REVIEW

Lipoprotein(a): Nonhuman Primate Models

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Lipoprotein(a) [Lp(a)] is a low density lipoprotein which has apo(a) disulfide-linked to apoB100. Apo(a) has recently been shown to have a striking homology with plasminogen, a knowledge that has stimulated a lot of interest in the mechanism of atherogenicity and thrombogenicity of this lipoprotein particle. Several studies have documented the presence of Lp(a) in nonhuman primates with particular reference to the rhesus monkeys and baboons. The Lp(a) of rhesus monkey is structurally very similar to that of humans, except for the absence of kringle V and the amino acid composition of the catalytic region. The Lp(a) of nonhuman primates, like their human counterparts, exhibit a wide range of interindividual plasma levels and also a wide size polymorphism of apo(a). Nonhuman primates appear to represent a good model for the study of the structure and biology of Lp(a).
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Lipoprotein(a) [Lp(a)], first described by Berg (1) as a genetic variant of low density lipoprotein (LDL), is considered to be an independent risk factor for atherosclerotic cardiovascular disease, (ACD) (2-5). The distinguishing feature of Lp(a) is to have apoB100 bound to apo(a) by a disulfide linkage (6,7). This protein has recently been shown to have a striking homology with plasminogen (8,9), which is a serine protease zymogen that promotes clot lysis on activation to plasmin. This knowledge has stimulated a lot of interest in the mechanism of atherogenicity and thrombogenicity of this lipoprotein particle.

Lp(a) is heterogeneous in size and density due to differences in protein/lipid mass ratio, apo(a) size polymorphism and apoB100/apo(a) molar ratio (10,11). Apo(a) size isoforms are under the control of a single gene which is localized in the long arm of chromosome 6 in proximity of the plasminogen gene (12,13). Family studies have shown a linkage between apo(a) and plasminogen genes (14).

The studies conducted thus far favor the concept that apo(a) is under a strict genetic regulation, a notion that is in keeping with the insensitivity of plasma Lp(a) to diets and hypolipidemic agents (15). However, data are emerging that post-translational events may be contributing factors in determining the plasma levels of Lp(a) (16). Most of the studies on Lp(a) have been carried out in human subjects. Lp(a) has been reported absent in the plasma of common laboratory animals (*e.g.*, dogs, rats, mice, etc.). On the other hand, several studies have documented the presence of Lp(a) in nonhuman primates. The purpose of this article is to provide an overview on these studies with particular emphasis on the Old World monkeys.

GENERAL OBSERVATIONS

Lp(a) immunoreactive materials were identified by Berg (17) in serum samples from rhesus monkey, baboon, chimpanzee and orangutan. Several reports have also identified Lp(a) in the plasma of patas monkeys (18,19), pig-tailed monkeys (19), mangabey monkeys (20) but not in common marmosets (21) which belong to the New World monkeys.[2]

Makino *et al.* (23) carried out a systematic, genealogical study on some of the immunological characteristics of Lp(a) or Lp(a)-like material in the serum samples of 77 nonhuman primates of 24 species (see Table 1). The immunological cross-reactives of Lp(a) or Lp(a)-like materials were studied by Ouchterlony's double diffusion using a rabbit anti-human Lp(a) serum (Fig. 1). Sera from great apes (orangutan and chimpanzee) exhibited a reaction of complete identity with human sera (Fig. 1D, E). Sera from Old World monkeys (savannah, patas, Assamese, rhesus, Formosan, pig-tailed, crab-eating, Japanese monkeys and hamadryas baboon) generated spurs when tested in adjacent wells against human sera, indicating that Lp(a) species of Old World monkeys are relatively deficient in antigenic determinants as compared with human Lp(a).

