

LP (a) levels and apo (a) phenotypes in urban black South African men

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Objective. To investigate the lipoprotein (a) (Lp (a)) levels and apolipoprotein (a) (apo (a)) phenotypes in a group of urban black South African men.

Design. Cross-sectional design.

Setting. Lead acid battery plant, East London, Eastern Cape.

Participants. Blood samples from a study on the association between lead and renal failure were kindly donated for the present study and 111 of the donors participated (K Steyn — personal communication).

Outcome measures. Lp (a) levels and apo (a) phenotypes.

Results. Three groups were identified: those with normal (< 300 U/l), intermediate (300 - 700 U/l) and high (> 700 U/l) plasma Lp (a) concentrations. Nine apo (a) phenotypes and 26 combinations thereof could be discerned. Apart from the single- and double-band phenotypes described before, triple-band phenotypes were also present. As the Lp (a) values increased, the relative frequency of the single-band phenotype decreased, whereas the relative frequency of the double-band phenotype increased. The relative frequency of the triple-band phenotype was highest in the group with high Lp (a) concentrations. No correlation was evident between the size of the apo (a) isoforms and the Lp (a) concentrations.

Conclusions. Raised plasma Lp (a) levels have been associated with coronary heart disease (CHD). In addition, it has been proposed that the apo (a) gene determined plasma Lp (a) concentrations. These studies were performed using plasma from white subjects. CHD is uncommon in black South Africans. The reason may be that, given the lack of relationship between the size of the apo (a) isoforms and the Lp (a) concentrations observed in the present study, factors other than the isoform size may determine the Lp (a) levels in this particular ethnic group.

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Lipoprotein (a) (Lp (a)) is a plasma protein similar in size and lipid composition to low-density lipoprotein (LDL).¹ In

addition to apo B100, the molecule contains another glycoprotein, apolipoprotein (apo (a)), which bears a strong resemblance to plasminogen.² Lp (a) exhibits both inter-individual and intra-individual heterogeneity, in terms of size (280 - 800 kD) and hydrated density.^{2,3} This heterogeneity is mostly accounted for by the striking size polymorphism of apo (a).^{2,3}

Extended family studies demonstrated that the Lp (a) trait was inherited⁴ and that Lp (a) represented a quantitative rather than a qualitative genetic trait.⁵

Gel electrophoresis and immunoblotting have identified between 6 and 34 apo (a) isoforms, depending on the method used.⁶⁻⁹

The factors controlling Lp (a) plasma levels have not been clearly defined. An inverse relationship between the apo (a) size and plasma Lp (a) levels has been documented;⁶ however, alleles of the same size may be associated with an up to 200-fold difference in Lp (a) concentrations.¹ In addition, ethnic differences in Lp (a) levels also occur for the same apo (a) size.^{10,11}

Lp (a) plays a role in the atherosclerotic process.¹² The lipoprotein accumulates in atherosclerotic plaques,¹³ stimulates smooth-muscle proliferation¹⁴ and promotes cholesterol accumulation in cells.¹⁵ It may also promote thrombosis because of its structural similarities to plasminogen.¹⁶

Black South Africans have been reported to have a protective lipid and lipoprotein profile associated with their low prevalence of coronary heart disease (CHD).¹⁷ The present study was therefore undertaken to investigate the Lp (a) values and apo (a) phenotypes in a group of black urban South African men.

Methods

Subjects

Plasma samples from black South African men working at a lead acid battery plant in the Eastern Cape were employed. Lp (a) values were determined immediately, after which the samples were stored at -20°C for approximately 1 year prior to the commencement of the investigation into the apo (a) phenotypes. It should be emphasised that storage of samples for up to 24 months at either -20°C or -80°C has no effect on the detection of apo (a).¹⁸ Of the samples received, 111 were used.

