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Genetics of the Lp(a)/apo(a) system in an autochthonous Black African population from the Gabon

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Plasma lipoprotein(a) (Lp(a)) is a quantitative trait associated with atherothrombotic disease in European and Asian populations. Lp(a) concentrations vary widely within and between populations, with Africans exhibiting on average two- to threefold higher Lp(a) levels and a different distribution compared to Europeans. The apo(a) gene locus on chromosome 6q26–27 (LPA, MIM 152200) has been identified as the major quantitative trait locus (QTL) for Lp(a) concentrations in Europeans and populations of African descent (North American and South African Blacks) but data on autochthonous Black Africans are lacking. Here, we have analysed Lp(a) plasma concentrations, apo(a) isoforms in plasma and four polymorphisms in the LPA gene in 31 African families with 54 children from Gabon. Weighted midparent–offspring regression estimated a heritability $h^2 = 0.76$. The correlation of Lp(a) levels associated with LPA alleles identical by descent (IBD) resulted in a heritability estimate of 0.801. Our data demonstrate that Lp(a) concentrations are highly heritable in a Central African population without admixture and high Lp(a) (median 43 mg/dl). LPA is the major QTL, explaining most or all of the heritability of Lp(a) in this population.

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Introduction

Lipoprotein(a) (Lp(a)) from human plasma consists of an LDL-like lipoprotein and the distinguished apolipoprotein(a) (apo(a)) (for a review, see Utermann¹). Apo(a) contains a 5' signal sequence, a 3' plasminogen(PLG)-like protease domain, one domain homologous to kringle (K) V from PLG and 10 different types of kringle IV domains (K IV-1 to K IV-10). The number of K IV-2 is variable, ranging from 1–42 copies in individual LPA alleles and apo(a) isoforms (apo(a) size polymorphism = K IV-2 VNTR).² Lp(a) is a

quantitative trait that shows considerable interindividual (from <0.1 to >200 mg/dl) and interethnic variation (from two- to three-fold). Africans, on average, exhibit two to threefold higher median Lp(a) plasma concentrations and a less skewed distribution of Lp(a) levels than populations of European descent.^{3–9} Individual Lp(a) levels are rather constant under physiological conditions and twin and family studies have shown that Lp(a) concentrations are under strong genetic control, with heritability estimates ranging from 0.51 to 0.98.^{10–15} Across all studied populations, an inverse correlation of the number of K IV-2 repeats in the LPA gene (or protein) with Lp(a) plasma levels has been demonstrated.^{3,10,13,15–17} However, Lp(a) concentrations still show substantial variation when matched for the K IV-2 VNTR, ie for alleles of identical K IV-2 repeat number.¹⁸ Estimates of the variance in Lp(a) levels explained by the K

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IV-2 VNTR range from 19% in Sudanese to 77% in Malays.³ In general, the association is weaker in Africans than in Europeans or Asians, explaining only 19–39% of the variance.^{3,5,19,20}

Family and sib-pair linkage analyses have demonstrated that LPA is the major QTL for Lp(a) levels in Europeans and explains from 70 to >95% of their population variance. Thus, the fraction of Lp(a) level variation explained by the LPA locus is larger than the one explained by the LPA K IV-2 VNTR, implying that other variations in the gene may also play a role. Other polymorphisms in the apo(a) gene have indeed been found to be associated with Lp(a) concentrations, some of which are likely to be causal.^{21,22} Frequencies of these polymorphisms vary among populations of different ethnic origins but none of them explains a major part of the marked differences in median Lp(a) levels and in the distribution of Lp(a) concentrations among populations.

Some studies have indicated that differences in Lp(a) concentrations between African and European populations may in part be due to environmental and/or transacting factors independent of the LPA locus.^{13,15,23} A comparative family study of Black South Africans, Khoi-San, and Europeans (Austrians) found that heritabilities of Lp(a) were considerably lower in Africans ($h^2=0.51$) than in Europeans ($h^2=0.71$) and that the LPA locus explained almost 100% of the genetic variance of the Lp(a) trait in the Austrians but less than 50% in the Africans.¹³

These data suggested that the contribution of the apo(a) locus on the quantitative Lp(a) trait might be much weaker in Black people and that the higher Lp(a) in Africans might be explained by factors other than the LPA locus. A population study conducted in African Americans and Nigerians indeed suggested a strong gene–environment interaction on Lp(a) in Black people.²³

To date, all family studies of apo(a)/Lp(a) in Black populations^{13,15} have been performed in populations which are living in an environment that considerably differs from the situation in most of Africa, and which have a white admixture that may well reach over 20% in African Americans.^{24,25}

To get a better understanding of the genetic architecture of the LPA trait in Africans, we have performed a family study in an autochthonous Black African population from rural Western Central Africa.

Subjects and methods

Participants were recruited exclusively for the purpose of this study between October 1998 and February 1999 in various villages located in a rural area approximately 25 to 48 km south of Lambaréné in Gabon (Figure 1). The study was introduced to village chiefs and heads of households by personal communication of KS during site visits. Participation was voluntary and based on informed con-

sent, and included a short medical checkup as a benefit for the participants. The study received the ethics commission's approval both at Innsbruck and Lambaréné. All participants were asked about their affiliation to an ethnic group; only individuals who claimed to have parents of Gabonese origin were included in our study. All individuals were of Bantu origin and all major ethnic groups forming the population of the province of Moyen-Ogooué were represented in the sample (Table 1).²⁶

As Lp(a) plasma concentrations have been shown to have risen to the adult level at the age of 3 years,²⁷ children had to be at least 4 years old to be included in the study.

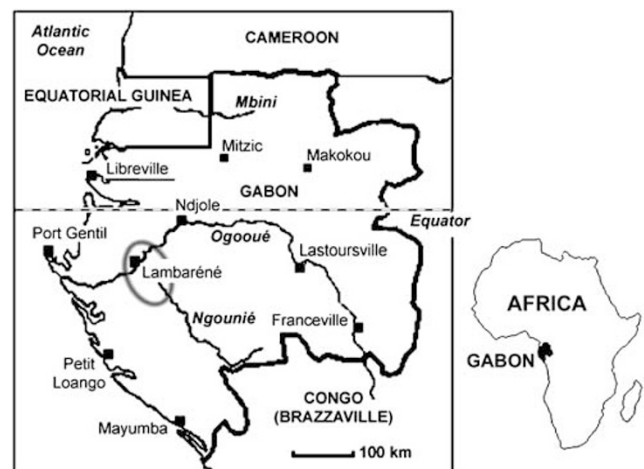


Figure 1 Geographic location of recruitment area for the study population.

