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Frequencies of *CETP* gene *Taq*I B and D442G polymorphisms in North Indian population

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In this study we have analysed the frequencies of *Taq*I B and D442G polymorphisms of *CETP* gene and lipid profile in 315 healthy individuals from North Indian population. For genotyping, PCR was followed by restriction digestion with *Taq*I enzyme. Frequencies of B1B1 : B1B2 : B2B2 genotypes were 25.4% : 54% : 20.6% for *Taq*I B polymorphism. Our study showed that B1B1 genotype frequency is lower and B1B2 frequency is higher than other populations. D442G polymorphism was absent in North Indian population. In spite of high frequency of B2 allele, the HDL-cholesterol levels were low. In conclusion, *CETP Taq*I B polymorphism shows distinct pattern of genotype and allele frequency in North Indians and D442G mutation is absent. However, *CETP Taq*I B polymorphism was not found to be associated with circulating lipid levels.

VARIATIONS in total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and triglyceride have been associated with increased risk of various diseases or complications associated with diseases like atherosclerosis and diabetes mellitus[1]. During the past 30 years, the rates of coronary artery disease (CAD) have doubled with privileged circumstances and urbanization in rural villages in India. Genetic preponderance may also alter risk for CAD by influencing lipid profile. Establishment of genetic risk factors may help in recognition of the population at risk and ultimately the management of disease.

Cholesterol ester transport protein (CETP) mediates the exchange of lipids between antiatherogenic HDL and atherogenic apolipoprotein B containing lipoproteins; thereby it potentially regulates steady-state levels of HDL-cholesterol as well as LDL-cholesterol. CETP can be antiatherogenic because of its roles in the removal of excess cholesterol from the body via LDL receptor-mediated uptake in the liver and excretion in the bile. On the other hand, it may lower the concentration of atheroprotective HDL-cholesterol[2,3].

The *CETP* gene encompasses 16 exons and has been localized on chromosome 16q21. Several polymorphisms of the *CETP* gene have been reported, which may be associated with alteration in CETP activity. *Taq*I B polymorphism is most widely studied, which is created by a silent mutation in nucleotide 277 in intron 1, resulting in two alleles B1 and B2. Allele B2 with low CETP mass and increased

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HDL-cholesterol, has been related to a decreased risk for coronary heart disease (CHD) in many studies, including the Framingham Offspring Study[4], but this observation is not consistent[5].

Other genetic variations include D442G mutation/polymorphism present in exon 15 of *CETP* gene close to the active site, leading to reduced plasma CETP mass and specific activity. The mutation is more prevalent in Japanese subjects with high HDL-levels (> 100 mg/dl)[6], but results are inconsistent from other countries[7].

The aim of the present study was to determine the frequency of *Taq*I B polymorphism and D442G mutation in North Indian population and the interindividual variability in lipoprotein subclasses determined by allele and genotype frequencies.

A total of 315 individuals (mean age 40.8 ± 12.5 yrs) were taken for the study. All subjects were either staff members of our institute or healthy individuals from the general population of the North India. No participant was an alcohol consumer or smoker. No history of any metabolic disorder was present, and mean BMI was 23.34 ± 2.68 kg/m^2. Among these, 140 were males (mean age 39.9 ± 12.7 yrs) and 175 were females (41.5 ± 12.2 yrs). From each individual, 5 ml blood was collected in EDTA after 12 h fast, plasma was isolated and palette was used for DNA extraction. DNA was extracted from lymphocytes present in blood pellet using salting-out method[8].

Both the polymorphisms were detected by PCR and RFLP (*Taq*I). For analysis of *TaqI* B polymorphism[9], 535 bp fragment of *CETP* gene was amplified (Thermal cycler, PTC-200, MJ Research, Inc) using forward primer 5′-CAC TAG CCC AGA GAG AGG AGT GCC-3′ and reverse primer 5′-CTG AGC CCA GCC GCA CAC TAA C-3′. For detection of D442G mutation of *CETP* gene[7], exon 15 flanking sequence was amplified using forward primer 5′-GTG TTT ACA GCC CTC ATG AAC-3′ and reverse primer 5′-AAG CCA AAG TCC ATC TCTGC AG-3′ in a reaction mix of 25 µl.

