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FULL LENGTH ARTICLE

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

Weaam Gouda [a], Esmat Ashour [a,*], Yehia Shaker [a], Wafaa Ezzat [b]

[a] *Biochemistry Dept., National Research Center, Dokki, Giza, Egypt*
[b] *Internal Medicine Dept., National Research Center, Dokki, Giza, Egypt*

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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

Abbreviations: MS, Metabolic syndrome; MTP -493G > T, Microsomal triglyceride transfer protein; NAFLD, Non-alcoholic fatty liver disease.

* *Corresponding author.*
E-mail address: esmatashour3@gmail.com (E. Ashour).
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metabolic syndrome.[4] 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.[5] Therefore, the possibility of NAFLD increases proportionately with the number of metabolic syndrome factors present.[6]

In general, NAFLD is a multifactorial disease produced by complex interactions between nutritional factors, lifestyle choices, and genetic determinants.[7] Previous studies suggested that genetic factors play an important role in NAFLD etiology by altering hepatic lipid metabolism.[8–11]

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.[12] Lower hepatic expression of MTP plays a crucial role in NAFLD development.[13] 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.[14,15] The data concerning the importance of the *MTP* -493G > T polymorphism in NAFLD development are inconsistent.[16–20,2] 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[21] 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[22] 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/m^2). 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.[23]

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.[24] Briefly, a 109 bp fragment in the MTP gene was amplified by PCR using, the following primers: F: 5′-AGTTTCACACATAAGGACAATCATCTA-3′ and R: 5′ GGATTTAAATTTAAACTGTTAATTCATATCAC-3′ (New England Biolabs, USA). PCR was performed using Taq PCR