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Apolipoprotein C3 (*Sst*I) Gene Variability in Northwest India: A Global Perspective

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KEYWORDS Protein; ethnic groups; polymorphism; heart disease; medical genetics

ABSTRACT Apolipoprotein C3 plays an important role in the receptor mediated hydrolysis of triglyceride rich lipoproteins (TRLs) by inhibition of lipoprotein lipase (LPL), delayed clearance of which causes hypertriglyceridemia (HTG). Indians are considered to be more vulnerable to the adverse effects of hypertriglyceridemia and consequently its probable sequel of cardiovascular disorders. Several studies have revealed the association of rare allele (S2) of APOC3 (*Sst*I) polymorphism with dyslipidemias and coronary artery diseases. In order to investigate the role and relevance of this polymorphism in Northwest India, the present study aimed to investigate the genetic variation of 3' untranslated region of APOC3 (*Sst*I) in 312 individuals belonging to four endogamous groups (Banias, Brahmins, Jatsikhs and Khatris) of Punjab. Uncommon *S2 allele frequency was 22.6%, 22.5%, 22.7% and 26.2% in Banias, Brahmins, Jatsikhs and Khatris respectively. Higher heterozygosity of 0.39 in Khatris reflected their greater variation at this locus than the other populations. Chi-square analysis did not reveal any significant differences between these populations and other studies from North India ($P > 0.05$). Comparative analysis of 66 other populations across the world revealed large heterogeneity at this locus whereby, Mongoloid populations have the highest frequencies of *S2 allele (0.19 to 0.48) followed by Indians (0.18 to 0.29), Africans (0.04 to 0.27) and Caucasian populations (0.01 to 0.12). Genetic distance and multivariate analyses showed that Indian population is quite distinct from other Caucasian and Oriental populations. Clinal heterogeneity of predisposing *S2 allele in Asia showed an increasing cline ($y = 0.0043x + 0.1209$, $R^2 = 0.1162$) towards North. As this allele is associated with HTG and other cardiovascular complications, differential variation in different populations may have insightful implications for association and medical genetic studies.

INTRODUCTION

APOC3 (protein: apoC-III; gene: APOC3) is a constituent of very low density lipoproteins (VLDL), chylomicron remnants and high density lipoprotein (HDL) particles (Mahley et al. 1984). It helps in the hepatic uptake of triglyceride (TG) rich lipoproteins (TRLs) and their remnants. Molecular perturbations of APOC3 gene hampers the function of receptor mediated hydrolysis of circulating TRLs which augments the development of hypertriglyceridemia (Zeng et al. 1995), myocardial ischemia (Ginsberg et al. 1995) and carotid artery atherosclerosis (Sharrett et al. 1995).

The APOC3 gene, flanked by APOA1 and APOA4 genes in a 15kb cluster, is localized on 11q23.3 (Bruns et al. 1984). The cytosine (C, *S1 allele) to guanosine (G, *S2 allele) substitution in 3'-untranslated region (3'UTR) of exon 4 in the APOC3 gene gives rise to an *Sst*I restriction site (Karathanasis 1985) which has been studied extensively in relation to various lipid disorders. The frequency of *S2 allele varies greatly among populations of the world ranging from 0% in Europeans (Stocks et al. 1987) to 39% in Japanese (Paul et al. 1987). The significant association of this allele with coronary heart disease (CHD) and diabetes (Rigoli et al. 1995) is partly attributed to the elevated triglycerides, plasma cholesterol, HDL and APOC3 levels (Rigoli et al. 1995; Ordovas et al. 1991) with some conflicting reports (Paul-Hayase et al. 1992; Tybjaerg-Hansen et al. 1993). As lipid levels are strongly related to cardiovascular disease risk (La Rosa et al.1990), it has been proposed that variation in the population frequency of *S2 allele may genetically modulate lipids and lipoproteins differently in various populations and hence, conferring different disease risks.

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A large amount of data has been generated globally but, studies documenting the APOC3 gene variability in Indian population are scarce. Therefore, the present study was carried out to enlarge our knowledge about the functional consequences of APOC3 polymorphism in four ethnic groups belonging to different social strata of Punjab, a Northwest state of India. In addition, we have carried out the global evaluation of these allele frequencies across 66 populations of the world and explored some plausible reasons of its wide differential disparity. These allelic dissimilarities may have insightful implications in disease association and medical genetic studies.

MATERIALS AND METHODS

***Study Subjects*:** The present study comprised of total 312 (228 males, 84 females) unrelated and apparently normal individuals ranging in age from 20 to 65 years (mean age ± SEM, 44.46 ± 1.28). The participants belonged to one of the four main ethnic groups, Banias (n = 73), Brahmins (n = 69), JatSikhs (n = 88) and Khatris (n = 82) from various places of Punjab representing social hierarchy and spoke both Punjabi and Hindi languages.

Ethnic Profiles of the Study Population: The people of Punjab are considered as the progeny of a mixture of many proto and post harappan invaders who entered this land from west and became original settlers of this area (Rose 1970). Banias in Punjab belong to Vaishya Varna of Indian caste system and mainly a mercantile community. Brahmins of Punjab are primarily Saraswat Brahmins and their vocation is teaching, performing priestly chores alongwith trade and industry. Khatri, a variant of the Sanskrit word Kashtrya were nomadic invaders who came and expanded into Central Asia and Europe, creating the similarities in Indo-European languages. The JatSikhs are the main agricultural community distributed all over Punjab. They are thought to be the progeny of Indo-Scythian / Indo-European / Indo-Aryan stocks. All these castes practise endogamy at the caste level and exogamy at gotra level. However, in the changing socio-economic circumstances, the rigidity in not having matrimonial alliances with the other castes is diluted.

