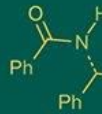


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## Correlation between TM6SF2 rs58542926 SNP with a risk of non-alcoholic fatty liver disease (NAFLD) in Iraqi patients

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### Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. In particular, the transmembrane 6 superfamily member 2 (TM6SF2) gene's rs58542926 (C>T) SNP has been connected to lipid metabolism and liver fat accumulation. The connection between the TM6SF2 rs58542926 polymorphism and some biochemical markers such as lipids profile and liver functions were investigated in patients with NAFLD from Iraq. Methods: A case-control study comprising of 60 NAFLD patients and 40 healthy controls. Anthropometric, biochemical, and lipid parameters were measured. TM6SF2 rs58542926 SNP and genotype analysis were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Standard t-test, Chi-square and ANOVA, with p-value less than 0.05 considered significant. Results: This study suggested that BMI, ALT, AST, TC, TG and LDL-C were found a higher levels while HDL-C was found lower levels in NAFLD patients (all  $p < 0.001$ ). Genotype distribution in NAFLD group were (CC: 33.3%, CT: 46.7%, TT: 20.0%) while in controls (CC: 62.5%, CT: 30.0%, TT: 7.5%) and this results shows statistically significant ( $p = 0.012$ ). TG, LDL-C levels were lower in T allele carriers ( $p < 0.05$ ). Conclusion: The TM6SF2 rs58542926 T allele is significantly associated with NAFLD in Iraqi patients and correlates with reduced serum TG and LDL-C. This study suggests that TM6SF2 rs58542926 SNP as a genetic risk factor of hepatic lipid metabolism and a potential marker for NAFLD risk assessment.

**Keywords:** NAFLD, TM6SF2, rs58542926, SNP, lipid profile, liver

### Introduction

A metabolic condition known as non-alcoholic fatty liver disease (NAFLD) causes the liver to abnormally of liver fat accumulation [1]. According to certain researchs, NAFLD is quite prevalent (> 20%) in adult general populations worldwide [2-4]. Non-alcoholic steatohepatitis (NASH) and, eventually, severe liver disorders including cirrhosis and hepatocellular carcinoma (HCC) can develop from non-symptomatic NAFLD [5]. Although the discovery of treatment drugs for NAFLD and its more severe symptoms has not been very successful, changing one's diet and exercise habits to lose weight can stop the disease's progression or even reverse it [6]. "NAFLD or its progression to severe liver disorders can be caused by a broad range of modifiers [7]. These include age, lifestyle, diabetes, genetic predisposition, and so forth [7]. Clarifying the part that genetic characteristics play in the pathophysiology of non-alcoholic fatty liver disease has garnered a lot of attention lately [8].

A group of liver diseases known as non-alcoholic fatty liver disease (NAFLD) are characterized by excessive hepatic fat accumulation without substantial alcohol consumption. It can progress from steatosis to cirrhosis, fibrosis, and non-alcoholic steatohepatitis (NASH) [9]. Its global incidence is rising alongside obesity, diabetes, and metabolic syndrome [10]. Dyslipidaemia is one of the characteristics of NAFLD, with high TG, high LDL-C, and low HDL-C levels [11].

The key genetic determinant of lipid metabolism has been identified as TM6SF2 gene. The rs58542926 (C>T, p.Glu167Lys) SNP affects VLDL secretion, hepatic lipid accumulation and plasma lipid levels [12]. T allele carriers have higher hepatic lipid levels but lower circulating TG and LDL-C as a result of diminished hepatic VLDL secretion [13]. Associations have been established in European and Asian cohorts, however there is little information among Middle Eastern groups.

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Therefore, this research paper was planned to investigation of the association between TM6SF2 rs58542926 polymorphism and lipid profile in NAFLD Iraqi patients.

### Materials and Methods

This study were carried out in the Clinical Biochemistry Department, college of medicine, university of Babylon for period from April 2025 to September 2025) and designed as case-control study. One hundred participants were recruited including 60 subjects diagnosis with non-alcoholic fatty liver disease (NAFLD) and 40 subjects healthy control persons. Both men and women were represented with ages of 25 to 65 yrs, respectively.

