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Apolipoprotein A-IV Polymorphism in Five Punjabi Populations: Gene Frequencies and Gene Diversities

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ABSTRACT Apolipoprotein A-IV genotypes were determined in five population groups (Brahmins, Banias, Jat sikhs, Khatris and 'Others') of Punjab (n=780) by two dimensional gel electrophoresis. The apo A-IV exhibits two common polymorphic alleles AIV-1 and AIV-2 with pooled frequencies 0.903 and 0.097 respectively. Distribution of apo A-IV isoforms highlight considerable variation among different ethnic groups across the world. Average heterozygosity of Punjabi population was 0.1753 ± 0.011 at apo AIV structural locus. 'Others' showed the higher heterozygosity (19.55%) than other groups, reflecting greater proportion of incongruence at this locus. The gene diversity among five population groups was 0.3% (H_s = 0.1753, H_T = 0.1765). Estimated allele frequencies of these groups have been compared with the data found in other population groups. The frequency of apo A-IV2 in 'Others' was observed to be the highest except Finns and Icelanders.

INTRODUCTION

Apolipoproteins play a pivotal role in secretion, processing and metabolism of various lipoprotein particles (Mahley, 1988; Balgir et al., 1997 and Singh et al., 1999). Various marker and non-marker epidemiological studies on apolipoproteins have been conducted elucidating the heterogeneity and it's significance in various pathological conditions (Boemi et al., 1993; Kervinen et al., 1994; Luc et al., 1994; Olson et al., 1995; Singh et al., 1998 and Kamboh et al., 1999). Of these apoA-IV have been studied extensively (Saha, 1991 and Menzel et al., 1995) because of it's polymorphic nature and susceptibility to various lipid disorders.

Apolipoprotein A-IV is 46kD glycoprotein containing 6% carbohydrate by weight (Weinberg and Scanu 1983). It is synthesised by intestinal epithelial cells and is a constituent of nascent lymph chylomicron (Green et al., 1979). In fasting plasma it's density varies from 14mg/ml to 37mg/ml in Humans (Beisiegel and Utermann 1979). ApoA-IV acts as allosteric cofactor for enzyme Lecithin:cholesterol acyltransferase (LCAT) in vitro (Steinmetz and Utermann 1984). It acts as a ligand for receptor mediated transport and distribution of lipid particles across the cell membranes (Stein et al., 1986). It also enhances lipase activity in the presence of apoliporotein C-II which is a cofactor for lipoprotein lipase (LPL) activity (Goldberg et al., 1990).

Genetic polymorphism of apoA-IV is controlled by two most common alleles apoAIV-1 and AIV-2 whereas the most common pattern designated as homozygous 1-1 consists of two major and three minor isoforms with isoelectric points (pI's 5.15-5.40). The other homozygous type designated 2-2 is composed of two major and three minor isoforms but has more basic pI's (5.25-5.65) as compared to 1-1 type (Kamboh et al.,1992).The apo A-IV1 wild type allele is by far the most common in all populations studied to date and A-IV2 (Gln_{360}-His) is the most frequent variant in Caucasians (Tenkanen et. al., 1992). Some rare alleles A-IV3 and A-IV4 have also been observed by Menzel et al. (1988). Family studies show autosomal codominant transmission of A-IV1 and A-IV2 coded by a single structural locus.

In the present study apoAIV genetic variability of five population groups of Punjab have been reported, gene diversity at this locus was analysed and estimated allele frequencies were compared with other population groups to fill the gap in this corner of the World.

MATERIAL AND METHODS

Blood samples were procured from 780 (males - 400, females - 380) unrelated and randomly selected individuals ranging in age

between 20-60 years from blood donation camps being organised at various places of Punjab. Plasma of each sample was separated and stored at -20°C until analysed. Data were selected from five major population groups (Banias, Brahmins, Jat Sikhs, Khatris and 'Others') of Punjab.The group "Others" comprised of Scheduled castes and Scheduled tribes. All the subjects were born in Punjab and their three grand parents were native to this area. People of Punjab are considered as the progeny of mixture of many proto and post Harappan invaders who entered this land from west and became original settlers of this area (Rose, 1970).

Apo A-IV genotyping was performed using isoelectricfocusing (IEF) and density gradient SDS polyacrylamide gel electrophoresis method by Manabe et al. (1987) with little modifications. IEF was carried out on 7.5% polyacrylamide gels using triton X-100 as denaturant in 8M urea with 5% ampholines (pH 3.5-10) at 8°C for 20 hrs at 250V. Prefocussing of the samples were done for 1 hr at 3W and 500 volts. In second dimension, density gradient (5-25%) was formed using SDS and IEF gels were electrophoresed. Genotypes of Apo A-IV were scored directly from the gels after silver staining.

Allele frequencies were estimated by allele counting. χ^2 (goodness of fit) test was performed to check the Hardy-Weinberg equilibrium. Average heterozygosities were calculated by gene identities. Gene diversity of the total, within and between subpopulation was calculated by the method given by Nei (1973).

