

Apolipoprotein E Polymorphism Among the Indian Populations and its Comparison with Other Asian Populations

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ABSTRACT The genetic polymorphism at apo E locus has been determined among the Kshatriya (n=182) and the Mala (n=38) populations from Andhra Pradesh, India. No significant difference was found in genotype frequencies between the two populations. The sample of the two populations were pooled and called as the Indian sample. The gene frequencies for apo ϵ 2, ϵ 3 and ϵ 4 alleles were 0.0500, 0.8636 and 0.0864, respectively. The allele frequencies of the Indian sample were compared with other Asian populations using χ^2 test and cluster analysis. The Indian sample differs significantly with most of the Chinese populations and shows similarities with Japanese populations and the Indian sample clusters with Japanese populations.

INTRODUCTION

The apolipoprotein (apo) E is a 299 amino acid, arginine rich glycoprotein with a molecular weight of 34,145 daltons (Rall et al. 1982). It is synthesised in many tissues, including notably the liver parenchyma cells (major source of plasma apo E), astrocytes of the brain (major source of cerebrospinal fluid apo E) and macrophages (Mahley, 1988; Linton et al. 1991). In plasma, apo E appears on chylomicron remnants, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and a fraction of high density lipoproteins (HDL) (Davignon 1993). Apo E plays a central role in the normal catabolism of triglyceride rich lipoprotein remnants and is probably involved in reverse cholesterol transport (Mahley 1988; Mahley and Rall 1989) and also plays an important role in determining interindividual differences in cholesterol levels.

In humans, the structural locus for apo E is polymorphic with three common apo ϵ 2, ϵ 3 and

ϵ 4 alleles and several other rare alleles. In population studies, the frequencies of the apo E common alleles range from 0 to 14% for apo ϵ 2, 48 to 91% for ϵ 3 and 5 to 39% for ϵ 4 (Davignon et al. 1988; Gerdes et al. 1992; Davignon 1993; Kamboh 1995). These allele frequencies are significantly heterogeneous among world populations. The three common codominant alleles apo ϵ 2, ϵ 3, and ϵ 4 at one autosomal locus are coding for the three apo E isoforms E2, E3 and E4, respectively. The three isoforms differ in P_i by a single charge unit, apo E4 being the most basic and E2, the most acidic isoform. The three isoforms E2, E3 and E4 determine six apo E phenotypes in the populations: E2/2, E4/4 and E3/3 in homozygotes and E3/2, E4/2 and E4/3 in heterozygotes.

The three alleles ϵ 2, ϵ 3 and ϵ 4 show differences in amino acid residues at two sites, 112 and 158 (Rall et al. 1982a). Apo ϵ 4 has arginines and apo ϵ 2 has cysteines at both sites, while apo ϵ 3 has a cysteine at residue 112 and an arginine at 158. In all populations studied so far, apo E3 has been the predominant isoform but the relative proportions of the three isoforms have shown variation among populations (Davignon et al. 1988; Sepehrnia et al. 1989; Hallman et al. 1991; Gerdes et al. 1992; Davignon 1993). The genetic variation at apo E locus and particularly the higher frequencies of ϵ 4 allele may be a contributory factor for coronary heart disease (CHD) risk in caucasians (Gerdes et al. 1992). India with its multiethnic composition is alarmingly showing an increasing trend in CHD prevalence (Gupta and Gupta 1996). The genetic polymorphism at this locus in Indian populations may provide an opportunity to understand the cause of CHD risk.

A number of studies were conducted on apo E polymorphism among European and mixed

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North American populations and very few studies in the remaining parts of the world. No study is available on Indian populations except one on the migrant Indians living in Singapore (Hallman et al. 1991). In the present study the genetic variation at apo E locus has been detected on two populations: the Kshatriyas and the Malas from rural areas of Andhra Pradesh state, India. The purpose of this paper is two fold: 1. to report the apo E genotypes and gene frequencies for the Indian sample and 2. to compare the results of the present study with the available studies on apo E polymorphism from other Asian populations.