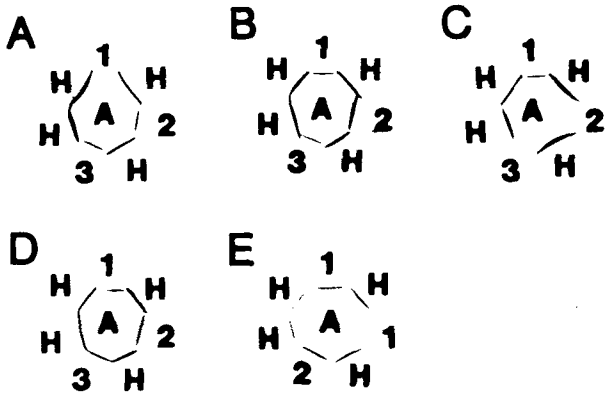

FIG. 1. Ouchterlony's double diffusion tests. The anti-human serum is in the central well (H, human serum). A: 1, Common marmoset; 2, savannah monkey; 3, patas monkey. B: 1, Assamese monkey; 2, Formosan monkey; 3, rhesus monkey. C: 1, pig-tailed monkey; 2, night monkey; 3, gibbon. D: 1, Japanese monkey; 2, crab-eating monkey; 3, chimpanzee. E: 1, Chimpanzee; 2, orangutan.

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Abbreviations: ACD, atherosclerotic cardiovascular disease; FH, familial hypercholesteremia; Lp(a), lipoprotein(a); LDL, low density lipoprotein; LDL-R, low density lipoprotein receptor.

[2] A very recent study, which appeared after this article had completed the review process, reported the presence of Lp(a) in the plasma of the common marmoset (Callithrix jacchus). A single apo(a) isoform the size of apoB100 was observed and associated with variable plasma Lp(a) levels (22).

TABLE 1

Species and Numbers of Nonhuman Primates Used in the Studies and Lp(a) Detection[a]

Species		No.	Lp(a)
Prosimiae:	Prosimians:		
Lemuridae			
Lemur catta	Ring-tailed lemur	3	ND
Galagidae			
Galago crassicaudatus	Grand galago	3	ND
Lorisidae			
Nycoticebus coucang	Slow loris	1	ND
Simiae:	Simians:		
Platyrrhina	New World monkeys		
Callithricidae			
Callithrix jacchus	Common marmoset	1	ND
Sanguinus oedipus	Cotton-headed tamarin	5	ND
Cebidae			
Aotes trivirgatus	Night monkey	2	ND
Cebus capucinus	White-throated capuchin	2	ND
Cebus apella	Tufted capuchin	3	ND
Ateles geoffroyl	Central American spider monkey	2	ND
Cattarrrhina	Old World monkeys		
Cercopithecidae			
Cercopithecus aethiops	Savannah monkey	3	D
Erythorcebus patas	Patas monkey	3	D
Macaca assamensis	Assamese monkey	4	D
Macaca mulatta	Rhesus monkey	5	D
Macaca cyclopis	Formosan monkey	3	D
Macaca arctoides	Stump-tailed monkey	4	D
Macaca nemestrina	Pig-tailed monkey	4	D
Macaca radiata	Bonnet monkey	3	D
Macaca fascicularis	Crab-eating monkey	5	D
Macaca fuscata	Japanese monkey	4	D
Papio hamadryas	Hamadryas baboon	4	D
Hylobatidae	Lesser apes		
Hylobates lar	White-handed gibbon	2	D
Hylobates agilis	Agile gibbon	1	D
Pongidae	Great apes		
Pongo pygmaeus	Orang-utan	1	D
Pan troglodytes	Chimpanzee	8	D

[a]ND, not detected; D, detected.

On the other hand, Lp(a) was undetectable in sera of Prosimians (ring-tailed lemur, grand galago and slow loris) and New World monkeys (common marmoset, cotton-headed tamarin, night monkey, white-throated capuchin, tufted capuchin and Central American spider monkey) by both the double diffusion method and a sensitive ELISA. It is worth noting that the New World monkeys are believed to have diverged from the Old World monkeys about 40 million years ago, which is the same time frame that has been estimated for a divergence of the apo(a) gene from the plasminogen gene (9).