### Inclusion and Exclusion Criteria

The inclusion criteria for NAFLD patients were Ultrasonography diagnosis of hepatic steatosis, not consumption of alcohol, and stable metabolic and liver function diseases at least three months before enrollment. Exclusion criteria were history of viral hepatitis (HBV, HCV), other immune liver diseases such as Wilson's disease, use of hepatotoxic or lipid lowering drugs, Alcohol intake above referred limits, Pregnancy, and severe systemic illness.

### Ethical Considerations

Depending on the instructions of college of medicine, university of Babylon and the written acceptance was taken before blood drawing from all subjects enrolled in this study.

### Clinical and Biochemical Assessment

All subjects underwent a complete clinical examination with anthropometric measures: a self-administered questionnaire including medical history and anthropometric measurements such as body mass index (BMI). After overnight fasting (10-12 hours), the venous blood samples were collected. For the biochemical analyses, This study examined the lipid profile and liver enzymes which included TC, TG, HDL-C, LDL-C, ALT, AST and blood sugar FBG which measured by using automated enzymatic and colorimetric techniques (e.g., Roche Cobas c311 analyzer, Roche Diagnostics, Germany). For individuals with TG < 400 mg/dL, the Friedewald formula was used to calculate the LDL-C and VLDL-C.

### DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercial extraction kit (e.g., GeneJET Whole Blood Genomic DNA Purification Kit, Thermo Fisher Scientific, USA) following the manufacturer's instructions. DNA purity and concentration were verified using a NanoDrop spectrophotometer (Thermo Scientific, USA).

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to genotype the TM6SF2 rs58542926 (C>T) polymorphism. The following primers were used for PCR amplification of a particular fragment containing the rs58542926 SNP: Forward primer: 5'-GTT GGG AGA CTG AAG GAG AA-3' and Reverse primer: 5'-TGC AGG AGT CTT TGA TGT TT-3'. PCR conditions illustrate in Table 1.

**Table 1:** The PCR conditions for rs58542926 SNP analysis

| Step                 | Temperature                     | Time   | Cycles |
|----------------------|---------------------------------|--------|--------|
| Initial Denaturation | 95 °C                           | 4 min  | 1      |
| Denaturation         | 95 °C                           | 45 sec | 35     |
| Annealing            | 58.2 °C (optimized for primers) | 45 sec |        |
| Extension            | 72 °C                           | 50 sec |        |
| Final Extension      | 72 °C                           | 7 min  | 1      |
| Hold                 | 4 °C                            | -      | -      |

The amplified products were visible under a UV lamp using ethidium bromide-stained 2% agarose gel electrophoresis.

### Restriction Enzyme Digestion:

PCR products (140 bp) were digested using *HhaI* restriction enzyme, which recognizes and cuts the wild-type allele (C) but not the mutant allele (T). The digestion products were separated on a 3% agarose gel and visualized under UV transillumination. Genotypes were classified as CC (wild type) (109+31 bp), CT (heterozygous) (140,109, 31 bp), and TT (homozygous mutant) (140 bp).

### Statistical Analysis

SPSS version 26.0 (IBM Corporation, USA) was used to process all of the data. Categorical data were reported as frequency and percentage, while continuous variables were reported as Mean  $\pm$  SD. The Chi-square test for categorical variables and the student t-test or ANOVA for continuous variables were used to analyze group differences. The genotype distribution's Hardy-Weinberg equilibrium (HWE) was examined. The associations between TM6SF2 genotypes and lipid metrics were ascertained using either linear regression analysis or Pearson's correlation coefficient. A statistically significant p-value was defined as less than 0.05.

### Results

Table 2 shows demographic and biochemical characteristics of study groups. NAFLD patients exhibited higher BMI, ALT, AST, TC, TG, and LDL-C levels and lower HDL-C compared with controls ( $p < 0.001$ ).