MATERIAL AND METHODS

The sample for the present study were drawn from two populations: the Kshatriya (n=182) and the Mala (n=38). The sample of Kshatriya was drawn from Chittoor (12.37-14.08N and 78.03 to 79.50E) and Cuddapah (13.14 -15.14N and 77.85-79.29E) districts and for the Mala from Chittoor district (12.37-14.08N and 78.03 to 79.50E) of Andhra Pradesh, India. The age of the subjects range from 20-70 years. Five ml of blood was drawn from each subject into tubes containing disodium ethylenediamine tetracetate (EDTA) as anticoagulant (1mg/ml). The buffy coat in between the plasma and red cells was transferred into another prelabelled tubes on centrifugation at 2500 rpm for 20 minutes. The buffy coat specimens were transported by air on dry ice to the Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh (USA). The DNA was extracted from these buffy coat samples on treating them with sodium dodecyl sulphate (SDS) (5%) and proteinase K (100 µg/ml) at 55°C for 16 hrs (Kan and Dozy 1978). After phenol extraction, the DNA was precipitated with ethanol and resuspended (1.0 mg/ml). The apolipoprotein E genotyping was determined by using restriction enzyme isoform genotyping method of Hixson and Vernier (1990).

Statistical Analyses

Gene frequencies were calculated using gene-counting method. A contingency χ^2 test was applied in between the sample of two Indian

populations to test for homogeneity in genotype frequencies. The difference in apo E genotype frequency distribution between the Indian sample and other Asian populations were determined by χ^2 test. A cluster analysis was performed by single linkage minimum distance method (Sokal and Sneath 1963) to suggest the clusters of different Asian populations. To perform cluster analysis, Euclidian distances based on ϵ_2 and ϵ_4 gene frequencies for 17 populations (including the present study sample) were estimated with the help of SPSSx package.

RESULTS AND DISCUSSION

Five genotypes in the Kshatriya (2/3, 2/4, 3/3, 3/4, 4/4) and three genotypes in the Mala (3/3, 3/4, 2/3) representing three common alleles, ϵ_2 , ϵ_3 , ϵ_4 were observed. In both the populations the 3/3 is the most common genotype followed by 3/4 and 2/3. In the Kshatriya the 4/4 and 2/4 were observed with a less frequency and in the Mala no case was found with 4/4 and 2/4 genotypes. The gene frequencies for the Kshatriya were $\epsilon_2 = 0.0522$, $\epsilon_3 = 0.8516$ and $\epsilon_4 = 0.0962$. The gene frequencies for apo ϵ_2 , ϵ_3 and ϵ_4 alleles in the Malas were 0.0395, 0.9210 and 0.0395. Based on the results of the contingency χ^2 test, it is observed that there is no significant difference between the genotype frequencies of the two populations, which might be probably due to the fact that the sample of the two populations were drawn from the same geographical region. The sample of the two populations were therefore pooled and treated as a single homogenous sample called the Indian sample and this is being used to compare with the results of the other Asian populations. The gene frequencies in the pooled sample for apo ϵ_2 , ϵ_3 and ϵ_4 alleles were 0.0500, 0.8636 and 0.0864, respectively. In the Kshatriya, the Mala and in the pooled sample, the observed genotypes were in Hardy-Weinberg equilibrium (Kshatriya: $\chi^2_3 = 0.14$; Mala: $\chi^2_3 = 0.04$ and Pooled sample: $\chi^2_3 = 0.1$).

Apolipoprotein E Gene Frequencies of the Present Study in Comparison with Other Asian Populations

The apo E gene frequencies for the Indian

and other Asian populations were presented in table 1. It was observed that most of the populations from Japan and the two Indian populations were characterized by the low frequency of apo $\epsilon 2$ allele compared to the populations from China. However, the frequency of $\epsilon 4$ allele was higher in most of the Japanese populations. Interestingly among other Asian populations the migrant Indians living in Singapore have the highest frequency of $\epsilon 4$ allele with 0.127 (Hallman et al. 1991).

The χ^2 values obtained between the Indian sample and the other Asian populations were also presented in table 1. The majority of the Asian populations from Japan and Indians living in Singapore (IND_(s)) showed an insignificant dif-

ference with the present study sample and most of the Chinese populations showed significant difference. The populations of China (CH2), Singapore (MAS, SIN) have showed comparatively higher χ^2 value which may be due to the greater frequency of the apo $\epsilon 2$ allele in those populations.

