STUDY OF APOLIPOPROTEIN3 SstI POLYMORPHISM IN HEALTHY VOLUNTEERS FROM NORTHERN INDIA

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ABSTRACT

Several studies including a small case-control (hypertriglyceridemic / normotriglyceridemic individuals) study by us revealed close association between rare S2 allele of APOC3 SstI polymorphism and hypertriglyceridemia. With the understanding that Asian Indians are highly vulnerable to the adverse effects of hypertriglyceridemia, we extended the investigation and studied the frequency distribution of this polymorphism in 216 healthy volunteers from Northern plains of India. We found that more than 50% of the study population had one or two S2 allele. This may suggest that a larger fraction of this population is genetically predisposed to hypertriglyceridemia.

KEY WORDS

APOC3, SstI polymorphism, CAD, India, Hypertriglyceridemia

INTRODUCTION

ApoCIII protein is a monomer of 79 amino acids, synthesized primarily in liver and to a lesser extent in small intestine (1). It circulates in plasma in association with TG rich lipoproteins and HDLs. ApoCIII inhibits the lipoprotein lipase and hampers the TG clearance. Overexpression of the human APOC3 gene causes hypertriglyceridemia in transgenic mice, whereas the absence of APOC3 in knockout mice reduces plasma TG to 70% of normal (1). An SstI polymorphism resulting from a C->G base transversion in 3' untranslated region of APOC3 gene had been studied extensively in relation to hypertriglyceridemia (2-23). Here, the rare S2 allele apparently predisposes an individual to hypertriglyceridemia (2-6,9,11,13-16,18,20-22). Asian Indians are reported to have high genetic predisposition to develop CAD. Hypertriglyceridemia appears to be a major risk factor for CAD in this population. As such Indians are considered to be

more sensitive to the adverse effects of the hypertriglyceridemia (24). Earlier, we found a significant association of S2 allele with hypertriglyceridemia in a paired study (25) comprising normotriglyceridemic (N=105) and hypertriglyceridemic (N=34) individuals from Northern India. Study also suggested a higher prevalence of S2 allele in our subjects when compared with the normal distribution of this allele in Caucasians. This was the first report from India. In the present paper, we report our findings on the normal distribution of APOC3 SstI polymorphism in a sample of 216 inhabitants of Northern plains of India.

MATERIALS AND METHODS

Two hundred and sixteen healthy volunteers with mean age - 52.2 ± 10.8 years were recruited in the study. All underwent routine biochemical tests (Hemoglobin, urea & sugar) and blood pressure measurement. Subjects having angina or any history of myocardial infarction were excluded. The research was undertaken with the approval of ethical committee set by All India Institute of Medical Sciences, New Delhi and its guidelines were observed.

Genomic DNA samples isolated from venous blood

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were amplified using forward primer: 5'-CAT GGT TGC CTA CAG GAG TTC-3' and reverse primer: 3'-TGA CCT TCC GCA CAA AGC TGT-5' (MWG Biotech GmbH, Germany) followed by digestion with SstI restriction enzyme (2). The digested PCR products were resolved on 3% Agarose gel and visualized by Ethidium Bromide staining. The alleles were defined as S1 and S2 based on absence or presence of the SstI restriction site respectively. The allele frequencies were estimated by gene counting. Chi-square goodness of fit was used to verify the agreement of the observed genotype frequencies with those expected (*Hardy-Weinberg equilibrium*).

RESULTS

The genotypic and the allelic distribution of *APOC3* in the study group are shown in Table-1. The most frequent genotype observed was S1S1 (48.6%) followed by S1S2 (43.5%) and S2S2 (7.9%) (Table-1). Genotypic distribution was in agreement with *Hardy-Weinberg equilibrium*. No significant difference existed between the observed and the expected genotype frequencies as shown in Table-1 ($p=0.5218$, non significant). The frequency of S1 allele was 0.7035 and S2 was 0.2965 (Table-1). The 95% confidence interval is also given in Table-1. Table-2 shows a comparison of the *APOC3* S2 allele in our population with those reported in other populations.