Table 1 Self-reported affiliation to ethnic groups^a

Main group ^b	Group ^b	n
Meriés	Bapunu	16
	Eshira	9
	Massango	9
	Bavungu	2
	Balumbu	1
Okandés	Mitsogo	12
	Puvi	1
Mbédé	Bandjabi	12
Fang	Fang	3
Akélé	Akélé	2
Myénés	Nkomi	1
Other/none given ^c		5

^aOnly the parents of the family study are included in the list.

^bOther spellings of the names are also in use.

^cEither the participant refused to affiliate to any 'tribal' group and preferred to be listed as Gabonese or the parents were of different ethnic groups and no preference of affiliation was given.

Blood was drawn by peripheral venipuncture and was collected in 9 ml sodium-EDTA and serum containers. Samples were transported in cooling bags to the International Research Laboratory at the Albert Schweitzer Hospital in Lambaréné within a maximum period of 16 h, for further investigations. GGT, creatinine, total bilirubin, sodium and urea were measured from fresh serum by the Vitros[®] analyser system from Ortho-Clinical Diagnostics (Johnson&Johnson). A giemsa-stained standard thick blood smear was analysed for presence of parasitemia.

Sodium-EDTA blood was centrifuged at 1000 rpm, then 1 ml plasma was taken off, and plasma and blood were stored at -80°C till February 1999, when all samples were transported on dry ice to Innsbruck, Austria, for apo(a)/Lp(a)-specific analyses in the same year. Lp(a) concentrations were measured by a sandwich-type ELISA using an antibody, both for capturing and detection, recognizing specifically the K IV-2 domain of apo(a).^{28,29} This ELISA has been used in several previous studies^{13,16,30–32} and showed good overall correlation with an ITA-based Lp(a) assay provided by Denka Seiken Co. Ltd., Japan (A Lingenhel, F Kronenberg, KS, unpublished), which had been tested with good results for apo(a) size independency of Lp(a) measurements.³³

DNA containing agarose plugs were prepared from leukocytes as described.⁵ Apo(a) size polymorphism was analysed by pulsed-field gel electrophoresis.³⁴ Three additional polymorphisms at the LPA locus were analysed, as described in the given references: a 5'pentanucleotide repeat polymorphism (g.-1417(TTTTA)4–12) at position -1232,³⁵ db SNP rs1853021 (g.-49T>C),³⁶ and db SNP rs1801693 in the IV-10 (g.115618T>C).³⁷

Apo(a) isoforms were determined by SDS gel electrophoresis and immunodetection, followed by densitometric semiquantitative determination of isoforms as described,³⁸ and the proportion of Lp(a) associated with each isoform was calculated (=K IV-allele-associated Lp(a) concentrations).^{18,39}

Statistical methods

Gene frequencies were estimated by gene counting. For individuals whose kinship was confirmed by short tandem repeats (STR) analysis,⁴⁰ alleles IBD for the LPA locus were identified by segregation analyses using information of the four analysed polymorphisms at that locus.

Data of Lp(a) concentrations were square-root transformed where statistical tests are based on the assumption of a normal distribution of parameters to better meet test assumptions.

Statistical tests were performed using SPSS[®] version 10.0 for Microsoft[®] windows.

The heritability h^2 , which represents the proportion of the phenotypic variance of the Lp(a) trait attributable to additive genetic factors (no dominance component has to

be assumed for the LPA trait^{11,12,14}), was estimated using the following approaches:

Parent-offspring regression analyses of offspring Lp(a) plasma concentrations on parental Lp(a) values were conducted on weighted and unweighted midparent-offspring, single-parent-offspring, and single-parent-midoffspring models. In case of midparent-offspring analysis, h^2 is represented directly by the slope of the regression line, the regression coefficient b , whereas for single-parent-offspring or single-parent-midoffspring analysis $h^2 = 2b$.⁴¹ Thus, values larger than 1 are possible results for h^2 . When mean values were used, data transformation was performed on the mean values and not on the original data, as the mean is regarded as a virtual measurement in these calculations. Weighted linear regression analysis (WLS – weighted least-squares model in SPSS) was used to account for the heterogeneous size of the families. The mean Lp(a) values of the offspring of each family were weighted with a factor w_n according to the family size n .⁴¹

The usual Haseman and Elston method of sib-pair analysis⁴² was tested for its applicability to estimate the fraction of the genetic variance of Lp(a) concentrations attributable to the LPA locus.

Correlation analysis of allele-associated Lp(a) plasma concentrations for alleles IBD was used to estimate the heritability by the Pearson correlation coefficient.

Pearson correlation coefficients r for allele-associated Lp(a) concentrations among full- and half-sib-pairs were compared.⁴³

Results

Recruitment of families

Blood samples were initially collected from 206 individuals thought to belong – according to anamnestic information of the participants – to 55 families with 104 children including families of three polygamous men who had two and three wives, respectively. Based on apo(a) and STR typing, several children and families had to be excluded for nonpaternity (25 children), nonmaternity (seven children) or because neither of the parents was a biological parent (17 children). Further, two families had to be excluded because biochemical markers of hepatic or renal function were abnormal in one parent. Finally, 31 families with 54 children remained for a midparent-offspring analysis. These included 18 families with one child, six families with two, six with three, and one with six children. In addition, there were 25 mother-child and six father-child pairs. Altogether, samples were available from 39 full sib-pairs and 32 half-sib-pairs (For family structures and details see Table 2).

