Highly polymorphic apolipoprotein A-IV locus in the baboon

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Abstract Apolipoprotein A-IV is found in mesenteric lymph chylomicrons, very low density lipoprotein particles, high density lipoprotein particles, and in the lipoprotein-free fraction of plasma. Apolipoprotein A-IV is polymorphic in a variety of species including human, dog, and horse. Efforts to estimate the impact of apolipoprotein A-IV structural variation on quantitative lipid levels in humans have been limited by the low frequency of the less common alleles. In the baboon, *Papio hamadryas anubis*, we have found apolipoprotein A-IV to be highly variable at the protein level with five alleles appearing at polymorphic frequency. We have confirmed the autosomal codominant inheritance of these five alleles in pedigreed baboons. The baboon has been shown to be a suitable animal model for the study of atherosclerosis, and the existence of a common, multi-allele apolipoprotein A-IV polymorphism in the baboon may be useful in elucidating the role of apolipoprotein A-IV in lipid metabolism. —**Ferrell, R. E., B. Sepehrnia, M. I. Kamboh, and J. L. VandeBerg.** Highly polymorphic apolipoprotein A-IV locus in the baboon. *J. Lipid Res.* 1990. **31:** 131–135.

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Apolipoprotein A-IV is a 46,000 dalton protein found in the lipoprotein-free fraction and in association with triglyceride-rich lipoprotein particles and high density lipoprotein particles in the plasma of rat, human and dog (1, 2). It is synthesized in the intestine and liver as preapolipoprotein A-IV, containing a 20 amino acid signal peptide which is cleaved cotranslationally to yield the mature apolipoprotein (3). While apoA-IV is relatively abundant in human plasma, with estimates ranging from 14 mg/100 ml (4–6) to 37 mg/100 (7), its role in lipoprotein metabolism has not been clearly elucidated. Steinmetz and Utermann (8) have presented evidence that apoA-IV is an in vitro activator of plasma lecithin:cholesterol acyltransferase. Stein et al. (9) showed that apoA-IV enhanced cholesterol efflux from human skin fibroblasts with an efficiency comparable to that of apolipoproteins E and A-I. Dvorin et al. (10) have suggested that apoA-IV is a ligand for the binding of rat HDL to primary rat hepatocytes. Finally, it has been suggested recently that A-IV has a modulatory role in the activation of lipoprotein lipase enzyme (11). These independently assayed functions of apoA-IV are probably interrelated.

In humans, genetically determined polymorphism at the APOA4 locus has been reported with two major alleles (frequency >0.01) and several minor alleles in Caucasian and Black populations (12–14). Insight into the role of ApoA-IV in lipoprotein metabolism might be gained using a quantitative genetic approach to estimate the impact of each allele on lipoprotein levels. This approach has contributed significantly to our understanding of the role of apolipoprotein E in determining cholesterol levels in the general population (15). However, the low frequency (<0.10) of the less common major allele in the human apoA-IV system restricts its usefulness to large population samples and has been the limiting factor in this approach. An alternative approach would be to determine the effect of genetically controlled apoA-IV variation on lipoprotein parameters in a nonhuman primate model. The baboon has been developed as such a model for genetic research on lipoprotein metabolism (16–18) and exhibits a wide array of lipoprotein phenotypes (19). Consequently, we surveyed a pedigreed baboon colony for genetically determined polymorphism at the APOA4 locus, and at the APOA1 and APOA2 loci as well.

MATERIALS AND METHODS

The population

The baboons involved in this investigation were 139 selected members of the breeding colony maintained at the Southwest Foundation for Biomedical Research. Most of them were olive baboons (*Papio hamadryas anubis*; classification according to refs. 20 and 21); the others were

Abbreviations: IEF, isoelectric focusing; TBS, Tris-buffered saline.

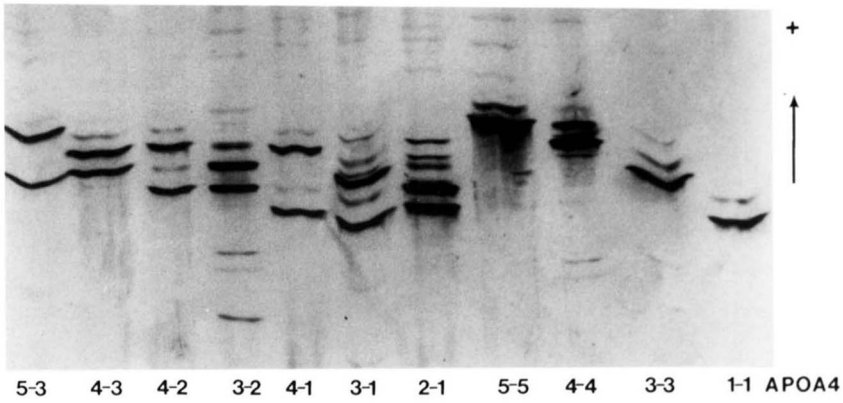

Fig. 1. IEF/immunoblotting patterns observed in unrelated baboon plasma samples. The corresponding phenotypes are shown below each lane.

yellow baboons (*P. h. cynocephalus*), red baboons (*P. h. papio*), or of mixed or unknown subspecies ancestry.