RESULTS AND DISCUSSION

Table 1 exhibits the apoA-IV genotype distribution, estimated allele frequencies, genic diversity and heterozygosities among five caste groups of Punjab. Three genotypes A-IV1-1 (81.92%), A-IV1-2 (16.79%) and A-IV2-2 (1.28%) were observed. None of the subject showed genotypic constitution of rare alleles(A-IV3, A-IV4 etc.). Apo A-IV1 fluctuates from 89% (Others) to 92.1% (JatSikhs). Khatris and Banias show trifle variation for apoA-IV1 allele (0.903 vs 0.902). ApoA-IV2 ranges from 0.079 (JatSikhs) to 0.110 (Others). Banias and Khatris show similitude for this allele (0.098 & 0.097 respectively). Average heterozygosity (0.1753 ± 0.0108) varies from 14.55% (JatSikhs) to 19.55% (Others). The within and among population gene diversities were apportioned as H_S=0.1753 and D_{ST}= 0.0006 respectively. The coefficient of gene diversity is G_{ST}=0.003 thus, the extent of gene diversity at apoA-IV locus between population relative to total diversity is 0.3% in sub divided Punjabi population.All the observed and expected values of apoA-IV in five caste groups are in accordance and statistically non-significant at 5% level (χ^2_2=1.2099,P< 0.05) thus, agreeing to the Hardy-Weinberg law of equilibrium.

Juxtaposing the allele frequencies of these caste groups with other ethnic groups of the World, table 2 illuminates the interallelic-interethnic variability at this locus. ApoA-IV1 allele shows the highest frequency in Japan (0.998 and

Table 1: Apolipoprotein A-IV genotypes,allele frequencies,heterozygosities and genetic diversity in five caste groups of Punjab

Locus	*Phenotypes*	*Banias n=148*	*Brahmins n=147*	*Jatsikhs n=165*	*Khatris n=175*	*Others n=145*	*Pooled n=780*
ApoAIV	AIV 1-1	121 (120.41)	119 (118.54)	140 (139.96)	144 (142.70)	115 (114.85)	639 (636.1)
	AIV 1-2	25 (26.17)	26 (26.93)	24 (24.01)	28 (30.65)	28 (28.39)	131 (136.64)
	AIV 2-2	2 (1.42)	2 (1.53)	1 (1.03)	3 (1.65)	2 (1.75)	10 (7.34)
Allele Frequencies	AIV 1	0.902	0.898	0.921	0.903	0.890	0.903
	AIV 2	0.098	0.102	0.079	0.097	0.110	0.097
	$\chi 2_2$	0.2921	0.1783	0.0009	1.3454	0.0610	1.2099
	P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
	H	0.1768	0.1832	0.1456	0.1752	0.1958	0.1753
	H±S.D			0.1753±0.011			
GeneDiversity		J_S	J_T	D_{ST}	H_T	H_S	G_{ST}
		0.8247	0.8241	0.0006	0.1765	0.1753	0.0033

D_{ST} is the inter population gene diversity,H_T is the gene diversity in total population,H_S is the intra population gene diversity and G_{ST} is the coefficient of gene differentiation.Values in parenthesis are expected numbers.

0.986) followed by Malayans of Singapore (0.967) and Amerindians of U.S.A. (0.967). ApoA-IV was found to be monomorphic in Korean (Kongju) population (Kim and Kamboh, 1998). The monomorphic status of apoA-IV gene in Korea and highest frequency of it's gene products in Japan, Singapore and India is anthropologically very important. One can speculate the origin of this gene in Asia but as the data is wanting, this is too early to say this. Jatsikhs and Germans show similitude for apo A-IV1 allele (0.921 vs 0.923). One of the Finland population displays lowest frequency of 78.8% for this allele. Interestingly, Punjabi "Others" highlights the highest frequency (0.110) of apo A-IV2 except Icelanders (0.112) and Finns (0.212). It is found to be absent in one of the Japanese group studied by Asakawa et. al. (1985), Ghanians and Amerindians of U.S.A.whereas it is found to be lowest in Japanese (0.002) and Nigerians (0.003). ApoA-IV variants and other isoforms are found only in few populations

Table 2: Apolipoprotein A-IV distribution in various population groups of the World