STUDIES IN THE RHESUS MONKEY

Early studies by Fless *et al.* (24) showed that plasma rhesus monkey Lp(a) is heterogenous in size and density and that such heterogeneity can also occur within individual monkeys. Those studies also showed that the concentration of Lp(a) in plasma is unaffected by diets, although some remodeling of the Lp(a) core components (*i.e.*, cholesteryl esters and triglycerides) can occur (25). From the structural standpoint, rhesus monkey Lp(a), like that of humans, represents an LDL-like particle having as a protein moiety apoB100 covalently linked to apo(a) (26,27). Tomlinson *et al.* (28) cloned and sequenced the 3′ 4.5kb region of the apo(a) cDNA from an individual rhesus monkey and found it to share 93% nucleotide homology and a predicted 90% amino acid identity with its human counterpart. The rhesus sequence was also composed of multiple, tandem repeats encoding plasminogen kringle IV-like sequences, but not a kringle V-like domain (Fig. 2).

Scanu and co-workers (29,30) recently described a rhesus monkey pedigree with members exhibiting a spontaneous hypercholesteremia associated with a low density lipoprotein receptor (LDL-R) deficiency (Fig. 3), assessed by [^{125}I]LDL binding to cultured skin fibroblasts, ligand and immunoblot analyses. By using the polymerase chain reaction in subsequent studies, they showed that the affected monkeys are heterozygous for a nonsense mutation (TGG→TAG) in position 284 of exon 6 of the LDL-R gene, potentially resulting in a truncated 283 (31) (Fig. 4). Using this *Spe I* site as a marker, this mutation was shown to

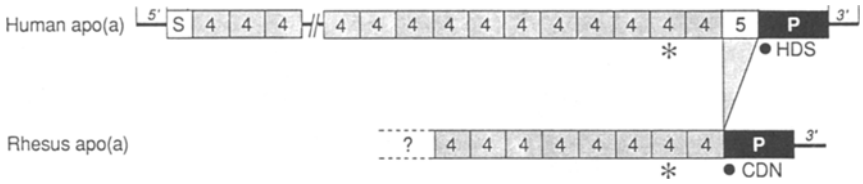

FIG. 2. Comparative representation of the structure of human and rhesus monkey apo(a). The sequence domains indicated are 5′ untranslated, signal peptide(S), plasminogen kringle IV- and V-like sequences of human and rhesus monkey apo(a), protease(P), and 3′ untranslated. Also indicated are the extra cysteine residue in kringle 36 that is a candidate for covalent linkage to apoB100 (*), the substitution of serine for the activation site arginine residue of plasminogen (●), and the catalytic triad of plasminogen (HDS) that is retained in human apo(a) and changed to CDN in rhesus monkey apo(a) (From Lawn, *et al.*; ref. 27).

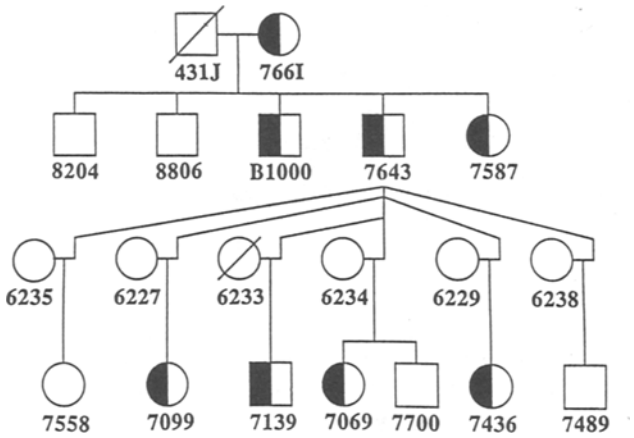

FIG. 3. Pedigree of the rhesus monkey family. Half-filled symbols indicate monkeys heterozygous for the mutation in exon 6 in the LDL-R gene as determined by the presence of an *Spe I* restriction site. Circles, females; squares, males; slashed lines, deceased animals.