Reaction mixture contained 12.5 pmol of each primer, 200 µM of each dNTP (Bangalore Genei, India), 200 ng genomic DNA, and 2U Taq polymerase (Bangalore Genei). The amplification protocol consisted of initial denaturation step (95°C for 5 min) followed by 30 cycles each with denaturation at 95°C for 1 min, annealing at 65°C for 1 min followed by extension at 72°C for 1 min, and final extension at 72°C. PCR products were digested at 68°C using 5U of *Taq*I restriction endonuclease (Fermantas INC, USA) for 3 h. Products for *Taq*I polymorphism were run on 2% agarose gel and visualized by staining with ethidium bromide. Presence of site (B1 allele) gave two bands of 174 and 361 bp and absence (B2 allele) showed one band of 535 bp.

For genotyping of D442G polymorphism, PCR products were run on 20% polyacrylamide gel. This product has two sites for *Taq*I. Homozygous subjects with DD showed two bands (218 and 69 bp). If the sample is heterozygous, it should show four bands (218, 69, 41 and 28 bp) and homozygous condition for G allele will result in three bands (218, 41 and 28 bp).

In plasma total cholesterol, triglyceride, and HDL-cholesterol were analysed using commercially available kits (Accurex Biomedical Pvt Ltd, Mumbai). For HDL-cholesterol, estimation selective precipitation of other lipoproteins was done using sodium tungstate and magnesium chloride. LDL-cholesterol was calculated from concentrations of other components[10].

Data were analysed using statistical software (SPSS Inc, Chicago). Direct gene counting method was used to determine the frequency of genotypes and alleles. The chi-square test was used to determine Hardy–Weinburg equilibrium. Continuous variables were expressed as mean ± SD. Comparisons among genetic subgroups were made by one-way variance analysis.

The population analysed was in Hardy Weinberg equilibrium. The distribution of *CETP Taq*I B genotype was B1B1 25.4%, B1B2 54%, B2B2 20.6% and allelic distributions were 52.38% and 47.62% respectively, for B1 and B2 (Table 1). Frequency of B1B1 genotype was found to be lowest and heterozygosity was highest. B1 allele frequency was also quite low and B2 was higher than in other populations. We studied D442G polymorphism in the Indian subcontinent and only D-allele was present at this position in our study.

Across B1B1, B1B2 and B2B2 genotypes, circulating levels of total cholesterol, HDL-cholesterol, triglyceride, and LDL-cholesterol were not significantly different. Even after stratification of the data into male and female (Table 2), no significant differences in lipid profile were found.

To see the effect of B1 or B2 allele on lipid profile, the study population was divided as B1B1 vs B1B2 + B2B2 and B1B1 + B1B2 vs B2B2, but no statistically significant differences were found (data not shown).

In this study, allele frequencies of *CETP Taq*I B and D442G polymorphisms along with their relationship with variation in circulating lipid levels were examined. This work describes frequencies of *Taq*I B and D442G polymorphisms in North India.

The *CETP* gene is highly polymorphic and many genetic variations have been identified in both coding and non-coding regions[11,12]. Various polymorphisms associated with

Table 1. *CETP* gene *Taq*I B polymorphism genotype and allele frequencies

Genotype/ allele	Total n = 315 (%)	Male n = 140 (%)	Female n = 175(%)
B1B1	80 (25.4)	34 (24.3)	46 (26.3)
B1B2	170 (54.0)	79 (56.4)	91 (52.0)
B2B2	65 (20.6)	27 (19.3)	38 (21.7)
B1	330 (52.38)	147 (52.5)	183 (52.29)
B2	300 (47.62)	133 (47.5)	167 (47.71)

Table 2. Circulating levels of plasma lipids in various genotypes of *CETP* gene *Taq*I B polymorphism

	Total			Male			Female		
	B1B1 (n = 38)	B1B2 (n = 85)	B2B2 (n = 29)	B1B1 (n = 18)	B1B2 (n = 38)	B2B2 (n = 13)	B1B1 (n = 20)	B1B2 (n = 47)	B2B2 (n = 16)
Total cholesterol[a] (mg/dl)	160.66 ± 36.94	152.02 ± 62.12	145.29 ± 43.61	153.08 ± 32.07	153.56 ± 52.61	138.90 ± 51.53	167.48 ± 40.42	150.77 ± 69.41	150.49 ± 36.90
HDL-cholesterol[a] (mg/dl)	32.48 ± 8.81	32.28 ± 10.64	31.92 ± 10.71	31.42 ± 8.28	30.80 ± 10.36	28.39 ± 12.51	33.44 ± 9.37	33.49 ± 10.81	34.78 ± 8.33
LDL-cholesterol[a] (mg/dl)	104.28 ± 33.15	98.21 ± 56.67	91.31 ± 39.14	99.53 ± 29.23	98.73 ± 44.55	89.37 ± 45.07	108.55 ± 36.54	97.78 ± 65.33	92.89 ± 35.05
Triglyceride[a] (mg/dl)	119.51 ± 69.08	107.64 ± 54.29	110.30 ± 41.05	110.68 ± 38.49	120.17 ± 60.00	105.68 ± 44.40	114.06 ± 39.19	97.50 ± 47.45	127.47 ± 88.47