Lp (a)

Lp (a) values were determined with a Pharmacia Apo (a) solid phase two-site immunoradiometric assay. The method is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the apo (a) molecule. During incubation apo (a) in the sample reacts with ¹²⁵I-anti-apo (a) antibodies and anti-apo (a) antibodies bound to sepharose particles. The antibody-antigen complex formed is separated from excess tracer by addition of decanting suspension, followed by centrifugation and decanting. The radioactivity in the pellet is proportional to the concentration of apo (a) in the sample. Normal values for this laboratory are < 300 U/l.

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Electrophoresis

Samples were diluted according to their Lp (a) values in 0.15M phosphate buffer pH 6.8, containing 12.5% glycerol, 2% sodium dodecyl sulphate (SDS), 5% β -mercapto-ethanol and 0.001% bromophenol blue. After boiling for 5 minutes in a boiling bath, aliquots ranging from 10 μ l to 25 μ l were electrophoresed.

An Lp (a) standard (Pharmacia Apo (a) RIA) was included occasionally to monitor the immunoblotting procedure, while apo B100 (prepared from plasma saturated with sodium chloride and spun on a saline gradient in an ultracentrifuge) was included in each run as a reference for the position of the different phenotypes on the gel.¹⁹

The gels used in the present study were gradient SDS-polyacrylamide gels (SDS-PAGE), 3 - 20%,²⁰ for the separation of large proteoglycans. An LKB 2001 (Bromma, Sweden) vertical gel electrophoresis unit was used.

Electrophoresis was performed overnight at a constant current of 17 mA, after which the gels were removed for electroblotting.

Electroblotting

Transfer of proteins from the gradient gels to nitrocellulose (Hybond-C, 0.45 μ , Amersham, UK) was performed according to a modification of the semi-dry method described by Lauriere.²¹ The system used was a Polyblot Transfer System, Model SBD-1000 (American Bionetics).

The buffer system included two anode buffers, 0.3M Tris, 20% methanol pH 10.4 and 25 mM Tris, 20% methanol pH 10.4, respectively, and a cathode buffer, 25 mM Tris, 40 mM 6-aminohexanoic acid, 20% methanol pH 9.4.

Blotting was carried out at 2.5 mA/cm² of gel area for 20 minutes. Subsequently the nitrocellulose was cut, one section containing the samples and the other the apo B100. The latter section was stained with amido black 10B (Sigma Chemical Company, St Louis, Mo.), 0.5% in 50% methanol, 10% acetic acid, and destained in 45% methanol, 10% acetic acid.

Immunoblotting

The method used for immunoblotting of the apo (a) phenotypes was essentially that of Huang *et al.*²² After the electroblotting, the nitrocellulose section bearing the electrophoresed samples was transferred to a solution of 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS) to saturate free protein-binding sites. After 5 hours the

nitrocellulose was incubated with human anti-Lp (a) (1 μ g/ml; Boehringer Mannheim, Germany) in TBS containing 1% BSA and 0.05% Tween 20 (dilution buffer) overnight; after extensive washing with TBS, the blot was incubated with anti-mouse IgG Biotin conjugate in dilution buffer (500 ng/ml; Sigma Chemical Company, St Louis, Mo.) for 2 hours. Once again the blot was washed with TBS and then transferred to streptavidin-peroxidase in dilution buffer (500 ng/ml; Sigma Chemical Company, St Louis, Mo.) for 1 hour. Finally, after washing with TBS, the substrate, diaminobenzidine (0.05%; Sigma Chemical Company, St Louis, Mo.) in TBS with CoCl₂ (0.002%), NH₄NiSO₄ (0.002%) and H₂O₂ (50 μ l of a 30% solution) was added to the blot for 20 minutes, after which the latter was rinsed with H₂O and the positions of the Lp (a) phenotypes noted and compared with that of apo B100.

Statistics

Comparison of Lp (a) concentrations was performed by means of the Mann-Whitney U-test. Comparison of apo (a) frequencies between groups with high (> 700 U/l), intermediate (300 - 700 U/l) and normal (< 300 U/l) Lp (a) values was done with the χ^2 -test.