IEF/immunoblotting

Apolipoprotein A-IV phenotypes were determined by the IEF/immunoblotting method of Kamboh and Ferrell (13). Thin-layer (0.5 mm) polyacrylamide gels with a total acylamide concentration of 5% (mono = 4.85%; bis = 0.15%) were prepared to contain 6 M urea, 2% Pharmalyte, pH 4–6.5, and 30 μl of 0.1% riboflavin solution, in a final volume of 20 ml, to initiate the polymerization process. The electrode solutions for the anode and cathode were 1 M H_3PO_4 and 1 M NaOH, respectively. Two-microliter plasma samples absorbed on 5 × 4-mm Whatman 3MM wicks were applied 1 cm from the cathode electrode. IEF was performed with an LKB 2217 Ultraphor electrofusing unit connected to a Lauda RM6 cooling unit operating at 10–14°C and an LKB 2197 power supply set at 30 W and 2,500 V (no set limits for mA). The sample wicks were removed after 20 min, and IEF was continued for an additional 160 min.

After IEF, proteins were transferred partially from the gel onto a 0.20-μm pore-size nitrocellulose membrane (Schleicher and Schuell BA83) through capillary action by maintaining the gel-membrane contact for 10 min at room temperature. After the passive transfer of proteins, the membrane was exposed to 5% (w/v) nonfat dry milk in TBS (0.25 M NaCl, 0.03 M Tris-HCl, pH 8.0) for 45 min to block the remaining protein-binding sites, followed by incubation with rabbit anti-human apoA-IV antiserum (1 μl/ml) (provided by Dr. K. H. Weisgraber, Gladstone Foundation Laboratories) for 30 min and then three 10-min washings in TBS buffer. The membrane was then exposed for 30 min to goat anti-rabbit IgG conjugated with alkaline phosphatase (1 μl 5/ml), followed by three 10-min washings. Finally apoA-IV heterogeneity was visualized by staining the membrane for alkaline phosphatase using 20 mg β-naphthyl phosphate, 20 mg Fast Blue BB salt, and 50 mg magnesium sulfate in 50 ml buffer (1.8 g NaOH, 3.7 g boric acid/liter, pH).

RESULTS

ApoA-I and apoA-II were monomorphic, but apoA-IV was highly variable. Some of the apoA-IV phenotypes observed in plasma samples from the 139 baboons that we typed are shown in **Fig. 1** with our suggested phenotype designations. Each homozygous phenotype is represented by the presence of one major isoprotein band and several minor species. Heterozygous phenotypes are characterized by two major bands representing the products of two alleles, and associated minor species. The exact combination of isoprotein patterns in heterozygotes is consistent with the monomeric structure of the apoA-IV molecule. Ten phenotypes representing combinations of the products of five putative alleles at the APOA4 structural locus

TABLE 1. Segregation data for 64 pedigreed baboon progeny of two sires which had apoA-IV phenotypes 1-1 and 2-1, respectively

Phenotypes		Number of Progeny of Each Phenotype[a]					
Sire	Dam	1-1	2-1	2-2	3-1	4-1	4-2
1-1	1-1	5	1[b]	0	0	0	0
1-1	2-1	2	1	0	0	0	0
1-1	2-2	0	11	0	0	0	0
2-1	1-1	7	13	0	0	0	0
2-1	2-1	2	11	7	0	0	0
2-1	3-2	0	0	0	2	0	0
2-1	4-2	0	0	0	0	1	1

[a]Sexes are combined because no significant differences between sexes were observed.

[b]This progeny also had a third component of complement phenotype that was not consistent with the recorded sire's phenotype; we presume that the pedigree record is incorrect.

