in non alcoholic fatty liver disease (NAFLD) in the past few years.

Although studies showed the strong relation between TM6SF2 and fibrosis (22), this study didn't show that it was related to fibrosis or activity in patients with CHC in Egyptians. This was in concordance with the study conducted by Urzua and his colleagues (23) and Coppola and his colleagues (24). But this was different from Milano et al (25) which showed marked necroinflammatory activity related to TM6SF2.

Urzua et al (23) didn't show statistical significance with the viral load which was opposite to this study which showed high statistical significance with  $p$  value of 0.002 with TM6SF2 but Mohammed et al

(26) had the same results. The same study had different viral load log concerning the TT genotype where TT had the lowest viral load but ours had the highest load, which we postulate that could be related to difference in the HCV genotype (G) in the two studies, as he studied G1 infected patients while our patients were G 4 which is the prevalent type in Egypt.

Diabetes mellitus and obesity are parts of metabolic syndrome and consequently are of the established causes of steatosis. Investigating DM and BMI with both genes didn't show statistical significance. Linking this finding with the high viral load with high statistical significance to TM6SF2 was explained by Salvatore and his colleagues (27). He postulated that TM6SF2 has less steatogenic effect in presence of CHC based on that viral infection by itself is a steatogenic factor (28) and silencing the gene in experimental studies (22), reduced the secretion of very low density lipoproteins and predispose to fatty liver. In case of presence of both TM6SF2 and HCV, the gene effect becomes less relevant.

NCAN is related to steatosis and fibrosis (29) and even to development of hepatocellular carcinoma (14). On examining the same points with NCAN, viral load didn't show statistical significance but the degree of activity was 0.01 which can be postulated to have the same explanation, that viral load is inversely proportional to degree of activity in presence of certain genes. Also, NCAN is related to steatosis by decreasing triglyceride peripheral levels either through increased uptake or decreased release by the liver causing steatosis (19, 7). This could emphasize our finding as activity was increased with NCAN and it may be related to induction of steatosis by viral infection leading to necroinflammation.

Laboratory data suggesting liver injury as liver enzymes, prothrombin concentration and bilirubin didn't show statistical significance with any of the examined genes which is in concordance with lacking association with liver fibrosis with both genes. This was not the case in the study conducted by Nischalke et al (14) concerning NCAN but the patients had alcoholic liver which may be a cause of lab affection. But our study was similar to that

carried by Eslam et al (26) which showed no statistical significance between liver markers and TM6SF2.

After adjusting different confounders, NCAN was related to degree of activity with p value of 0.024. On examining the viral load, it was related to alpha fetoprotein with high statistical significance of 0.00, activity of p-value of 0.024, genes 3 with p value of 0.045 and fibrosis of p value 0.03. Univariate analysis of degree of fibrosis, BMI had p value of 0.015. This was against Eslam et al (26) which didn't show statistical significance with BMI.

Our studied patients were mainly heterozygous which makes it difficult to predict the effect of homozygous groups on fibrosis and activity in case of CHC. This is in concordance with studies of Urzua, Coppola, Milano and Salvotare (23,24,25,27) who reported also low number of TT carrier patients indicating similar prevalence of this polymorphism among different ethnicities.

Worth mentioning, that, up to our knowledge, this is the first work recording the prevalence of TM6SF2 (rs58542926) and NCAN (rs2228603) polymorphism in upper African HCV patients.

Our points of weakness are the small sample size of studied group in spite of the high prevalence of HCV in Egypt and being a retrospective study with no follow up for the patients after treatment to study the effect of genetic factors on sustained virological response and the degree of improvement of fibrosis or activity after cure.

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None.

#### **Conflict of Interest:**

Authors declare that they have no conflict of interest.

#### **Ethical approval:**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Table 1:TM6SF2 polymorphism and demographic data

	CC(n:110)	CT(n:35)	TT(n:2)	P value
Gender				
MALE	56	18	2	0.387
Female	54	17	0	
DM				
Positive	17	8	0	0.491
Negative	78	23	2	
Smoking				
Positive	10	4	0	0.936
Negative	77	30	1	
Age (years)	47.36 ± 10.29	44.23 ± 8.57	52 ± 1.41	0.199
BMI (Kg/m <sup>2</sup> )	28.38 ± 3.97	29.8 ± 3.85	28 ± 0	0.177
Hb (g/dl)	13.49 ± 1.62	13.63 ± 1.48	14.4 ± 0.71	0.661
Creatinine (mg/dl)	0.92 ± 0.59	0.84 ± 0.19	0.9 ± 0	0.730
Albumin (g/L)	3.94 ± 1.01	3.93 ± 0.47	4.65 ± 0.64	0.535
PC (%)	86.44 ± 12.76	86.43 ± 16.35	90 ± 8.49	0.936
INR	1.12 ± 0.18	1.14 ± 0.14	1.12 ± 0.11	0.913
Platelets (10 <sup>3</sup> /mmc)	180000 (44000-350000)	170000 (150-298000)	257000 (233-281000)	0.183
AST (IU/L)	63 (10-241)	58 (23-190)	76.5 (59-94)	0.735
ALT(IU/L)	58 (15-370)	65 (22-179)	156.5 (80-233)	0.176
WBC (x10 <sup>9</sup> /L)	6000 (2.3-10200)	4750 (4.1-8900)	6650 (5-8300)	0.171
AFP (ng/ml)	7.74 (0.01-195.75)	3.8 (0.83-34)	4.13 (1.3-6.96)	0.047*
Total bilirubin(mg/dl)	0.8 (0.14-6.53)	0.8 (0.12-2)	0.7 (0.6-0.8)	0.742
Viral load (IU/L)	300674.5 (8-23300000)	83000 (59-1165000)	463109 (379000-547218)	0.002*
Activity	64.6/27.8/7.6	75/25/0	2/0/0	0.584
A1/A2/A3 (%)				
Fibrosis	31.2/19.2/31.2/18.3	25.7/20/22.9/31.4	100/0/0/0	0.270
F1/F2/F3/F4				