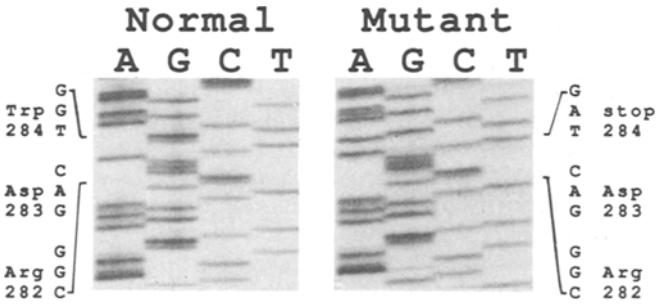

FIG. 4. Identification of the mutation in exon 6 of the rhesus monkey LDL-R gene. Exon 6 was amplified from genomic DNA of monkey 7643. PCR products were clones into M13 and sequenced. Five of thirteen clones had the sequence TGG at a position corresponding to amino acid 284 of the human protein (left). The remaining clones had a G→A transition which causes replacement of a tryptophan residue with a translation termination codon (right).

be present in all of the hypercholesteremic members of the pedigree and absent in unaffected members through three generations. Quantitative analyses of RNA obtained from liver biopsies showed that the abundance of the LDL-R RNA of the affected monkeys was also reduced by about 50% (Fig. 5).

All of the animals studied had levels of plasma Lp(a) protein ranging between 1.0 mg/dL and 57.5 mg/dL that were only weakly correlated with total plasma cholesterol, LDL cholesterol and apoB. LDL-R deficiency correlated with plasma LDL but not Lp(a). A seven-week fat challenge (16.5% lard, 0.64% cholesterol) that markedly raised the plasma LDL levels had no effect on plasma Lp(a) (30), suggesting that in the rhesus monkey the LDL receptor plays either only a limited or no functional role in the metabolism of Lp(a).

As in the human model, rhesus monkey apo(a) exhibits size polymorphism. A total of six isoforms were detected in all animals studied and classified by relating the R_f of each isoform to that of the apoB100 band. However, each animal presented either one or two isoforms (Fig. 6). About 25% of the animals studied exhibited a single fast band with an R_f of 1.13. About 5% of the animals were homozygous for the slow apo(a) isoform with an R_f of 0.80. The majority of the animals were heterozygous; the most frequent phenotypes were 0.64/1.13 and 0.80/1.13. The apo(a) isoforms with a migration faster than apoB100 (low molecular weight) had a preference for affiliation with the lighter lipoprotein particles and *vice versa*. As shown in Figure 7, animals with phenotype 1.13/1.13 had a tendency to have the highest plasma levels of Lp(a), whereas those with phenotype 0.80/0.80 had the lowest plasma concentrations. However, in spite of the same apo(a) phenotype, the plasma Lp(a) levels varied widely, supporting the concept that factors other than apo(a) phenotype participate in the regulation of these levels. In animals that were heterogeneous of the apo(a) isoforms the relation with plasma Lp(a) levels was equivocal, indicating that in the presence of heterozygosity it is difficult to assess the role that the apo(a) gene plays in the overall biology of Lp(a).

More recent studies showed the accumulation of Lp(a) in atherosclerotic arteries of rhesus monkeys with diet-induced atherosclerosis (32). Immunohistochemistry with monospecific Lp(a) antisera revealed striking accumulation of Lp(a) in atherosclerotic coronary artery lesions, whereas no Lp(a) was detected in the normal, nonatherosclerotic arteries. Analysis of paired tissue and serum samples from 17 male hyperlipoproteinemic monkeys revealed a significant correlation between the concentrations of aortic wall Lp(a) and serum Lp(a) levels. The serum cholesterol level failed to correlate with either aortic Lp(a) or serum Lp(a). These results add further evidence for the potential role of Lp(a) in the pathogenesis of atherosclerosis.