Parasitemia with *Plasmodium spp.* (mostly low grade) and/or microfilaria of *Loa loa* or *Mansonella perstans* was detected in many individuals (Table 3). We tested for an

Table 2 List of all families included in the study

Family	Sex	Age (years)	K IV-VNTR			Family	Sex	Age (years)	K IV-VNTR				
			DNA	Protein	Lp(a) (mg/dl)				DNA	Protein	Lp(a) (mg/dl)		
1	Father	M	33	10/25	10/26	35.5	11	Father	M	28	21/27	21/27	39.1
	Mother	F	31	19/31	20/31	48.6		Mother	F	23	22/25	n/25	20.9
	1. Child FM	F	11	25/31	26/31	58.7		Child FM	F	4	21/22	21/22	22.4
	2. Child FM	M	7	19/25	20/26	57.6		1. Child F	F	10	27/(29)	28/30	29.7
								2. Child F	M	5	27/(32)	28/33	54.7
2	<i>a</i>						12	Father	M	61	30/39	??/?	4.2
	Father	M	55	31/37	28/32	4.5		Mother	F	36	23/27	24/28	44.1
	Mother a	F	41	22/30	21/28	89.2		1. Child FM	F	16	23/39	24/n	22.8
	Child FM a	M	5	30/31	28/30	21.3		2. Child FM	M	8	23/30	24/30	27.3
	Child M	F	21	22/(27)	21/(27)	136.0		3. Child FM	F	5	23/30	24/30	18.8
	Child F	F	27	(19)/37	(20)/35	54.0							
	<i>b</i>						13	Father	M	26	21/32	21/31	48.9
	Father	M	55	31/37	28/32	4.5		Mother	F	24	24/29	23/29	46.4
	Mother b*	F	49	17/34	17/33	(12.0)		Child FM	F	4	21/24	21/23	57.6
	Child FM b	F	16	17/31	17/31	101.1		Child M	F	9	29/(29)	29	48.9
3	Father	M	33	20/27	20/27	155.0	14	Mother	F	31	23/24	24/25	59.3
	Mother	F	29	23/26	22/26	35.4		1. Child M	M	11	24/(37)	25/36	58.8
	Child FM	F	5	23/27	22/28	29.8		2. Child M	M	9	23/(31)	25/32	54.7
4	Father	M	64	23/24	23/25	33.7	15	Father	M	33	24/32a	25/n	29.5
	Mother	F	62	22/33	22/33	56.3		Mother	F	24	23/32b	24/33	44.4
	Child FM	M	16	22/24	22/25	36.6		1. Child FM	M	9	32a/32b	33	21.7
5	<i>a</i>							2. Child FM	F	5	23/32a	24/n	21.8
	Father	M	62	23/25a	23/25	59.0	16	Father	M	32	20/29	20/n	75.1
	Mother a	F	56	22/25b	22/25	68.8		Mother	F	31	28/32	28/32	22.1
	Child FM a	M	20	22/25a	22/25	63.4		1. Child FM	F	9	28/29	28/29	13.9
	<i>b</i>							2. Child FM	F	7	28/29	27/29	11.0
	Father	M	62	23/25a	23/25	59.0		3. Child FM	M	5	20/32	21/32	87.9
	Mother b	F	32	30a/30b	29	59.5	17	Father	M	52	32a/32b	31	8.9
	Child FM b	M	10	25a/30x	25/29	32.0		Mother	F	39	21/25	21/25	106.0
6	Father	M	35	29/37	28/36	12.3		1. Child FM	M	11	25/32x	25/n	43.8
	Mother	F	29	17/22	17/22	133.0		2. Child FM	F	7	25/32x	24/n	14.8
	Child FM	M	5	17/29	18/30	160.0		Child M	F	14	21/(30)	21/30	114.0
7	Father	M	46	23/27	23/27	115.0	18	Father	M	55	23a/23b	23	58.7
	Mother	F	43	24/28	25/28	85.8		Mother	F	55	27/31	27/30	59.6
	Child FM	M	10	23/28	23/28	104.0		Child FM	M	18	23x/27	23/27	47.0
8	Father	M	32	28/31	28/31	21.2	19	Mother	F	30	24/40	25/n	35.2
	Mother	F	29	24a/24b	24	40.9		Child M	M	14	(23)/40	23/n	24.7
	Child FM	M	6	24x/28	24/28	40.0	20	Father	M	28	26/37	26/n	39.1
9	Father**	M	64	25/47	25/n	(54.4)		Mother	F	28	13/38	13/36	41.4
	Mother	F	62	19/31	18/29	12.5		Child FM	F	4	37/38	34/35	9.2
	Child FM	M	30	31/47	29/n	4.7	21	Mother	F	23	30/32	30/32	31.2
10	Father	M	38	25/29	25/29	38.5		Child M	F	8	(24)/32	24/32	15.4
	Mother	F	37	27/31	27/n	38.2	22	Father	M	29	26/37	26/n	37.1
	1. Child FM	M	7	25/31	25/31	29.8		Mother	F	24	27/32	26/32	53.4
	2. Child FM	M	4	25/27	25/27	72.7		Child FM	M	5	26/32	26/32	99.5
	1. Child M	M	15	31/(33)	30/31	4.4							
	2. Child M	F	11	27/(28)	27/28	82.8							

Table 2 (Continued)

