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Association of plasma cholesteryl ester transfer protein activity and polymorphism with coronary artery disease extent in Tunisian type II diabetic patients

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Abstract

Introduction: Cholesteryl ester transfer protein (CETP), a key protein in reverse cholesterol transport, has a controversial role in atherosclerosis.

Objectives: We investigated CETP activity and polymorphism in Tunisian type II diabetes and its relationship with coronary artery disease (CAD).

Design and methods: 173 type II diabetic patients with or without CAD were compared to 67 controls.

Results: The HDL cholesterol concentration was low in a Tunisian population. The B1 allele of the CETP gene was associated with a low concentration of HDL cholesterol and was more frequent in Tunisians than in other populations. In type II diabetic patients, the B1 allele was associated with increased prevalence of CAD only in men (OR = 0.357, CI = 0.161–0.791, $P = 0.01$). The CETP activity increased in type II diabetic patients compared to controls ($P = 0.05$). Furthermore, the CETP activity was increased in patients with double or triple vessel disease compared to those with single vessel disease ($P = 0.025$).

Conclusions: Our data are in favour of an association between CETP and developing CAD, as well as the extent of CAD.

Keywords: Cholesteryl ester transfer protein (CETP); CETP activity; Coronary artery disease (CAD); CAD extent; HDL cholesterol; Taq IB polymorphism; Tunisian; Type II diabetes

Introduction

A strong inverse relationship exists, in general, between plasma HDL cholesterol levels and coronary heart disease [1]. Plasma HDL levels are regulated by genes and environmental factors. The cholesteryl ester transfer protein (CETP) is a modulating factor of HDL cholesterol concentration [2,3]. CETP mediates the transfer of cholesteryl ester from high density lipoprotein (HDL) to TG-rich lipoproteins as well as the transfer of TG in the opposite way [4]. It plays a key role in cholesterol reverse transport by facilitating cholesterol excess removal. Thus, theoretically, CETP may be either proatherogenic or antiatherogenic: (i) in rabbits, a CETP inhibitor attenuates atherosclerosis [5]; (ii) mutations in the CETP gene are associated with increased coronary heart disease despite increased HDL level [6]. Thus, the CETP gene was considered to be one of the candidate genes involved as a cardiovascular risk factor [7].

Diabetes mellitus is a strong risk factor for the development of atherosclerosis [8]. Despite intensive study, it

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remains controversial whether plasma CETP level and activity are abnormal in type II diabetes. Several mutations in the CETP gene have been reported, most of them influence the HDL cholesterol concentration [2,3,9] and may modulate the risk of myocardial infarction [10,11]. The Taq IB polymorphism identified in intron 1 was the most frequently reported polymorphism in the CETP gene [10]. The association of the CETP Taq IB polymorphism with plasma HDL cholesterol and coronary heart disease may be population-specific, and highly influenced by environmental factors [10,11].

We, therefore, employed the study to elucidate the effects of the Taq IB polymorphism on serum CETP activity and HDL cholesterol level, and overall coronary artery disease in Tunisian patients with type II diabetes mellitus.

Research design and methods

Subjects

172 type II diabetic patients (73 with CAD and 99 without CAD) were recruited in the Department of Cardiology and Internal Medicine in the Teaching Monastir Hospital, then compared to 67 healthy subjects. Written or oral consent was obtained from all the patients and healthy subjects before the study. Written consents were not possible in all cases because the majority of eligible subjects in our study are unable to read or write. The diagnosis of diabetes was based on a previous history of diabetes based on American Diabetes Association criteria [12]. The diabetic patients without CAD had a normal ECG and no history or clinical signs of CAD with a maximal negative exercise test. The patients with CAD were defined as those having clinical history of stable angina pectoris, previous acute coronary syndromes with or without ST segment elevation, and this CAD was confirmed by coronary angiography. The extent of CAD was assessed by the number (1–3) of coronary vessels with more than 50% reduction in the luminal diameter. Exclusion criteria were taking insulin or lipid lowering drugs, having a renal or liver failure or thyroid disease, alcohol consumption 3 days prior to the study or less, a body mass index (BMI) more than 35 kg/m^2 and glycosylated hemoglobin (Hb A_{1c}) more than 12%. Plasma triglyceride concentration was under 10 mmol/L in type II diabetes and under 2.3 mmol/L in healthy controls. Post menopausal women had no hormone replacement therapy. Body mass index (BMI) was calculated using the formula: weight (kg) / height^2 (m^2). Obesity was defined as BMI > 30 kg/m^2. The waist-to-hip ratio was calculated from measurements of the waist circumference taken at the midpoint between the umbilicus and xiphoid and the hip circumference, at the widest point around the hips, respectively. Blood samples were drawn after subjects had fasted overnight (12 h) into tubes containing EDTA, and plasma was immediately separated by centrifugation. In patients with CAD, the blood samples were not collected during acute coronary syndrome.

Laboratory procedures

Plasma glucose, glycosylated hemoglobin (HbA1c), plasma creatinine, lipid, lipoproteins and apolipoproteins were determined as described by Smaoui et al. [13]. The measure of CETP activity was done essentially as described by Tall et al. [14]; CETP activity (nmol CE/mL/2 h) was defined as the quantity (nmol) of total tritiated cholesteryl ester (^{3}H-CE) transferred from HDL (donor lipoproteins) to LDL and VLDL (acceptor lipoproteins) in the presence of a small volume of the patient's plasma (10 μL). The intra-assay coefficient of variation was 1.5 to 6.5%. The inter-assay coefficient of variation was 4.5 to 7%.

Taq IB polymorphism of the CETP gene

After DNA extraction, amplification of a fragment encompassing 535 bp in intron 1 was obtained by polymerase chain reaction (PCR) according to the method of Fumeron et al. [10]. 0.5 μg of DNA, 50 pmol of each primer, 5 μL of PCR buffer and 0.25 U of Taq DNA polymerase (Q Biogene, France) were mixed in a final volume of 50 μL. The amplification program was 5 min at 95°C, with the following cycle repeated 35 times: 30 s at 62°C, 1 min at 72°C and 30 s at 95°C; finally 3 min at 72°C. The PCR product was hydrolyzed with 10 U of Taq I (Appligene, 10 U/μL) at 65°C for 2 h. After separation by electrophoresis on a 2% agarose gel, the digestion products were revealed by ethidium bromide staining.

Statistical analyses

Data management and statistical analyses were performed using SPSS 10.0 software. Results are summarized as mean ± SD. Logarithmic transformation of CETP activity and TG concentration was performed. A Student's t test was used to compare means between two groups. Comparison in continuous variables among three groups was made using the one-way ANOVA. A Chi-square (χ^2) test was used to examine the distribution of males and smokers in selected groups. A value of $P < 0.05$ was considered significant.

Results

Our study, conducted in the central part of Tunisia, showed that total cholesterol and HDL cholesterol concentrations are lower in Tunisians compared to occidental populations (Table 1). This profile was similar to another Arab population (Saudi Arabia).

To study the effect of CETP Taq IB polymorphism, we pooled the data for B2B2 and B1B2 genotypes because of