Results

The Lp (a) values of the sample population varied tremendously and it was therefore decided to distinguish three groups and to investigate the apo (a) phenotypes in each group. The groups included Lp (a) values < 300 U/l ($N = 36$), 300 - 700 U/l ($N = 40$) and > 700 U/l ($N = 35$). The difference between the former two groups, as well as the latter two, was highly significant ($P = 0.0$, Mann-Whitney U-test). Total cholesterol and LDL cholesterol values for the different groups were as follows: 4.8 ± 1.00 and 2.6 ± 1.00 , respectively (< 300 U/l, mean \pm SD), 4.8 ± 0.94 and 2.8 ± 0.88 , respectively (300 - 700 U/l, mean \pm SD) and 4.9 ± 0.88 and 2.9 ± 0.93 , respectively (> 700 U/l, mean \pm SD).

As far as the apo (a) phenotypes are concerned, Fig. 1 is a representation of an immunoblot. Lane a shows a position of apo B100, lane b phenotypes F1, B and S3 (Lp (a): 984 U/l), lane c phenotypes S2 and S3 (Lp (a): 1 564 U/l), lane d phenotypes S1, S2 and S3 (Lp (a): 654 U/l), lane e phenotype S1 (Lp (a): 591 U/l), lane f phenotype S1 (Lp (a): 239 U/l) and lane g phenotypes S1 and S3 (Lp (a): 123 U/l).

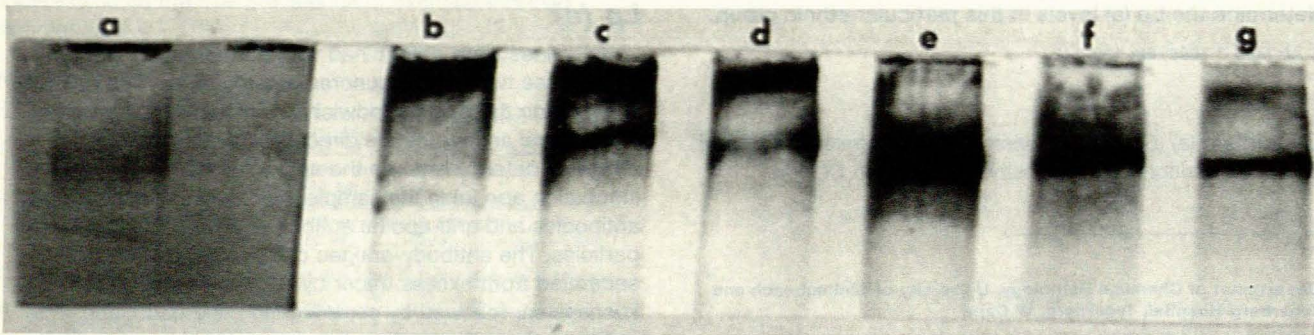


Fig. 1. A representative photograph of an immunoblot. (Lane a: apo B100; b: phenotypes F1, B and S3; Lp (a) 984 U/l; c: phenotypes S2 and S3; Lp (a) 1 564 U/l; d: phenotypes S1, S2 and S3; Lp (a) 654 U/l; e: phenotype S1; Lp (a) 591 U/l; f: phenotype S1; Lp (a) 239 U/l; g: phenotypes S1 and S3; Lp (a) 123 U/l.)

An interesting observation was the large number of phenotypes, i.e. 9, and the even larger number of combinations, i.e. 26, present in the test sample (Table I). Unfortunately, because of this, as well as the fact that the frequencies were low, the χ^2 -test was unable to establish significant differences between groups. However, an interesting observation was the presence of triple-band phenotypes, apart from the single- and double-band phenotypes described before (Table I).¹⁹ These samples were repeated; however, the results remained the same. In each group, one or more null phenotypes were present, despite the fact that Lp (a) levels were high in some of the samples. Repeating the electrophoresis and blotting steps once again yielded similar results.