***APOC3 Genotyping*:** Blood was drawn from an antecubital vein into tubes containing disodium ethylenediamine tetra acetate (EDTA) to a final concentration of 1.5 mg/ml. DNA was extracted from whole blood following standard phenol chloroform method. For *Sst*I polymorphic site, 596 bp fragment of APOC3/3'UTR was amplified using forward primer: 5'-CAT GGT TGC CTA CAG GAG TTC-3' and reverse primer: 5'-TGT CGA AAC ACG CCT TCC AGT-3'. The PCR amplification was carried out in a final reaction volume of 25µl containing ~100ng of genomic DNA, 0.4mM primers (Bangalore Genei, India), Genei PCR master mix (containing 0.2mM each of dATP, dCTP, dGTP, and dTTP, 1.25 units Taq DNA polymerase, 2.5mM $MgCl_2$). After the initial denaturation at 95°C for 5 minutes, 35 PCR cycles were carried out as 95°C = 1 minute, 58°C =45 seconds, 72°C =1 minute with the final extension at 72°C for 7 minutes. The PCR products were digested with 10 units of *Sst*I (New England Biolabs Inc., USA) in the presence of 1 µl of 10X buffer provided with the restriction enzyme for 3 hours. The restricted products were electrophoresed on a 3% agarose gel prepared with TBE buffer and 0.5mg/ml of ethidium bromide and visualized in a UV inspection unit. Internal validation of these polymorphisms was done by reanalysing some samples to avoid bias in genotyping error.

***Statistical Analyses*:** Allele frequencies were estimated by gene counting method. The departure from Hardy-Weinberg equilibrium (D_A) along with the statistical significance of RFLP was calculated according to the formulae

$$D_A = P_{2/2} - p_2^2 \text{ and } \chi^2 (D_A) = n D_A^2 / p_1^2 p_2^2$$

given by Haviland et al. (1991), where *P* denotes genotype frequency; *p* denotes relative allele frequency; 1 and 2 denote the frequent and rare allele respectively; n is the number of subjects studied. Average heterozygosities were calculated by gene identities. Gene diversity analysis of H_T, H_S and G_{ST} statistics were calculated following Nei's (Nei et al. 1983) method. DA genetic distance (Nei et al. 1983) was calculated and the multidimensional distance matrix was reduced into UPGMA and Neighbor joining dendrograms. Correspondence analysis was carried out using NTSYSpc programme.

RESULTS

The distribution of APOC3 genotypes, estimated allele frequencies, heterozygosities (H) and their departure from Hardy Weinberg equilibrium (D_A) is summarized in table 1. Allele frequencies of *S1 and *S2 in all the studied

Table 1: Genotype and allele frequencies of APOC3 (*Sst*I) in four ethnic groups of North India.

Populations (Numbers)	*S1S1*	*S1S2*	*S2S2*	*S1 ± S.E*	*S2 ± S.E*	*H*	D_A	χ^2
Banias (73)	45 (43.73)	23 (25.54)	5 (3.73)	0.774 ± 0.049	0.226 ± 0.049	0.35	0.017	0.723
Brahmins (69)	41 (41.48)	25 (24.04)	3 (3.48)	0.775 ± 0.05	0.225 ± 0.05	0.35	-0.007	0.111
Jatsikhs (88)	54 (52.54)	28 (30.91)	6 (4.54)	0.773 ± 0.045	0.227 ± 0.045	0.35	0.017	0.78
Khatris (82)	47 (44.64)	27 (31.73)	8 (5.64)	0.738 ± 0.049	0.262 ± 0.049	0.39	0.029	1.82
Pooled (312)	187(182.31)	103(112.37)	22 (17.31)	0.764 ± 0.024	0.236 ± 0.024	0.36	0.015	2.17

Values in parenthesis are expected numbers, H-Expected Heterozygosity, D_A- Departure from Hardy Weinberg Equilibrium

populations showed trifle variations except Khatris where *S1 and *S2 showed slightly higher dissimilarity than Banias, Brahmins and JatSikhs. All the populations were in Hardy Weinberg equilibrium and did not show any significant variation (P > 0.05). Khatris showed higher heterozygosity (39%) than other ethnic groups which reflected their higher heterogeneity and more gene flow than Banias, Brahmins and JatSikhs. However, the mean estimated heterozygosity 0.36 ± 0.009 in Northwest Indian populations (pooled) showed similitude with the Asian value (≈ 37%). The overall pattern of allele frequency in the present study groups was found to be comparable with Asians but quite dissimilar to Caucasians.

The estimated allele frequencies and heterozygosities of APOC3 in various populations across the world are given in table 2. An examination of this table clearly shows that the APOC3 polymorphism shows greater variation in Asian populations and demonstrates relatively lower polymorphism in Europeans. In Caucasians *S2 allele was found to be the highest in Greeks (0.16) and lowest in Caucasians of UK (0.01) whereas, Mongoloids were found to exhibit the highest *S2 allele frequency**,** (0.48) in Chinese followed by Japanese (0.39) and Koreans (0.30). Average allele frequencies weighted upon sample size along with gene diversity analysis has been compiled in table 3. Here, the Japanese (0.36) but not the Chinese (0.298) showed highest weighted frequencies of *S2 followed by Koreans (0.278), Indians (0.268) and least in Caucasians (0.079). Chinese (44.3%), Japanese (44%), Indians (36.7%) and Asians (32.1%) showed higher

Table 2: Allele frequencies and heterozygosities of APOC3 (*Sst*I) polymorphism in various populations of the World.