Cluster Analysis

India, due to historical, cultural, religious and linguistic differences, shows wide genetic diversity, it is of interest to investigate the status of Indian sample for apo E polymorphism among the Asian populations. Therefore, for the purpose of comparison the present study population along with 16 other Asian populations for

Table 1: Apolipoprotein E allele frequencies among the Asian populations

Population Studied	N	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	χ^2 (df=2)	Reference	Definition of the study population	Code
Indians	220	0.050	0.863	0.086		Present study	Unrelated individuals from two populations of Andhra Pradesh state	IND
Indians (Singapore)	142	0.046	0.827	0.127	3.16	Hallman et al., 1991	Unrelated blood bank donors, immigrants from India living in Singapore	IND (S)
Chinese (Singapore)	190	0.097	0.829	0.074	6.92*	Hallman et al., 1991	Unrelated blood bank donors, immigrants from China living in Singapore	CH1
Chinese (Beijing)	507	0.124	0.806	0.070	18.80**	Bao-Sheng et al., 1988	Randomly selected population	CH2
Chinese (Beijing)	95	0.053	0.883	0.064	0.89	Wang, 1986	Healthy volunteers	CH3
Chinese (Montreal)	101	0.114	0.822	0.064	9.21**	Wang, 1986	Immigrants from China living in Montreal	CH4
Malaysians (Singapore)	118	0.114	0.767	0.119	12.12**	Hallman et al., 1991	Immigrants from Malaysia living in Singapore	MAS
Singapore	188	0.122	0.782	0.096	14.47**	Utermann, 1987	Unrelated individuals of Chinese, Indians and Malays	SIN
Japanese (Kyushu & Fukuoka)	319	0.081	0.849	0.067	4.98	Hallman et al., 1991	Unrelated medical professional students	JA1
Japanese (Osaka)	208	0.067	0.829	0.103	2.01	Yamamura et al., 1990	CHD case-control study.	JA2
Japanese (Niigata)	129	0.093	0.787	0.120	7.60*	Miida, 1990	CHD case-control study	JA3
Japanese (Kuamoto)	188	0.035	0.872	0.093	1.19	Kabori et al., 1988	Hyperlipidemic case-control study	JA4
Japanese (Sendai)	107	0.061	0.822	0.117	2.05	Sano et al., 1988	Hyperlipidemic case-control study	JA5
Japanese (Asahikawa)	576	0.037	0.846	0.117	4.26	Eto et al., 1986	Apparently healthy unrelated volunteers	JA6
Japanese	305	0.037	0.861	0.102	1.54	Horita et al., 1993	Randomly selected civil service workers	JA7
Japanese (Hiroshima & Nagasaki)	110	0.023	0.891	0.086	2.74	Asakawa et al., 1985	Healthy volunteers	JA8
Japanese (Tokyo)	197	0.038	0.843	0.119	2.20	Tsuchiya et al., 1985	Healthy outpatients	JA9

Note: P values shown in column 6 are < 0.05 (*) and < 0.01 (**)

which the data on apo E polymorphism were available have been considered. The dendrogram was constructed based on $\epsilon 2$ and $\epsilon 4$ allele frequencies to suggest the cluster of different Asian populations (Fig.1). The dendrogram depicts three clusters and were named, A, B and C. The cluster A includes three populations one each from Singapore (SIN), Malaysia (MAS) and Japan (JA3). These populations form a cluster though they are not closely related. The cluster B has four populations, of which three are from China (CH1, CH2 and CH4) and one is from Japan (JA1). In cluster B the two Chinese populations (CH2 and CH4) are closely related. The cluster C includes 10 populations and it is further divided into three sub clusters. The sub cluster C_1 has two (JA2 and JA5) and C_2 has seven populations (IND_s, JA9, JA6, JA5, JA4, JA7 and IND). One population from China (CH3) has been formed into a separate cluster and named as C_3 . The Indian sample from Andhra Pradesh shows close relationship with Japanese

populations due to closer allele frequencies.

