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The Apo(a) Gene is the Major Determinant of Variation in Plasma Lp(a) Levels in African Americans

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Summary

The distributions of plasma lipoprotein(a), or Lp(a), levels differ significantly among ethnic groups. Individuals of African descent have a two- to threefold higher mean plasma level of Lp(a) than either Caucasians or Orientals. In Caucasians, variation in the plasma Lp(a) levels has been shown to be largely determined by sequence differences at the apo(a) locus, but little is known about either the genetic architecture of plasma Lp(a) levels in Africans or why they have higher levels of plasma Lp(a). In this paper we analyze the plasma Lp(a) levels of 257 sibling pairs from 49 independent African American families. The plasma Lp(a) levels were much more similar in the sibling pairs who inherited both apo(a) alleles identical by descent (IBD) (r = .85) than in those that shared one (r = .48) or no (r = .22) parental apo(a) alleles in common. On the basis of these findings, it was estimated that 78% of the variation in plasma Lp(a) levels in African Americans is attributable to polymorphism at either the apo(a) locus or sequences closely linked to it. Thus, the apo(a) locus is the major determinant of variation in plasma Lp(a) levels in African Americans, as well as in Caucasians. No molecular evidence was found for a common "high-expressing" apo(a) allele in the African Americans. We propose that the higher plasma levels of Lp(a) in Africans are likely due to a yet-to-be-identified *trans*-acting factor(s) that causes an increase in the rate of secretion of apo(a) or a decrease in its catabolism.

Introduction

Lipoprotein(a), or Lp(a), is an atherogenic lipoprotein composed of a particle of LDL to which is attached a highly polymorphic glycoprotein—apolipoprotein(a), or apo(a). In all populations studied to date, plasma levels of Lp(a) vary over a much broader range than do plasma concentrations of LDL (1,000-fold range vs. ~3-fold range) (Albers et al. 1990). The distribution of plasma Lp(a) levels differs significantly between ethnic groups (Guyton et al. 1985; Sandholzer et al. 1991; Cobbaert and Kesteloot 1992). In Caucasians, Orientals, and Hispanics, the distribution is highly skewed toward lower levels, whereas in Africans and African Americans the distribution is significantly less skewed, and the median plasma Lp(a) level is two- to threefold higher (Guyton et al. 1985; Sandholzer et al. 1991; Haffner et al. 1992).

High plasma levels of Lp(a) are associated with premature atherosclerosis (Rader and Brewer 1992), so factors that contribute to its plasma levels have been extensively investigated. In Caucasians, plasma levels of Lp(a) are highly heritable (Albers et al. 1974; Austin et al. 1992; Boomsa et al. 1993). It has been estimated that 40%–70% of the interindividual variation in plasma Lp(a) levels is attributable to allelic differences in the sizes of the apo(a) gene and glycoprotein (Boerwinkle et al. 1989, 1992; Trommsdorff et al. 1995). This size polymorphism is due to variations in the number of a tandemly repeated sequence in the apo(a) gene encoding a cysteine-rich motif called "kringle 4" (K4) (Lackner et al. 1993; van der Hoek et al. 1993). In general, there is an inverse relationship between the number of K4 repeats in the apo(a) gene and the plasma level of Lp(a) (Boerwinkle et al. 1989); however, some apo(a) alleles of identical sizes may be associated with widely different plasma Lp(a) levels (Lackner et al. 1991; Cohen et al. 1993; Perombelon et al. 1994). These size-independent differences in plasma Lp(a) levels are largely due to yet-to-be-identified sequence differences linked to the apo(a) locus (Boerwinkle et al. 1992). Analysis of multiple sequence polymorphisms at the apo(a) locus in Caucasian populations has revealed a high degree of linkage disequilibrium across the entire gene (Mancini et al. 1995; Mooser et al. 1995). Apo(a) haplotypes have been defined that are consistently associated with characteristic plasma levels of Lp(a) (Mancini et al. 1995; Mooser et

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al. 1995); however, the *cis*-acting sequences that are responsible for the observed variations in plasma levels of Lp(a) among individuals with apo(a) alleles of the same size remain to be identified.

In contrast to the situation in Caucasians, little is known about the genetic architecture of plasma Lp(a) levels in Africans and African Americans. The frequencies of smaller apo(a) alleles, which tend to associate with higher plasma levels of Lp(a), are not higher in Africans (Helmhold et al. 1991; Sandholzer et al. 1991; Marcovina et al. 1993*b;* Gaw et al. 1994). Thus, the higher plasma levels of Lp(a) in individuals of African descent are not due to a greater proportion of apo(a) alleles containing fewer K4 repeats. As a first step in defining the genetic contribution to plasma Lp(a) levels in African Americans, we have performed family studies to determine how much of the interindividual variation in plasma Lp(a) levels is due to sequences within or closely linked to the apo(a) gene.

Subjects and Methods

Subjects

A total of 307 individuals, from 35 independent African American families living within 50 miles of Dallas and 17 families from New Orleans, were recruited into the study. Informed consent was obtained from each participant, and the study protocol was approved by the institutional review boards. The age, sex, apo(a) isoforms, and apo(a) genotypes for each family member are given in table 1. Of the 52 families, 48 were nuclear families, and 4 were three- or four-generation families (table 1, pedigrees 15, 38, 44, and 49). Both parents were studied in 44 families, whereas only a single parent was available in 8 families (families 10, 12, 24, 30, 31, 44, 47, and 49). In addition, four in-laws from the three-generation families were sampled.

The 31 women who were postmenopausal are identified in table 1 (each of these women is denoted by means of a superscript "d" appended to the "Age"-column entry), as are those who are on hormone-replacement therapy (each of these women is denoted by a superscript "e" appended to the "Age"-column entry). All but three of the postmenopausal women were in the parental generation.

Fasting venous blood was collected from each family member. Plasma was isolated <1 h after collection, and aliquots were stored at −80°C. Plasma lipoproteins were quantitated in the laboratory of Dr. Scott Grundy, according to the procedures of the Lipid Research Clinic (Lipid Research Clinic Program 1982). The plasma levels of Lp(a) were assayed <3 mo after collection, by use of a sensitive sandwich enzyme–linked immunoabsorbent assay (ELISA), exactly as described elsewhere (Marcovina et al. 1995). The ELISA assay employs IgG-a6, a mouse monoclonal antibody directed against the type 2 K4 repeats, as the capture antibody and employs IgG-a40, whose epitope is in the type 9 K4 repeat, as the detecting antibody. The assay is not influenced by the number of K4 repeats in the apo(a) glycoprotein (Marcovina et al. 1995). In the present paper the amount of plasma Lp(a) is expressed as total Lp(a) mass.

Analysis of Length Polymorphism in Apo(a) Gene and Protein

The number of K4 repeats in the apo(a) alleles, which varied from 13 to 41 in our sample, was determined by Southern blot analysis after size fractionation of *Hpa*I-digested high-molecular-weight genomic DNA, by use of pulsed-field gel electrophoresis (PFGE) (Lackner et al. 1993). The size of the apo(a) isoforms was determined by size fractionating 3 μl of plasma on a 2% (w/v) SDS-agarose gel, transferring the plasma proteins to a nitrocellulose membrane, and then immunoblotting with an apo(a)-specific monoclonal antibody against an epitope in the type 1 and type 2 K4 repeats (IgG-a5). The apo(a) isoforms were classified on the basis of the number of K4 repeats contained within each allele, which was determined by their migration relative to standards described elsewhere (Lackner et al. 1993).

Analysis of the Segregation of Apo(a) Alleles in the Families

PFGE analysis of the size of the apo(a) gene was fully informative in 45 of the African American families. In three families, one of the parents was homozygous for two apo(a) alleles with the same number of K4 repeats (table 1, families 11, 21, and 33); in all three of these parents the two alleles could be distinguished by use of other sequence polymorphisms at the apo(a) locus. One individual was heterozygous for a length polymorphism characterized elsewhere (Wade et al. 1993; Mooser et al. 1995) (table 1, family 11, individual I.2), and two parents were heterozygous for a 1-bp polymorphism revealed by use of the SSCP technique (Cohen et al. 1993) (table 1, individuals I.1 and I.2 in families 21 and 33, respectively). In three of the families (table 1, families 10, 12, and 49) with only a single parent, just one of the two apo(a) alleles of the missing parent was identified in the offspring. In one family (table 1, family 15), the parents were heterozygous for apo(a) alleles of the same sizes, but the parental alleles could be distinguished by an SSCP polymorphism.