**Table 2:** Demographic and biochemical characteristics of study groups

| Parameter                | Control (n = 40) | NAFLD (n = 60)   | p-value |
|--------------------------|------------------|------------------|---------|
| Age (years)              | 44.5 $\pm$ 8.7   | 46.8 $\pm$ 9.2   | 0.214   |
| BMI (kg/m <sup>2</sup> ) | 25.6 $\pm$ 2.9   | 30.8 $\pm$ 3.4   | <0.001  |
| ALT (U/L)                | 24.2 $\pm$ 6.8   | 58.9 $\pm$ 14.3  | <0.001  |
| AST (U/L)                | 22.6 $\pm$ 5.7   | 49.5 $\pm$ 12.9  | <0.001  |
| ALP (U/L)                | 78 $\pm$ 14      | 102 $\pm$ 22     | <0.001  |
| TC (mg/dL)               | 176.3 $\pm$ 28.1 | 205.7 $\pm$ 33.9 | <0.001  |
| TG (mg/dL)               | 132.5 $\pm$ 26.7 | 187.2 $\pm$ 41.5 | <0.001  |
| HDL-C (mg/dL)            | 51.8 $\pm$ 8.2   | 41.4 $\pm$ 7.5   | <0.001  |
| LDL-C (mg/dL)            | 102.4 $\pm$ 22.8 | 132.1 $\pm$ 28.7 | <0.001  |
| VLDL-C (mg/Dl)           | 26.5 $\pm$ 5.3   | 37.4 $\pm$ 8.3   | <0.001  |

Table 3 and figure 1 presents genotype frequencies, showing higher T allele occurrence among NAFLD patients ( $p = 0.012$ ). In the NAFLD group, genotype distribution was CC = 33.3%, CT = 46.7%, and TT = 20.0%, while in controls it was CC = 62.5%, CT = 30.0%, and TT = 7.5%. A significant difference in genotype distribution was observed between groups ( $p = 0.012$ ), with the T allele being more frequent in NAFLD patients.

**Table 3:** The genotype frequencies of study groups

| Genotype | Controls (n = 40) | NAFLD (n = 60) | $\chi^2$ | p-value |
|----------|-------------------|----------------|----------|---------|
| CC       | 62.5%             | 33.3%          | 6.31     | 0.012   |
| CT       | 30.0%             | 46.7%          |          |         |
| TT       | 7.5%              | 20.0%          |          |         |
| C allele | 77.5%             | 56.7%          | 6.87     | 0.009   |
| T allele | 22.5%             | 43.3%          |          |         |

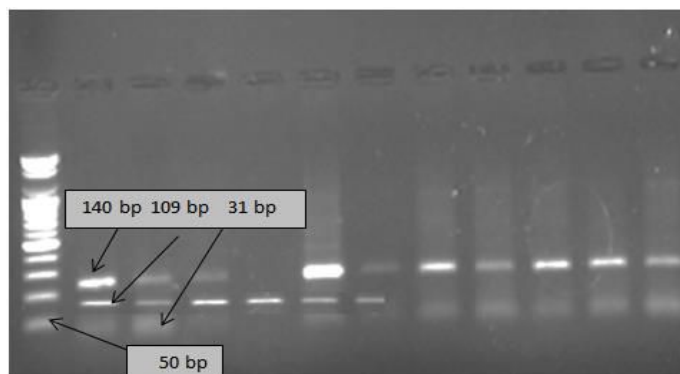
**Fig 1:** PCR products (140 bp) were digested using *HhaI* restriction enzyme, which recognizes and cuts the wild-type allele (C) but not the mutant allele (T) and (CC, CT, and TT) genotypes

Figure 2 shows genotype distribution differences between groups and depicts the frequency of TM6SF2 rs58542926 genotypes (CC, CT, TT) in NAFLD and control groups. The CT and TT genotypes were found to be significantly more common among NAFLD patients compared to controls

( $p < 0.05$ ), suggesting a strong association between the T allele and NAFLD risk in the Iraqi population

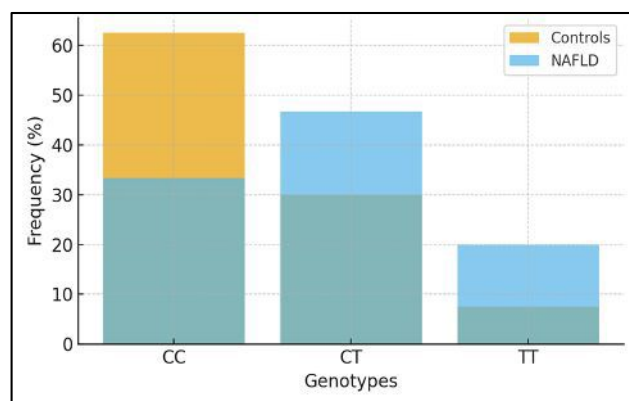
**Fig 2:** Genotype Distribution of TM6SF2 rs58542926 SNP in NAFLD and Control Groups