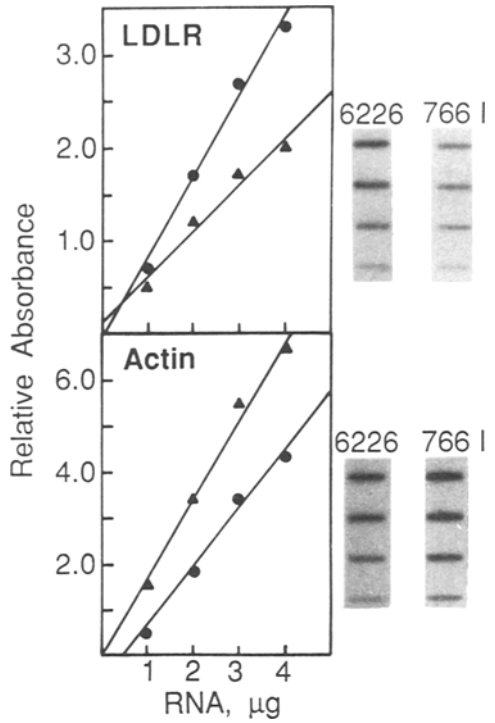

FIG. 5. Slot blot analysis of total cell RNA isolated from 766I, a hypercholesteremic monkey, and 6226, a normocholesteremic control. RNA (1, 2, 3 or 4 µg) from each monkey was applied to Zeta-Probe filter in triplicate. The filter was hybridized to a human LDL-R cDNA probe and scanned by laser densitometry. The average value for each point was calculated and the slope was determined by linear regression. One set of these data is shown at the right. The slopes are 0.50 (r=0.98) and 0.88 (r=0.99) for 766I and 6226, respectively. The filter was stripped and rehybridized with a mouse β-actin cDNA probe. Slopes of 1.77 (r=0.99) and 1.29 (r=0.99) were calculated for 766I and 6226, respectively. ●, 6226; ▲, 766I.

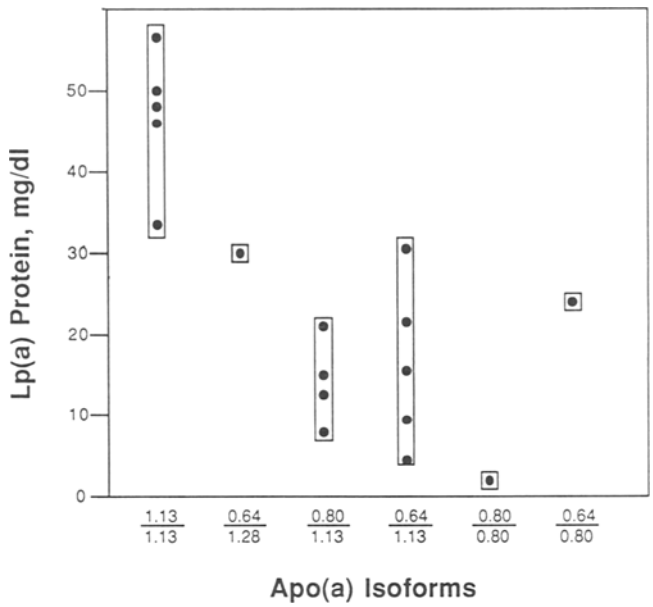

FIG. 7. Relation between apo(a) isoform phenotypes and plasma Lp(a) protein levels.

STUDIES IN THE BABOON

Studies by Rainwater and colleagues (33-38) have demonstrated that baboons possess Lp(a) that is similar to human Lp(a) and that the gene encoding apo(a) is closely linked to plasminogen (34). The mean serum level of 80 unrelated baboons was 4.7 mg/dL, with the distribution skewed toward the lower levels (35). Moreover, primary baboon hepatocytes were found to synthesize Lp(a) (36).

Baboon apo(a) exhibited at least nine isoforms distinguishable by size (36). Thirty-one different apo(a) isoform phenotypes were detected in a population of 165 unrelated baboons and found to be unaffected by changes in diet. The analysis of two large families suggested that apo(a) isoform patterns and serum Lp(a) concentrations are inherited. Putative parental alleles responsible for specific Lp(a) concentration was estimated to be 0.95 ± 0.04.

In a survey of 22 baboons, Hixson *et al.* (16) found a correlation between size of liver apo(a) transcripts (5.2∼11.2 kilobases) and size of serum apo(a) isoforms (r^2=0.996, $p<0.001$). However, the correspondence between length of the liver apo(a) messages and relative mobility of each specific isoform by gradient gel electrophoresis was not always observed, suggesting that post-transcriptional events might affect apo(a) production.

