MTP genetic variants associated with non-alcoholic fatty liver in metabolic syndrome patients

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Abstract This study was performed for investigation the relationship between variants of MTP gene polymorphism and the development of NAFLD in patients with and without MS. The study was included 174 NAFLD patients (106 with MS and 68 without MS), and 141 healthy control subjects. The 493 G/T polymorphism of MTP gene was evaluated by PCR-RFLP method. The frequency of MTP TT genotype and T allele were significantly higher in NAFLD patients when compared to healthy controls. Moreover, a significant association in MTP gene polymorphism was observed in NAFLD patients with MS compared to NAFLD patients without MS and controls. Our study suggested that MTP 493 G/T gene polymorphism may act as susceptibility biomarker for NAFLD and MS.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a universal disorder which is considered as the most common liver disease worldwide; it is defined as the accumulation of excessive fat in the liver in the absence of excessive drinking of alcohol and any secondary cause.¹ Numerous risk factors have been suggested in NAFLD pathogenesis, including advanced age, dietary habits, obesity, and some traits of metabolic syndrome (MS), such as insulin resistance and dyslipidemia.²,³

Non-alcoholic fatty liver disease is no longer considered to be a primary liver disease, but rather a constituent of...
metabolic syndrome. Epidemiologic studies support belief to the relation between NAFLD and MS, the latter may be the etiologic agent that triggers the pathophysiological cascade of NAFLD. Therefore, the possibility of NAFLD increases proportionately with the number of metabolic syndrome factors present.

In general, NAFLD is a multifactorial disease produced by complex interactions between nutritional factors, lifestyle choices, and genetic determinants. Previous studies suggested that genetic factors play an important role in NAFLD etiology by altering hepatic lipid metabolism.

Microsomal triglyceride transfer protein (MTP, or MTTP), a lipid transfer protein involved in apolipoprotein B (apoB) assembly, is localized to the endoplasmic reticulum in hepatocytes and enterocytes. Lower hepatic expression of MTP plays a crucial role in NAFLD development. Although a large number of single-nucleotide polymorphisms in the MTP gene have been identified, -493G>T (rs1800591) is one of the most common and widely investigated polymorphisms. The data concerning the importance of the MTP -493G>T polymorphism in NAFLD development are inconsistent. Therefore, we performed this study to investigate whether MTP -493G>T polymorphism contributes to the risk of NAFLD and to investigate its relation with metabolic syndrome in NAFLD patients. Additionally, we studied the relationship between gene variants and lipid profile of NAFLD patients with MS.

**Subjects and methods**

**Subjects**

A total of 174 patients with non-alcoholic fatty liver disease who were newly diagnosed by liver ultrasonography using established criteria were recruited from the liver clinic of the Medical Service Unit at the National Research Center, Egypt. The NAFLD patients were subdivided according to metabolic syndrome criteria into 106 patients with MS and 68 without MS. In addition, 141 control healthy subjects with no detectable fatty liver disease or metabolic syndrome were also recruited from the same center during the same study period. They were frequency matched with the NAFLD patients regarding sex, age, ethnicity, occupation and area of residence according to the propensity score matching method. This research was approved by the Human Ethics Committee of the National Research Center. Written informed consent was obtained from all participants before their participation in the study.

**Diagnosis of NAFLD**

The diagnosis of NAFLD was based on abdominal ultrasound examinations without including other causes of chronic liver disease (liver cirrhosis, hepatic carcinoma, hepatitis history, impaired hepatic function (alanine transaminase > 2.0 times upper limit of normal), hepatitis B, hepatitis C virus infection, drugs for liver damage, and excessive drinking (>20 g/d)). Abdominal ultrasonographic examinations were performed by the same physician for all patients and controls using SonoAce R5 (6 MHz; Samsung).

**Definition of the metabolic syndrome**

Metabolic syndrome was defined using a previously published modification of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines as having 3 or more of the following risk determinants: (1) waist circumference (WC) ≥ 90 cm; (2) raised triglyceride (TG) ≥ 150 mg/dL; (3) reduced high-density lipoprotein (HDL) < 40 mg/dL or lipid medication use; (4) raised blood pressure (BP), systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg; (5) fasting blood glucose (FBG) ≥ 100 mg/dL.

**Anthropometric measurements**

Body mass index (BMI) was determined by dividing weight by square height (kg/m²). Waist circumference (WC) was obtained from each subject by measuring at the midpoint between the lower rib margin and the iliac crest using a conventional tape graduated in centimeters (cm). Hip circumference (HC) was measured as the greatest circumference at the level of greater trochanters. Waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference.