Table I. Apo (a) phenotypes and their respective frequencies observed in urban black South African men

No.	Apo (a) phenotypes	Observed frequencies Lp (a) (U/l)		
		< 300	300 - 700	> 700
1	B	3	3	2
2	S1	3	7	3
3	S2	3	2	1
4	S3	2	1	1
5	S4	2		2
6	0	3	2	1
7	F1		1	1
8	F2	3	1	
9	F3			
10	B/S1	1		1
11	B/S2		1	1
12	B/S3		1	
13	B/S4			
14	S1/S2	1	2	3
15	S1/S3		1	2
16	S1/S4			
17	S2/S3	1	3	2
18	S2/S4		1	1
19	S3/S4			
20	B/F1	1		2
21	B/F2			
22	B/F3			
23	F1/F2		2	
24	F1/F3		1	
25	F2/F3	1		
26	F1/S1		2	2
27	F1/S2	1		
28	F2/S1	2		
29	F2/S2		2	
30	F3/S3			1
31	F3/S4			1
32	F1/S2/S4	1		1
33	S1/S2/S3		1	
34	B/F1/F2			1
35	B/F1/S3			1
		28	34	30

Table II shows the different phenotype groups and the relative frequencies in each of the three sample groups. An interesting observation was that as the Lp (a) values increased, the relative frequency of the single-band phenotype decreased and, inversely, the relative frequency

of the double-band phenotype increased. The relative frequency of the triple-band phenotype was highest among those men with the high Lp (a) values, while the relative frequency of the null phenotype was highest among the low Lp (a) samples.

Table II. Relative frequencies of groups of apo (a) phenotypes observed in urban black South African men

Apo (a) phenotype groups	Relative frequencies (%) Lp (a) (U/l)		
	< 300	300 - 700	> 700
Single-band	57	44	33
Double-band	29	50	53
Triple-band	4	3	10
Null	11	6	3

Although it has previously been suggested that the size of the apo (a) isoforms correlated negatively with the plasma Lp (a) concentrations,²³ this was not evident in the present study.

Discussion

In the present study, plasma samples, kindly donated by the lead study, were evaluated for Lp (a) values and apo (a) phenotypes in urban black South African men. The Lp (a) concentrations in the samples varied and three groups were therefore identified: those with normal (< 300 U/l), intermediate (300 - 700 U/l) and high (> 700 U/l) values. Apo (a) phenotypes in each of these groups were determined by SDS-PAGE, electro- and immunoblotting. Although the sample size in each group was small as a result of the cost involved, especially that of SDS-PAGE, interesting observations were made. The large number of apo (a) phenotypes observed (9) and the subsequent combinations (26) are in agreement with results from a similar study.⁹ These authors identified 34 different apo (a) isoforms in a sample of black Americans with a high-resolution SDS-agarose gel electrophoretic method. Another group¹⁰ also reported the presence of 101 distinct phenotypes in a group of American blacks, using SDS-agarose gel electrophoresis. Other studies have reported smaller phenotype numbers,^{8,19} however, the method of separation of very-high-molecular-weight proteins plays a role in the outcome of the results. The gradient gels used in the present study²⁰ have particularly been suggested for the apo (a) proteins of which the molecular weights range between 200 and 800 kD.²

Another interesting observation was the decrease in the relative frequency of single-band phenotypes with increasing concentrations of Lp (a), while the relative frequency of the double-band phenotype showed an increase with increasing Lp (a) concentrations. As far as each single-band phenotype was concerned, however, only the S1 tended to be more frequent among the mid-range of Lp (a) concentrations. In addition, this is the first study to report the possible existence of a triple-band phenotype. At least one was observed in each of the groups studied. Since Lp (a) is a single allele, it would be expected that either one or two isoforms would be present in a patient. The third band observed in the present study could be a true novel

observation, or may be due to a combination of two isoforms. With regard to the null phenotype, we are not able to explain its existence, despite the fact that in each case Lp (a) was present.