Study Populations	*S1*	*S2*	*N*	*H*	*Authors*	*Label*
Caucasians in Europe						
Austrians	0.890	0.110	118	0.196	Paulweber et al. 1988	Au
Belgians	0.890	0.110	162	0.196	Paul-Hayase et al. 1992	Be
Danes	0.910	0.090	261	0.164	Tyajaerg-Hansen et al.1993	Da
Finland 1	0.920	0.080	61	0.147	Aalto-Setala et al. 1987	Fi1
Finland 2	0.860	0.140	50	0.241	Miettinen et al. 1994	Fi2
France (Lille)	0.931	0.069	152	0.128	Kee et al. 1999	Frl
France (Strasbourg)	0.906	0.094	208	0.17	Kee et al. 1999	Frs
France (Toulouse)	0.912	0.088	215	0.161	Kee et al. 1999	Frt
Greeks	0.840	0.160	50	0.269	Vavatsi et al. 1995	Gr
Italians 1	0.920	0.080	107	0.147	Xu et al. 1990	It1
Italians 2	0.920	0.080	314	0.147	Marasco et al. 1993	It2
Italians 3	0.919	0.081	62	0.149	Rigoli et al. 1995	It3
Ireland (Belfast)	0.944	0.056	186	0.106	Kee et al. 1999	Irb
Norway	0.880	0.120	33	0.211	Kessling et al. 1986	No
Scotland	0.880	0.120	117	0.211	Morris and Price, 1985	Sc
UK (Bristol)	0.920	0.080	90	0.147	Kessling et al. 1988	Uk2
UK (Caucasians)	0.990	0.010	92	0.02	Paul et al. 1987	Uk3
UK (Caucasians)	0.980	0.020	74	0.039	Ferns and Galton 1986	Uk4
UK (London)	0.980	0.020	35	0.039	Trembath et al. 1987	Uk6
UK (London)	0.980	0.020	47	0.039	Ferns et al. 1985	Uk7
UK (London)	0.980	0.020	31	0.039	Shoulders & Baralle, 1986	Uk8
UK (London)	0.960	0.040	81	0.077	Vella et al. 1985	Uk9
UK (London)	0.940	0.060	73	0.113	Kessling & Humphries, 1985	Uk10
UK (London)	0.930	0.070	123	0.13	Ferns and Galton, 1986	Uk11

Table 2: Contd....

Study Populations	*S1*	*S2*	*N*	*H*	*Authors*	*Label*
Mediterraneans	0.910	0.090	129	0.147	Antonarakis et al. 1988	Me
Caucasians Elsewhere						
Canada (Quebec)	0.907	0.093	252	0.169	Haviland et al. 1991	Ca1
Canada (Vancouver)	0.950	0.050	38	0.095	Hayden et al. 1987	Ca2
US (Boston)	0.940	0.060	160	0.113	Hegele et al 1989	Us1
US(Boston)	0.920	0.080	145	0.147	Ordovas et al. 1991	Us2
US (Boston)	0.896	0.104	371	0.186	Liu et al. 2004	Us3
US (Iowa)	0.920	0.080	36	0.147	Anderson et al. 1986	Us4
US (Seattle)	0.940	0.060	101	0.113	Deeb et al. 1986	Us5
US (Seattle)	0.910	0.090	366	0.164	Thompson et al. 1988	Us6
US (Whites)	0.905	0.095	823	0.172	Hallman et al. 2006	Us7
Australia-Anglo Irish	0.940	0.060	47	0.113	Buzza et al. 2001	AAI
Australia-Greeks 1	0.970	0.030	80	0.058	Buzza et al. 2001	AG1
Austrlalia-Italians 4	0.880	0.120	76	0.211	Buzza et al. 2001	AIt4
Chinese						
Chinese 1	0.770	0.230	151	0.354	Saha et al. 1995	Ch1
Chinese 2	0.610	0.390	73	0.476	Xiang et al. 1989	Ch2
Chinese 3	0.520	0.480	20	0.499	Rees et al. 1985	Ch3
Japanese						
Japanese 1	0.810	0.190	21	0.308	Rees et al. 1985	Jp1
Japanese 2	0.650	0.350	34	0.455	Paul et al. 1987	Jp2
Japanese 3	0.631	0.369	65	0.466	Bai et al. 1995	Jp3
Japanese 4	0.630	0.370	68	0.466	Thompson et al. 1988	Jp4
Japanese 5	0.620	0.380	74	0.471	Aburatani et al. 1988	Jp5
Japanese 6	0.610	0.390	35	0.476	Rees et al. 1986	Jp6
Koreans						
Korea (Seoul)	0.755	0.245	92	0.37	Hong et al. 1997	Ko1
Korea (Seoul)	0.699	0.301	131	0.421	Song et al. 1998	Ko2
Africans						
Africa (Blacks)	0.850	0.150	20	0.255	Rees et al. 1985	Af1
African- Americans	0.832	0.168	360	0.28	Hallman et al. 2006	Af2
Blacks	0.950	0.050	67	0.077	Antonarakis et al. 1988	Af3
South Africa	0.940	0.060	42	0.113	Henderson et al. 1987	Sa
UK (Blacks)	0.870	0.130	53	0.226	Thompson et al. 1988	UkB
UK (Negroes)	0.730	0.270	28	0.394	Paul et al. 1987	UkN
Asians						
Arabs 1	0.980	0.020	31	0.039	Tas 1989	Ar1
Arabs 2	0.840	0.160	63	0.269	Johansen et al. 1991	Ar2
Arabs 3	0.96	0.04	69	0.077	Johansen et al. 1990	Ar3
India (Punjabi Khatris)	0.738	0.262	82	0.387	Present study	Ipk
India (Punjabi Banias)	0.775	0.225	69	0.349	Present Study	Ipb
India (Punjabi Brahmins)	0.774	0.226	73	0.35	Present study	Iph
India (Punjabi JatSikhs)	0.773	0.227	88	0.351	Present study	Ipj
North West India 1	0.764	0.236	312	0.361	Present study	Nwi1
Indian Asians	0.820	0.180	28	0.295	Rees et al. 1985	Ia
North India 2	0.704	0.296	216	0.417	Chhabra et al. 2003	Ni2
North India 3	0.688	0.312	151	0.425	Chhabra et al. 2004	Ni3
UK (Indian Asians)	0.81	0.19	24	0.308	Paul et al. 1987	UkI
Philipinos	0.770	0.230	84	0.354	Johansen et al. 1990	Ph
Taiwanese	0.736	0.264	159	0.389	Huang et al. 2006	Ta
Vietnamese	0.674	0.326	351	0.439	Thu et al. 2006	Vi
Other Ethnic Groups						
Brazil	0.890	0.110	100	0.196	Relvas et al. 2005	Br
Dogrib Indians	0.750	0.250	130	0.375	Cole et al. 1989	Di

average heterozygosities than Africans (22.4%) and Caucasians (13.7%) which manifest their greater differentiation at this locus (Table 3). It has been delineated that genetic diversity at APOC3 locus in Caucasians has the lowest total ($H_T = 0.141$) and within subpopulation ($H_S = 0.137$) gene diversities whereas Chinese showed these indices to be the highest ($H_T = 0.464$, $H_S = 0.443$). The percent of genetic differentiation (G_{ST}) attributable between populations relative to total