Values are mean ± SD.
[a]As fasting blood was not available for all cases, lipid profile was analysed in 152 individuals.

Table 3. Genotype and allele frequencies of *CETP* gene *Taq*I B polymorphism in various populations of the world

Population	B1B1 (%)	B1B2 (%)	B2B2 (%)	B1 (%)	B2 (%)	Reference
Amsterdam	32	49	19	56.5	43.5	5
Finland	32	49	19	56.5	43.5	22
Glasgow (Caucasians)	27	54	19	54	46	23
Japan	38	41	21	58.5	41.5	24
Saudi Arabia	32	46	22	55	45	25
China	32.2	51	16.8	57.7	42.3	26
Greece	37.1	41.2	21.6	57.7	42.2	27
UK	31	49	20	55.5	44.5	14
African-Americans	56	37	7	74.5	25.5	28
USA, American Whites (Caucasians)	33	45	22	55.5	44.5	28
Mumbai, India	27	46	27	50	50	13
Lucknow, India	25.4	54	20.6	52.38	47.62	Present study

CETP have been studied; among them *Taq*I B is the most widely studied. Frequency of B2 allele varies worldwide (25.5%, in African-Americans to 46% in Caucasians, Table 3). In our study we found that frequency of B1B1 genotype is lowest (25.4%) and B1B2 is highest (54%) in North Indians (Table 3). At the allelic level also, frequency of B1 allele is low and B2 is high but the difference is less prominent. Recently, a study from western India (Mumbai) reported slightly higher frequency of B2 allele (50% vs 47.62%). The frequency of homozygous B2B2 was also higher (27% vs 20.6%) than the present study[13]. It also supports that the frequencies of B2 allele/B2B2 genotype are higher in Indians than in other populations.

In our population, exonic mutation D442G of *CETP* gene was not observed. In other populations of the world, frequency of D442G varies greatly (0–5.3%)[14]. In Japan, D442G mutation in *CETP* gene is around 7% in random male samples[15]. However, we did not observe any individual having the mutation either in homozygous or heterozygous state. Studies in the Chinese population show enormous variation (0.2–5%)[16,17]. This may be due to different ethnic groups studied. The same is true for studies from other countries. Absence of D442G polymorphism shows similarity of our population with Caucasians.

Several studies have reported association of *Taq*I B polymorphism with lipid variation; B2 allele has been shown to be associated with higher HDL-cholesterol levels[5,18]. However, a study on the Singapore population comprising Chinese, Malays and Asian Indians showed highest frequency of B2 allele in Asian Indians, but lowest HDL-cholesterol levels[19]. In the present study also, the frequency of the B2 allele did not correlate with low levels of HDL-cholesterol (32.26 ± 10.16 mg/dl) found in North Indians. We believe this discrepancy may be due to very high frequency of B1B2 heterozygotes (Table 3).

Considering that CETP deficiency is the main cause of high HDL-cholesterol in some populations, there must be some other genetic variants of the *CETP* gene which also contribute in lipid variation. Several studies, particularly from Japan, have attributed D442G mutation as the major factor in high HDL-cholesterol. The D442G change occurs near the carboxy terminal region of CETP, which has been shown to be essential for its function. The mutation has been shown to behave in dominant negative manner and presence of G-allele even in heterozygous state, is a risk factor for coronary atherosclerosis in certain populations in Japan[20,21]. Absence of G-allele rules out any role of the mutation in the Indian population.

In India, there is a heterogeneous population and it would be desirable to find out the gene frequency and lipid profile in sub-populations of the country. Early establishment of population at risk and control of high-risk environmental factors should be helpful in lowering the burden of disorders associated with dyslipidaemia.

In conclusion, the frequency of *CETP Taq*I B polymorphism is distinct in Indians. The *CETP* gene D442G mutation is absent or extremely rare. However, *CETP Taq*I B polymorphism was not found to be associated with circulating lipid levels.

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