DISCUSSION

APOC3 SstI polymorphism has gained much attention since Rees et al (3) reported an association between TG homeostasis and this polymorphism in British population. The distribution of various genotypes (S1S1, S1S2 and S2S2) varied in different populations, S1S1 being the major genotype in most of them. Rare S2 allele appears to be more frequent in non Caucasians like Chinese (4), Mayans (5), Japanese (6-8), Koreans (9), Taiwanese (10), Arabs (11,12) as compared to the Belgian (2), British (3,13), Danish (14), Dutch (15), Finnish (16), Scottish (17), American (18) and French (Canada)(19) populations. Most of these studies found a relationship between the presence of S2 allele and hypertriglyceridemia (2-6,9,11,13-16,18,20-22). An association of this allele with CAD was also reported (26-28). Paul-Hayase et al (29) conducted a study on the SstI polymorphism on Asian Indians residing at U. K. ($S2=0.19$). However, the sample size was very small ($N=24$). We were the first ones to report an association of S2 allele with hypertriglyceridemia in Asian Indians (25). Our data also suggested a high prevalence of S2 allele

in this population. In the present study, we have elucidated the normal distribution of *APOC3* SstI polymorphism in a comparatively larger sample ($N=216$) of residents of Northern plains of India. The study subjects represented mixed ethnicity with common socio cultural background and dietary habits. We found that the distribution of different genotypes in our study subjects was closer to those reported for Chinese (4), Mayans (5), Japanese (6,7,8), Koreans (9), Taiwanese (10) Arabs (11,12). Frequency of S2 allele was also comparable which is higher in these populations as compared to those from different parts of Europe (2,3,13-17), USA (18) and French (Canada)(19) (Table-2). The results corroborates with our previous findings (25). Though association of apoCIII with hypertriglyceridemia is well documented, role of S2 allele in this context is only partially explained. This polymorphism may directly modulate the expression of the *APOC3* gene (3). Increased levels of apoCIII has been reported in S2 carriers(20-23). It is possible that this site may be involved in mRNA stability because 3' untranslated sequences have been shown to influence the mRNA turnover (30). Esterbauer et al showed that mRNA transcribed from the S2 allele displayed a higher abundance than S1 allele in 3 out of 5 subjects (30). Since the SstI polymorphism is in the 3' untranslated region of the *APOC3* gene, it is more likely to be in linkage disequilibrium with an unknown causative mutation(s) in *APOC3* or nearby genes involved in the lipid homeostasis. However, other studies carried out on Caucasians (17,19), Taiwanese(10), Japanese(7,8) and Arabs(12) did not show any significant association of S2 allele with hypertriglyceridemia. It was speculated that linkage disequilibrium between S2 allele and causative mutations is weakened or absent in these populations. Other possibility could be different environmental and unrelated genetic effects in these populations.

To conclude, the distribution of *APOC3* SstI polymorphism in our population resembled that in non-Caucasians and over 50% of the study population had one or two S2 allele, and thus, are genetically predisposed to hypertriglyceridemia. This finding is important especially because Asian Indians are prone to get adversely affected by even moderately high levels of TG and lifestyle in India is fast shifting to sedentary affluent habits (24).

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Table 1

Frequency distribution of various genotypes and alleles of *APOC3* SstI polymorphism

Genotype	Observed genotype frequency (%)	Expected genotype frequency (%)	Allelic Frequency (95% confidence Interval)
S1S1	105 (48.6%)	106.96 (49.52 %)	S1 = 0.7035(0.65-0.74)
S1S2	94 (43.5%)	90.08 (41.70%)	
S2S2	17(7.9%)	18.96 (8.78%)	S2 = 0.2965(0.26-0.34)
Total	216		

In Hardy-Weinberg equilibrium; Chi-square=0.4103 df=1, p=0.5218

Table 2

Frequency of *APOC3* S2 allele in different populations

Populations	S2 allele	Reference
<u>Non –Caucasians</u>		
Present Study	0.2965	
Chinese	0.29	4
Mayans	0.28	5
Japanese	0.25	6
	0.369	7
	0.39	8
Koreans	0.24	9
Taiwanese	0.26	10
Arabs	0.37	11
	0.21	12
<u>Caucasians</u>		
Belgians	0.11	2
British	0.02	3
	0.00	13
Danish	0.09	14
Dutch	0.09	15
Finnish	0.08	16
Scottish	0.09	17
American	0.083	18
French (Canadians)	0.091	19

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