Table 3: Compiled data showing genetic diversity analysis of 70 populations across the world

Population groups (number)	*Average estimated allele frequencies*		*Average frequencies weighted upon sample size*		*Gene Diversity Analysis*		
	**S1*	**S2*	**S1*	**S2*	G_{ST}	H_T	H_S
Caucasians in Europe (25)	0.924	0.076	0.921	0.079	0.026	0.141	0.137
Caucasians elsewhere (12)	0.926	0.074	0.914	0.086	0.011	0.138	0.136
Chinese (3)	0.633	0.367	0.702	0.298	0.046	0.464	0.443
Japanese (6)	0.658	0.342	0.637	0.363	0.021	0.450	0.440
Koreans (2)	0.727	0.273	0.722	0.278	0.004	0.397	0.396
Africans (6)	0.855	0.145	0.851	0.149	0.094	0.247	0.224
Arabs (3)	0.880	0.120	0.846	0.154	0.048	0.211	0.201
Asians (11)	0.88	0.12	0.846	0.154	0.028	0.380	0.369
Indians (8)	0.752	0.248	0.732	0.268	0.035	0.374	0.360
All populations (70)	0.844	0.156	0.854	0.146	0.108	0.263	0.234

genetic diversity was observed to be the highest in Africans (0.094) followed by Arabs (0.048), Chinese (0.046) and Indians (0.035). Considering all populations together only 10.8% of the total genetic diversity has been apportioned to between population variation.

The unbiased genetic distance (DA) was calculated for all 70 populations but the relationship was difficult to display and interpret. Therefore, allele frequencies for major groups were pooled and used for genetic distance calculations. In this analysis, all Caucasians (Europe and elsewhere) were pooled. The resultant multidimensional data matrix is reduced into UPGMA and Neighbor-joining dendrograms (Fig. 1A and 1B). Contrary to the expectations, Northwest Indian populations (pooled) form a distinct single point cluster separated widely from particularly the Caucasians and aligned as outer element to the cluster of East Asian populations.

Correspondence analysis of 70 populations is presented in figure 2. In this plot, Caucasian

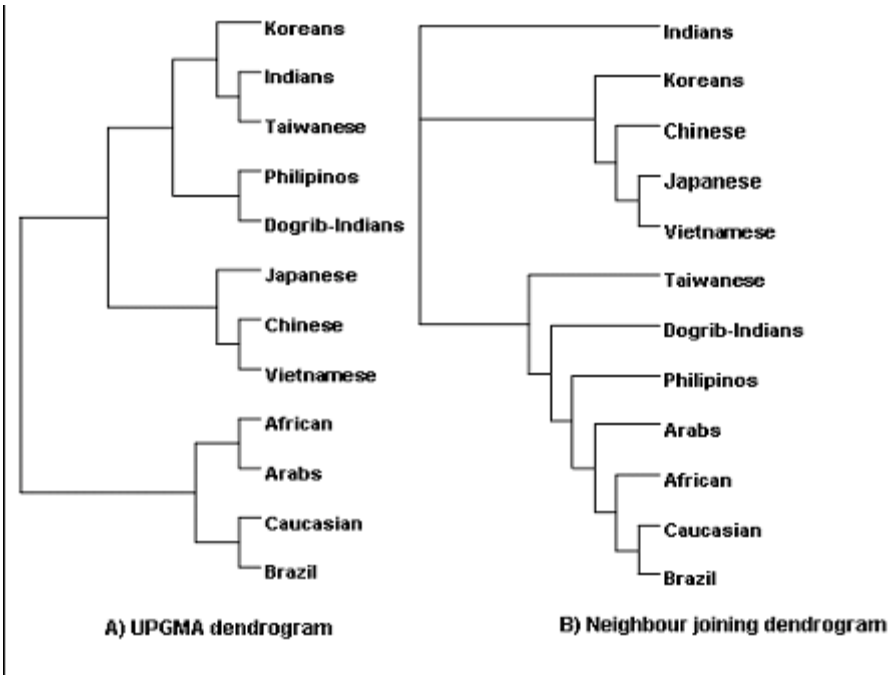

Fig. 1. UPGMA and neighbour joining dendrograms of main populations based upon APOC3 *Sst*I polymorphism