Coronary artery disease is a major cause of death all over the world. South Asians have been found to have the highest mortality rates due to coronary artery disease amongst all ethnic groups so far studied (Dhawan and Petkar 1998). The conventional risk factors of the disease, namely high cholesterol, smoking and high blood pressure do not explain all the differences in mortality due to this disease. South Asians do tend to suffer more from diabetes, higher insulin levels, abdominal obesity, low high density lipoprotein levels, higher triglyceride levels, lower levels of physical exercise and higher lipoprotein (a) levels. While the exact cause of this increased rate of mortality due to coronary artery disease has yet to established. It appears that both genetic and environmental factors play a role. When the contribution of apo E polymorphism to the incidence of cardiovascular disease across populations of the world was considered, most of the available data are suggestive of a beneficial in-

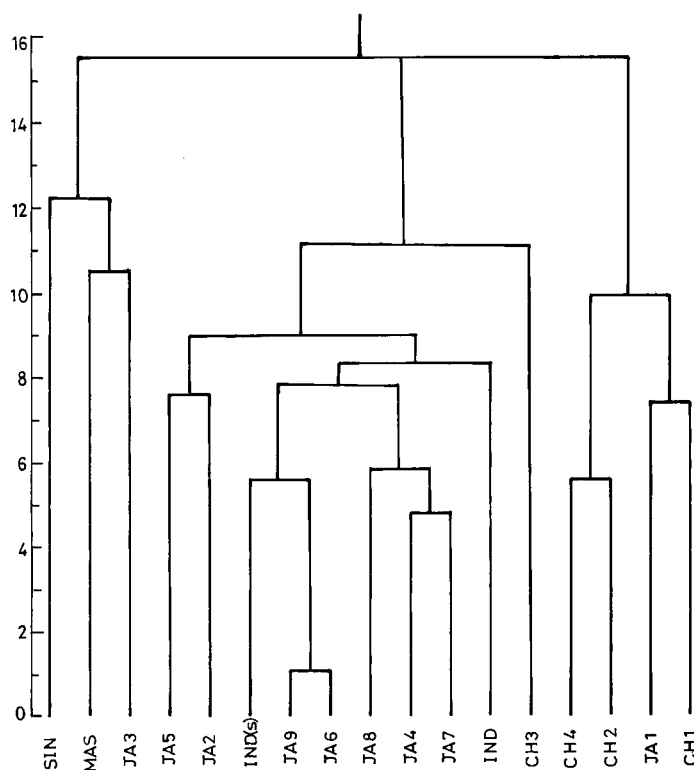


Fig. 1. Dendrogram showing the cluster of 17 Asian Populations based on Apo $\epsilon 2$ and $\epsilon 4$ allele frequencies

fluence of the $\epsilon 2$ allele on cardiovascular disease, while the $\epsilon 4$ allele appears to predispose to cardiovascular disease (Davignon et al. 1988; Davignon 1993). The studies on caucasian populations have showed that the apo E polymorphism, and particularly the population frequency of $\epsilon 4$, may contribute to the interpopulation variability in CHD mortality rates (Gerdes et al. 1992; Sing and Moll 1989). The frequency of the $\epsilon 4$ allele tends to be higher in populations with higher CHD mortality rates eg., the Finns (Ehnholm et al. 1986) and lower in those with lower rates (eg., the Japanese, Chinese).

Epidemiological studies in migrant Indians have clearly demonstrated that people of South Asian origin particularly the emigrant Indians have one of the highest incidence of coronary heart disease in the world (Mc Keigue 1989; Vardan et al. 1995). Epidemiological surveys in native Indians have showed that the prevalence of CHD though increasing it is low among rural populations (Gupta and Gupta 1988). Since the frequency of $\epsilon 4$ allele has been a contributory factor for the differences in CHD mortality rates in caucasians (eg., Finns) the allelic variation at apo E locus may provide an opportunity to understand the cause of this CHD mortality in Indians. The frequency of $\epsilon 4$ allele in the present study is lower (0.086) than in migrant Indians (0.127) (Hallman et al. 1991). The rural Indians are having lower prevalence of CHD and its associated risk factors compared to urban ones (Gupta and Gupta. 1998; Venkatramana and Chengal Reddy 1999). Since the present study sample has been drawn from rural areas, the low frequency of $\epsilon 4$ allele in the present study sample might be one of the reasons for the lower frequency of CHD prevalence in rural Indian populations. Also unlike most western societies, Indian populations rely on a diet that is low in animal fat (Mitchell et al. 1993). To understand more clearly about the contribution of apo E genetic variation to the cardiovascular diseases in Indian populations, further research is required using prospective study designs and a multi-regional community based study sample.

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