Therefore, all four parental alleles could be distinguished in 49 families, and these families were included in the sibling-pair analysis. A total of 21 offspring from an additional eight sibships in the 2d and 3d generations of the multigenerational families were also included in the sib-pair analysis. In seven of the offspring, neither apo(a) allele matched the paternal alleles, and these indi-

Table 1

Plasma Apo(a) Genotypes, Apo(a) Isoforms, and Lp(a) Levels, in 52 African American Families

Pedigree and Subject[a]	Age (years)	Sex	Apo(a) Size (No. of K4 Repeats)		Lp(a) (mg/dl)
			Isoform[b]	Genotype[c]	
1:					
I.1	37	M	26/n	26/32	5
I.2	33	F	22/24	22/24	45
II.1	7	F	22/n	22/32	2
II.2	3	M	24/n	24/32	18
II.3	2	M	24/26	24/26	39
2:					
I.1	39	M	21/27	21/27	97
I.2	36	F	13/24	13/24	37
II.1	17	F	13/27	13/27	56
II.2	14	F	21/24	21/24	87
II.3	10	M	24/27	24/27	45
3:					
I.1	46	M	22/n	22/23	93
I.2	40[d,e]	F	19/23	19/23	83
II.1	20	F	23/23	23/23	38
II.2	15	F	19/22	19/22	71
II.3	10	F	22/23	22/23	96
4:					
I.1	43	M	30/33	30/33	8
I.2	42	F	28/n	28/38	10
II.1	21	M	30/n	30/38	7
II.2	17	F	28/n	28/33	3
II.3	12	M	30/n	30/38	2
5:					
I.1	33	M	24/31	24/31	13
I.2	35	F	26/28	26/28	16
II.1	7	F	24/28	24/28	32
II.2	5	M	28/31	28/31	22
II.3	3	M	24/28	24/28	39
6:					
I.1	60	M	16/28	16/28	44
I.2	59[d,e]	F	30/40	30/40	16
II.1	35	M	16/30	16/30	30
II.2	30	F	16/40	16/40	34
II.3	25	F	16/40	16/40	34
7:					
I.1	33	M	n/25	21/25	18
I.2	33	F	21/22	21/22	86
II.1	14	F	21/n	21/21	75
II.2	10	F	22/25	22/25	100
II.3	6	M	21/n	21/21	57
8:					
I.1	65	M	n/29	26/29	29
I.2	60[d]	F	24/n	24/36	58
II.1	38	M	24/n	24/26	49
II.2	33	F	NA	26/36	1
II.3	23	M	24/n	24/26	55
9:					
I.1	35	M	23/25	NA	47
I.2	35	F	n/38	NA	1
II.1	10	F	n/23	NA	70
II.2	NA	NA	23/38	NA	73
II.3	7	M	25/38	NA	49
10:[f]					
I.1[g]	35	M	(24/n)	(24/–)	NA
I.2	35	F	19/31	19/31	74
II.1	15	M	24/n	24/31	3
II.2	13	F	24/31	24/31	34
II.3	8	F	n/31	24/31	10
11:					
I.1	47	M	24/n	(24/35)	16
I.2	47	F	22/22	22a/22b[h]	96
II.1	26	F	22/24	22a/24	58
II.2	21	F	22/n	22b/35	3
II.3	16	M	22/24	22a/24	128
12:[f]					
I.1[g]	NA	NA	NA	(21/–)	NA
I.2	63[d]	F	n/n	26/28	2
II.1	45	M	21/28	21/28	87
II.2	44	F	21/28	21/28	65
II.3	35	F	21/26	21/26	83
13:					
I.1	70	M	n/n	23/34	1
I.2	70[d]	F	19/23	19/23	125
II.1	38	F	23/n	23/34	38
II.2	36	M	23/n	23/23	38
14:					
I.1	55	M	26/29	26/29	32
I.2	50[d]	F	n/25	23/25	43
II.1	25	M	25/29	25/29	37
II.2	23	F	n/26	23/26	31
II.3	19	M	25/26	25/26	34
II.4	16	F	25/26	25/26	42
15:					
I.1	59	M	28/n	28a/29b[i]	26
I.2	60[d,e]	F	n/29	28b/29a[i]	5
II.1	37	F	n/29	28a/29b	16
III.1	15	M	29/32	29/32	6
III.2	10	M	28/n	28/28	3
II.2	35	F	28/n	28b/29a	8
III.1	13	M	23/29	23/29	63
III.2	8	M	n/n	29/32	6
II.3	25	F	28/28	28a/28b	13
16:					
I.1	51	M	23/27	23/27	33
I.2	44	F	27/n	27/30	12
II.1	19	F	23/27	23/27	25
II.2	16	F	27/27	27/27	14
II.3	14	F	27/27	27/27	14
17:					
I.1	39	M	26/27	26/27	33
I.2	34	F	29/n	29/38	8
II.1	11	M	27/n	27/38	24
II.2	10	M	27/29	27/29	22
II.3	8	M	26/29	26/29	22

(*continued*)

Table 1 (continued)

Pedigree and Subject[a]	Age (years)	Sex	Apo(a) Size (No. of K4 Repeats) Isoform[b]	Apo(a) Size (No. of K4 Repeats) Genotype[c]	Lp(a) (mg/dl)
18:					
I.1	45	M	15/23	15/23	117
I.2	38	F	18/22	18/22	36
II.1	18	M	15/22	15/22	55
II.2	15	F	15/18	15/18	133
II.3	14	F	15/18	15/18	127
19:					
I.1	36	M	25/28	25a/28	16
I.2	35	F	25/n	25b/31	5
II.1	17	M	25/n	25a/31	10
II.2	14	F	25/28	25a/28	16
II.3	13	M	28/n	28/31	5
20:					
I.1	39	M	n/23	21/23	27
I.2	37	F	27/n	27/28	21
II.1	13	F	n/n	21/28	4
II.2	11	F	n/n	21/28	1
II.3	9	F	n/n	21/28	3
II.4	6	M	n/27	21/27	21
21:					
I.1	50	M	26/26	26a/26b[i]	35
I.2	46	F	23/26	23/26	59
II.1	23	M	23/26	23/26b	40
II.2	20	M	26/26	26/26b	39
II.3	13	F	26/26	26/26b	40
22:					
I.1	53	M	26/n	26/41	21
I.2	50[d,e]	F	n/n	22/34	1
II.1	29	M	n/n	34/41	1
II.2	27	M	n/26	22/26	22
II.3	22	F	n/26	22/26	11
23:					
I.1	36	M	23/27	23/27	78
I.2	35	F	26/32	26/32	22
II.1	15	F	23/32	23/32	41
II.2	13	F	23/26	23/26	82
II.3	6	M	23/32	23/32	40
24:					
I.1[g]	NA	NA	NA	(26/36)	NA
I.2	72[d]	F	15/23	15/23	23
II.1	49	M	23/n	23/26	5
II.2	37	F	23/36	23/36	11
II.3	31	F	23/n	23/26	5
II.4	29	F	15/n	15/26	23
25:					
I.1	57	M	26/29	26/29	30
I.2	51[d]	F	27/29	27/29	34
II.1	30	M	29/29	29/29	9
II.2	29	F	26/27	26/27	50
II.3	28	M	29/29	29/29	20
II.4	26	M	26/27	26/27	21
II.5	23	M	27/29	27/29	20
II.6	22	M	29/29	29/29	9
26:					
I.1	52	M	26/n	26/34	20
I.2	49	F	24/n	24/28	20
II.1	23	F	24/26	24/26	46
II.2	17	M	24/26	24/26	48
II.3	14	M	26/n	26/28	34
27:					
I.1	46	M	25/29	25/29	49
I.2	45[d]	F	22/n	22/34	25
II.1	15	F	25/n	25/34	32
II.2	13	F	22/29	22/29	40
II.3	10	F	22/25	22/25	78
28:					
I.1	40	M	26/28	26/28	45
I.2	32	F	24/32	24/32	44
II.1	14	F	26/32	26/32	26
II.2	13	F	24/28	24/28	59
II.3	9	M	28/32	28/32	24
29:					
I.1	38	M	n/27	23/27	19
I.2	36	F	25/28	25/28	61
II.1	17	F	n/25	23/25	60
II.2	17	F	n/25	23/25	57
II.3	19	F	n/28	23/28	16
II.4	15	M	27/28	27/28	23
II.5	15	M	27/28	27/28	47
30:					
I.1[g]	NA	NA	NA	(27/29)	NA
I.2	32	F	25/28	25/28	38
II.1	14	M	25/27	25/27	32
II.2	13	F	25/29	25/29	42
II.3	11	M	n/28	27/28	10
II.4	7	M	25/n	25/27	43
31:					
I.1[g]	NA	M	(26/28)	NA	NA
I.2	43	F	17/25	NA	43
II.1	30	M	17/26	NA	29
II.2	29	M	25/28	NA	29
II.3	23	M	25/28	NA	24
II.4	15	F	25/28	NA	15
32:					
I.1	45	M	27/29	27/29	65
I.2	46	F	25/28	15–17[j]/28	11
II.1	19	F	28/28	28/29	19
II.2	17	F	25/29	15–17/29	63
II.3	15	F	27/29	27/28	29
33:					
I.1	50	M	30/n	30/40	5
I.2	44	F	26/26	26a/26b[i]	12
II.1	19	F	n/30	26a/30	9
II.2	15	M	26/n	26b/40	2