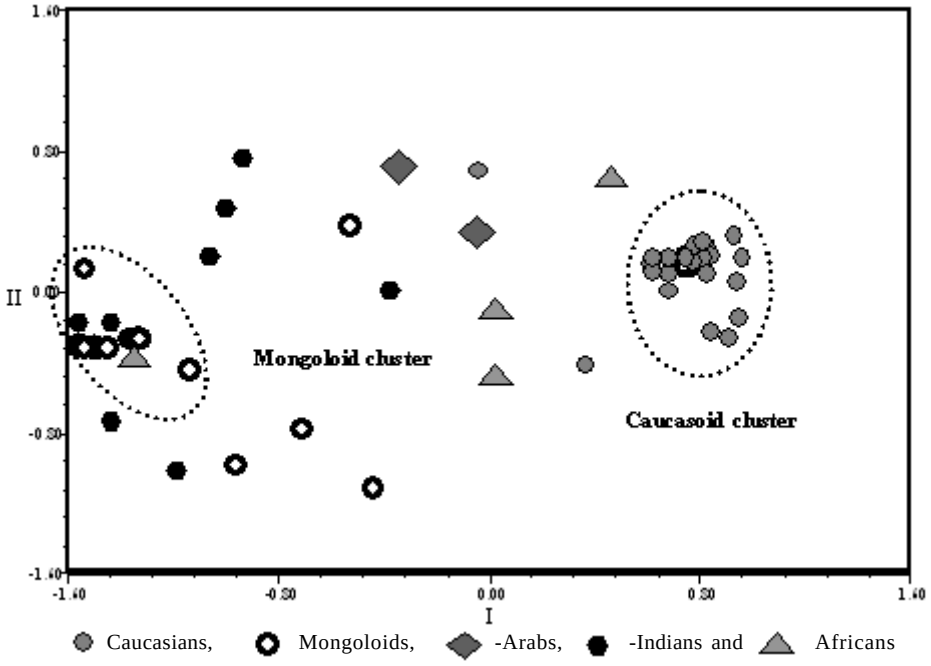

Fig. 2. Correspondence Analysis Plot of APOC3 allele frequencies

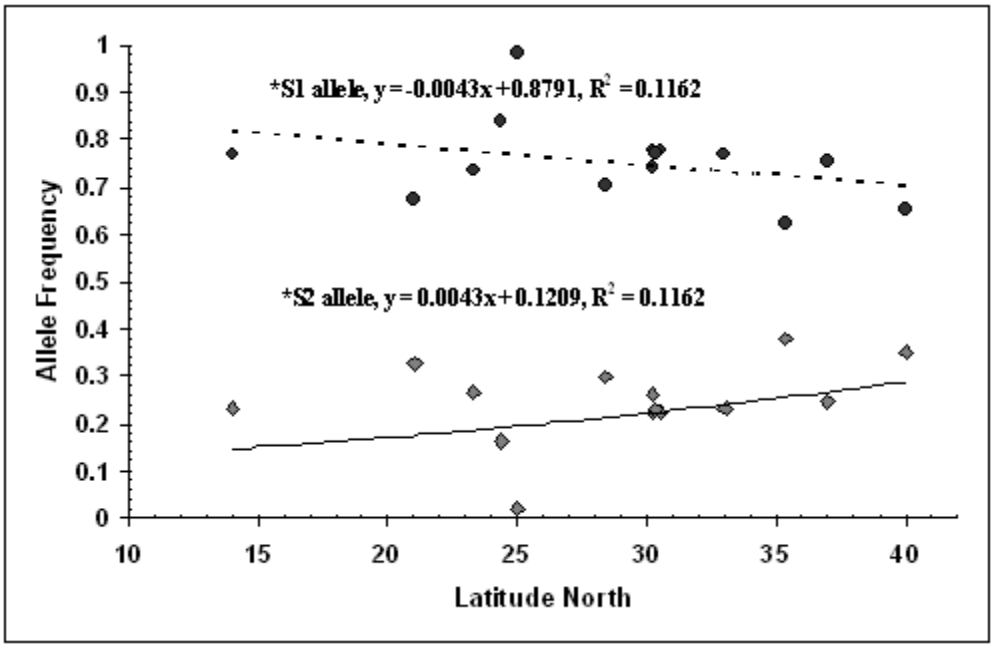

Fig. 3. Clinal heterogeneity of APOC3 in Asia

populations from all over the world constitute a tight cluster on the right-hand side, while majority of Mongoloid along with Northwest Indian groups are on the left hand side. The African population groups along with Arabs are scattered in the middle. Three populations (Brahmins, Banias and JatSikhs) of Punjab form their own loose cluster on top left-hand side of the plot. Punjabi Khatri who showed slightly higher frequency of *S2 allele is isolated from other Punjabi populations and is aligned towards Mongoloid populations (lower left hand side on the plot). The CA plot needs caution in interpretations as it is based only on single locus. Overall, the plot clearly shows that Indians form a distinctive group with reference to this locus.

Latitudinal correlated APOC3 polymorphism in Asia has been shown in figure 3 where decreasing cline of *S1 allele ($y = - 0.0043x + 0.8791$, $R^2 = 0.1162$) and increasing cline of *S2 allele ($y = 0.0043x + 0.1209$, $R^2 = 0.1162$) against the function of latitude North are evident which is found to be opposite in case of Europe (not shown).

DISCUSSION

The main aim of this study was to investigate the genetic heterogeneity of APOC3 *Sst*I locus in four ethnically well defined populations i.e. Banias, Brahmins, Jat, Sikhs and Khatris of Punjab, a Northwest state of India which is considered to be the high incidence CHD belt. The alleles at APOC3 *Sst*I locus display considerable variation between the present study and various other populations of the world confirming the wide range of incongruity at this locus both at regional and inter-regional level.

It is important to investigate different kinds of polymorphisms in different genes to understand spectrum and functional consequences of mutations. Therefore, it is recognized that investigation of APOC3, 3'UTR polymorphism is important as it has been associated with elevated serum triglycerides as well as increased risk of CHD in many populations and can be employed as an adjunct in CHD risk calculations, though not directly influencing or causing these disorders. Higher *S2 allele frequencies in various ethnic groups of Punjab, a high fat consuming belt of India, may shed light on the adverse effects of *S2 allele on these diseases. In the present sample, 40% of the individuals carried one or two copies of the susceptible allele and are therefore more susceptible for HTG and this may have public health consequences.

Regression analysis of APOC3 allele frequencies from 14 geographically well defined populations of Asia showed a contrast cline to that of European populations but none of the R^2 values are statistically significant, so the interpretation needs prudence. Such clinal heterogeneity lenses the evolutionary factors like selection of the gene, which may be informative in dealing with overseas patients of different regions. Due to different linkage disequilibrium scores and their expression, selection may favour one genotype or allele at one extreme and disfavours it at other.

Continentally in Asia, when percent CHD mortality was compared with *S2 allele frequency, its association with CHD is consistent in China, India and Korea but not compatible with Taiwan, Japan and S. Arabia. In Europe also, there are instances where *S2 allele is low in the areas where death certification (per 100, 000) because of CHD is high like Ireland, UK and Denmark. The above comparisons need caution as direct correlations with a multifactorial disease like CHD are unlikely to be consistent in all populations. These imperfect associations of *S2 allele expression with CHD in different populations of the world may have many unforeseen mechanisms involved. To begin with, it is possible that the influence of *Sst*I RFLP on plasma TG could be potentially confounded by other neighboring loci such as APOA5, a newly identified TG regulator and modifier gene localised in the close proximity of ApoAI-CIII-AIV gene cluster. Secondly, it is also possible that *Sst*I RFLP of APOC3 gene may be involved in modulating TGs differently in different ethnic groups because of its different linkage disequilibrium scores with other gene variants (coding or regulatory regions). Furthermore, as some haplotypes of APOC3 *Sst*I and promoter variants affects TG homeostasis, therefore; this polymorphism may have some impact on mRNA stability as suggested by Smith et al. (1992).