**Sample collection**

A venous blood sample of 6 mL was drawn from each subject after an overnight fast, 3 mL of which were collected in a glass tube for serum lipid determination, 2 mL were collected in EDTA containing tube for DNA extraction to determine MTP gene polymorphism, while the remaining 1 mL was transferred to a tube with a mixture of EDTA and fluoride for plasma separation to measure fasting plasma glucose.

**Biochemical analyses**

Fasting plasma glucose (FPG) was determined using a modified hexokinase technique; total cholesterol (TC), triglycerides (TGs) and high-density lipoprotein (HDL) were measured enzymatically with commercially available kits from STANBIO, USA; low-density lipoprotein (LDL) was calculated by the Friedewald equation.

**Genotyping**

Genomic DNA was extracted and purified from whole peripheral blood samples using QiAamp DNA extraction kit (Qiagen Inc., Valencia, CA, USA). The MTP 493G/T polymorphism was genotyped by a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay as described by Karpe et al. Briefly, a 109 bp fragment in the MTP gene was amplified by PCR using the following primers: F: 5' - AGTTTTCACACATAAAGGACAATCATCTA-3' and R: 5' - GGATTAAAAATTAAACTGTATAATTATAC-3' (New England Biolabs, USA). PCR was performed using Taq PCR...
Master Mix (Qiagen, Valencia, CA, USA) and T-Gradient thermal cycler (Biometra, Germany). The cycle profile was as follow: predenaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. PCR products (10 μL) were incubated with 2.5 U HphI (New England Biolabs, USA) at 37 °C for 1 h. Electrophoresis was then performed on 3% agarose gel stained with Red-Safe and then the electrophoretic products were visualized using ultra-violet light transillumination. A "G" at position -493 yielded bands of 89 and 20 bp, whereas a "T" at position -493 yielded a band of 109 bp, thus an individual with band(s) at 89 and 20 bp, at 109 bp only, and at 109, 89 and 20 bp was defined as GG homozygotic genotype, TT homozygotic genotype, and GT heterozygotic genotype, respectively (Fig. 1).

Statistical analysis

Data are expressed as means ± standard deviation for quantitative variables, and as frequency for qualitative variables. Qualitative variables were compared using the Chi-square ($\chi^2$) test or Fischer's exact test. One-way ANOVA was used to compare the clinical and laboratory characteristics of patients divided according to genotypes. The Statistical Package for the Social Sciences software (SPSS 17.0, Chicago, IL, USA) was used. P ≤ 0.05 was considered significant. Sample size calculation was done using Stats Direct statistical software version 2.8 for MS Windows (Stats Direct Ltd., Cheshire, UK).

Results

Table 1 shows a significant difference in age distribution between NAFLD patients and controls according to MTP -493G/T genotype (TT = 39.35 ± 0.82, G/T = 37.6 ± 1.19, G/G = 40.65 ± 1.67, T/T = 37.8 ± 2.37; G/T = 35.68 ± 1.29; and G/G = 35.45 ± 1.22, years respectively). Additionally, fasting plasma glucose (FPG), insulin and homoeostasis model assessment-insulin resistance (HOMA-IR) index were significantly higher in NAFLD than healthy subjects at P value ≤ 0.05.

Serum concentrations of lipids in NAFLD subjects according to MTP -493G/T genotypes are shown in Table 1. Non-alcohol fatty liver homozoygous subjects for the rare MTP -493T variant had lower serum triglyceride concentration compared to subjects carrying 1 or 2 copies of the common G allele (T/T, 204.63 ± 11.35; G/T, 221.85 ± 12.15; and G/G, 235.65 ± 15.49 mg/dL). Also in NAFLD patients, lipid profile parameters (total cholesterol, triacylglycerol, LDL-cholesterol and very low-density lipoprotein (VLDL)-cholesterol) were significantly higher when compared to controls, while there was significant decrement in HDL between NAFLD and controls (P = 0.000).

Twenty percent of NAFLD GG, 44% NAFLD GT and 36% NAFLD TT had hypertension (systolic/diastolic blood pressure >130/85 mm Hg), 22.6% of NAFLD GG, 43.4% of NAFLD GT and 34% of NAFLD TT had the whole picture of the metabolic syndrome.

The results regarding the relationship between the frequency of MTP -493G/T polymorphism and the risk of NAFLD are presented in Table 2.