Family	Sex	Age (years)	K IV-VNTR			Lp(a) (mg/dl)	Family	Sex	Age (years)	K IV-VNTR				
			DNA	Protein	Lp(a) (mg/dl)					DNA	Protein	Lp(a) (mg/dl)		
23	Mother	F	27	<u>23/30</u>	22/30	60.3	32	Mother	F	38	<u>30/31</u>	29/30	72.9	
	1. Child M	M	7	<u>23/30</u>	22/30	115.0		1. Child M	F	8	(25)/ <u>31</u>	25/30	13.8	
	2. Child M	F	5	23/(32)	22/31	22.5		2. Child M	F	5	(24)/31	24/30	22.4	
24	Father	M	39	<u>35/50</u>	31/n	7.1	33	Mother	F	26	<u>22/25</u>	23/25	70.9	
	Mother	F	34	<u>25/43</u>	24/37	43.5		1. Child M	F	5	(18)/22	19/23	84.0	
	1. Child FM	F	18	<u>43/50</u>	36/n	0.5		2. Child M	M	4	22/(27)	23/29	60.5	
	2. Child FM	M	17	43/50	37/n	0.3	34	Father	M	31	<u>24/26</u>	24/n	41.4	
	3. Child FM	F	14	25/35	24/31	56.5		Mother	F	29	<u>20/28</u>	20/28	55.0	
	4. Child FM	M	12	43/50	36/n	6.2		Child FM	F	5	<u>20/24</u>	20/24	57.7	
	5. Child FM	F	9	25/35	24/n	86.5	35	Mother	F	37	<u>33a/33b</u>	32	31.1	
	6. Child FM	M	6	43/50	36/n	2.3		Child M	F	5	(16)/ <u>33x</u>	10/n	3.5	
25	Father	M	32	<u>25/35</u>	25/34	55.7	36	Father	M	62	<u>23/32</u>	23/31	46.6	
	Mother	F	28	<u>24a/24b</u>	24	92.0		Mother	F	52	<u>19/25</u>	19/25	35.8	
	Child FM	F	4	<u>24a/25</u>	24/25	55.6		1. Child FM	F	16	<u>19/23</u>	19/31	25.4	
	Child F	M	10	25/(31)	25/31	41.2		2. Child FM	M	9	19/23	19/23	48.0	
	1. Child M	M	12	<u>24a/(30)</u>	24/30	51.1	37	Mother	F	51	<u>31/36</u>	30/34	6.1	
	2. Child M	F	5	<u>24a/(32)</u>	24/31	25.7		Child M	M	18	(30)/ <u>36</u>	29/34	13.5	
26	Father	M	31	<u>31/32</u>	31/32	26.5	38	<i>a</i>	Father	M	23	<u>25/29</u>	24/28	35.8
	Mother	F	35	<u>22/25</u>	23/25	61.9		Mother <i>a</i>	F	21	<u>32/39</u>	28/35	5.6	
	1. Child FM	F	11	<u>25/32</u>	24/32	37.3		Child FM <i>a</i>	M	5	<u>25/39</u>	26/37	35.9	
	2. Child FM	F	9	22/32	24/32	16.0		<i>b</i>	Father	M	23	<u>25/29</u>	24/28	35.8
	3. Child FM	F	5	22/32	23/32	23.6		Mother <i>b</i>	F	21	<u>24/26</u>	23/25	75.1	
27	Father	M	50	<u>25a/32</u>	24/n	19.7		Child FM <i>b</i>	M	4	<u>24/29</u>	24/29	43.9	
	Mother	F	44	<u>25b/25c</u>	24	25.2	39	Mother	F	61	<u>21/31</u>	21/31	87.3	
	1. Child FM	M	9	<u>25x/32</u>	25/n	41.7		1. Child M	F	30	<u>21/(27)</u>	21/27	61.9	
	2. Child FM	M	5	<u>25x/32</u>	20/28	0.1		2. Child M	M	28	21/(27)	21/27	39.1	
	Child M	M	11	<u>25x/(32)</u>	24/31	45.0	40	Mother	F	27	<u>33/34</u>	23/33	29.1	
28	Father	M	39	<u>22/28</u>	22/28	80.2		Child M	F	6	(18)/ <u>34</u>	18/33	65.4	
	Mother	F	26	<u>27/35</u>	26/34	31.5	41	Father	M	33	<u>28/32</u>	26/30	32.7	
	1. Child FM	M	11	<u>27/28</u>	27/28	60.4		Mother	F	25	<u>18/30</u>	17/27	29.3	
	2. Child FM	M	6	22/35	22/n	79.6		1. Child FM	M	7	<u>18/28</u>	18/27	38.2	
	3. Child FM	M	4	28/35	28/34	33.5		2. Child FM	F	5	18/32	18/30	41.2	
29	Father	M	53	<u>27/34</u>	26/34	19.2		3. Child FM	F	4	30/32	26/30	7.7	
	Mother	F	49	<u>30/31</u>	30/31	18.7		Child M	F	9	18/(35)	17/30	14.7	
	1. Child FM	F	12	<u>27/30</u>	26/30	20.2	42	Mother	F	53	<u>27/31</u>	27/31	59.5	
	2. Child FM	M	10	27/31	26/31	17.7		Child M	M	19	(19)/27	20/26	70.5	
	3. Child FM	M	7	30/34	30/34	34.5	30	Father	M	56	<u>20/31a</u>	19/31	18.9	
	Father	M	56	<u>20/31a</u>	19/31	18.9		Mother	F	44	<u>28/31b</u>	27/31	25.6	
	Mother	F	44	<u>28/31b</u>	27/31	25.6		Child FM	F	21	<u>20/28</u>	19/27	20.2	
	Child FM	F	21	<u>20/28</u>	19/27	20.2	31	Father	M	50	<u>29/35</u>	28/34	49.0	
	1. Child F	M	15	<u>29/(30)</u>	28/29	59.5		1. Child F	M	15	<u>29/(30)</u>	28/29	59.5	
	2. Child F	M	14	29/(31)	28/30	60.3		2. Child F	M	14	29/(31)	28/30	60.3	

Notes: Only family members whose lineage was confirmed by 10 STR analyses are listed and were included in the study. Given are the structures of the family (child FM: father and mother of the child are included in the study; child F, child M: only the father, only the mother of the child is included in the study), sex, age, the size of the apo(a) alleles by the KIV-VNTR (equals total number of all KIV elements) as derived from PFGE ('DNA'), the apo(a) isoform size ('Protein') (n: not expressed; one number only: both isoforms of same size), and the total Lp(a) plasma concentration.

Underlined are the parental alleles passed to children. 'a', 'b', or 'c' is used to distinguish alleles of the same size; if an allele in a child could not be assigned to one of these parental alleles, it was marked with 'x'. Alleles put in parentheses don't descend from the individuals listed as parents.

Individuals were excluded from analyses concerning Lp(a) concentration due to abnormal biochemical markers for hepatic () or renal (**) function. Individuals were excluded from analyses concerning Lp(a) concentration due to abnormal biochemical markers for renal (**) function.

influence of parasitemia on Lp(a) levels but could not detect an effect in our sample (unpublished). Thus, impaired hepatic or renal function remained our only criterion for exclusion from the study.