Ordovas et al. (2002) has demonstrated that APO A-I gene of its Apo AI-CIII-AIV gene complex may influence response to environmental factors such as dietary fats. Differences in diet can be important link in the wide variability of APOC3 *Sst*I polymorphism because dietary habits influence the same phenotypic CHD risk factor

as minor allele *S2 of this polymorphism (TG levels). From the last many years, it is well understood that it is better to monitor TG than cholesterol levels in determining hyperlipidemic status in countries like India, where generally low protein and high carbohydrate diet is consumed. In addition, Indians are considered to be more sensitive to the exacerbating effects of hypertriglyceridemia (Enas and Mehta 1995).

In the genetic landscape of APOC3 SstI polymorphism across various populations of the world, the nutritional behavior of some populations can be attributable to higher prevalence of *S2 allele despite having low CHD mortality for instance in Japan, China and S. Arabia. In these countries high protein and low carbohydrate/fat diet is consumed in comparison to India, Taiwan and Korea.

Perez-Martinez et al. (2001) has shown that *S2 allele may modulate the magnitude of atherogenesis and dyslipidemic state significantly ($P < 0.039$) when patients changed their diet from saturated fatty acids (SFA) to Mediterranean diet (olive oil). Moreover, APOC3 polymorphism affects insulin response to oral glucose tolerance test (OGTT) in persons consuming high saturated fats (Salas et al. 1998). Thus, APOC3 *Sst*I polymorphism plays an imperative role in determining the levels of lipid response to the dietary changes. Such inter-actions may be helpful to expose the link between inter population variability of CHD prevalence and APOC3 *Sst*I polymorphism in relation to the dietary behavior.

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REFERENCES

Aalto-Setala K, Kontula K, Sane T, Nieminen M, Nikkila E 1987. DNA polymorphisms of apolipoprotein A-I/C-III and insulin genes in familial hypertriglyceridemia and coronary heart disease. *Atherosclerosis,* **66:** 145-152.

Aburatani H, Matsumoto A, Itoh H, Murase T, Takaku F, Itakura H 1988. Deoxyribonucleic acid (DNA) polymorphism in the apolipoprotein AI gene: A study in a Japanese population. *Jpn J Med,* **27:** 56-59.

Anderson RA, Benda TJ, Wallace RB, Eliason SL, Lee J, Burns TL 1986. Prevalence and associations of apolipoprotein A-I linked DNA polymorphisms: results from a population study. *Genet Epidemiol,* **3:** 385-397.

Antonarakis SE, Oettgen P, Chakravarti A, Halloran SL, Hudson RR, Feisee L, Karathanasis SK 1988. DNA polymorphism haplotypes of the human apolipoprotein APOA1-APOC3-APOA4 gene cluster. *Hum Genet,* **80:** 265-273.

Bai H, Saku K, Liu R, Imamura M, Arakawa K 1995. Association between coronary heart disease and the apolipoprotein AI-CIII-AIV complex in a Japanese population. *Hum Genet,* **95:** 102-104.

Bruns GA, Karathanasis SK, Breslow JL 1984. Human apolipoprotein A-I—C-III gene complex is located on chromosome 11. *Atherosclerosis,* **4:** 97-102.

Buzza M, Fripp Y, Mitchell RJ 2001. Apolipoprotein AI and CIII gene polymorphisms and their association with lipid levels in Italian, Greek and Anglo-Irish populations of Australia. *Ann Hum Biol,* **28:** 481-490.

Chhabra S, Narang R, Krishnan LR, Vasisht S, Agarwal DP, Srivastava LM, Manchanda SC, Das N 2003. Apolipoprotein C3 SstI polymorphism and triglyceride levels in Asian Indians. *BMC Genet,* **6:** 3-9.

Chhabra S, Narang R, Lakshmy R, Vasisht S, Agarwal DP, Srivastava LM, Manchanda SC, Das N 2004. Apolipoprotein C3 SstI polymorphism in the risk assessment of CAD. *Mol Cell Biochem,* **259:** 59-66.

Cole SA, Szathmary EJ, Ferrell RE 1989. Gene and gene-product variation in the apolipoprotein A-I/C-III/A-IV cluster in the Dogrib Indians of the Northwest Territories. *Am J Hum Genet,* **44:** 835-843.

Deeb S, Failor A, Brown BG, Brunzell JD, Albers JJ, Motulsky AG 1986. Molecular genetics of apolipoproteins and coronary heart disease. *Cold Spring Harb Symp Quant Biol,* **1:** 403-409.

Enas EA, Mehta J 1995. Malignant coronary artery disease in young Asian Indians: thoughts on pathogenesis, prevention, and therapy. Coronary Artery Disease in Asian Indians (CADI) Study. *Clin Cardiol,* **18:** 131-135.

Ferns GAA, Galton DJ 1986. Haplotypes of the human apoprotein AI-CIII-AIV gene cluster in coronary atherosclerosis. *Hum Genet,* **73:** 245-249.

Ferns GAA, Stocks J, Ritchie C, Galton DJ 1985. Genetic polymorphism of Apolipoprotein CIII and insulin in survivors of myocardial infarction. *Lancet,* **2:** 300-303.

Ginsberg HN, Jones J, Blaner WS, Thomas A, Karmally W, Fields L, Blood D, Begg MD 1995. Association of postprandial triglyceride and retinyl palmitate responses with newly diagnosed exercise-induced myocardial ischemia in middle-aged men and women. *Arterioscler Thromb Vasc Biol,* **15:** 1829-1838.

Hallman DM, Srinivasan SR, Chen W, Boerwinkle E, Berenson GS 2006. Longitudinal analysis of haplotypes and polymorphisms of the APOA5 and APOC3 genes associated with variation in serum triglyceride levels: The Bogalusa Heart Study. *Metabolism,* **55:**1574-1581.

Haviland MB, Kessling AM, Davignon J, Singh CF 1991.

Estimation of Hardy-Weinberg and pairwise disequilibrium in the apolipoprotein AI-CIII-AIV gene cluster. *Am J Hum Genet,* **49:** 350-365.

Hayden MR, Kirk H, Clark C, Frohlich J, Rabkin S, McLeod R, Hewitt J 1987. DNA polymorphisms in and around the Apo-A1-CIII genes and genetic hyperlipidemias. *Am J Hum Genet,* **40:** 421-430.

