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The influence of apolipoprotein A5 T-1131C and apolipoprotein E common genetic variants on the levels of hemostatic markers in dyslipidemic patients

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ABSTRACT

Objectives: The aim of this study was to evaluate the relationships of the T-1131C (rs662799) polymorphism variants of apolipoprotein A5 (Apo A5) gene and variants of apolipoprotein E (Apo E) gene common polymorphism (rs429358, rs7412) to selected hemostatic markers.
Study design and methods: We examined 590 asymptomatic dyslipidemic patients, subsequently divided into MetS+ (n = 146) and MetS− (n = 444) groups according to the criteria for identification of the metabolic syndrome (MetS). We compared variant frequencies and differences in levels of hemostatic markers according to Apo A5, Apo E and Apo A5/Apo E common variants.
Results: The −1131C Apo A5 minor variant was associated with elevated tissue plasminogen activator (tPA) in comparison to TT genotype ($p < 0.001$), but not in the MetS+ group. The analysis of Apo A5/Apo E common variants in all subjects revealed that the presence of −1131C minor allele has always been associated with higher levels of tPA in comparison with T allele, regardless of Apo E genotype. Also the presence of minor Apo E2 allele led to elevated tPA concentrations in both T and C carriers. In addition, common −1131C/E2 variant was associated with the highest tPA levels.
Conclusion: We demonstrated a remarkable association especially between the −1131C Apo A5 variant and increased tPA levels in asymptomatic dyslipidemic patients.

1. Introduction

Recent epidemiological and clinical studies support a connection between obesity and thrombosis represented by an increased expression of the prothrombotic markers and platelet activation. In obese patients, clinical markers of a prothrombotic state might indicate a risk for the development of complication of metabolic syndrome [1].

The metabolic syndrome is a common metabolic disorder associated with an increased risk of type 2 diabetes mellitus and cardiovascular diseases [2]. The association with elevated risk of atherothrombotic cardiovascular events and venous thromboembolism is described as well [3]. In MetS, enhanced coagulation as well as impaired fibrinolysis was observed. High levels of fibrinogen and plasminogen activator-inhibitor 1 (PAI-1), together with increased von Willebrand factor (vWF) and tissue plasminogen activator (tPA) concentrations could reflect endothelial dysfunction [4]. This process is regarded as an early step in the development of atherosclerosis, and is characterized by an increased permeability of endothelium, tendency to vasospasm and thrombosis.

Apolipoprotein A5, a minor plasma apolipoprotein, has been documented to play a major role in triglyceride metabolism by the enhancement of very low density lipoprotein (VLDL) lipolysis and clearance, and inhibition of VLDL production [5]. The Apo A5 gene was identified 30 kb upstream of the well-characterized Apo A1/C3/A4/A5 gene cluster on chromosome 11q23, and comprises 3 exons encoding 366 amino acids. The −1131C Apo A5 allele of polymorphism in the gene promoter was identified as a susceptibility variant for development of MetS in recent studies in different populations [6–11], although the results are not fully consistent [12]. Nevertheless, the association between Apo A5 T-1131C (rs662799) polymorphism and hemostatic markers, and their potential relationship to MetS has not been evaluated so far.

Abbreviations: MetS, metabolic syndrome; NCEP, National Cholesterol Education Program; ATP, adult treatment panel; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; CRP, C-reactive protein; TC, total cholesterol; TG, triglycerides; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; nonHDL, TC − HDLc; AI, atherogenic index of plasma (log TG/HDLc); Apo, apolipoprotein; Lp(a), lipoprotein (a); vWF, von Willebrand factor; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; T, C, alleles of Apo A5 gene at position −1131; E22, E23, E24, E33, E34 and E44, Apo E genotypes.

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Apolipoprotein E is a structural component of triglyceride-rich lipoproteins serving as a high-affinity ligand for low density lipoprotein (LDL) receptor and related proteins. It plays also an important role in the catabolism of remnant lipoprotein particles [13]. Apo E4 (Cys112Arg, rs429358) and Apo E2 (Arg158Cys, rs7412) variants differ from common Apo E3 isoform by a single amino acid substitution and the variants vary on both structural and functional levels. In recent studies in different populations, Apo E4 variant was associated with both carotid and coronary atherosclerosis [14–18], carotid plaque formation [17], cerebral infarction [18], and other atherosclerosis-linked syndromes, although the results are conflicting in some works (e.g. [19]). Several studies also evaluated an association between ApoE polymorphism and metabolic syndrome [20–23]. Nevertheless, very little is known about the relationship of hemostatic markers to Apo E polymorphism in asymptomatic dyslipidemic patients.

Therefore, our study aimed to evaluate the relationship of T-1131C Apo A5 (rs662799) variants, and variants of Apo E common polymorphism (rs429358, rs7412) to fibrinogen, von Willebrand factor, tissue plasminogen activator, and plasminogen activator–inhibitor 1 levels in dyslipidemic patients, subsequently divided according to the presence/absence of MetS. Together with these parameters, lipid and lipoprotein analytes as well as markers of insulin resistance were determined.