Parents of the family study and those individuals excluded from the family study owing to the STR analysis were included in a population-based study. In this sample ($n = 119$, mean Lp(a) 47.8 mg/dl, median 42.9 mg/dl), the effect of the K IV-2 VNTR (expressed as the sum of the K IV-2 repeats from both alleles) on the total variability of Lp(a) concentrations was estimated at 43.8% (general linear model, $P = 0.001$).

Heritability of Lp(a)

We first performed midparent–offspring regression analysis of Lp(a) plasma concentrations in offspring on parental Lp(a) concentrations using different approaches (Table 4). Unweighted analysis of the mean Lp(a) in offspring on the

mid-parent values estimated h^2 at 0.765 (Figure 2). Weighted analysis considering the different sizes of families did not change the result ($h^2 = 0.763$). Hence mid-parent–offspring analysis suggests that heritability of Lp(a) in the Gabonese is around $h^2 = 0.76$.

All other calculations using various settings and thus sometimes larger sample size resulted in no smaller and most often very similar heritability estimates (Table 4).

Contribution of the LPA locus to the heritability of Lp(a) – sib-pair linkage analysis

Next, we investigated whether apo(a) is a major determinant of Lp(a) heritability in Gabonese, using sib-pair linkage analysis. Apo(a) genotypes (defined by K IV-2 numbers) were determined in all family members, which allowed us to categorize sib-pairs into those with two, one, or no apo(a) allele IBD. A total of 39 full sib-pairs from 13 families were available for this analysis.

Including all possible full-sib-pairs into the analysis showed a significantly higher correlation for the group of sibs sharing two alleles IBD ($n = 10$ pairs; $r = 0.901$, $P < 0.001$) than for those with one allele IBD ($n = 14$; $r = 0.176$, $P \gg 0.05$). The group with no allele IBD showed a strong and significant negative correlation ($n = 13$; $r = -0.727$, $P = 0.005$). However, IBD groups contained sib-pairs from families that differed considerably in size: 13 sib-pairs from six different families shared no alleles IBD, 16 pairs from 11 families shared one, and 10 from four families shared two alleles IBD at the LPA locus. It is

Table 3 Prevalence of parasitemia as detected in thick blood smears from included participants ($n = 159$) of the family study

Type of parasite	Percentage of positive individuals		
	All ages	≤ 14 a	≥ 15 a
<i>Plasmodium spp.</i>	52.8	79.7	32.2
<i>Loa loa</i>	11.3	1.4	18.9
<i>Mansonella perstans</i>	32.1	21.7	40.0

Table 4 Linear regression parameters and heritabilities of Lp(a) plasma concentrations for different family kinships and various settings regarding the inclusion of the number of children per family in the regression analysis

Type of regression analysis	n	Heritability h^2 (relation to regression coefficient b)	SD of b	95% confidence interval of regression coefficient b	R	R^2 (corrected)
Midparent–offspring (unweighted mean of all children per family)	31	0.77 (= b)	0.21	0.33–1.20	0.559	0.288
Midparent–offspring (weighted mean of all children per family)	31	0.76 (= b)	0.19	0.38–1.15	0.599	0.336
Midparent–offspring (unweighted, each child with parents as separate family, all children)	54	0.89 (= b)	0.22	0.44–1.34	0.484	0.220
Midparent–offspring (one child per family by chance)	31	0.74 (= b)	0.27	0.19–1.29	0.466	0.190
Single-parent–midoffspring (all parents, unweighted mean of all children per parent)	73	0.82 (= $2b$)	0.1	0.21–0.61	0.434	($P = 0.011$) 0.177
Single-parent–midoffspring (all parents, each child with parent as separate family, all children)	141	0.78 (= $2b$)	0.09	0.21–0.57	0.334	0.105
Single-parent–offspring (all parents, one child by chance per parent)	73	1.06 (= $2b$)	0.12	0.28–0.78	0.454	0.195
Father–mother	31	no ($b = -0.10$)	0.15	-0.42–0.21	0.122	-0.019 ($P = 0.515$)

All Lp(a) values have been square root transformed (if mean values have been used, the mean values and not the original data were transformed). P -values for the regressions were < 0.001 if not stated otherwise.

noteworthy, that in the group of sib-pairs sharing two alleles IBD, three out of the total of six pairs derived from the same family and two of them shared two very large apo(a) alleles (43 and 50 K IV repeats), which are associated with low Lp(a) levels. Consequently, the group with two alleles IBD had significantly lower Lp(a) levels than the other groups ($P=0.001$, Kruskal–Wallis test).

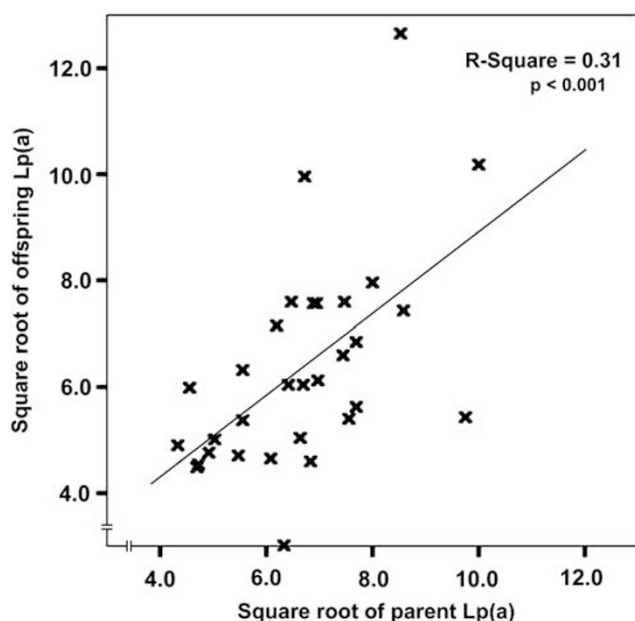


Figure 2 Regression of the mean of offspring Lp(a) plasma concentrations (all children of each family) on the mean of the parental Lp(a) concentrations. Mean Lp(a) concentrations were square-root transformed. Some dots are overlapping. The regression equation is: offspring Lp(a) = $0.765 \times$ parent Lp(a) + 1.27.

