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Apolipoprotein Polymorphism in Man - It's Role in Health and Disease

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ABSTRACT Apolipo proteins play an important role in lipid transport in human body; starting from absorption of processed dietary lipids from the exogenous pathway in intestines to the endogenous transport involving extra hepatic cells and processing them in the liver. Along with various enzymes the binding characteristics of apolipoproteins are the main determinants in lipid metabolism in the body. The binding characteristics are inturn dependant on the amino acid sequence and molecular structure of the apolipoprotein. The various apo proteins identified to date, their structure and genetics are discussed. The polymorphisms at the apo protein loci detected at biochemical level and reported for various world populations are listed. The effect to genetic heterogeneity on the lipid level in health and disease is also delineated.

INTRODUCTION

The plasma lipid levels in man are determined by the net turnover of all the exogenous and endogenous lipid transport and metabolism. This lipid transport as well as metabolism are effected through the intervention of various proteins viz. the apolipoproteins, the lipo protein modifying enzymes, the receptors and the enzymes involved in cellular lipid metabolism. The complex interaction between all these genes and environmental factors viz. diet, exercise and life style etc. account for the quantitative variation in plasma lipid levels as well as the susceptibility to various diseases. It is only recently that we have been able to delineate some of these interactions. This review is confined to the genetic heterogeneity of apolipoproteins at biochemical level, reported among various populations and its role in the quantitative variability observed in lipid level. Firstly an outline of the role of apolipoproteins in lipid transport in man.

Exogenous lipid transport involves the absorption of processed dietary triglycerides (TG) and cholesterol (Ch) in the intestines and their packaging into the intestinal epithelial cells as large TG and Ch containing chylomicrons. The chylomicrons are constituted from intestinal forms of apolipoproteins apoB-48, A-I and A-IV. In the blood circulation they acquire apo C-II, which is a cofactor for lipo protein lipase (LPL) enzyme. The LPL present in the endothelial cells of the capillaries, hydrolyses the chylomicron core TG to cholesteryl ester (CE)-rich chylo micron remnant; with transfer of apoE from HDL to the remnant. Other surface components of chylomicron viz. phospholipids and apolipo proteins get transfered to HDL during lipolysis. The chylomicron remnants are catabolised in the hepatic cells. The catabolism is facilitated by the recognition of apoE present on the surface of the remnant by its receptors on the hepatic cells.

Endogenous lipid transport comprises of production and secretion of very low density lipoproteins (VLDL) by liver and their transport in the plasma. The VLDL are constituted from the liver form of apoB-100, C-II and apoE. The VLDL triglycerides are hydrolysed by LPL forming intermediate density lipo proteins (IDL) which are either cleared from plasma by hepatic LDL receptors which recognize and bind apoE or IDL. IDL are lipolysed by hepatic triglyceride lipase (HTGL) resulting in formation of Low Density Lipo proteins (LDL). The LDL generated is either cleared by liver which recognizes apoB and the mediation of LDL receptors on liver or it is modified. The modified LDL is then lipolysed by mediation of receptors on macrophages and endothelial cells (these two types of cells are also involved in the initial stages of atheros clerosis).

Reverse Cholesterol transport refers to the liver as the main clearing house for cholesterol. The process involves nascent HDL attracting free Ch from extra hepatic cells. The Ch is

esterified in the plasma by lecithin cholesterol acyltransferase (LCAT), which utilizes apoAI from HDL as a çofactor. Along with Cholesteryl ester (CE), new surface components are added to HDL to form mature HDL. The HDL-CE is transferred to VLDL, IDL to LDL with the mediation of chloresteryl ester transfer protein (CETP). The lipoproteins are inturn catabolised by liver (Breslow, 1988).

The molecular structure of the apo protein determines its lipid binding characteristics and consequently the fate of the lipid. Lipid transport be it in the form of VLDL, LDL or HDL thus involves genetic endowment of the individual to a significant extent. The apolipoprotein constituents delineated so far are listed in table 1 according to their occurrence in various lipoprotein fractions obtained by ultra centrifugations viz. the very low density lipoprotein (VLDL), the low density lipoprotein (LDL) and the high density lipoprotein (HDL).

The distinctive apolipoproteins characterized to date on the basis of their molecular weight, amino acid composition, Isoelectric point, concentration in plasma and the chromosomal localisation of their genes are listed in table 2. Their physio-chemical characteristics and genetic heterogeneity is discussed by listing them in an alphabetical order. The generally accepted 'ABC' nomenclature (Alaupovic 1971) as listed in table 3 has been followed. The table also gives synonyms used in the literature.