Hegele RA, Hennekens CH, Breslow JL. 1989. Allele frequencies of apolipoprotein A-I and A-II gene locus DNA polymorphisms in Boston-based whites. *Hum Hered,* **39:** 174-178.

Henderson HE, Landon SV, Michie J, Berger GM 1987. Association of a DNA polymorphism in the apolipoprotein C-III gene with diverse hyperlipidaemic phenotypes. *Hum Genet,* **75:** 62-65.

Hong SH, Park WH, Lee CC, Song JH, Kim JQ 1997. Association between genetic variation of apoAI-CIII-AIV cluster gene and hypertryglyceridemic subjects. *Clin Chem,* **43:** 13-17.

Huang MC, Wang TN, Liu YL, Pa TH, Tu HP, Huang YC, Chang WT, Ko YC 2006. Effect of SstI polymorphism of the apolipoprotein CIII gene and environmental factors on risks of hypertriglyceridemia in Taiwan aborigines. *Circ J,* **70:** 1030-1036.

Johansen K, Dunn B, Tan JC, Kwaasi AA, Skotnicki A, Skotnicki M 1991. Coronary artery disease and apolipoprotein A-I/C-III gene polymorphism: A study of Saudi Arabians. *Clin Genet,* **39:** 1-5.

Johansen K, Skotnicki A, Tan JC, Kwaasi AA, Skotnicki M 1990. Apolipoprotein A-I/C-III gene cluster polymorphism in Saudi Arabians, Filipinos and Caucasians. *Clin Genet,* **37:** 194-197.

Karathanasis S K 1985. Apolipoprotein multigene family: tandem organization of human Apolipoprotein AI, CIII and AIV genes. *Proc Natl Sci USA,* **82:** 6374-6378.

Kee F, Amouyel P, Fumeron F 1999 Lack of association between genetic variations of apo A-I-C-III-A-IV gene cluster and myocardial infarction in a sample of European male: ECTIM study. *Atherosclerosis,* **145:** 187-195.

Kessling AM, Berg K, Mockleby E, Humphries SE 1986. DNA polymorphisms around the apo AI gene in normal and hyperlipidaemic individuals selected for a twin study. *Clin Genet,* **29:** 485-490.

Kessling AM, Humphries SE 1985. Interpretation of presence of S2 allele. *Lancet,* **2:** 510-511.

Kessling AM, Nanjee MN, Miller NE, Humphries SE 1988. Variations in the apolipoprotein AI-CIII-AIV gene region and in lecithin:cholesterol acyltransferase concentration are determinants of plasma cholesterol concentrations. *Atherosclerosis,* **70:** 13-19.

LaRosa JC, Hunninghake D, Bush D, Criqui MH, Getz GS, Gotto AM Jr, Grundy SM, Rakita L, Robertson RM 1990. The cholesterol facts. A summary of the evidence relating dietary fats, serum cholesterol and coronary heart disease. A joint statement by the American Heart Association and the National Heart, Lung and Blood Institute. The task force on cholesterol issues, American Heart association. *Circulation,* **81:** 1721-1723.

Liu S, Song Y, Hu FB, Niu T, Ma J, Gaziano M, Stampfer MJ 2004. A prospective study of the APOA1 XmnI and APOC3 SstI polymorphisms in the APOA1/C3/A4 gene cluster and risk of incident myocardial infarction in men. *Atherosclerosis,* **177:** 119-126.

Mahley RW, Innerarity TL, Rall SC Jr, Weisgraber KH 1984. Plasma lipoproteins: apolipoprotein structure and function. *J Lipid Res,* **25:** 1277-1294.

Marasco O, Melina F, Mele E, Quaresima B 1993. Linkage disequilibrium of three polymorphic RFLP markers in the Apolipoprotein AI-CIII gene cluster on chromosome 11. *Hum. Genet,* **91:** 169-174.

Miettinen HE, Korpela K, Hamalainen L, Kontula K 1994. Polymorphisms of the apolipoprotein and angiotensin converting enzyme genes in young North Karelian patients with coronary heart disease. *Hum Genet,* **94:** 189-192.

Morris SW, Price WH 1985. DNA sequence polymorphisms in the apolipoprotein A-I/C-III gene cluster. *Lancet,* **2:** 1127-1128.

Nei M, Tajima F, Tateno Y 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J Mol Evol,* **19:** 153-170.

Ordovas JM, Civeira F, Genest J Jr, Craig S, Robbins AH, Meade T, Pocovi M, Frossard PM, Masharani U, Wilson PW, Salem DN, Ward RH, Schaefer EJ 1991. Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus. Relationships with lipids, apolipoproteins, and premature coronary artery disease. *Atherosclerosis,* **87:** 75-86.

Ordovas JM, Corella D, Cupples LA, Demissie S, Kelleher A, Coltell O, Wilson PW, Schaefer EJ, Tucker K 2002. Polyunsaturated fatty acids modulate the effects of the APOA1 G-A polymorphism on HDL-cholesterol concentrations in a sex-specific manner: the Framingham Study. *Am J Clin Nutr,* **75:** 38-46.

Paul H, Galton D and Stocks J. 1987. DNA polymorphic patterns and haplotype arrangement of the apoAI, ApoCIII and apoA-IV gene cluster in different ethnic groups. *Human Genetics,* **75:** 264-268.

Paul-Hayase H, Rosseneu M, Robinson D, Van Bervliet JP, Deslypere JP, Humphries SE 1992. Polymorphisms in the apolipoprotein (apo) AI-CIII-AIV gene cluster: detection of genetic variation determining plasma apo AI, apo CIII and apo AIV concentrations. *Hum Genet,* **88:** 439-446.

Paulweber B, Friedl W, Krempler F, Humphries SE, Sandhofer F 1988. Genetic variation in the apolipoprotein AI-CIII-AIV gene cluster and coronary heart disease. *Atherosclerosis,* **73:** 125-133.