In a further approach, we tried to correct for family size by randomly selecting one sib-pair from each family. Each individual was allowed only in one pair per group (Figure 3). However, this still resulted in significantly different mean Lp(a) concentrations between the groups with two, one or no LPA allele IBD. Also, in this analysis, the pairs with two alleles IBD showed the highest correlation of the square-root transformed data with a Pearson correlation coefficient of $r=0.911$ ($P=0.011$). In the groups with one or no allele IBD, no significant correlation was detectable ($r=0.054$ and $r=-0.349$, respectively).

Taken together, the sample particularities bias any sib-pair analysis. Mathematical assumptions for classical sib-pair analysis as proposed by Haseman and Elston were not fulfilled by the data as standard deviations of the standardized residuals showed too large differences among the groups. Another approach using variance components as proposed by Amos⁴⁴ also did not yield significant results (data not shown). Thus, no quantification of the impact of the LPA locus on Lp(a) levels could be obtained by classical sib-pair analysis.

Heritability estimates from the correlation of allele-associated Lp(a)

As the failure to obtain meaningful data from the sib-pair analysis was due to the small sample size in combination with the structure of families, we decided to increase the number of observations by analysing the effect of apo(a) alleles rather than apo(a) genotypes on Lp(a) levels. This was possible because, in addition to the apo(a) genotype (K IV-2 repeats) and total plasma Lp(a) concentrations, we had data on the quantitative distribution of apo(a) isoforms in all individuals from immunoblots of plasma

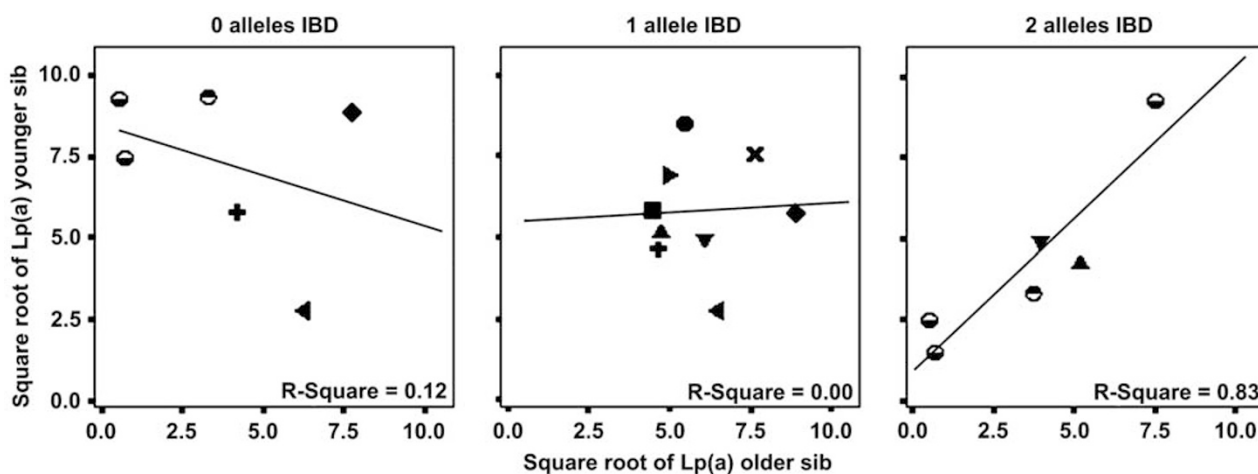


Figure 3 Correlation of square-root transformed Lp(a) concentrations between full-sib-pairs depending on the number of apo(a) alleles IBD. The full-sib-pairs are from five (zero alleles IBD), nine (one allele IBD), and four (two alleles IBD) families, respectively, as represented by different symbols in the graphics. All sib-pairs have been chosen randomly from all possible sib-pairs with identical number of alleles IBD in any family.

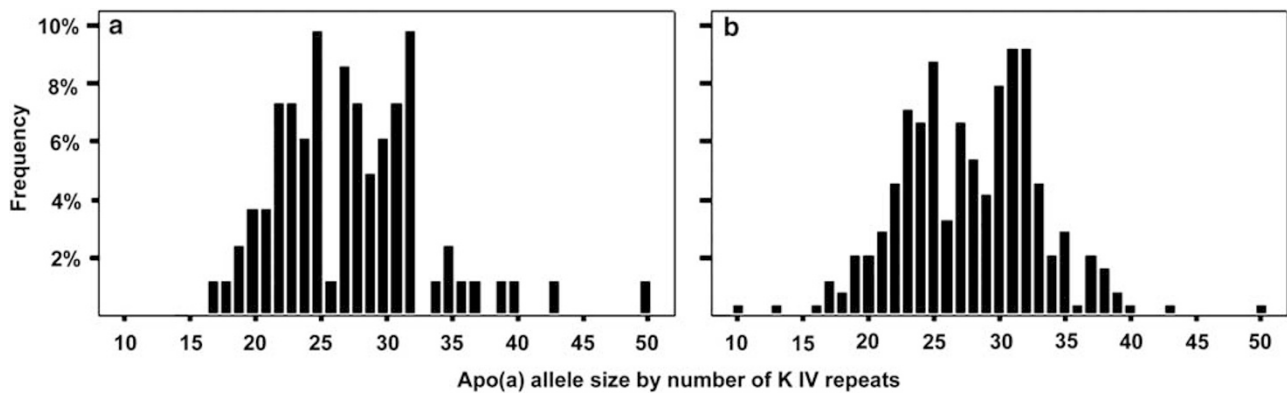


Figure 4 Apo(a) allele size (by total number of Kringle IV repeats) distribution of (a) 81 alleles for which allele-associated Lp(a) concentrations were available for analysis in the family study, and (b) 238 alleles from 119 unrelated individuals.

apo(a) except for those individuals carrying two apo(a) alleles of the same or very similar size.

In all, 27 out of 221 alleles from the family study had to be excluded for this reason. Altogether, 194 observations of allele-associated Lp(a) concentrations remained and were included in these analyses, 81 different alleles IBD with known allele-associated Lp(a) concentrations could be traced unambiguously in parents and children (see Table 2); 56 of them could be traced in two, 20 in three, three in four, and two in five individuals of the same family, thus allowing for 154 pairwise comparisons in total.