Country Population	*A-IV1*	*A-IV2*	*A-IV Others*	*No. tested*	*Authors*
AUSTRIA					
Tyrol	0.918	0.077	0.004	473	Menzel et al., 1988
DENMARK					
Dutch	0.901	0.079	0.020	1939	Knijff et al., 1988
EUROPE					
5Pop.Combined	0.919	0.081	00	1261	Enholm et al., 1994
FINLAND					
——	0.942	0.058	00	387	Lukka et al., 1988
——	0.788	0.212	00	---	Tenkanen et al., 1992
——	0.925	0.075	00	188	Boerwinkle & Sing, 1986
FRANCE					
Nancy	0.943	0.057	00	158	Visvikis et al., 1990
GERMANY					
——	0.923	0.075	0.002	1000	Menzel et al., 1982
Turks	0.967	0.033	00	240	Malle et al., 1996
GHANA	0.948	0.00	0.037	99	Menzel et al., 1988
HUNGARY					
——	0.959	0.039	0.002	202	Csaszar, 1995
Budapest	0.950	0.039	0.011	---	Menzel et al., 1995
ICELAND	0.855	0.112	0.003	188	Menzel et al., 1990
INDIA(Punjab)					
Banias	0.902	0.098	00	148	Present study
Brahmins	0.898	0.102	00	147	Present study
JatSikhs	0.921	0.079	00	165	Present study
Khatris	0.903	0.097	00	175	Present study
Others	0.890	0.110	00	145	Present study
JAPAN					
Tokyo	0.998	0.002	00	---	Akiyama et al., 1989
—-	0.986	0.00	0.014	110	Asakawa et al., 1985
NIGERIA	0.894	0.003	0.103	171	Siphernia et al., 1988
NORWAY	0.950	0.05	00	124	Schamaun et al., 1985
SINGAPORE					
Chinese	0.959	0.010	0.031	455	Saha, 1991
Malayans	0.967	0.011	0.022	45	Saha, 1991
Dravidians	0.933	0.043	0.024	127	Saha, 1991
U.S.A					
U.S.Whites	0.909	0.088	0.003	159	Kamboh & Ferrell, 1987
U.S.Blacks	0.961	0.035	0.004	127	Kamboh & Ferrell, 1987
Amerindians	0.967	0.00	0.033	105	Asakawa et al, 1985
Texas	0.928	0.066	0.006	---	Hannis et al, 1991

Full references can be provided by the corresponding author on written request.

(Table 2). Studies on these unique alleles and their association with lipid parameters may serve as a tool to study ethnic differences in lipid metabolism and cardiovascular disease risks. Though we are not aware of any such study being conducted on apoA-IV locus in India, therefore the estimated allele frequencies of apoA-IV as the mean estimates weighted on sample size of various population groups are compared with frequencies of Punjab (India) in figure 1. It exposes contrastive interallelic variations and throws light on the current status of Punjab at this locus in the World.

Despite genetic polymorphism at this locus the influence of allelic variation at apoA-IV on plasma lipoprotein parameters have been studied extensively (Malle et al., 1996 and Singh et al., 1999). Menzel et al.(1988, 1990) demonstrated the significant triglyceride (TG) lowering or high density lipoprotein cholesterol (HDL-c) elevating effect of apoA-IV2 allele.Reduced levels of apoA-IV have been reported in abetalipoproteinemia (Menzel et. al., 1988). ApoA-IV is thought to be required for the efficient release of apoC-II from HDL to triglyceride rich lipoprotein particle and therefore structural changes in the apoA-IV molecule may play an important role in regulating the postparandial metabolism of lipoprotein but it's integration with environmental determinants cannot be ruled out.

Many studies have been conducted on the genetic variation of apoA-IV and it's effect on lipid parameters but to generalise the positive and negative correlation of this gene with cardiovascular diseases is cumbersome.More epidemiological marker studies are required in this part of the World to elucidate the physiological role of apoA-IV and it's linkage with other candidate genes vis-a-vis their manifestations for various lipid disorders, which we wish to persue in future.

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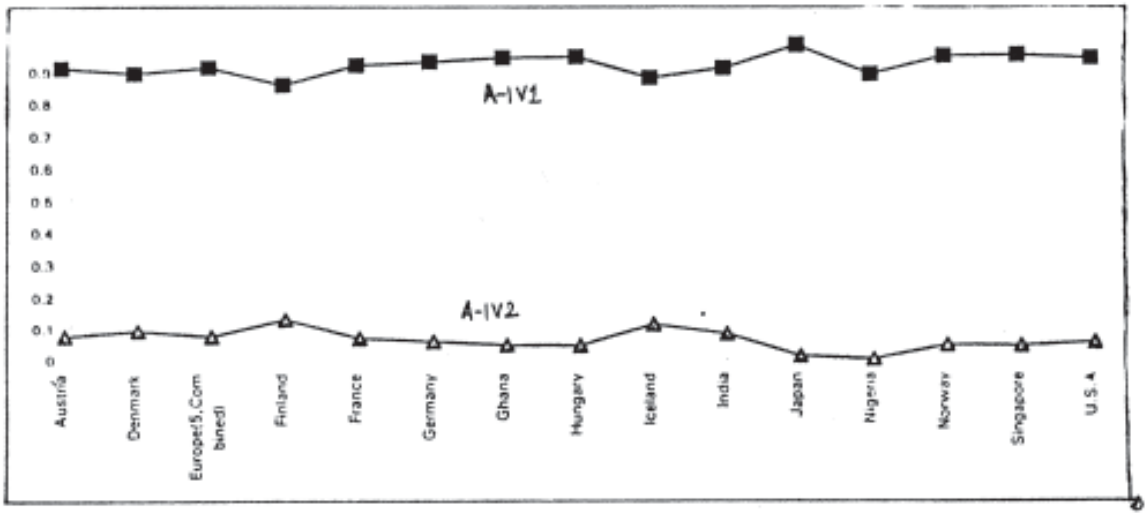

Fig. 1. Apo AIV allele frequency distribution in the world

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