Apo(a) is a major apolipo protein found in the Lp(a) lipo protein particle (Uterman et al. 1989). The Lp(a) particle is a genetic variant of LDL, in which ApoB is associated with apo(a) which carries the antigen Lp(a). Lp(a) gene has been localised to chromosome 6 and found to be linked to the plasminogen gene. At structural level too it shows sequence homology to plasminogen. Its structure shows 38 blocks of repeating units of 114 amino acids. The first 37 resemble closely the kringle IV and repeat 38 resembles kringle V of plasminogen. After the repeats lie the protease domain which has the His/Asp/Ser catalytic domain as seen in plasminogen. In addition to their structural homology the two genes are linked on the chromosome 6(Lindal et al. 1989). Several studies have reported an association between elevated levels of Lp(a) and premature development of athero- sclerosis (Brown and Goldstein, 1987).

Table 1: Constituents of various lipoprotein fractions

	Very low density lipoprotein (VLDL)	*Low density lipoprotein (LDL)*	*High density lipoprotein (HDL)*
Electrophoretic mobility	Pre-β	β	β
Apolipo-protein composition	ApoB-100 ApoC-I ApoC-II ApoC-III ApoD ApoE ApoH	Apo(a) ApoB-100 ApoC-I ApoC-II ApoC-III	ApoA-I ApoA-II ApoA-IV ApoC-I ApoC-II ApoC-III ApoD ApoE ApoJ
Triglyceride	~60%	~10	~10.5%
Cholesaterol	~17%	~60%	~32%
Phospholipid	~8%	~30%	~50%

Table 2: Properties of human serum apolipoproteins

Apolipoprotein	*Molecular weight (D)Isoforms*	*pI of major (Mg/dl)*	*Conc. in Plasma location*	*Chromosomal*
Apo A-I	28,016	5.6	100-150	11q13
Apo A-II	17.414	4.9	50-70	1q21-22
Apo A-IV	46,000	5.15-5.65	4-15	11q13
Apo B-100	500,000	-	80-120	2q24
Apo B-48	250,000	-	50	2q24
Apo C-I	6,630	8.0	5-10	19cen-q13.2
Apo C-II	8,824	4.7	3-5	19cen-q13.2
Apo C-III	8,764	4.9	7-12	11q13
Apo D	19,303	5.3	7-12	3
Apo E	34,145	5.5	4-14	19cen-q13.2
Apo H	43,000	9.5	70	-
Apo J	34,000-39,000		4.9-5.4	10
Apo (a)	-		1-150	6

Table 3: Classification of Apolipoproteins

	ABC nomen-clature	*C-Terminal*	*Synonyms*
Apo A-I	Glutamine	Fraction III	apo-LP-Gln-I
Apo A-II	Glutamine	Fraction IV	apo-LP-Gln-II
Apo B	Serine		apo-LP-Ser
Apo C-I	Serine	Fraction V	apo-LP-Ser
Apo C-II	Glutamic acid	Fraction V	apo-LP-Glu
Apo C-III	Alanine	Fraction V	apo-LP-Ala
Apo D		Thin line polypeptide	
Apo E	Alanine	Arginine rich peptide	

Quantitative Lp(a) levels in human plasma varies from 1-15 mg/dl, determined directly by a multiallelic polymorphism at the APO(a) structural locus. Kamboh et al. (1991) detected at least 24 alleles with an estimated mean heterozygosity of 94%, which remains the highest value yet reported for any protein coding locus due to an expressed hyper variable region within the coding region of the gene (Table 4). In a recent Kikuchi et al. (1993) have reported 26 alleles at the locus amongst Japanese and Chinese populations using gradient PAGE. Apo(a) genetic variants have been observed to effect HDL levels (Hegele et al., 1991).

Apo-AI is a major component of the HDL fraction. Its serum concentration have been found to be inversely correlated with atherosclerosis (Blangero et al., 1990). It is one of the genes of the AI-CIII-AIV cluster on Chromosome 11. Study of rare mutations at AI locus indicate that apo AI is required for normal HDL assembly (Karathanasis, 1992). Apo-AI is secreted as pro apo AI and matured by extacellular cleavage of an amino terminal hexapeptide by hetherto unknown propeptidase. By studying Apo - AI variants in which proline residues at positions 3 or 4 were substituted by other aminoacids it was established that these were important structural requirements for the normal processing of the apo-AI (von Eckardstein et al., 1989. The Apo-AI has been found to be monomorphic in all the populations studied todate. Its variants have been associated with high triglyceride levels and CHD.