The distribution of apo(a) alleles by size of the K IV-2 VNTR did not differ significantly in this sample from that of the total population sample (Figure 4) ($P=0.241$, Mann-Whitney U -test). We used this data set to perform correlation analysis of apo(a) allele-associated Lp(a) concentrations for apo(a) alleles IBD in different kinds of kinship and for all alleles IBD in the families. In this analysis, the correlation of allele-associated Lp(a) concentrations for alleles IBD is a direct measure of the heritability h^2 and the contribution of the LPA locus. The Pearson correlation coefficient for allele-associated Lp(a) concentrations (square-root transformed) was estimated at $r=0.776$ for mother-offspring, at $r=0.828$ for father-offspring pairs, and at $r=0.805$ for all parent-offspring pairs (all $P<0.001$) (Table 5). Thus, according to this type of analysis, heritability of Lp(a) levels is independent of parental factors ($P>0.23$ for the difference between $r_{\text{father-offspring}}$ and $r_{\text{mother-offspring}}$, one-sided). Considering all alleles IBD in the sample (number of pairs $n=154$), a Pearson correlation coefficient of $r=0.801$ (equal to $h^2=0.801$) was obtained (Figure 5). To control for any influence of the larger number of pairwise comparisons possible for those alleles IBD that could be traced in more than two individuals of the same family, we also calculated the correlation in a sample that included each allele IBD in only one randomly

Table 5 Comparison of Pearson coefficients of correlation for allele-associated Lp(a) concentrations between pairs of individuals in different kinds of kinship

Pairs of alleles	Pearson coefficient of correlation ^a	Number of pairs
Parent-offspring	0.805*	107
Mother-offspring	0.776*	56
Father-offspring	0.828*	51
Full-sibs	0.853*	33
Half-sibs	0.585 [†]	14
All alleles IBD ^b	0.801*	154
All alleles IBS ^b	0.374*	958

* $P<0.001$; [†] $P=0.028$.

^aAll allele-associated Lp(a) levels have been square-root transformed prior to analysis to approach a Gaussian distribution. All possible pairwise correlations per family were included, meaning that more than one child per parent was allowed for. The setting of any allele in each pair as variable 1 or variable 2 for the calculation was done randomly.

^bFor control, the correlations of allele-associated Lp(a) levels for all possible pairs of alleles IBD and of all possible pairs of alleles identical by state (IBS) in the K IV-VNTR (the later from 119 unrelated individuals from a Gabonese population sample) were calculated, too.

selected pair of individuals ($n=81$). The result ($r=0.779$, $P<0.001$) was in the same range as all others in this set of analyses.

When the same kind of analysis was performed for all possible pairs of alleles identical by state for the K IV-2 VNTR from the population sample (194 independent alleles with allele-associated Lp(a) concentrations), the correlation was 0.374 ($P<0.001$) (Table 5).

As a considerable number of half-sib-pairs were available, it was possible to compare the pairwise correlation of allele-associated Lp(a) concentrations for LPA alleles IBD of half- and full-sib-pairs. If the LPA locus was the sole genetic component influencing Lp(a) levels, then these correlations should not be significantly different. Otherwise, if

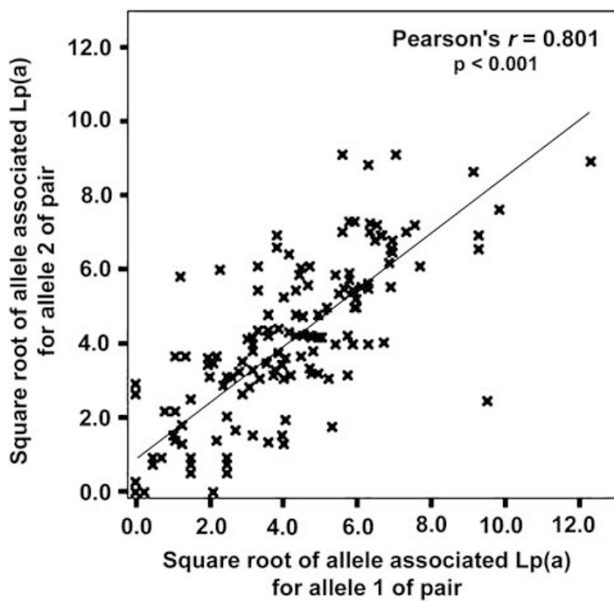


Figure 5 Correlation of allele-associated Lp(a) concentrations for 154 pairs of apo(a) alleles IBD in Gabonese families. Lp(a) concentrations were square-root transformed prior to analysis.

correlation among full-sibs was higher than among half-sib-pairs, other gene loci are likely to contribute to the genetic determination of Lp(a) levels as full-sib-pairs share, on average, 50% and half-sib-pairs only 25% of their genes. A total of 33 full- and 14 half-sib-pairs could be compared. Indeed, the Pearson correlation coefficient for full-sib-pairs was higher than for half-sib-pairs ($r=0.853$, $P<0.001$ vs $r=0.585$, $P=0.028$), with the difference reaching a significance level of $P=0.048$ (one-sided).

Discussion

Our data clearly show that Lp(a) levels are highly heritable in the Gabonese from Western Central Africa, which is so far the first autochthonous African population without admixture that has been analysed for this trait in a family study.

This result was somewhat unexpected since a previous population-based study had suggested a larger effect of diet on Lp(a) in Africans⁴⁵ and since the effect of the apo(a) K IV-2 VNTR on Lp(a) is comparatively small, explaining only 19 to 43.8% of the trait variance in populations of African descent;^{3,5,19,20} and in this study. For Europeans and Asians, much higher values have been reported (63–77%).^{3,5} If the effect of the LPA K IV-2 VNTR is smaller in Africans, but the effect of the locus is not different from Europeans, we have to conclude that other types of variation, for example, SNPs at the LPA locus, have a greater impact in Africans.