The results showed that the prevalence of -493 MTP G/G carriers was 42.6% in controls versus 19.6% in non-alcoholic fatty liver disease (P = 0.007), heterozygous G/T carriers were 46.8% in controls and 44.8% in NAFLD (P = 0.317), and homozygous TT carriers were 10.6% in controls versus 35.6% in NAFLD (P = 0.000). The results revealed that there was a significant difference in the genotype distribution of MTP -493G/T polymorphism between the NAFLD patients and healthy controls.

The results also revealed that there was an association between the presence of the T allele (58.3%) in the MTP -493G/T polymorphism and the incidence of NAFLD (P = 0.000). There was significant difference in -493 MTP allelic frequency (G allele versus T allele) between the NAFLD and control groups (OR = 0.369, 95% CI = 0.266–0.511, P = 0.000).

Additionally, the results demonstrated that the homozygous T/T carrier for -493 MTP genotype (adjusted odds ratio [OR]: 0.137; 95% confidence interval [CI]: 0.068–0.277; P = 0.000) was associated with increased risk of NAFLD, compared with the wild-type homozygous of -493 MTP G/G carriers genotype. These associations were further demonstrated using a dominant model: for -493G/T genotypes GT + TT versus GG, the OR: 0.328; 95%CI: 0.198–0.542 and P = 0.000. In addition there was significant difference in recessive model GG + GT versus TT with P value of 0.000; OR = 0.215; 95%CI = 0.116–0.399. However, there was no significant difference in over-dominant model, GG + TT versus GT at P-value = 0.726; OR = 1.083; 95%CI = 0.694–1.691 (Table 2).

The prevalence of MTP polymorphism in NAFLD patients, with and without metabolic syndrome, compared to healthy controls is shown in Table 3. MTP -493T/T genotype was significantly different in controls and NAFLD subjects, with and without metabolic syndrome, at P value = 0.000. Also, the allele frequencies were different among the studied groups and were respectively G: 66%, 44.3%, 38.2% and T: 34%; 55.7%; 61.8% (P = 0.000). Furthermore, using the recessive (GT + TT vs GG) and dominant model (GG + GT vs TT) a significant difference was found between the studied groups with P value = 0.000.
As shown in Tables 4 and 5, we analyzed the influence of -493 MTP genotypes on lipid profile; the TG and VLDL levels of the MTP TT carriers were significantly lower than those of GT/GG carriers among NAFLD and NAFLD with MS groups (P = ≤0.05 for each).

### Discussion

Non-alcoholic fatty liver disease (NAFLD) is becoming a worldwide epidemic and one of the most important global health issues. NAFLD is associated with metabolic syndrome (MS), including obesity, hyperlipidemia, hypertension, and diabetes. Therefore, NAFLD is considered part of MS. Several genes have been identified as potential candidates in the pathogenesis and progression of fatty liver, genes involved in mechanisms of liver injury and genes influencing lipid metabolism like microsomal triglyceride transfer protein (MTP). Many previous studies have reported that a common polymorphism (-493G > T, rs1800591 G/T) in the MTP gene may be implicated in the susceptibility to NAFLD, but individually published data are inconclusive. Although the exact function of MTP genetic polymorphism in the development of NAFLD is not fully elucidated yet, a possible cause might be due to low hepatic expression of MTP induced by inherited variants in the MTP gene, which are associated with changes in hepatic lipid metabolism resulting in its low plasma level, thereby possibly explaining inter-individual differences in disease incidence of NAFLD.

The finding of our study suggested that MTP -493G > T polymorphism might contribute to an increased risk of NAFLD, implying that MTP -493G > T polymorphism may be an important causative factor for the incidence of NAFLD. Besides, MTP -493G > T polymorphism could be associated with metabolic syndrome in non-alcoholic fatty liver disease. Our finding is in agreement with Bernard et al; Husain et al; Peng et al; Zheng et al; Miyakawa and Nakao who reported that MTP -493G/T polymorphism may be used for early detection of NAFLD. This might appear in contrast with a study in a Brazilian population which demonstrated that, the frequency of the G allele or of the G/G genotype for MTP was not different from that of controls without fatty liver.

Additionally, in the current study, the TT individuals in both NAFLD and NAFLD with MS had lower TG and VLDL levels in comparison with GT and GG carriers. Therefore, NAFLD is considered part of the metabolic syndrome (MS), including obesity, hyperlipidemia, hypertension, and diabetes. Therefore, NAFLD is considered part of MS. Several genes have been identified as potential candidates in the pathogenesis and progression of fatty liver, genes involved in mechanisms of liver injury and genes influencing lipid metabolism like microsomal triglyceride transfer protein (MTP). Many previous studies have reported that a common polymorphism (-493G > T, rs1800591 G/T) in the MTP gene may be implicated in the susceptibility to NAFLD, but individually published data are inconclusive. Although the exact function of MTP genetic polymorphism in the development of NAFLD is not fully elucidated yet, a possible cause might be due to low hepatic expression of MTP induced by inherited variants in the MTP gene, which are associated with changes in hepatic lipid metabolism resulting in its low plasma level, thereby possibly explaining inter-individual differences in disease incidence of NAFLD.