(*continued*)

Table 1 (continued)

Pedigree and Subject[a]	Age (years)	Sex	Apo(a) Size (No. of K4 Repeats) Isoform[b]	Apo(a) Size (No. of K4 Repeats) Genotype[c]	Lp(a) (mg/dl)
34:					
I.1	48	M	25/29	25/29	34
I.2	47[d]	F	22/23	22/23	26
II.1	18	F	23/29	23/29	34
II.2	17	F	22/25	22/25	24
II.3	14	F	23/29	23/29	48
35:					
I.1	40	M	n/n	22/36	4
I.2	39	F	28/31	28/31	15
II.1	16	M	31/n	31/36	5
II.2	11	F	31/n	31/36	12
II.3	8	M	31/n	31/36	6
36:					
I.1	67	M	27/39	27/39	43
I.2	58[d]	F	18/n	18/28	19
II.1	37	M	18/n	18/39	22
II.2	36	F	n/30	28/30[k]	2
II.3	33	F	n/n	28/39	1
II.4	25	M	19/n	19/28[k]	58
37:					
I.1	68	M	17/24	17/24	145
I.2	55[d]	F	n/28	16/28	4
II.1	38	M	n/28	27/28[k]	38
II.2	37	M	n/28	21/28[k]	3
II.3	35	F	n/25	24/28	20
II.4	31	M	n/17	16/17	84
II.5	28	M	17/28	17/28	92
38:					
I.1	61	M	n/25	24/25	18
I.2	60[d]	F	25/30	25/30	49
II.1a[g]	NA	NA	NA	(27/–)	NA
II.1	39	F	n/30	29/30[k]	37
III.1	16	F	27/29	27/29	53
III.2	15	M	n/30	29/30[k]	36
III.3	10	F	27/29	27/29	41
II.2	38	M	25/25	25/25	60
II.3	NA	F	n/25	24/25	32
II.4	25	M	n/25	24/25	43
II.5	23	M	n/30	24/30	14
II.6	22	M	25/25	25/25	58
39:					
I.1	69	M	22/27	22/27	67
I.2	63[d]	F	21/23	21/23	113
II.1	39	M	21/22	21/22	127
II.2	36	M	21/27	21/27	66
II.3	34	F	23/27	23/27	68
II.4	32	F	21/22	21/22	198
II.5	30	F	21/22	21/22	114
II.6	27	M	22/23	22/23	62
II.7	25	F	21/27	21/27	76
II.8	20	M	23/27	23/27	52
40:					
I.1	31	M	n/25	24/25	27
I.2	31	F	n/n	18/26	19
II.1	10	M	n/25	18/25	51
II.2	9	M	24/n	24/26	14
II.3	8	F	n/24	18/24	17
41:					
I.1	68	M	29/30	29/30	20
I.2	56[d]	F	21/24	21/24	232
II.1	36	F	24/30	24/30	32
II.2	35	F	24/30	24/30	35
II.3	33	F	21/29	21/29	98
42:					
I.1	42	M	26/n	26/36	37
I.2	46	F	18/27	18/27	32
II.1	24	F	n/22	18/22[k]	38
II.2	20	M	26/27	26/27	26
II.3	16	M	27/36	27/36	10
II.4	15	F	n/27	26/27	44
43:					
I.1	45	M	23/31	23/31	23
I.2	43[d]	F	19/26	19/26	78
II.1	25	F	19/31	19/31	83
II.2	22	M	19/31	19/31	65
II.3	18	M	19/23	19/23	89
44:					
I.1[g]	NA	NA	NA	(30/36)	NA
I.2	73[d]	F	n/29	27/29	5
II.1	51[d,e]	F	n/30	27/30	4
III.1	33	M	30/n	30/32	2
III.2	31	F	n/32	27/32	1
III.2a	35	M	28/n	28/33	5
IV.1	9	M	n/n	27/33	.3
IV.2	6	F	32/n	32/33	.3
III.3	27	M	26/30	26/30	15
II.2	50	M	29/30	29/30	11
II.3	42	M	29/n	29/36	2
II.3a	37[d]	F	27/30	27/30	40
III.1	12	M	29/30	29/30	7
III.2	6	F	29/30	29/30	6
II.4	40[d]	F	n/30	27/30	4
III.1	22	F	23/n	23/27	24
III.2	19	F	28/30	28/30	7
III.3	10	F	28/30	28/30	9
45:					
I.1	41	M	23/29	23/29	30
I.2	38[d]	F	24/28	24/28	59
II.1	12	F	24/29	24/29	45
II.2	10	M	23/24	23/24	67
II.3	7	M	24/29	24/29	48
II.4	6	M	23/24	23/24	49

(*continued*)

Table 1 (continued)

Pedigree and Subject[a]	Age (years)	Sex	Apo(a) Size (No. of K4 Repeats) Isoform[b]	Apo(a) Size (No. of K4 Repeats) Genotype[c]	Lp(a) (mg/dl)
46:					
I.1	52	M	21/n	21/40	63
I.2	47	F	n/24	23/24	21
II.1	29	F	n/n	23/40	2
II.2	25	M	21/24	21/24	74
II.3	18	F	21/24	21/24	109
II.4	17	M	21/n	21/23	53
II.5	16	F	n/n	23/40	7
47:					
I.1[g]	NA	NA	NA	(17/40)	NA
I.2	50[d]	F	18/21	18/21	127
II.1	32	F	21/n	21/40	42
II.2	30	F	17/21	17/21	72
II.3	27	F	17/21	17/21	118
II.4	25	M	17/18	17/18	129
II.5	21	F	18/n	18/40	56
II.6	19	F	17/21	17/21	124
II.7	16	F	21/n	21/40	28
II.8	10	M	17/18	17/18	108
48:					
I.1	62	M	n/n	25/28	1
I.2	62[d]	F	24/n	24/27	37
II.1	36	F	24/n	24/28	38
II.2	27	F	24/n	24/28	42
II.3	19	F	n/n	27/28	.3
49:[f]					
I.1[g]	NA	NA	(17/–)	NA	NA
I.2	69[d]	F	15/29	15/29	79
II.1	47[d]	F	17/29	17/29	112
II.1a	46	M	25/n	25/29	40
III.1	21	M	17/25	17/25	96
III.2	8	F	17/n	17/29	78
II.2	45	M	17/29	17/29	90
II.2a	47[d]	F	23/31	23/31	40
III.1	20	F	n/31	27/31[k]	2
III.2	19	F	23/29	23/29	46
50:					
I.1	57	M	19/n	19/24	113
I.2	56[d,e]	F	n/23	17/23	33
II.1	33	F	n/24	17/24	31
II.2	32	F	23/24	23/24	51
II.3	29	M	n/19	17/19	96
51:					
I.1	49	M	21/26	21/26	26
I.2	46[d,e]	F	23/27	23/27	40
II.1	19	F	26/27	26/27	18
II.2	17	M	26/27	26/27	27
II.3	16	M	21/27	21/27	22
52:					
I.1	63	M	22/24	22/24	61
I.2	58[d,e]	F	20/28	20/28	81
II.1	40	F	22/28	22/28	47
II.2	38	M	24/28	24/28	38
II.3	30	F	22/28	22/28	81

NOTE.—NA = not available.

[a] Third-generation individuals are listed immediately below their parent(s). An "a" appended to the designation denotes that the individual is an in-law.

[b] n = null (no apo[a] protein was visible for an apo[a] allele) or low-expressing allele. Independent apo(a) alleles are underlined. If only a single apo(a) isoform was visible on immunoblot analysis, and if the genotype analysis showed that the individual was homozygous for the same-size apo(a) allele, it was inferred that the individual was homozygous for the same-size isoform.

[c] Independent apo(a) alleles are underlined. If a parent was dead and the genotype could be inferred from the genotypes of the offspring, the genotype of the surviving parent is shown (in parentheses).

[d] Individual was postmenopausal.

[e] Individual was postmenopausal and on hormone-replacement therapy.

[f] None of the four parental apo(a) alleles could be unambiguously distinguished.

[g] Individual deceased or sample not obtained.

[h] TTTTA length polymorphism (Mooser et al. 1995) was used to follow segregation of apo(a) alleles. "a" and "b" denote that alleles are different from one another.

[i] SSCP1 polymorphism (Mooser et al. 1995) was used to follow segregation of apo(a) alleles.

