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CETP TaqIB polymorphisms and CETP activity in normolipidemic healthy northern Indians

Kiran Meena [a], Anoop Misra [b], R.M. Pandey [c], Kalpana Luthra [a,*]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*
[b] *Diabetes Unit, B-1, Fortis Hospital, Vasant Kunz, Delhi, India*
[c] *Department of Biostatistics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*

KEYWORDS
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High-density lipoprotein cholesterol (HDL-C);
Total cholesterol (TC);
Lecithin-cholesterol acyltransferase (L-CAT);
TaqIB polymorphisms;
Normolipidemic

Summary

Background and aim: Asian Indians have relatively lower high-density lipoprotein cholesterol (HDL-C) as compared to the other populations. Cholesterol ester transfer protein (CETP) is involved in the transfer of cholesterol esters from HDL-C to other lipoproteins in the plasma. In order to assess the role of *CETP* polymorphisms and their association with HDL-C, if any, we have studied the TaqIB polymorphism in intron 1 of *CETP* and its correlation with plasma lipids in 158 normolipidemic healthy subjects.
Methods and results: Plasma lipids levels were estimated using commercially available kits from Randox (India) and *CETP* TaqIB polymorphism were assayed by PCR-RFLP in 158 healthy normolipidemic individuals. The observed allelic frequencies of TaqIB polymorphisms, B1 and B2 were 43.0% and 56.9%, respectively. Lipid parameters were comparable between individuals with the different *CETP* TaqIB polymorphisms. The individuals categorized on the basis of their HDL-C levels ($<$ or $>$1.04 mmol/l), had comparable distribution of *CETP* genotypes. CETP activity was assessed in 84 of the total 158 individuals included in the study and did not vary among the *CETP* TaqIB genotypes. CETP activity in the females (n = 39) negatively correlated with HDL-C levels.
Conclusion: This study reveals that *CETP* TaqIB polymorphisms are not associated with the decreased levels of HDL-C in healthy normolipidemic individuals. We are presently involved in studies to assess the precise role of *CETP* TaqIB polymorphism and CETP activity in hyperlipidemia in Asian Indians.

Introduction

Since the discovery of cholesterol ester transfer protein (CETP) and its identification as a modulator of high-density lipoprotein cholesterol (HDL-C) levels, there has been much speculation about the role of CETP in lipid metabolism and related vascular diseases in humans. There are well-docu-

* Corresponding author.
E-mail address: kalpanaluthra@rediffmail.com (K. Luthra).

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mented biochemical studies that demonstrate the ability of CETP to affect lipid transport [1,2].

The natural genetic variation at the *CETP* locus is useful as a tool to understand its impact on lipid levels and diseases. With the early detection of genetic variants among families deficient in CETP, genetic studies have played a significant role in their attempt to understand the association of CETP with lipids and diseases.

Several studies show a clustering of risk factors for coronary heart disease (CHD), diabetes and obesity in the Asian Indian population [22,26,31]. Although changes in lifestyle are significant, genetic factors may also contribute to increased risk. It has been reported that low levels of HDL-C contribute to higher rate of CHD in Asian Indians [17,22,23]. Only 14% men and 5% women of Asian Indian origin in USA had optimum levels of HDL-C in CADI study [16]. Gupta et al. [15] reported that lower levels of HDL-C were found among native Asian Indian population. In addition, several studies have reported the prevalence of higher serum triglyceride (TG) levels in Asian Indian population [3,12,13,23]. High levels of TG, low levels of HDL-C, increased abdominal adiposity and insulin resistance are reported to be more prevalent among South Asians [9,24]. Genetic factors play a significant role in the predisposition and development of these metabolic abnormalities. In previous case—control studies, we have investigated the status of ApoE, a genetic determinant of plasma lipid levels and CHD, in Asian Indians [21,22].

Several proteins (ApoB, CETP, etc.) are involved in maintaining levels of lipoproteins in plasma. Hyperlipidemia and other similar metabolic disorders are often modulated by gene—gene and gene—environment interactions. Various candidate genes, which are being investigated for their role in hyperlipidemia, include *Apo B*, *Apo A-1/CIII*, *A-IV*, *Apo E*, *CETP* and *paraoxygenase*, etc. Mutations in the *CETP* gene resulting in complete loss of CETP activity are some of the causes of hyperalphalipoproteinemia [36]. *CETP* gene spans 25,000 bp and is composed of 16 exons and 15 introns localized on chromosome 16 in the 16q-12-21 adjacent to the lecithin-cholesterol acyltransferase (*LCAT*) gene [1,29]. CETP, a high molecular weight (74 kDa), acidic glycoprotein [34] is involved in transfer cholesterol esters (CE) from HDL to VLDL and LDL with a reciprocal exchange of TG from VLDL and LDL to HDL [18,34]. Thus, CETP mediates one of the steps of reverse cholesterol transport (RCT), an anti atherogenic process that channels cholesterol from peripheral tissues back to liver. CETP may therefore play a crucial role in the development of atherosclerosis. Several DNA polymorphisms of *CETP* gene have been described [8,10,37]. A number of studies suggest that plasma CETP concentrations, CETP activity and HDL-C levels are related to common variations in *CETP* gene such as the TaqIB polymorphism [14,19,20]. A strong association has been observed between the BI allele of TaqIB polymorphism of *CETP* with high plasma CETP concentrations and hypoalphalipoproteinemia in white healthy subjects. The presence of B1allele has also been related to the progression of coronary atherosclerosis [20]. Individuals with the B2B2 genotype of *CETP* are reported to have higher HDL-C concentrations than individual carrying B1B1 genotype [11].

Till date, only two studies have been reported on the status of *CETP* TaqIB polymorphisms in Asian Indians. First is a case—control study of *CETP* TaqIB polymorphisms in type 2 diabetic and non diabetic Asian Indians in north India [7] and the second study has been conducted in healthy Tamilian volunteers in south India [30]. The association, if any, of *CETP* TaqIB polymorphisms with CETP activity has not been assessed in the Asian Indians. We have therefore undertaken the present study to determine the frequency distribution of the *CETP* TaqIB polymorphisms and to check for the association of the polymorphisms with CETP activity and lipids in a healthy normolipidemic Asian Indians in north India [15].

Materials and methods

Subjects

An approval of institutional ethical committee was taken. One hundred and fifty eight normolipidemic subjects randomly selected from 250 healthy volunteers were included in the study. The criteria for inclusion were total cholesterol (TC) $<$5.2 mmol/l, triglycerides (TG) $<$1.69 mmol/l.

Collection and storage of samples

Ten millilitres fasting blood was collected in 15 ml poly-vinyl tubes containing dry EDTA (2 mg/ml of blood). The EDTA blood was centrifuged at 800 $\times$ *g* for 15 min to separate the plasma and the blood cells. Plasma was divided into two [30] aliquots and stored frozen at -20°, to determine the lipid profile and to estimate CETP activity using CETP activity assay kit (Biovision Research Product, Catalog no. K601-100 Assays, Germany). Blood cells separated after centrifugation, were used for genomic DNA isolation. DNA Samples were stored at $-20\ ^{\circ}$C.

Estimation of lipid profile

TC, TG and HDL-C levels were estimated using commercially available kits (Randox, India). Estima-