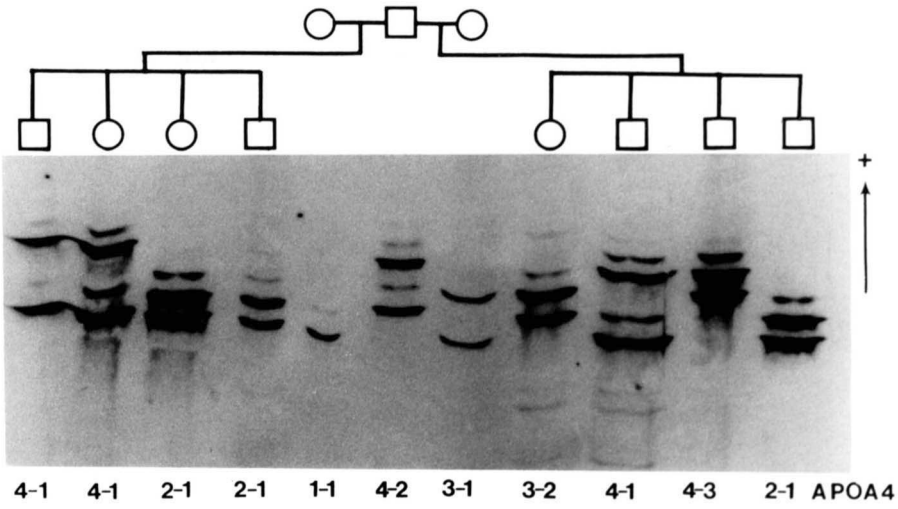

Fig. 2. Pedigree showing the autosomal codominant segregation of the *APOA4*1, APOA4*2, APOA4*3, APOA4*4* alleles in baboons.

were observed. Because of the low frequency of some alleles and the limited sample size, not all of the 15 phenotypes expected from the random combination of five alleles were encountered. To establish that these phenotypes are genetically determined and not due to sample degradation or post-translational modification of the primary APOA4 gene product, we typed 64 offsping of parents of the various phenotypes. All of the results were consistent with autosomal codominant inheritance, with one exception (see **Table 1**). The segregation pattern of four of the five alleles is shown in **Fig. 2**. Segregation of the remaining allele, *APOA4*5*, was observed in a separate pedigree (not shown). Based on their frequency and Mendelian segregation we have designated these alleles *APOA4*1–APOA4*5* in order of their focusing positions from the cathode to anode on IEF gels. ApoA-IV phenotype distribution and gene frequencies observed in 61 unrelated *P. h. anubis* are shown in **Table 2**.

TABLE 2. Distribution of apolipoprotein A-IV phenotypes and allele frequencies in unrelated *P. h. anubis*

Phenotype	Observed Number	Expected Number[a]	Allele Frequencies
1-1	23	21.81	
3-3	7	7.60	
2-1	2	1.82	*APOA4*1* = 0.598
3-1	25	25.75	*APOA4*2* = 0.025
3-2	1	1.08	*APOA4*3* = 0.353
4-3	2	0.69	*APOA4*4* = 0.016
5-3	1	0.34	*APOA4*5* = 0.008
Total	61	59.09	

[a]Expectation based on the assumption of Hardy-Weinberg equilibrium.

DISCUSSION

Quantitative genetic studies of polymorphic genetic variation in human apolipoprotein E has provided insight into the role of apoE in lipoprotein metabolism (15). Similar approaches to the study of apoA-IV are limited by the low frequency of alleles at the human APOA4 locus. Menzel et al. (22) have recently suggested that apoA-IV variation has an impact on HDL cholesterol levels in man. The present study documents the occurrence of five alleles at the APOA4 structural locus in the baboon. Each is present at polymorphic frequency (>1%), and shows discrete autosomal codominant segregation in pedigrees. This level of polymorphism in apoA-IV is much higher than has been observed in any other species. Indeed the average heterozygosity at the APOA4 locus in baboons is 0.494. This variation may serve as a tool to determine the role of apoA-IV in lipid metabolism in baboon model. Like human apoA-IV, baboon's apoA-IV major isoforms are also associated with several corresponding minor isoforms in each phenotype observed. This interindividual microheterogeneity in baboon's plasma could be due to post-synthetic modiffications in the apoA-IV molecule similarly as suggested in human apoA-IV (13). Each allele has characteristic associated minor bands that cosegregate with the major isoforms suggesting that the minor bands are derived from the primary apoA-IV gene product and are not the product of an independent genetic locus.

Two other apolipoproteins have been examined for evidence of genetic variation in our baboon population. No variation was detected in baboon apoE by IEF that resolves the human apoE isoforms (D. Hewett-Emmett, personal communication); but the specific apolipoprotein moiety of baboon Lp[a] is highly polymorphic and exists

in many discrete size isoforms that can be resolved by SDS-electrophoresis and are inherited as autosomal codominant traits (23).

Research at the DNA level also has revealed polymorphisms in baboon apolipoprotein genes. Two restriction fragment length polymorphisms (RFLPs) have been detected in apoA-I (24, 25) and four in apoB (26). However, a search for baboon apoE RFLPs did not reveal any variation (27).

The existence of genetic variation in several apolipoprotein genes, as well as in the LDL receptor (J. E. Hixson, personal communication) and the lipolytic enzyme lecithin:cholesterol acyltransferase (LCAT) (28) in a nonhuman primate model provides new opportunities for research. In particular, interactions among these loci in determining lipoprotein characteristics can now be investigated in a primate model that closely resembles humans in its characteristics of lipoprotein metabolism and atherosclerosis. In addition, because environmental variables can be carefully controlled from birth, dietary manipulations will enable the characterization of genotype-environment interactions that influence lipoprotein phenotypes.

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