In more recent studies, twelve different size isoforms (including the null) of apo(a) were identified across the five subspecies (*Papio hamadryas hamadryas, P.h. cynocephalus, P.h. ursinus, P.h. papio* and *P.h. anubis*) and these isoforms acted as alleles (38). Significant differences in apo(a) isoform frequencies were found between subspecies. Plasma levels of Lp(a) also differed among subspecies. The genetic distances between the baboon

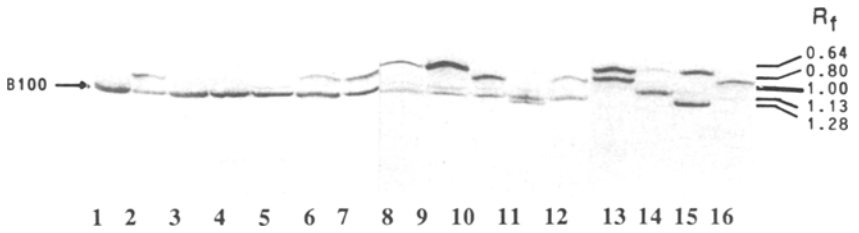

FIG. 6. Apo(a) isoform patterns of animals from a rhesus monkey pedigree with FH (see text). Some animals exhibited a single band and some two main bands. Each isoform was classified as ratio between their R_f and that of apoB100 by 2-16% polyacrylamide gel electrophoresis. 1 #766, 2 #431, 3 #1000, 4 #7643, 5 #8204, 6 #8806, 7 #7587, 8 #7099, 9 #7139, 10 #7436, 11 #7489, 12 #7558, 13 #6229, 14 #6234, 15 #6238, and 16 #7587.

subspecies based on the apo(a) isoforms were consistent with geographic relationships between the subspecies and previously assessed genetic marker information. However, the genetic distances based on apo(a) isoforms did not exhibit the same pattern of between-subspecies variation as phenotypic distances based on Lp(a) levels, suggesting that multiple genes might be involved in the determination of Lp(a) levels.

GENERAL COMMENTS

The results of the studies reviewed in the previous sections indicate that the Lp(a) of rhesus monkeys and baboons has structural properties very close to those of humans. It is apparent that like in human subjects, the plasma Lp(a) levels and apo(a) isoforms of these nonhuman primates are highly inherited traits, although this does not rule out the action of other factors, either genetic or environmental. Significant in this regard are the studies of Scanu and colleagues (29,30), showing that members of the same rhesus monkey family, although exhibiting the same fast apo(a) phenotype, had varying plasma levels of Lp(a). The studies on rhesus monkeys have also indicated that the plasma levels of Lp(a) do not closely correlate with LDL-R function, implying that the LDL receptor might have a very limited or no role in Lp(a) degradation. This conclusion is in accord with turnover studies showing a plasma residence time for Lp(a) significantly longer than that of LDL (unpublished data) and also with the dietary experiments documenting diverging effects on the plasma levels of Lp(a) and LDL.

While recognizing that nonhuman primates represent a good model for the study of the structure and biology of Lp(a), it is also important to stress that nonhuman primates offer unique features complementary to those in man. A case in point is absence of kringle V in rhesus monkey apo(a) that should permit an assessment of the functional significance of this kringle domain in Lp(a). Moreover, nonhuman primates offer the possibility of a comparatively wider experiment latitude like turnover studies, performance of serial liver biopsies, testing new pharmacological agents or potential drug toxicity. Not of lesser importance is the possibility of breeding studies to further elucidate the genetic basis of the inheritance of the apo(a) isoforms and also permit a careful analysis of the effect of development on plasma Lp(a) levels and distribution. These are just a few advantages that the nonhuman primate model offers in the study of Lp(a). An additional dimension is offered by the rhesus monkey family with FH described by Scanu and colleagues (29,30), since the members of this family provide an ideal setting for examining the potential role of the LDL receptor on Lp(a) metabolism.

ACKNOWLEDGMENTS

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