[j] Contains an extra *Hpa*I site in K4-coding region and cosegregates with an apo(a) isoform with 25 K4 repeats.

[k] False paternity.

viduals (in table 1, each of these individuals is denoted by a superscript "k" appended to the appropriate "Genotype"-column entry) were not included in the sibling-pair analysis. Of the 57 total sibships, 8 families had two children, 37 families had three children, 7 families had four children, 2 families had five children, 1 family had six children, and 2 families had eight children. The number of sibships ($n = 57$) is greater than the number of families ($n = 49$) because more than one sibship was obtained from some of the multigenerational families. Our analysis of the contribution of independent apo(a) alleles to plasma Lp(a) levels in the African American

families was compared with the results of a similar analysis performed elsewhere on 43 independent nuclear Caucasian families (Boerwinkle et al. 1992).

Estimation of Plasma Lp(a) Level Associated with Independent Apo(a) Alleles

Genomic DNA was extracted from the white blood cells of each family member by use of a genomic DNA extractor (Applied Biosystem), and PCR was used to characterize a $(TTTTA)_{5-12}$ length polymorphism located within the 5′ flanking region of the apo(a) gene (Mooser et al. 1995). Haplotypes were constructed by following the segregation of the TTTTA and K4 repeat polymorphisms in the families. The plasma levels of Lp(a) associated with each independent apo(a) allele were estimated by a comparison of the results of the immunoblot analysis with the results of PFGE analysis, as described elsewhere (Mooser et al. 1995). In individuals who were heterozygous for an apo(a) allele with no detectable plasma apo(a), the amount of Lp(a) associated with the "expressing" allele was considered to be equal to the total plasma Lp(a), as measured by the ELISA assay. By means of this methodology, the plasma Lp(a) level associated with each of the four parental alleles could be estimated in 23 of the African American families. In eight families, only three of the parental alleles could be included, in one family only two alleles. Finally, in 20 families all four parental alleles were associated with immunodetectable plasma apo(a), so the relative contribution of each allele could not be accurately estimated. The contribution of independent apo(a) alleles to plasma Lp(a) level could be estimated for 118 alleles.

These results were compared with the results for 43 Caucasian families that were collected and analyzed in an identical fashion (Mooser et al. 1995). A plasma Lp(a) level could be assigned to all four parental alleles in 34 families and to three apo(a) alleles in 4 families, for a total of 148 apo(a) alleles. In five of the Caucasian families, all four parental alleles were associated with significant amounts of apo(a) protein, so the plasma Lp(a) associated with each allele could not be accurately estimated; these alleles were not included in the analysis.

Statistical Methods

Statistical computing was performed by use of SPLUS. The frequency distributions of plasma Lp(a) levels and apo(a) allele sizes were compared in African Americans and Caucasians by the Kolmogorov-Smirnov test (Kendall and Stuart 1979). The frequency of "low-expressing" apo(a) alleles was compared in African Americans and Caucasians by Pearson's χ^2 test. Correlations between the Lp(a) concentrations of spouses and between the concentrations in parents and those in their offspring were estimated by Pearson's correlation. Correlations between the plasma Lp(a) concentrations of siblings were estimated by the intraclass correlation.

The heritability index of plasma Lp(a) in African Americans was estimated by regression of the average of the offspring plasma Lp(a) on the median parent values, by weighted least squares. Weights to adjust for unequal family sizes were calculated as suggested by Falconer (1989, pp. 148–186), with an unweighted least-squares estimate used as the initial estimate of heritability in the weights.

The observed variation in plasma Lp(a) levels that could be accounted for by polymorphism in the apo(a) gene was estimated by a sibling-pair regression procedure developed by Haseman and Elston (1972). This procedure was chosen for the following reasons: First, simulation studies (Blackwelder and Elston 1982; Amos et al. 1989; Wan et al. 1997) have suggested that the method is robust to nonindependent sibling pairs, which occur in all sibships larger than three. Second, although the procedure was originally based on a model under which genetic variance was due to a single biallelic gene, we have shown that the procedure is also valid when genetic variance is due to multiple genes each having two or more alleles (Stoesz et al., in press). Third, the procedure has provided consistent estimates of the Lp(a) variation that can be accounted for by the apo(a) gene, in three different Caucasian populations (Boerwinkle et al. 1992; Kraft et al. 1992; DeMeester et al. 1995).

This procedure models the trait value x of an individual as an additive combination of genetic and environmental effects:

$$x = \mu + g + e\,, \tag{1}$$

where μ is an overall mean, g is the effect due to a given candidate gene, and e is the residual environmental and additional polygenic factors that influence the trait. If x_i ($i = 1,2$) denotes the trait value of the ith sib in a sib pair, the procedure regresses the squared sib-pair differences $(x_1 - x_2)^2$ on the proportion π of trait alleles that the sib pair shares IBD. The expectation, E, conditional on π, of $(x_1 - x_2)^2$ is

$$E[(x_1 - x_2)^2] = \alpha + \beta\pi\,, \tag{2}$$

with α and β satisfying the equations

$$\begin{aligned} \alpha &= \sigma_\delta^2 + 2\sigma_g^2\,, \\ \beta &= -2\sigma_g^2\,, \end{aligned} \tag{3}$$

where σ_g^2 is the additive variance attributable to polymorphism at the candidate-gene locus g and σ_δ^2 is the variance attributable to sib-pair residual differences—that is, $\sigma_\delta^2 = \mathrm{var}(e_1 - e_2)$. If a common variance σ_e^2 is

assumed for the individual residual effects e, $\text{var}(e_1 - e_2) = 2\sigma_e^2 - 2\text{cov}(e_1 - e_2)$. If the covariance is zero, it follows that $\alpha = 2\sigma_e^2 + 2\sigma_g^2$. The contribution by g to the interindividual variation in the quantitative trait x is denoted by h_g^2 and is defined as $h_g^2 = \sigma_g^2/(\sigma_\delta^2 + 2\sigma_e^2)$. Estimates of the variance components σ_g^2 and $2\sigma_e^2$ are obtained by fitting the regression model (1) by least squares and solving for these components in the system of equations (2) and (3).

Results

As a first step in determining why African Americans have higher plasma levels of Lp(a), we determined both the overall heritability of plasma levels of Lp(a) and the contribution of the apo(a) gene to plasma levels of Lp(a) in this population. Blood was obtained from 48 nuclear African American families, 3 three-generation African American families, and 1 four-generation African American family that live within 50 miles of either Dallas or New Orleans. The age and sex, as well as the apo(a) isoforms and genotypes, are given in table 1. Both parents were included in 44 of the 52 families, whereas only a single parent was available in 8 families.

The plasma levels of Lp(a) were not significantly different between men and women ($P = .40$) and were not correlated with age ($P = .15$), plasma LDL-cholesterol ($P = .35$), plasma HDL-cholesterol ($P = .72$), or plasma triglyceride level ($P = .40$). The distribution of plasma levels of Lp(a) in the sample was similar to those of African American samples reported elsewhere (Guyton et al. 1985; Helmhold et al. 1991; Sandholzer et al. 1991; Gaw et al. 1994; Kraft et al. 1996; Marcovina et al. 1996). The frequency distribution of plasma Lp(a) levels among the 96 unrelated African American parents plus 4 unrelated spouses (i.e., in-laws) is shown in figure 1*A* (*top*) and is compared with that 96 parents among 48 unrelated Caucasian families analyzed by use of the same assay (fig. 1*A*, *bottom*). The distributions of plasma Lp(a) concentrations in these two samples were significantly different from each other ($P < .001$). The distribution of plasma Lp(a) levels was less skewed in the African American than in the Caucasian sample, and the mean (41.3 mg/dl vs. 16.9 mg/dl) and median (32 mg/dl vs. 9 mg/dl) plasma Lp(a) levels were shifted to higher values, and the median levels were significantly different ($P < .0001$).

The sizes of the 224 independent apo(a) alleles in the African American families were compared with those of 192 independent alleles from 48 Caucasian families, by use of pulsed-field gel electrophoresis (PFGE) and genomic blotting (fig. 1*B*). The mean (median) numbers of K4 repeats were remarkably similar in the two samples—26 (26) and 26.4 (27) in the African Americans and Caucasians, respectively.

Immunoblot analysis was also performed, by use of an equal volume of plasma from each family member, to assess the size and relative amount of apo(a) protein circulating in plasma. We did not estimate the amount of apo(a) associated with each apo(a) allele by use of densitometry, because of our inability to develop a system that was reproducibly quantitative with a nonradioactive immunoblotting-detection system. The very wide range in plasma apo(a) levels associated with different apo(a) alleles makes it very difficult to remain within the linear range for the chemiluminescence detector. This is particularly problematic at higher concentrations of apo(a), because of signal quenching. Also, there are problems with differences in the efficiency of transfer of apo(a) isoforms of different sizes, from the gel to the nitrocellulose membrane.

