Short report

Study of apolipoprotein E polymorphism in normal healthy controls from Northern India

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1. Introduction

Asian Indians have been reported to have higher prevalence of coronary artery disease (CAD) amongst any known ethnic group in the world. The hypothesis has been raised that genetic variations of apolipoproteins are the major determinants of inter-individual variation in the susceptibility to CAD. The most common variation is that of apolipoprotein E (apo E) gene. There exist three common allelic variants of apo E gene – $\varepsilon 2$, $\varepsilon 3$ & $\varepsilon 4$. This can result in six genotypes – $\varepsilon 2/2$, $\varepsilon 3/3$ & $\varepsilon 4/4$ (homozygous) and $\varepsilon 2/4$, $\varepsilon 3/2$ & $\varepsilon 3/4$ (heterozygous) [1]. Most population studies have shown consistent association of $\varepsilon 2$ allele with hypocholesterolemia and $\varepsilon 4$ with hypercholesterolemia [1,2]. Accordingly, individuals homozygous or heterozygous for $\varepsilon 4$ allele are at highest risk of CAD. Heterozygous individuals with $\varepsilon 4/2$ genotype are somewhat protected, but may be less than those with $\varepsilon 3/2$ genotype. Therefore, individuals with $\varepsilon 3/2$ are maximally protected whereas $\varepsilon 4/3$ are at the high risk, followed by $\varepsilon 3/3$ [1–4].

Apo E polymorphism is extensively studied in different ethnic groups residing in different parts of the world with the notion that this may aid in the identification of high risk and low risk groups, no data have so far been available from India in this regard.

2. Study group

122 normal healthy volunteers with mean age 47 ± 11 were recruited. All underwent routine biochemical tests (hemoglobin, urea and sugar) and blood pressure. Subjects having angina or any history of myocardial infarction were excluded.

3. Methods

DNA samples from venous blood were amplified using specific primers followed by digestion with HhaI enzyme [5]. The digested PCR products were resolved on 8% polyacrylamide gel electrophoresis and visualized by silver staining. The allele frequencies were estimated by gene-counting. The conventional Monte Carlo method was used to verify the agreement of the observed genotype frequencies with those expected (*Hardy-Weinberg equilibrium*) [6]. This method was preferred over chi-square goodness-of-fit because some of the genotype frequencies were small or null.

4. Results

The genotypic and the allelic distribution of apo E in the study group are shown in Table 1. The most frequent genotype was $\varepsilon 3/3$(72.13%) followed by $\varepsilon 3/4$(17.21%)

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Table 1
Distribution of genotypes and alleles of apolipoprotein E

Genotype	Frequency	Genotype frequency (percentage)	Allelic frequency (95% confidence interval)
ε3/3	88	72.13%	$\varepsilon 3 = 0.8527$ (0.801 − 0.893)
ε3/2	11	9.02%	
ε3/4	21	17.21%	$\varepsilon 2 = 0.0573$ (0.032 − 0.096)
ε2/2	1	0.82%	
ε2/4	1	0.82%	$\varepsilon 4 = 0.0900$ (0.058 − 0.135)
ε4/4	0	0%	
Total	122		

Table 2
Observed and expected frequency of apolipoprotein E genotypes

Genotype	Observed frequency	Expected frequency	Level of significance
ε3/3	88	88.6547	
ε3/2	11	11.9344	$p = 0.4712$
ε3/4	21	18.7541	Standard error
ε2/2	1	0.4016	$= 0.00558$
ε2/4	1	1.2623	Non Significant
ε4/4	0	0.9918	
Total	122	121.99	

and ε3/2 (9.02%) (Table 1). The frequencies of rare genotypes ε2/2, ε2/4 and ε4/4 were 0.82%, 0.82% and 0% respectively (Table 1). These were found to be in *Hardy-Weinberg equilibrium*. No significant difference existed between the observed and the expected genotype frequencies as shown in Table 2 ($p = 0.4712$, non significant). The apo E allele frequencies in decreasing order were $\varepsilon 3 > \varepsilon 4 > \varepsilon 2$ (Table 1).

5. Discussion

Variation of apo E gene is believed to provide a strong basis for susceptibility to CAD. The cardiovascular risk of various genotypes with respect to total cholesterol (TC) and low density lipoprotein (LDL) decreases in the following order: $\varepsilon 4/4 > \varepsilon 4/3 > \varepsilon 3/3 > \varepsilon 4/2 > \varepsilon 3/2 > \varepsilon 2/2$ [1–4]. Only 10.66% of the individuals in our study group carried ε2/2, ε3/2 & ε4/2 and hence, may be considered to be at a lower risk of CAD. The remaining 89.34% (ε3/3, ε3/4 & ε4/4) appear to be at a higher risk of developing this disease. Out of this vulnerable group, 19.26% (ε4/4 & ε3/4) are at the highest risk. In India, the threshold at which TC and LDL pose as a risk factor for CAD appears to be lower [7]. Therefore, although ε4 is considered to be the most predominant susceptibility factor, ε3 may also act as a disease allele.

The major risk factor associated with the genetic variability of apo E is hypercholesterolemia. This effect is however modifiable by environmental factors especially the dietary regimen [4]. It is worth noting that in the affluent west, populations with high risk of CAD (e.g. Finnish) have higher prevalence of ε4 allele and lower prevalence of ε2 allele [1]. Conversely, populations like Pygmies and Khoi San with high frequency of ε4 allele have low prevalence of CAD [4]. Average cholesterol level in urban males in Delhi increased from 160 mg/dl to 199 mg/dl between 1982 and 1994, whereas in rural populations it was found to be 20 mg/dl lower than in urbans [8]. Indians living in Singapore, who enjoy a comparatively affluent lifestyle, show a similar trend in mean cholesterol level (197.5 mg/dl) [2]. The apo E allele and genotype distribution in these Indians is similar to the pattern revealed in our study [2]. Another study reported that Indians in Singapore are at a higher risk of CAD than other ethnic groups residing there (Malays and Chinese) [9].

In India, the incidence of CAD is on an exponential rise at an alarming rate. This is disturbing. It may be due to economic growth and subsequent shift to affluent lifestyle [8]. With greater awareness of these risk factors, identification of the genetically predisposed, and adaption to appropriate lifestyle and dietary habits, the prevalence of CAD is progressively declining in the established risk prone countries like Finland and United States [8].

This report from us, for the first time, suggests that the great majority of even normal controls (about 89%) from India are genetically predisposed to CAD.

Whether the genotypic and allelic distribution of apo E is different in CAD patients remains to be elucidated. Nevertheless, in India, implementation of population based preventive strategies for CAD is urgently needed.

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