Perez-Martinez P, Gomez P, Paz E, Marin C, Gavilan Moral E, Lopez-Miranda J, Ordovas JM, Fernandez de la Puebla RA, Perez-Jimenez F 2001.Interaction between smoking and the Sstl polymorphism of the apo C-III gene determines plasma lipid response to diet. *Nutr Metab Cardiovasc Dis,* **11:** 237-243.

Rees A, Stocks J, Sharpe CR, Vella MA, Shoulders CC, Katz J, Jowett NI, Baralle FE, Galton DJ 1985. Deoxyribonucleic acid polymorphism in the apolipoprotein A-1-C-III gene cluster. Association with hypertriglyceridemia. *J Clin Invest,* **76:** 1090-1105.

Rees A, Stocks J, Paul H, Ohuchi Y, Galton D 1986. Haplotypes identified by DNA polymorphism at the apolipoprotein AI and CIII loci and hypertriglyceridemia. *Hum Genet,* **72:** 168-171.

Relvas WG, Izar MC, Helfenstein T, Fonseca MI, Colovati M, Oliveira A, Ihara SS, Han SW, Las Casas AA Jr, Fonseca FA 2005. Relationship between gene polymorphisms and prevalence of myocardial infarction among diabetic and non-diabetic subjects. *Atheroscelrosis,* **178:** 101-105.

Rigoli L, Raimondo G, Di Benedetto A, Romano G, Porcellini A, Riccardi G, Squadrito G, Cucinotta D 1995. Apolipoprotein AI-CIII-AIV genetic polymorphisms and coronary heart disease in type 2 diabetes mellitus. *Acta Diabetol,* **4:** 251-256.

Rose HA 1970. A glossary of the tribes and castes of Punjab and Northwest frontier province. *Census Report, Language Department Punjab India*, **1:** 55-59.

Saha N, Tay J S, Low P S, Basair J, Hong S 1995. Five restriction fragment length polymorphism of ApoA1-C3 gene and their influence on lipid and apolipoproteins in healthy Chinese. *Human Heredity,* **45:** 303-310.

Salas J, Jansen S, Lopez-Miranda J, Ordovas JM, Castro P, Marin C, Ostos MA, Bravo MD, Jimenez-Pereperez J, Blanco A, Lopez-Segura F, Perez-Jimenez F 1998. The SstI polymorphism of the apolipoprotein C-III gene determines the insulin response to an oral-glucose-tolerance test after consumption of a diet rich in saturated fats. *Am J Clin Nutr,* **68:** 396-401.

Sharrett AR, Chambless LE, Heiss G, Paton CC, Patsch W 1995. Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol,* **15:** 2122-2129.

Shoulders CC, Baralle FE 1986. Genetic polymorphism in the ApoA-I/C-III complex. *Methods Enzymol,* **128:** 727-745.

Smith JD, Brinton EA, Breslow JL 1992. Polymorphism in the human apolipoprotein A-I gene promoter region. Association of the minor allele with decreased production rate in vivo and promoter activity in vitro. *J Clin Invest,* **89:** 1796-1800.

Song J, Park JW, Park H, Kim JQ 1998. Linkage disequilibrium of Apo AI-CIII-AIV gene cluster and their relationship to plasma triglyceride, Apolipoprotein AI and CIII levels in Koreans. *Mol Cells,* **8:** 12-18.

Stocks J, Paul H and Galton D 1987. Haplotypes identified by DNA restriction-fragment-length polymorphism in the AI-CIII-AIV gene region and hypertriglyceridemia. *Am J Hum Genet,* **41:** 106-118.

Tas S 1989. Strong association of a single nucleotide substitution in the 3'-untranslated region of the apolipoprotein-CIII gene with common hypertriglyceridemia in Arabs. *Clin Chem,* **35:** 256-259.

Thompson EA, Deeb S, Walker D, Motulsky AG. 1988. The detection of linkage disequilibrium between closely linked markers: RFLPs at the AI-CIII apolipoprotein genes. *Am J Hum Genet,* **42:** 113-124.

Thu NN, Mai TT, Ohmori R, Kuroki M, Chuyen NV, Hung NT, Kawakami M, Kondo K 2006. Plasma triglyceride and HDL-cholesterol concentrations in Vietnamese girls are affected by lipoprotein lipase, but not apolipoprotein CIII polymorphism. *Circ J,* **136:** 1488-1492.

Trembath RC, Thomas DJ, Hendra TJ, Yudkin JS, Galton DJ 1987. Deoxyribonucleic acid polymorphism of the apoprotein AI-CIII-AIV gene cluster and coronary heart disease in non-insulin-dependent diabetes. *Br Med J,* **294:** 1577-1588.

Tybjaerg-Hansen A, Nordestgaard BG, Gerdes LU, Faergeman O, Humphries SE 1993. Genetic markers in the apo AI-CIII-AIV gene cluster for combined hyperlipidemia, hypertriglyceridemia, and predisposition to atherosclerosis. *Atherosclerosis,* **100:** 157-169.

Vavatsi NA, Kouidou SA, Geleris PN 1995. Increased frequency of the rare PstI allele (P2) in a population of CAD patients in northern Greece. *Clin Genet,* **47:** 22-26.

Vella M, Kessling A, Jowett N, Rees A, Stocks J, Wallis S, Galton D 1985. DNA polymorphisms flanking the apo A-1 and insulin genes and type III hyperlipidaemia. *Hum Genet,* **69:** 275-276.

Xiang KS, Cox NJ, Sanz N, Huang P, Karam JH, Bell GI 1989. Insulin-receptor and apolipoprotein genes contribute to development of NIDDM in Chinese Americans. *Diabetes,* **38:** 17-23.

Xu CF, Nanjee MN, Savill J, Talmud PJ, Angelico F, Del Ben M, Antonini R, Mazzarella B, Miller N, Humphries SE 1990. Variation at the apolipoprotein (apo) AI-CIII-AIV gene cluster and apo B gene loci is associated with lipoprotein and apolipoprotein levels in Italian children. *Am J Hum Genet,* **47:** 429-439.

Zeng Q, Dammerman M, Takada Y, Matsunaga A, Breslow JL, Sasaki J 1995. An Apolipoprotein CIII marker associated with hypertriglyceridemia in Caucasians also confers increased risk in west Japanese populations. *Human Heredity,* **95:** 371-375.