2. Materials and methods

2.1. Study design and subjects

The study was performed with asymptomatic dyslipidemic subjects (individuals without a history of clinically manifest atherosclerosis — coronary artery disease, heart failure, cerebrovascular ischemic disease and peripheral vascular disease, with altered plasma lipids, i.e. with total cholesterol (TC) ≥5.0 mmol/l and/or triglycerides (TG) ≥1.5 mmol/l). They had been examined for the first time in the Lipid Centre of the 3rd Department of Internal Medicine, University Hospital Olomouc, Czech Republic, during the period from January 2006 to March 2011. All subjects were tested for the signs of secondary hyperlipidemia: diabetes mellitus, hypothyroidism, renal or hepatic diseases and nephrotic syndrome. Other exclusion criteria were as follows: history of clinically manifested atherosclerosis presented by coronary artery disease, cerebrovascular disease and peripheral arterial disease, hypolipidemic therapy in the previous 8 weeks, hormone therapy and the clinical presence of acute infections. The subjects with E24 genotype of Apo E polymorphism were also excluded because of the opposite effects of E2 and E4 alleles on lipid levels. All individuals filled out a questionnaire on their previous medical history, especially cardiovascular status, medication and smoking habits. Body mass index and systolic and diastolic blood pressures (SBP, DBP) were also determined. The study was reviewed and approved by the Ethics Committee of the Medical Faculty and University Hospital Olomouc and informed consent was obtained from all participants.

Individuals who met the criteria mentioned above (n = 590, 288 males, 302 females) were subsequently divided into two groups: patients with the presence of metabolic syndrome (MetS+, n = 146, 77 males, 69 females), and individuals with the absence of metabolic syndrome (MetS−, n = 444, 211 males, 233 females). NCEP ATPIII Panel 2001 criteria were used for identification of MetS and the presence of at least three of the factors was sufficient for the diagnosis of MetS [24].

2.2. Laboratory analysis

Venous blood samples were drawn in the morning after a 12-h fast. After centrifugation, the serum was used for other analyses. For the assessment of hemostatic markers, venous blood was collected in 3.8% sodium citrate tubes and plasma was obtained after centrifugation.

Routine serum biochemical parameters were analyzed on a Modular SWA system (Roche, Basel, Switzerland) in the same day of blood collection. Concentrations of other special analytes were measured in the sample aliquots stored at −80 (−20) °C, no longer than 6 months — see below in the text.

Total cholesterol, TG and HDLc levels were determined enzymatically on a Modular SWA system (Roche, Basel, Switzerland). Low density lipoprotein cholesterol (LDLc) levels were calculated using the Friedewald formula (for TG less than 4.5 mmol/l). Other calculated parameters were as follows: nonHDL-cholesterol (nonHDLc = TC − HDLc), atherogenic index of plasma (AI) (logTG/HDLc), and homeostasis model assessment (HOMA-R = glucose × insulin/22.5). Concentrations of Apo B and Apo A1 were determined immunoturbidimetrically using Tina-Quant ApoB and ApoA-1 kits (Roche, Basel, Switzerland). Lipoprotein (a) [Lp(a)] was measured immunoturbidimetrically using Tina-Quant lipoprotein(a) TQ kit (Roche, Basel, Switzerland). C-reactive protein (CRP) was assessed by an ultrasensitive immunoturbidimetric method using the kit Tina-Quant (Roche, Basel, Switzerland). Glucose was determined using the GOD-PAP method (Roche, Basel, Switzerland). All tests were measured from fresh sera in the same day of blood collection.

Insulin was determined by the commercially available kit (Immunotech, Marseille, France) using specific antibodies by the IRMA method. C-peptide and proinsulin (PINS) were determined using the following kits: C-peptide (Immunotech, Marseille, France), and proinsulin (DRG Instruments GmbH, Marburg, Germany), by the IRMA method, and RIA method, respectively. The sample aliquots were stored at −20 °C, no longer than 6 months.

The following hemostatic markers were examined from human plasma stored at −20 °C: fibrinogen (function coagulation method by Clauss, Technoclone, Vienna, Austria), von Willebrand factor (immunoturbidimetric assay, Instrumentation Laboratory Spa, Milan, Italy), plasminogen activator inhibitor-1, and tissue plasminogen activator (ELISA, Technoclone, Vienna, Austria).

2.3. Genotyping

DNA was extracted from the peripheral leukocytes of all subjects using a standard commercial kit (QIAamp DNA blood mini-kit, Qiagen, Germany). After isolation, the extracts were stored at −20 °C, no longer than 3 months. Apo A5 genotypes were determined by a melting curve analysis after a real time PCR method adapted from the work of Frances et al. [25]. Genotyping of Apo E alleles was performed using a commercially available Apo E LightMix kit (Roche, Basel, Switzerland) by a melting curve analysis after real time PCR. The 228 bp fragments were amplified with specific primers and PCR amplicons then analyzed using SimpleProbe and hybridization probes.

2.4. Statistical analysis

All values of quantitative parameters are expressed as means ± standard deviation (SD) and parameters with skewed distribution also expressed as medians. The Kolmogorov–Smirnov test was used to check for normal distribution. Variables with skewed distribution [CRP, TG, AI, Lp(a), tPA, C-peptide] were log transformed in order to normalize their distribution before statistical analysis. Differences in variables between individual groups were analyzed with ANOVA, after adjustment for age and sex. Statistical analysis was performed by SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). Probability values of $p < 0.05$ were considered as statistically significant.

The genotype and allele frequencies for the Apo A5 and Apo E genes among all subjects together with MetS+ and MetS− groups were determined by gene counting method, and then evaluated by performing Pearson Chi-square statistical analysis to evaluate whether followed polymorphisms were consistent with Hardy–Weinberg equilibrium expectation.