The heritability of plasma Lp(a) concentrations in the African American families was estimated by regression of the mean plasma levels of Lp(a) in the offspring on the mid parent-plasma-Lp(a) level (fig. 2 and table 2). The heritability of plasma Lp(a) levels in the families was estimated to be .77, which is similar to the estimated heritability in the Caucasian population (Boerwinkle et al. 1992). The heritability estimate increased to .96 if a single family (pedigree 41; table 1) was excluded; in this family, the mother (individual I-2 in family 41) had an unusually high plasma level of Lp(a) (232 mg/dl), which was almost twice the level corresponding to the 99th percentile in the sample (134 mg/dl). No correlation was found between the plasma levels of Lp(a) in the spouses ($r = -.073$), so the high heritability is unlikely to be due to shared environmental factors.

To determine how much of the heritable variation in plasma Lp(a) levels is conferred by polymorphism at the apo(a) locus, the siblings were paired on the basis of the number of parental apo(a) alleles shared in common. The apo(a) length polymorphism revealed by PFGE was able to distinguish all four parental alleles in 45 of the 52 families (the exceptions were families 10, 11, 12, 15, 21, 33, and 49). As described in the Subjects and Methods section, other sequence polymorphisms in the apo(a) gene, including a TTTTA length polymorphism (Wade et al. 1993; Mooser et al. 1995) and a 1-bp polymorphism (Cohen et al. 1993), were used to distinguish the four parental apo(a) alleles in families in which one parent was homozygous for the same-size apo(a) allele ($n = 3$; families 11, 21, and 33) or in which both parents were heterozygous for identical apo(a) alleles ($n = 1$; family 15). In three families (10, 12, and 49), none of the sequence polymorphisms in the apo(a) gene were fully informative, and no subjects from these families were included in the sibling-pair analysis.

A total of 58 sibling pairs shared both parental alleles IBD, and the plasma Lp(a) levels were remarkably similar ($r = .85$; 95% confidence interval [CI] .76 to .91)

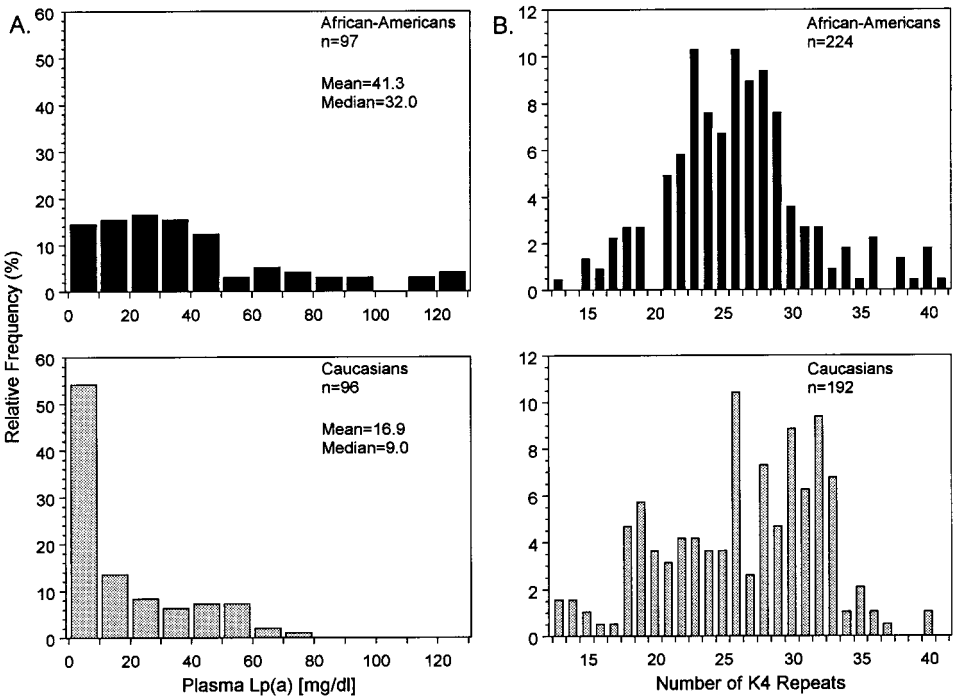

Figure 1 Distribution of plasma Lp(a) levels (*A*) and apo(a) alleles according to the number of K4 repeats (*B*), in parents of 52 African American families (*top panels*) and in parents of 48 unrelated Caucasian families (*bottom panels*). The plasma Lp(a) levels were determined by use of an ELISA assay (Marcovina et al. 1995), and the size of apo(a) alleles was determined by PFGE of *Hpa*I-digested high-molecular-weight genomic DNA and genomic blotting with an apo(a) K4-specific probe. The total number of K4 repeats was estimated by comparison of the migration of the bands, relative to size standards (Lackner et al. 1993).

(fig. 3, *right*). In contrast, the siblings who inherited no apo(a) alleles in common ($n = 70$ pairs) had much more dissimilar plasma levels of Lp(a) ($r = .22$; 95% CI −.066 to .39) (fig. 3, *left*). The siblings who shared only one allele in common ($n = 129$ pairs) had an intermediate correlation coefficient ($r = .48$; 95% CI .33 to .6) (fig. 3, *middle*). These correlations did not change when the three postmenopausal siblings were excluded from the analysis. No correlation was found between the plasma Lp(a) levels of African American spouses ($r = -.073$; $P = .63$) (table 2).

Figure 4 shows the relationship between the number of alleles shared IBD and the squared differences between the plasma levels of Lp(a) in the sibling pairs. Least-squares regression analysis of these relationships revealed that 78% of the interindividual variation in plasma levels of Lp(a) in African Americans can be attributed to the apo(a) gene or sequences closely linked to it (Boerwinkle et al. 1992). After a log transformation was performed on the data, the estimate was 81%, which is almost identical to that obtained when a normal scale is used. To assess the amount of within-genotype variation in plasma Lp(a) levels, we compared the median plasma Lp(a) levels in sibling pairs that inherit both apo(a) alleles IBD and in those that inherit no apo(a) alleles IBD. The median plasma levels of Lp(a) were 39.5 mg/dl and 38.5 mg/dl in the two groups, respectively. The median square differences in the plasma levels of Lp(a) were significantly lower in the sibling pairs inheriting both apo(a) alleles in common (56 mg/dl) than in those sharing no apo(a) alleles in common (842 mg/dl) ($P < .001$), again reflecting the major role that sequence variations linked to the apo(a) locus play in contributing to plasma levels of Lp(a).

These data are consistent with the apo(a) gene being the major determinant of the plasma Lp(a) levels in the African American as well as Caucasian population. To determine whether the higher plasma Lp(a) levels in African Americans were due to the presence of fewer "null" alleles, we examined the plasma for the presence or absence of immunodetectable apo(a) protein. The same volume of plasma was analyzed from each family member by immunoblotting without correction for the plasma level of Lp(a). For many of these apo(a) alleles, apo(a) protein was immunodetected when a larger volume of plasma was loaded onto the gel (data not shown). Therefore, the apo(a) alleles associated with no detectable apo(a) are classified as low-expressing rather than as null alleles. Elsewhere we have shown that apo(a) alleles associated with ≤ 0.1 mg/dl plasma Lp(a) are not detected by use of our immunoblotting system (Gaw et al. 1994).

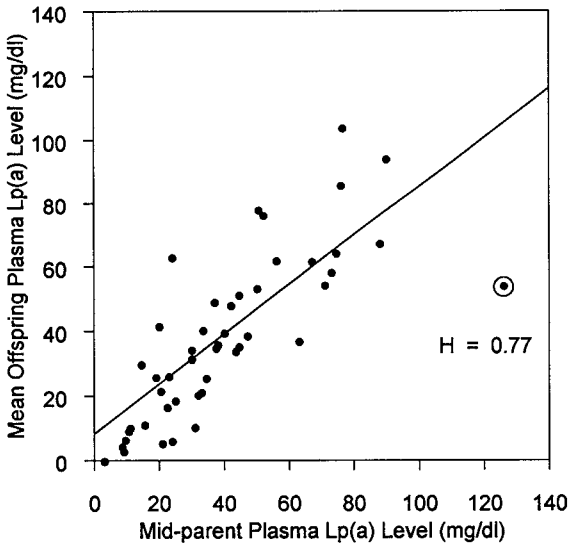

Figure 2 Heritability of plasma Lp(a) levels. The mean parent plasma levels of Lp(a) (*x*- axis) are plotted against the mean offspring plasma levels of Lp(a) (*y*-axis). The slope of the line is .77. If the single outlying family (*circled*) is removed from the analysis (the data from this family are circled), the heritability (H) increases from .77 to .96.