Table 4 shows the distribution of lipid profiles according to various TM6SF2 rs58542926 genotypes in NAFLD patients. Subjects with the TT genotype had significantly decreased levels of TC, TG, and LDL-C compared to subjects carrying the CC allele ( $p < 0.05$ ). There was no difference between groups with respect to HDL-C levels. Our results underscore that the T allele might mitigate lipid metabolism by reducing hepatic VLDL secretion leading to reduced circulating lipid levels, and it may thereby contribute to a modulatory effect on NAFLD risk.

**Table 4:** Lipid Profile According to TM6SF2 rs58542926 Genotypes in NAFLD Patients

| Parameter     | CC (n = 20) | CT (n = 28) | TT (n = 12) | F-value | p-value |
|---------------|-------------|-------------|-------------|---------|---------|
| TC (mg/dL)    | 210.6±32.4  | 200.2±30.8  | 189.3±29.6  | 3.47    | 0.037*  |
| TTG (mg/dL)   | 192.8±41.5  | 180.6±39.7  | 168.4±36.1  | 4.01    | 0.022*  |
| HDL-C (mg/dL) | 39.8±7.2    | 42.1±6.5    | 43.9±5.8    | 2.13    | 0.126   |
| LDL-C (mg/dL) | 136.7±27.4  | 128.5±26.2  | 121.3±24.9  | 3.09    | 0.045*  |

## Discussion

In investigating the association of the TM6SF2 rs58542926 (C>T) single-nucleotide polymorphism (SNP) with serum lipid profile, this study was performed on Iraqi NAFLD patients and a control group. The main results included that the T allele was found to be more frequent in NAFLD patients compared with controls, within NAFLD population carriers of T allele had lower circulating TG and LDL-C levels in comparison to non-carrier counterparts, these grew data are consistent with a role of TM6SF2 rs58542926 as risk factor for hepatic fat accumulation and contributor modulating serum lipids. The elevated frequency of the T allele in the NAFLD group suggests that the TM6SF2 rs58542926 variant may contribute to disease susceptibility in the Iraqi population. This is consistent with previous meta-analyses showing that the rs58542926 T allele is associated with greater risk of NAFLD in adults (OR  $\approx$  1.62) and in children (OR  $\approx$  2.87) [14]. The present results thus extend these observations into a Middle-Eastern context, where data have been more limited [15]. The paradoxical association we observed — T allele carriers having lower serum TG and LDL-C despite increased NAFLD risk — aligns with the mechanistic understanding of TM6SF2 function. TM6SF2 is expressed in hepatocytes and the small intestine and plays a role in the assembly and secretion of very-low-density lipoproteins (VLDL). Loss-of-function of TM6SF2 (as in the T-allele) appears to impair

VLDL export from the liver, leading to intrahepatic triglyceride accumulation and hepatic steatosis, while simultaneously reducing the amount of TG-rich lipoproteins released into the circulation [16]. Thus, the lower circulating lipids in T-allele carriers may reflect reduced hepatic lipoprotein secretion rather than a benign lipid metabolic profile.

The results of this study support the model in which TM6SF2 variants contribute to hepatic lipid overload by limiting export of triglyceride-rich VLDL particles. The resulting accumulation of hepatic lipids may drive the progression of steatosis and possibly fibrosis. A meta-analysis noted that rs58542926 T allele was associated not just with NAFLD presence but also severity of steatosis and fibrosis (e.g., OR for fibrosis stage  $\sim$ 1.50) [17, 18]. In this research, the higher T allele frequency among NAFLD patients may imply similarly increased risk of more advanced disease, although we did not measure fibrosis stage here.

## Conclusion:

This study shows the fact that subjects with T-allele carriers have lower TG and LDL-C emphasizes that circulating lipid levels in NAFLD cannot be interpreted in isolation. Indeed, a normal or low serum TG/LDL-C does not necessarily imply lower risk of hepatic lipid accumulation or liver injury in carriers of TM6SF2 risk variants.

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