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levels, similar to investigators who demonstrated an association between the MTP -493T allele and hepatic lipids\textsuperscript{39} \textsuperscript{41}; however, another investigator detected no relationship between this polymorphism and any lipid phenotype.\textsuperscript{42} Since it is widely accepted that hepatic lipid and lipoprotein abnormalities accompanied by chronic inflammation may play a vital role in the progression of NAFLD,\textsuperscript{43} excessive accumulation of triglycerides in hepatocytes is the hallmark of NAFLD.\textsuperscript{44} In general, hepatic triglycerides are exported from the liver in the form of VLDL particles mediated through plasma apoB-lipoprotein and MTP.\textsuperscript{45} Further, lipid transfer activity of MTP is required for the assembly and secretion of apoB-lipoproteins.\textsuperscript{46} However, MTP -493G > T polymorphism may result in a decrease in its protein and aberrant alterations of MTP synthesis and secretion, influence the capacity for lipid export, and induce dysregulation of hepatic lipid metabolism, thus contributing to the development of NAFLD.\textsuperscript{13,47}

### Table 2

| MTP (-493G/T) | NAFLD (n = 174) | Controls (n = 141) | $P^2$ values | $P$ values | OR | 95%CI |
|--------------|----------------|-------------------|--------------|----------|----|-------|
| Additive model |               |                   |              |          |    |       |
| GG/GT/TT    | 34/78/62       | 60/66/15          | 0.000        |          |    |       |
| Co-dominant model |            |                   |              |          |    |       |
| GG          | 34 (19.6%)     | 60 (42.6%)        | 0.007        |          |    |       |
| GT          | 78 (44.8%)     | 66 (46.8%)        | 0.317        | 0.006    | 0.479 | 0.281–0.817 |
| TT          | 62 (35.6%)     | 15 (10.6%)        | 0.000        | 0.000    | 0.137 | 0.068–0.277 |
| Recessive model |            |                   |              |          |    |       |
| GG          | 34 (19.6%)     | 60 (42.6%)        | 0.007        |          |    |       |
| GT + TT     | 140 (80.4%)    | 81 (57.4%)        | 0.000        | 0.000    | 0.328 | 0.198–0.542 |
| Dominant model |            |                   |              |          |    |       |
| GG + GT     | 112 (64.4%)    | 126 (89.4%)       | 0.364        |          |    |       |
| TT          | 62 (35.6%)     | 15 (10.6%)        | 0.000        | 0.000    | 0.215 | 0.116–0.399 |
| Over-dominant model |        |                   |              |          |    |       |
| GG + TT     | 96 (55.2%)     | 75 (53.2%)        | 0.108        |          |    |       |
| GT          | 78 (44.8%)     | 66 (46.8%)        | 0.317        | 0.726    | 1.083 | 0.694–1.691 |
| Allele model |              |                   |              |          |    |       |
| G           | 145 (41.7%)    | 186 (66%)         | 0.024        |          |    |       |
| T           | 203 (58.3%)    | 96 (34%)          | 0.000        | 0.000    | 0.369 | 0.266–0.511 |

Data are number (%), variables were compared using chi square ($\chi^2$) test or Fischer’s exact test.

$P^2$ values for the frequency of each genotype within the studied groups.

$P$ values for comparison between NAFLD and Controls; OR: odd ratio; CI: confidence interval.

Bold values indicate significant difference.

$P$ value $\leq 0.05$ was considered significant.

MTP: Microsomal triglyceride transfer protein.