Shown in figure 5 (*top*) is both the distribution of apo(a) alleles classified on the basis of the number of K4 repeats (*x*-axis) and the relative number of high-expressing (dark shading) and low-expressing (light shading) apo(a) alleles. Overall, 31% of the Caucasian alleles and 25% of the African American alleles were associated with very low levels of circulating apo(a), which is not a statistically significant difference (P = .28). As noted elsewhere (Gaw et al. 1994), the low-expressing apo(a) alleles were not confined to the larger-sized alleles but rather were seen over the entire size range of apo(a) alleles, in both the African American sample and the Caucasian sample. The distribution of nonexpressing alleles was not statistically significantly different between the two groups.

The proportions of low-expressing alleles within each size range, for both samples, are given in the lower panel of figure 5. In both samples, only a small number of apo(a) alleles had 12–14 K4 repeats; all of these alleles were expressing in the African American sample. In the Caucasian sample, 50% of these small alleles were not associated with detectable plasma protein. Between 10% and 25% of the apo(a) alleles with 15–23 repeats were low-expressing in both samples. In the Caucasian population, the percentage of low-expressing alleles tended to increase progressively in the apo(a) alleles with >24 K4 repeats. In contrast, in the African American sample, the proportion of nonexpressing alleles with >24 K4 repeats remained steady, at 20%, with increasing allele size, until reaching 32 K4 repeats. A lower proportion of the intermediate-size (24–29 K4 repeats) apo(a) alleles are nonexpressing in the African American sample (P = .028). No Caucasian apo(a) alleles with >39 K4 repeats were associated with detectable plasma Lp(a). A significant proportion (25%) of the African American apo(a) alleles with >32 repeats had detectable apo(a) protein in plasma, even in the largest size range. However, the significance of any differences between the two groups, in the proportion of expressing alleles within each size class, will have to be confirmed by use of larger samples, because of both the large number of different apo(a) allele sizes and the problems associated with the arbitrary binning of alleles.

One possible etiology for the higher plasma Lp(a) levels in the African American population is the presence of a common apo(a) sequence variant, of African origin, that is associated with higher plasma levels of Lp(a). As a first step in molecular definition of such a sequence difference, a 5-bp tandem repeat (TTTTA) length polymorphism located 1.3 kb 5′ of exon 1 of the apo(a) gene (fig. 6) was examined in the entire African American family sample. Apo(a) allele haplotypes were constructed by following the cosegregation of the TTTTA and K4 alleles in the families. The amount of plasma Lp(a) associated with each apo(a) allele was estimated as described in the Subjects and Methods section.

The distributions both of apo(a) alleles and of the associated plasma Lp(a) levels were examined in 118 African American apo(a) alleles and in 148 apo(a) alleles from 43 Caucasian families, which were analyzed in an identical fashion (Boerwinkle et al. 1992; Mooser et al. 1995) (fig. 6). The $(TTTTA)_6$ and $(TTTTA)_7$ allele were found only in the African American population. The most frequent apo(a) allele in both the African American sample and the Caucasian sample was $(TTTTA)_8$. There was an inverse relationship between the number of K4 repeats in the apo(a) gene and the plasma level of Lp(a) in the subset of apo(a) alleles with eight TTTTA repeats, in both the African American sample and the Caucasian

Table 2

Correlations of Plasma Lp(a) Concentrations, between Family Members

	n	*r*	*P*
Spouses[a]	48	−.073	.63
Parent-offspring[a]	304	.47	<.001
Mid parent–mean offspring[a]	47	.78	<.001
Siblings pairs (all pairs)[b]	259	.49	<.001
Sibling pairs sharing no alleles IBD[b]	70	.22	<.068
Sibling pairs sharing one allele IBD[b]	129	.48	<.001
Sibling pairs sharing two alleles IBD[b]	58	.85	<.001

[a] Data were calculated by use of Pearson's correlation coefficient.
[b] Data were calculated by use of Pearson's intraclass correlation coefficient.

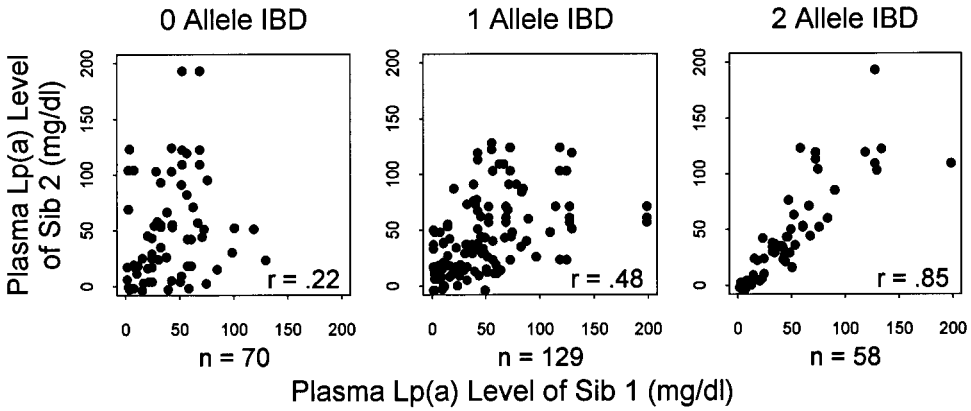

Figure 3 Comparison of plasma Lp(a) levels in sibs sharing 0, 1 or 2 apo(a) alleles IBD, in 49 African American families. "Sib 1" denotes the younger sibling, and "Sib 2" denotes the older sibling.

American sample, although the slope of this relationship was steeper in the African Americans than in the Caucasian Americans. If we include all the data, the slope estimated by regression analysis in the Caucasian sample is -2.27 (95% CI -2.67 to -1.87), whereas that for the African Americans is -3.13 (95% CI -4.09 to -2.18). These slope estimates are confounded by the presence of the null alleles. If we exclude the apo(a) alleles associated with <0.1 mg/dl, then the slope estimate for Caucasians is -2.52 (95% CI -2.96 to -2.08), and that for the African Americans is -4.57 (95% CI -5.80 to -3.35). Similar results were achieved by use of a log scale. The median plasma levels of Lp(a) were higher in the African Americans than in the Caucasians, in the subset of alleles with <24 repeats (56 vs. 39 mg/dl) and in the subset with 24–30 repeats (11 vs. 0 mg/dl) but was comparable for apo(a) alleles >30 repeats (0 in both groups).

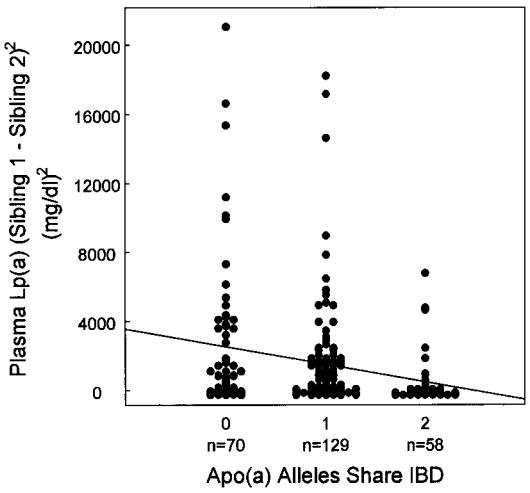

Figure 4 Variance in plasma Lp(a) levels in sibling pairs who share 0, 1, or 2 apo(a) alleles IBD.

The sample sizes in the apo(a) alleles with $(TTTTA)_9$, $(TTTTA)_{10}$, and $(TTTTA)_{11}$ were too small for us to see a systematic difference between the plasma levels of Lp(a) associated with apo(a) alleles of the same haplotype, although, for $(TTTTA)_9$ repeats, there was a trend toward higher plasma levels of Lp(a) in the African American apo(a) alleles than in the Caucasian apo(a) alleles. Most of the apo(a) alleles with 10 and 11 TTTTA repeats in the African American sample were associated with very low levels of Lp(a), as has been described elsewhere (Mooser et al. 1995). A single apo(a) allele in the African American sample had 12 TTTTA repeats (not shown); this allele had 16 K4 repeats and was associated with an easily detected plasma Lp(a) level, but the exact level could not be estimated, since both apo(a) alleles were associated with significant amounts of apo(a) glycoprotein in the individuals with this allele.

The low-expressing alleles were found over the entire size range of apo(a) alleles, in both samples. The fact that these alleles have multiple different haplotypes suggests either that they share a similar very ancient sequence variant that is responsible for the low plasma level of apo(a) or, more likely, that multiple different mutations are responsible for this phenotype. This analysis provided no evidence for a common apo(a) allele haplotype being responsible for the higher plasma Lp(a) levels in the African American sample.