\*p value: significant value, AFP: alpha feto protein, ALT: alanine transaminase, AST: aspartate transaminase, BMI: body mass index, HB: hemoglobin, INR : international normalized ratio, PC: prothrombin concentration, WBC: white blood cells

Table 2: NCAN polymorphism and demographic data

parameters	CC	CT	TT	P value
Gender				0.902
Male	12	65	0	
Female	11	63	0	
DM				0.752
Positive	3	23		
Negative	15	93		
Smoking				0.192
Positive	0	13		
Negative	13	98		
Age (years)	44.13 ± 15.1	47.67 ± 8.49		0.111
BMI(Kg/m <sup>2</sup> )	28.07 ± 3.84	28.6 ± 3.85		0.544
Hb (g/dl)	13.64 ± 1.74	13.51 ± 1.56		0.705
Creatinine (mg/dl)	0.86 ± 0.3	0.9 ± 0.52		0.805
Albumin (g/L)	0.85 ± 0.23	0.86 ± 0.19		0.878
PC (%)	86.34 ± 16.74	86.33 ± 13.04		0.998
INR	1.21 ± 0.42	1.12 ± 0.12		0.191
Platelets (10 <sup>3</sup> /mmc)	169000 (50000-313000)	180000 (150-360000)		0.844
AST (IU/L)	72 (19-221)	60.5 (10-241)		0.31
ALT (IU/L)	84 (15-191)	59 (17-370)		0.123
WBC (x10 <sup>9</sup> /L)	6200 (2400-9400)	5700 (2.3-10200)		0.704
AFP (ng/ml)	5.45 (0.8-33)	6 (0.01-195.75)		0.087
Total bilirubin (mg/dl)	0.8 (0.4-1.55)	0.8 (0.12-6.53)		0.929
Viral load (IU/L)	133223 (100-4240000)	247178.5 (59-23300000)		0.128
Activity				
A1/A2/A3 (%)	47.4/31.5/21.1	72.9/23.6/3.5	0/0/0	0.011*
Fibrosis				
F1/F2/F3/F4(%)	26.1/8.7/52.2/13	31.2/19.5/26.6/22.7	0/0/0/0	0.090



Table 3: regression analysis for activity, fibrosis and viremia with both studied genes

Variables	Univariate			Multivariate				
	Odds ratio	CI at 95%		p value	Odds ratio	CI at 95%		p value
<b>Activity</b> (A1 vs A2-A3)		Upper-lower				Upper-lower		
Gender	0.959	0.445	2.066	0.915	1.656	0.675	4.062	0.271
BMI (Kg/m <sup>2</sup> )	1.031	0.935	1.136	0.542	1.005	0.888	1.138	0.936
AFP (ng/ml)	0.989	0.964	1.015	0.420	0.990	0.965	1.017	0.481
NCAN	0.334	0.120	0.926	0.035*	0.274	0.089	0.846	0.024*
<b>viremia</b> (<=226000 vs > 226000)								
BMI(Kg/m <sup>2</sup> )	0.986	0.913	1.065	0.722	0.897	0.762	1.055	0.189
AFP (ng/ml)	1.190	1.105	1.282	0.001*	1.483	1.237	1.777	0.000*
Activity	1.624	0.910	2.898	0.101	4.373	1.209	15.814	0.024*
Fibrosis	0.854	0.647	1.126	0.262	0.397	0.173	0.913	0.030*
TM6SF2	0.472	0.227	0.982	0.045*	0.659	0.196	2.221	0.502
NCAN	1.605	0.648	3.972	0.306	5.244	0.657	41.876	0.118
<b>Fibrosis</b> (F1-2 vs F3-4)								
Gender	1.605	0.862	2.987	0.136	1.451	0.693	3.039	0.324
BMI (Kg/m <sup>2</sup> )	1.110	1.020	1.208	0.015*	1.095	0.986	1.216	0.089
AFP (ng/ml)	0.996	0.983	1.010	0.616	1.008	0.987	1.028	0.463
TM6SF2	0.940	0.473	1.869	0.861	0.799	0.365	1.753	0.576
NCAN	0.517	0.205	1.304	0.162	1.060	0.345	3.262	0.919