The weighted midparent–offspring analysis of Lp(a) heritability in the Gabonese is methodically very similar to that used by Mooser *et al*¹⁵ in their study of African American families and the result was nearly identical to that of Mooser *et al* with $h^2=0.77$ for the African Americans.¹⁵ Hence the high heritability of Lp(a) in African Americans can not be explained by admixture. However, Scholz *et al* calculated a heritability of only 0.51 for South African Blacks using weighted midparent–offspring regression (unweighted result: 0.65).¹³ These lower values might be considered as indicative of a difference, especially as the results of Scholz *et al*¹³ were obtained by the same laboratory using the same apo(a) detection assay. However, in view of the broad 95% confidence intervals for the heritability estimates (Table 1), differences of heritabilities between the Gabonese and South African Blacks or even Europeans cannot be considered significant.^{13,15} Furthermore, while in our study the probability of kinship was assured by STR analyses to be greater than 99.87%, Scholz *et al* relied only on the information revealed by four polymorphisms at the LPA locus. In our sample, 7% of alleles would have been falsely classified as IBD if we had relied on these polymorphisms at the LPA locus only. Thus, false assumptions on kinship might have lowered the heritability estimates in the South African sample.

Our data also indicate that the LPA locus is the major locus determining Lp(a) levels in the Gabonese. Given that the results from the midparent–offspring regression give heritability estimates of around $h^2=0.76$ and that the heritability attributed to the LPA locus is of the same order of magnitude (ie 0.776 for mother–offspring and 0.828 for father–offspring), it seems reasonable to assume that the LPA locus explains the entire genetic variability in this Central African family sample. There are, however, some caveats when comparing heritabilities estimated by different methods.⁴¹ Hence, our analysis does not exclude effects of minor loci. The observed differences in the correlation of allele-associated Lp(a) for alleles IBD between full- and half-sib-pairs in our sample could be indicative of such loci, but this result was only of borderline significance. Two studies on populations of European descent have reported other, but different, gene loci to be associated with Lp(a), one on chromosome 18,⁴⁶ the other on chromosome 1.⁴⁷ However, both studies had limitations (founder population,⁴⁶ and comparatively weak LOD score⁴⁷), and the results have not yet been confirmed in other studies.

A high impact of diet composition on Lp(a) concentration was shown for Bantu in Tanzania, with the median of Lp(a) plasma concentration being 48% lower in a population on fish diet than in a mainly vegetarian population.⁴⁵ No such division in dietary habits was to be expected in our family sample, as all families were living in a very similar environment. The observed dietary impact on Lp(a) levels in the Tanzanian Bantu population does not oppose the high heritability of the LPA trait in our study of West

African Bantus, as the two populations compared in Tanzania were exposed to population-specific different environmental factors (the diet was associated with the different geographical locations of their settlements). As heritability estimates the proportion of the phenotypic variance attributable to genetic factors, a lower phenotypic variance due to a shared environment will increase the heritability compared to a higher phenotypic variance due to differing environments.⁴¹ Thus, the high heritability of Lp(a) estimated for the Gabonese population does not exclude that nongenetic parameters influence Lp(a) concentrations.

The high heritability in the Gabonese was observed even though a high percentage of individuals suffered from parasitemia and other health impairments typical – and thus nearly impossible to evade in a family study – of a rural community in tropical Africa. This might either indicate that these factors do not influence Lp(a) plasma concentrations to a great extent, thus not much increasing the phenotypic variance of this trait, or that certain health impairments are so omnipresent in this population that their influence on the phenotypic variance was nearly uniformly present. We tested for any influence of parasitemia on the LPA trait in the Gabonese population sample. However, no simple significant relation could be detected (unpublished data). The prevalence of different kinds of parasitemia is in the same order of magnitude, when stratified for age groups as those of epidemiological screenings conducted in the same region.^{48–50} Hence, the prevalence data of parasitemia in our sample support the notion that there was no sampling bias due to the offered medical checkup, leading to an excess of sick persons in our study.

A new aspect of our study is the calculation of heritability from the allele-associated trait levels, which was possible because the unique features of the apo(a)/Lp(a) system allow for the determination of Lp(a) levels for each allele separately. The direct observation of Lp(a) levels for 81 different apo(a) alleles IBD, covering the complete range of KIV-2 VNTR sizes, which allowed for a total of 154 pairwise comparisons for allele-associated Lp(a) levels has given our study considerable power compared to previous family studies that associate total Lp(a) concentrations with diploid apo(a) genotypes and have relied on less than 60 families per population to determine heritability by midparent–offspring regression only.^{13,15} Classical midparent–offspring or single-parent–(mid)offspring and our new approach of heritability estimates from correlation of allele-associated Lp(a) for alleles IBD gave similar results in our study. This fact validates our approach even more. Our approach also allowed us to show that there is no significant influence of parental factors (eg imprinting) on the Lp(a) trait, which has not been demonstrated before. Allele-associated Lp(a) concentrations have been densitometrically measured in previous studies,^{18,38,39} but

ours is the first to use the results in order to calculate heritabilities and the effect of the apo(a) locus on Lp(a) levels.

As the mathematical assumptions for sibling-pair regression procedures, for example, those of Haseman and Elston,⁴² frequently seem to be violated by the structure of the data,^{13,15} the direct assessment of the effect of a locus on trait levels through the correlation of allele-associated trait concentrations among sibs could prove to be a valuable method for other QTLs.

Apart from the possible methodical impact on Lp(a) family studies in general, we consider the heritability estimates based on the densitometrically determined allele-associated Lp(a) concentrations the most robust and informative analyses of our study. These analyses estimate the variability explained by the LPA locus in the same range as found previously for populations of European descent (71–91%).^{13,16} Hence, the higher Lp(a) in Black Africans is likely due to differences in sequence variation at the LPA locus between Africans and Europeans. It is noteworthy that these sequence variations must be different from the apo(a) size polymorphism, as differences in the frequency distribution of the KIV-2 VNTR between Europeans and Africans are by far not large enough to explain the two- to threefold higher Lp(a) levels in the Africans^{5,6,20} and average Lp(a) concentrations for alleles of the same size are markedly different between world populations.^{5,20}

Thus, ongoing studies focusing on sequence differences at the LPA locus between world populations might reveal the reason for the higher Lp(a) concentrations in Africans.

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