Discussion

This is the first study to examine the segregation of the apo(a) gene in African American families and to analyze its relationship with plasma Lp(a) levels. In Caucasians, the plasma levels of Lp(a) are highly heritable (Albers et al. 1974; Hewitt et al. 1977), and sequence differences at or closely linked to the apo(a) locus are the major genetic determinants of plasma Lp(a) levels (Boerwinkle et al. 1992). As a first step toward under-

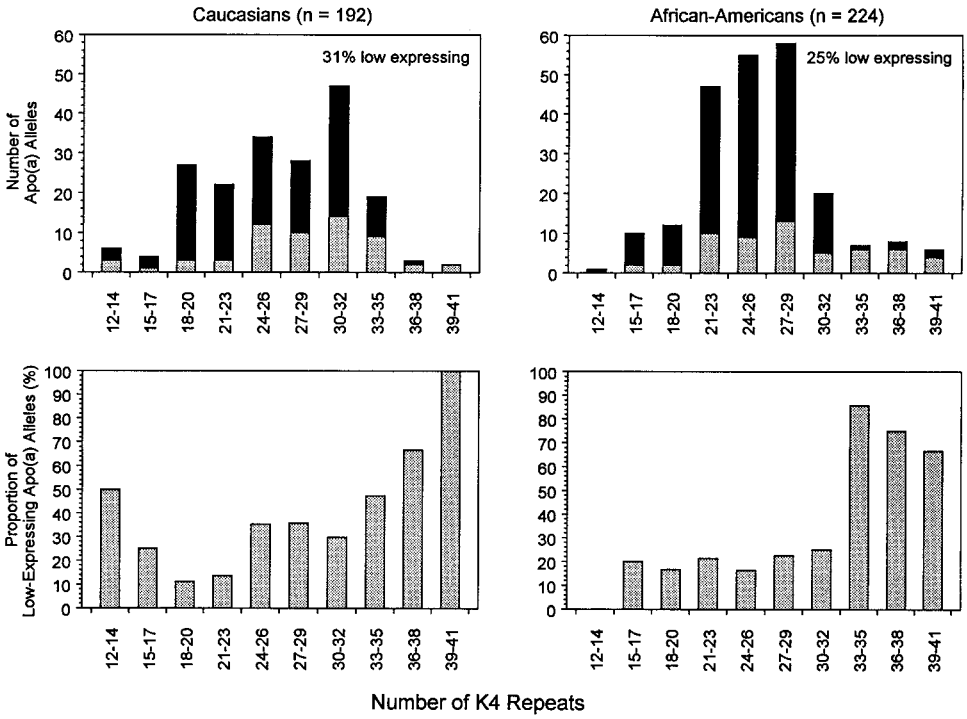

Figure 5 Distribution (*top panels*) and relative frequency (*lower panels*) of low-expressing apo(a) alleles in 48 Caucasian families (*left panels*) and 52 African American families (*right panels*). The contribution of apo(a) alleles to plasma Lp(a) level was determined by comparing the size of the apo(a) alleles, as determined by PFGE of high-molecular-weight genomic DNA and the immunoblot analysis of plasma apo(a) isoforms. Low-expressing apo(a) alleles were defined as those apo(a) alleles associated with no detectable apo(a) protein on immunoblot analysis of plasma (Gaw et al. 1994) and are indicated by gray shading. The black shading indicates apo(a) alleles with easily detectable apo(a) in the plasma.

standing why individuals of African descent have higher plasma levels of Lp(a), we compared the plasma Lp(a) levels in 257 sibling pairs from 52 African American families who shared none, one, or both apo(a) alleles IBD. In the African American families, the plasma levels of Lp(a) also were much more similar in the sibling pairs who inherited both alleles IBD (r = .85) than they were in those who inherited no apo(a) alleles in common (r = .22). The amount of interindividual variation in the plasma Lp(a) level attributable to the apo(a) gene (or closely linked sequences) was estimated to be 78%. The relative contribution of the apo(a) gene itself to variation in plasma levels of Lp(a) in African Americans is lower than what we elsewhere had reported for Caucasians (~91%) (Boerwinkle et al. 1992), but in both groups the apo(a) gene is the major determinant of variation in the plasma Lp(a) levels.

Why, then, do African Americans have higher plasma levels of Lp(a) than Caucasians? We cannot formally rule out the possibility that the higher plasma levels of Lp(a) are due to an environmental effect, but comparison of the plasma Lp(a) levels among African American and African populations demonstrates that median plasma Lp(a) levels are elevated in all populations of African descent that have been sampled, irrespective of these populations' diverse geographical locations and dietary habits. The ratios of the median plasma level of Lp(a), in individuals of African descent and in various other ethnic groups, to that in Caucasians are shown in figure 7. Figure 7 includes studies in which the plasma levels of Lp(a) were analyzed at the same time, by use of the same assay, in both ethnic groups. The median plasma levels of Lp(a) were two to three times higher in all of the African and African American samples. No consistent differences in median plasma Lp(a) levels were found between populations from the different geographic regions, including the Congo (Parra et al. 1987), South Africa (Kraft et al. 1996), Ghana (Helmhold et al. 1991), and different regions of North America (Guyton et al. 1985; Srinivasan et al. 1991; Marcovina et al. 1993*a,* 1996; Gaw et al. 1994), which strongly argues against the notion that higher plasma levels of Lp(a) in individuals of African descent are due to environmental factors.

Another possible explanation for the higher plasma levels of Lp(a) in the African American population is the existence of a common African apo(a) sequence variant that is associated with higher plasma levels. This sequence variant could either increase the production and/or secretion of Lp(a) by the liver or retard its rate

of clearance from the circulation. Elsewhere, we found both a high degree of linkage disequilibrium over the entire apo(a) locus and that particular apo(a) allele haplotypes were associated with similar plasma levels of Lp(a) (Mancini et al. 1995; Mooser et al. 1995). As a first step in determining whether there is a common, shared sequence among the African American apo(a) alleles that is responsible for the higher plasma levels of Lp(a), we analyzed a second length polymorphism in the 5′ flanking region of the apo(a) gene and then constructed haplotypes. We found no evidence for the presence of an apo(a) allele associated with high plasma levels of Lp(a). For apo(a) alleles of a given size and haplotype, the plasma levels of Lp(a) tended to be higher in the African American population, over a wide range of apo(a) allele sizes. Thus, if the higher plasma Lp(a) levels are due to a common sequence variant in the African American population, it must be ancient and must predate the generation of the size polymorphism in the apo(a) gene. Although, in the absence of more detailed molecular analyses of multiple African apo(a) alleles, this possibility cannot be formally ruled out, we think that it is highly unlikely.

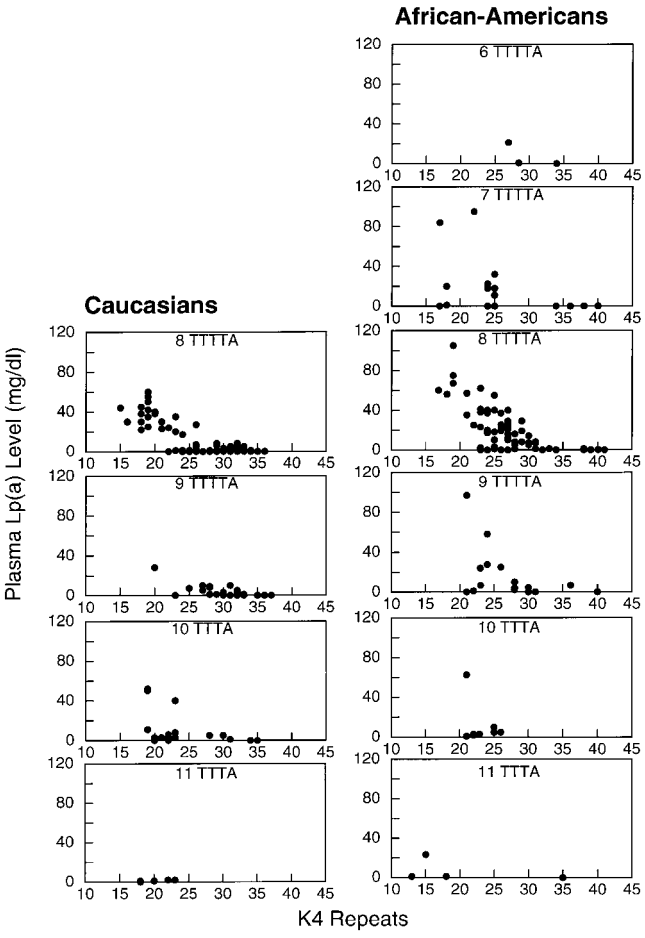

Figure 6 Relationship between the number of K4 repeats and plasma Lp(a) levels, according to the number of TTTTA repeats. The number of TTTTA repeats in the 5′ flanking region of the apo(a) gene was determined as described elsewhere (Mooser et al. 1995). The relationship between this TTTTA polymorphism and the number of K4 repeats was determined by analysis of the cosegregation of these markers in nuclear families. The plasma Lp(a) level attributed to individual apo(a) alleles was determined as described in the Subjects and Methods section. An unambiguous plasma Lp(a) level could be attributed to 148 apo(a) alleles in the Caucasian families or to 118 apo(a) alleles in the African American families. In addition, one apo(a) allele with 12 repeats was seen in the African American sample (not shown).