Apo-AIV another component of HDL fraction may be involved in the transfer of Apo-CII to chylomicrons (Goldberg et al., 1990) and thus in the reverse cholesterol transport. Its level were observed to be reduced in abetalipoproteinemia and Tangier's disease. It is also observed to be an activator for plasma enzyme lecithin cholesterol acyltransferase (LCAT). Sepehrnia and coworkers (1988) have reported polymorphism at the locus amongst various populations of the world (Table 4). Apo-AIV exhibits genetic

Table 4: Distribution of apolipoprotein (a) allele frequencies amongst various populations of the world

Population	*Allele frequencies*			
U.S.A	(null) N=0.061;	1=0.007	2=0.043;	
Apo(a)	3=0.014;	4=0.061;	5=0.089;	6=0.043;
	7=0.025;	8=0.118;	9=0.039;	10=0.093;
	11=0.046;	12=0.029;	13=0.057;	14=0.021;
	15=0.082;	16=0.032;	17=0.054;	18=0.046;
	19=0.014;	20=0.004;	21=0.004;	22=0.014;
	23=0.004.			
Japanese	(null) N=0.098;	3=0.007;	7=0.009;	
	8=0.009;	9=0.009;	10=0.025;	11=0.018;
	12=0.042;	13=0.029;	14=0.031;	15=0.076;
	16=0.059;	17=0.130;	18=0.084;	19=0.105;
	20=0.103;	21=0.064;	22=0.063;	23=0.024;
	24=0.012;	25=0.002.		
Chinese	(null) N=0.094;	1=0.005;	3=0.019;	
	4=0.005;	5=0.005;	6=0.005;	7=0.015;
	8=0.005;	9=0.034;	10=0.034;	11=0.029;
	12=0.019;	13=0.025;	14=0.035;	15=0.048;
	16=0.066;	17=0.065;	18=0.104;	19=0.130;
	20=0.081;	21=0.095;	22=0.048;	23=0.024;
	24=0.010.			

polymorphism. The two most common alleles show a single G to T substitution at codon 360 leading to a replacement of glutamine with a histidine (Lohse et al., 1990). Other less frequent alleles identified are Apo-AIV0, Apo-AIV3, Apo-, Apo-AIV5, Apo-AIV6 and Apo-AIV7 among the Nigerian population (Menzel et al., 1988). Apo B is a major apo protein in the LDL, VLDL and Chylomicron fraction. Apo B occures naturally in two closely related forms apo B-100 and apoB-48 encoded by the same gene. Apo B-100 is synthesized by the liver and is required for the assembly and secretion of the VLDL. It is also a constituent of the LP(a) particle where it is covalently linked to the apo (a) (Gaubatz et al., 1983). It also functions as a physiological ligand for LDL receptor and is responsible for receptor mediated catabolism of LDL. The apo B-100 gene shows only partial homology to the soluble apolipoproteins (De Louf et al., 1987). Apo B-48 associated mainly with chylomicrons and chylomicron remnants is synthesized solely by intestines. Apo B-100 consists of 4536 amino acids in a single polypeptide chain. The Apo B-8 represents the amino-terminal 47% of Apo B-100. Genetic abnormalities of Apo B-100 result in a decreased "in vitro" binding of LDL to their receptor and hence reduced "*in vivo*" catabolism of LDL as identified in some hyper cholesterolemic patients (Innerarity et al., 1987). Among these patients it was identified that a single substitution at base 11039 (G to A) leads to an arginine to glutamine change in a mature protein at aa 3500.

Apo-CI is a constituent common to all the lipo protein fractions of the serum and is a member of the apolipoprotein cluster II located on chromosome 19 cen-q 13.2. It is a 7KD protein with a pI 7.5 and a serine residue at its c-terminal (Herbert et al., 1971). It has been assigned the function of LCAT (Lecithin cholesterol acyltransferase) activation.

Apo C-II is a 9KD protein with a pI 4.9, found in the HDL and VLDL fractions. Its carboxy terminal amino acid is glutamic acid (Herbert et al., 1971). It is an essential cofactor for hydrolysis of triglyceride rich particles by LPL as they are responsible for activation of LPL (Goldberg et al., 1990). Mutation at this locus may result in Familial hyper chylomicronemia (Type 1). The protein has been found to be monomorphic amongst the Caucasian populations. Four alleles Apo C-II 1,2,3 and 4 (Table 4) unique to Nigerian Blacks and U.S. Blacks have been reported (Sephernia et al., 1988a). Apo C-III is a 9KD protein with its isoforms at pI 4.7-5.0. Its gene is a member of the Apo A-I, C-III, and A-IV gene cluster (McKusick, 1987) on chromosome 11q13. It functions as a Lipase inhibitor. In a family exhibiting Hyper alphalipo proteinemia a lowered serum C-III was observed. The apo C-III variant (Lys 58-Glu) isoprotein was present at a lowered concentration resulting in increased lipolysis and a consequential increase in HDL.