### Table 3

| MTP -493G > T | NAFLD with MS (n = 106) | NAFLD without MS (n = 68) | Controls (n = 141) | $X^2$ | $P$ value |
|--------------|-------------------------|---------------------------|--------------------|------|----------|
| Genotype     |                         |                           |                    |      |          |
| GG/GT/TT     | 36/46/24                | 26/32/10                  | 15/66/60           | 35.1 | 0.000    |
| Allele       |                         |                           |                    |      |          |
| G            | 94 (44.3%)              | 52 (38.2%)                | 186 (66%)          |      |          |
| T            | 118 (55.7%)             | 84 (61.8%)                | 96 (34%)           | 37.2 | 0.000    |
| Co-dominant model |               |                           |                    |      |          |
| GG           | 24 (22.6%)              | 10 (14.7%)                | 60 (42.6%)         |      |          |
| GT           | 46 (43.4%)              | 32 (47.1%)                | 66 (46.8%)         | 8.6  | 0.014    |
| TT           | 36 (34%)                | 26 (38.2%)                | 15 (10.6%)         | 35.2 | 0.000    |
| Recessive model |             |                           |                    |      |          |
| GT + TT      | 82 (77.4%)              | 58 (85.3%)                | 81 (57.4%)         | 20.9 | 0.000    |
| GG           | 24 (22.6%)              | 10 (14.7%)                | 60 (42.6%)         |      |          |
| Dominant model |             |                           |                    |      |          |
| TT           | 36 (34%)                | 26 (38.2%)                | 15 (10.6%)         | 26.7 | 0.000    |
| GG + GT      | 70 (66%)                | 42 (61.8%)                | 126 (89.4%)        |      |          |
| Over-dominant model |       |                           |                    |      |          |
| GT           | 46 (43.4%)              | 32 (47.1%)                | 66 (46.8%)         | 0.35 | 0.84     |
| GG + TT      | 60 (56.6%)              | 36 (52.9%)                | 75 (53.2%)         |      |          |

Data are number (%), variables were compared using Chi square ($\chi^2$) test.

$P$ values for comparison between the studied groups.

OR: odd ratio; CI: confidence interval.

Bold values indicate significant difference at $P \leq 0.05$.
are many reports of gene polymorphisms involved in lipid metabolism that are associated with NAFLD and NAFLD progression. Microsomal triglyceride transfer protein has a pivotal role in metabolizing hepatic TG and VLDL for lipid export from the liver. Therefore, MTP plays an important role in lipid metabolism.

In conclusion, our findings demonstrate that MTP -493G > T polymorphism might be correlated with the risk of NAFLD and MS. Thus, MTP -493G > T polymorphism could be used as a valuable and practical biomarker for early diagnosis of NAFLD. However, further studies in larger samples of different populations are required to elucidate the participation of MTP polymorphisms in NAFLD susceptibility.

**Author contributions**

- Conceived and designed the study: Yehia M. Shaker, Esmat Ashour and Wafaa Ezzat.
- Diagnosis and selection all participants in the study: Wafaa Ezzat.
- Contributed reagents/materials/analysis tools: Weaam Gouda and Esmat Ashour.
- Weaam Gouda and Esmat Ashour Contributed to the analysis of the results and to the writing of the manuscript.
- All authors provided critical feedback and helped shape the research, analysis and approved the final version submitted for manuscript.

**Conflicts of interest**

The authors declare that they have no conflict of interest.

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**Table 4** Lipid profile of NAFLD patients according to the genotypes of MTP gene.

|                        | TT (n = 62) | GT + GG (n = 112) | P value |
|------------------------|------------|-------------------|--------|
| Serum cholesterol (mg/dL) | 263.9 ± 8  | 268.3 ± 4.9     | 0.708  |
| Serum triglycerides (mg/dL) | 202 ± 7.9  | 228 ± 6.8       | 0.05   |
| HDL cholesterol (mg/dL)  | 48.5 ± 2.4 | 47.9 ± 1.4     | 0.833  |
| LDL cholesterol (mg/dL)  | 175.4 ± 7.9| 175 ± 4.9      | 0.967  |
| VLDL (mg/dL)            | 40.8 ± 1.6 | 45.3 ± 1.3     | 0.05   |

Data are mean ± SE, ANOVA test was used for comparison. Bold values indicate significant difference at P ≤ 0.05.

**Table 5** Lipid profile of NAFLD patients with MS according to the genotypes of MTP gene.

|                        | TT (n = 36) | GT + GG (n = 70) | P value |
|------------------------|------------|------------------|--------|
| Serum cholesterol (mg/dL) | 274.2 ± 9.4| 270.5 ± 6.3     | 0.74   |
| Serum triglycerides (mg/dL) | 216.5 ± 5.6| 242.6 ± 7.4     | 0.022  |
| HDL cholesterol (mg/dL)  | 43.6 ± 2   | 43.2 ± 1.3      | 0.882  |
| LDL cholesterol (mg/dL)  | 187.3 ± 9.3| 178.7 ± 6.5     | 0.452  |
| VLDL (mg/dL)            | 43.3 ± 1.1 | 48.5 ± 1.5      | 0.021  |

Data are mean ± SE, ANOVA test was used for comparison. Bold values indicate significant difference at P ≤ 0.05.
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