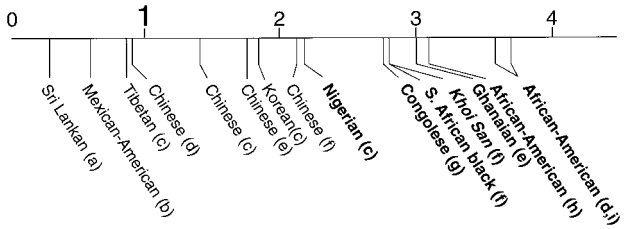

Figure 7 Comparison of median plasma level of Lp(a), in various populations. The ratio of the median plasma level of Lp(a) in each ethnic group was divided by the median plasma Lp(a) level in a sample of Caucasians. The only studies included were those in which the plasma Lp(a) levels were measured at the same time by use of the same assay and for which the median plasma levels were given for both groups. References (given in parentheses) are as follows: a = Jungner et al. (1995); b = Haffner et al. (1992); c = Cobbaert and Kesteloot (1992); d = Gaw et al. (1994); e = Helmhold et al. (1991); f = Kraft et al. (1996); g = Parra et al. (1987); h = Marcovina et al. (1993*a*); and i = Marcovina et al. (1996).

If there were a common apo(a) sequence variant that elevated plasma Lp(a) levels in individuals of African descent, African Americans would be expected to have lower plasma levels of Lp(a) than is seen in Africans, because of racial admixture. The proportion of Caucasian genes in our sample of African Americans is not known and cannot be easily determined, because of lack of knowledge regarding the genetic origins of both the Caucasian genes and the African genes in this region. It has been estimated that ~25% of the genes in the African American population are of Caucasian origin (Steinberg 1969; DeCroo et al. 1991; Chakraborty et al. 1992). Since plasma levels of Lp(a) are largely determined by the apo(a) gene and are codominant in their inheritance pattern, the distribution of plasma Lp(a) levels would be expected to be shifted toward lower values in African Americans compared with Africans. The median plasma levels of Lp(a) in the African and African American populations are remarkable similar (fig. 7), which argues against the notion that higher plasma Lp(a) levels are due to a race-specific common sequence variant associated with higher plasma levels of Lp(a).

The higher plasma levels of Lp(a) in the African Americans could be due to a lower frequency of low-expressing alleles. We have ruled out this explanation, since the percentage of low-expressing alleles in the Afri-

can Americans was not significantly different from that in Caucasians (25% vs. 31%; $P = .28$). Nor was there a significant difference between the size distribution of low-expressing alleles in the African American sample and that in the Caucasian sample ($P = .62$).

Alternatively, the higher plasma levels of Lp(a) in African Americans may be due to the action of factors acting in *trans* that affect the metabolism of either apo(a) or Lp(a). It has been shown in Caucasians that differences in plasma Lp(a) levels, even in individuals with apo(a) isoforms of the same size, are due to differences in the rate of Lp(a) synthesis, rather than to differences in the rate of degradation (Krempler et al. 1980; Rader et al. 1993). Unfortunately, no metabolic studies have been performed in individuals of African descent. A number of metabolic differences between Africans and Caucasians have been identified, and it is possible that one of these has an impact on the rate of either synthesis or degradation of Lp(a). As an example, growth hormone–secretion rates are higher in African American men than in Caucasian men (Wright et al. 1995). Growth-hormone administration to either growth hormone–deficient Caucasians adults (Edén et al. 1992) or normal children (Hershkovitz et al. 1996) is associated with increased plasma levels of Lp(a). Therefore, the increased growth-hormone levels in African Americans may contribute to the higher plasma Lp(a) levels in African Americans (B. Angelin, personal communication).

Increased plasma levels of Lp(a) in African Americans may be due to an increase in either the transcription of the apo(a) gene or the translation of the apo(a) mRNA. Alternatively, in African Americans there may be an increase in the efficiency of intracellular transport of apo(a) from the hepatic endoplasmic reticulum (ER) to the cell surface. Apo(a) is a massive glycoprotein that contains many cysteine-rich repeats, which must be properly folded before the protein is transported out of the ER. In an elegant series of studies in primary culture of baboon hepatocytes, White and Lanford (1994) have shown that >120 min can be required for newly synthesized apo(a) to leave the ER, move to the Golgi complex, and then be secreted. The efficiency of this process is inversely related to the size of the apo(a) isoform, thus contributing to the observed inverse relationship between apo(a) allele size and plasma level of Lp(a).

The data of Marcovina et al. (1996) suggest that the major plasma Lp(a)–level differences between African Americans and Caucasians are in the subset of apo(a) alleles that contain an intermediate number of K4 repeats. In both populations, the plasma levels of Lp(a) that are associated with the smaller apo(a) alleles vary over a wide range but tend to be associated with similarly high plasma Lp(a) levels. To date, in all populations studied, the plasma Lp(a) levels associated with the very large apo(a) alleles are uniformly low (Kraft et al. 1996; Marcovina et al. 1996), as we originally had reported elsewhere (Gaw et al. 1994). It is the level of plasma Lp(a) associated with the intermediate-size apo(a) alleles (~24–32 K4 repeats) that differs most markedly between ethnic groups (Marcovina et al. 1996). For this size range, Blacks tend to have higher plasma levels of Lp(a) (fig. 1) and smaller proportions of low-expressing alleles than are seen in Caucasians (fig. 5). The higher plasma Lp(a) levels in Africans may be due to a more efficient transport of apo(a) through the hepatic biosynthetic machinery, possibly because of differences in the complement of ER chaperone proteins. In protein transport within hepatocytes, there is evidence of interindividual differences within the same ethnic background (Wu et al. 1994), so it is not unreasonable to suspect that such differences may occur between different ethnic groups. Why is the effect most prominent in the intermediate-size alleles? Secretion efficiency is probably not the rate-limiting step in the production of small apo(a) isoforms, which exit the ER as soon as 30 min after synthesis (White and Lanford 1994). In contrast, large apo(a) isoforms require an extended time in order to undergo intracellular maturation, and a large portion of these proteins are degraded intracellularly. A difference in the complement of ER chaperones in African Americans would thus have little effect on the secretion of small apo(a) isoforms and may be sufficient to overcome the secretion defect for the intermediate-size apo(a) proteins, which undergo posttranslational processing with an intermediate efficiency.

One approach that could be used to determine whether major monogenic or polygenic *trans*-acting factors are responsible for the higher plasma Lp(a) levels in Africans would be to examine the segregation of the apo(a) gene and plasma Lp(a) levels in interracial families. An apo(a) allele transmitted from a Caucasian parent to an interracial offspring would be expected to result in a higher plasma Lp(a) concentrations if major common Lp(a)-raising factors were present in Africans. We have examined the segregation of the apo(a) gene and protein in four interracial families and have compared the plasma Lp(a) levels associated with each parental allele versus those of the interracial offspring (authors' unpublished data). We found no consistent effect of the introduction of ~50% African American alleles on the level of expression of a Caucasian parental apo(a) allele, but more families need to be analyzed in order to determine whether there are common polygenic or monogenic *trans*-acting factors responsible for the higher plasma levels of Lp(a) in Africans.

Finally, it is of interest that, despite having higher mean and median plasma levels of Lp(a), African Americans do not have a higher incidence of coronary artery disease than is seen in Caucasians (Heiss et al. 1984; Keil et al. 1993). Only two studies have been performed

to analyze the relationship between plasma levels of Lp(a) and coronary artery disease in African Americans (Sorrentino et al. 1992; Moliterno et al. 1995), and in neither study were plasma Lp(a) levels an independent risk factor for the presence of significant coronary artery disease. Interestingly, Caucasian, but not African American, children with a parental history of myocardial infarction have significantly higher plasma levels of Lp(a) (Srinivasan et al. 1991). Additional studies will be required before it can be concluded that plasma Lp(a) levels are not a risk factor in Blacks. But, if these results are verified, elucidation of the mechanism responsible for the relative protection that African Americans enjoy from the atherogenic effects of high plasma levels of Lp(a) may provide much-needed insights into the atherogenicity of Lp(a).

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