Apo D is a 33KD protein with isoproteins in the range of pI5.0-5.2. Its function is not very well understood but it has a stabilising effect on LCAT and hence may be involved in cholesterol esterification and its transport to liver for catabolism. The gene has been localized to chromosome 3. The protein has been found to be monomorphic amongst all the populations studied so far except Nigerian and U.S. Blacks (Kamboh et al., 1991a) amongst whom two alleles Apo D1 and Apo D2 were observed (Table 4).

Apo-E is an arginine rich glycoprotein made up of a single polypeptide chain of 38KD with isoproteins with the pI 5.7-6.0 (Rall et al., 1981). Apo E is a component of VLDL, IDL and HDL fractions of the plasma. It serves as a ligand for the chylomicron remnant receptor and LDL receptor present on the liver cellplasma membrane (Pitas et al., 1981). The gene for Apo E is located in the apolipoprotein cluster II on chromosome 19 cen-13.2. Three common alleles (Table 4) have been detected at the locus by isoelectric focusing Apo E2, Apo E3 and Apo E4 (Kamboh et al., 1988). Apo E2 frequency was found to be increased in hyper lipoproteinemia Type III (Utermann et al., 1978). Apo E genotype has been observed to effect the plasma cholesterol level in man. In all reported studies amongst various populations, the average effect of Apo E2 allele is to lower total cholesterol and that of Apo E4 to raise total cholesterol level (Hallman et al., 1991; Kniff et al., 1993).

APO E4 allele has been implicated in the common late onset familial and sporadic forms of Alzheimer's disease. The risk was found to increase from 20% to 90% and mean age at onset

Table 5: Distribution of apolipoprotein allele frequencies amongst various populations of the world

Locus	*Population*	*Alleles*			
Apo A-IV		Apo A-IV1	Apo A-IV2	Others	
	U.S. Whites	0.909	0.088	0.003	
	U.S. Blacks	0.961	0.035	0.004	
	Germans	0.923	0.075	0.002	
	Norwegians	0.950	0.050	-	
	Nigerians	0.984	0.003	0.003 A-IV4	
				0.015 A-IV5	
				0.003 A-IV6	
				0.082 A-IV7	
Apo C-II		Apo C-II1	Apo C-II2	Apo C-II3	Apo C-II4
	Nigerian Blacks	0.941	0.054	0.004	0.001
	Whites	Monomorphic			
Apo D		Apo D1	Apo D2		
	U.S. Blacks	0.987	0.013		
	Nigerian Blacks	0.978	0.022		
	U.S. Whites)				
	Dogrils Indians)				
	Mayan Indians)	Monomorphic			
	Aleuto)				
	Eskimos)				
Apo E		Apo E2	Apo E3	Apo E4	
	German	0.080	0.770	0.150	
	U.S.A.	0.009	0.770	0.140	
	New Zealand	0.120	0.740	0.140	
	Scotland	0.080	0.770	0.150	
	Finland	0.040	0.730	0.130	
	Japanese	0.040	0.850	0.110	
	Negroes	0.027	0.677	0.296	
	Nigerian Blacks	0.027	0.677	0.296	
Apo H		Apo H1	Apo H2	Apo H3	Apo H4
	U.S. Whites	0.060	0.880	0.060	-
	U.S. Blacks	0.020	0.900	0.070	0.010
	Nigerian Blacks	0.011	0.895	0.073	0.021
Apo J		Apo J1	Apo J2	Apo J3	
	U.S. Whites	Monomorphic			
	U.S. Blacks	0.760	0.237	0.003	
	Nigerian Blacks	0.720	0.280	0.000	

from 84 to 68 years with increasing number of Apo E4 alleles. Homozygosity for Apo E4 was seen as a sure indicator of development of the condition by age 80.

Apo H gene has been provisionally localised to chromosome 17. The protein has been found to be polymorphic (Table 4) amongst various populations studied (Breslow, 1988).

Apo J is found associated with HDL fraction in the plasma (de Silva et al., 1990). Its a glycoprotein with two sulfide linked subunits apo Já (34-36KD) and apo Já (36-39KD) with a pI 4.9-5.4. The genetic polymorphism was detectable only amongst the populations of African origin possessing two unique alleles Apo J1 and Apo J2 (Table 5), for other populations the loeus was found to be monomorphic (Kamboh et al., 1991b).

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