Although high levels of Lp (a) have been found to be associated with increased cardiovascular risk,² this does not seem to be the case in this particular ethnic group. Results from the lead study clearly showed that total cholesterol levels in urban black South Africans fell within the normal range and were lower than in other groups including whites, coloureds and Asians.¹⁷ In addition, the LDL-cholesterol levels of the black men from the lead study also fell within the normal range. Heterozygotes for familial hypercholesterolaemia have been shown to have about a threefold increase in plasma Lp (a) concentrations.⁴ It has subsequently been proposed that for Lp (a) to exert its adverse effects, a concomitantly raised LDL-cholesterol level was important.¹²

A possibility may be that Lp (a), because of its structural similarities to plasminogen¹⁶ and its having been shown to inhibit activation of the latter,²⁴ prevents clot dissolution in the blood vessel wall. LDL is known to enter macrophages in an oxidised form; these in turn enter vessel endothelial cells, forming foam cells.²⁵ These foam cells cause damage to the vessel wall with subsequent clot formation.²⁵ Because of the damage caused to the vessel wall, Lp (a) is able to enter¹⁶ and in that way prevent clot dissolution. Since their total and LDL-cholesterol levels are normal,¹⁷ this may protect black South Africans from the risk of CHD, even in the presence of such high Lp (a) levels.

Another possible reason for CHD's being rather uncommon in black South Africans may be that their Lp (a) genetics differ from those of other ethnic groups, including whites and Asians, known to have a high risk of CHD.¹⁷ It has been suggested that in some non-Caucasian ethnic groups Lp (a) levels may be determined by factors that are different from the apo (a) size polymorphism.⁴ However, it should be mentioned that although the blacks in the present study seem to be protected against CHD, it has been shown in another study²⁶ that all the elements of a potential epidemic of atherosclerotic cardiovascular disease were present in black populations, urban and rural, indicating the advanced stage of westernisation. Future studies should be able to cast light on the possible involvement of Lp (a) in CHD in this ethnic group.

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REFERENCES

- Perombelon YFN, Soutar AK, Knight BL. Variation in lipoprotein (a) concentration associated with different apolipoprotein (a) alleles. *J Clin Invest* 1994; **93**: 1481-1492.
- Klezovitch O, Scanu AM. Heterogeneity of lipoprotein (a): growing complexities. *Curr Opin Lipidol* 1995; **6**: 223-228.
- Fless GM, Roli CA, Scanu AM. Heterogeneity of human plasma lipoprotein (a). *J Biol Chem* 1984; **259**: 11470-11478.
- Utermann G. Lipoprotein (a): a genetic risk factor for premature coronary heart disease. *Curr Opin Lipidol* 1990; **1**: 404-410.
- Utermann G. The mysteries of lipoprotein (a). *Science* 1989; **246**: 904-910.
- Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp (a) glycoprotein phenotype: inheritance and relation to Lp (a) - lipoprotein concentrations in plasma. *J Clin Invest* 1987; **80**: 458-465.
- Gaubatz LW, Ghanem KI, Guevera J jun, Nava ML, Patsch W, Morrisett JD. Polymorphic forms for human apolipoprotein (a): inheritance and relationship of their molecular weights to plasma levels of lipoprotein (a). *J Lipid Res* 1990; **31**: 603-613.
- Kamboh MI, Ferrell RE, Kottke BA. Expressed hypervariable polymorphism of apolipoprotein (a). *Am J Hum Genet* 1991; **49**: 1063-1074.
- Marcovina SM, Zhang ZH, Gaur VP, Albers JJ. Identification of 34 apolipoprotein (a) isoforms: differential expression of apolipoprotein (a) alleles between American blacks and whites. *Biochem Biophys Res Commun* 1993; **191**: 1192-1196.
- Kamboh MI, Svitko CM, Williams E, Ferrell RE, Pollitzer WS. Hypervariable polymorphism of apo (a) in blacks and whites reflected by phenotyping. *Chem Phys Lipids* 1994; **67/68**: 283-292.
- Gaw A, Boerwinkle E, Cohen JC, Hobbs HH. Comparative analysis of the apo (a) gene, apo (a) glycoprotein, and plasma concentrations of Lp (a) in three ethnic groups. Evidence for no null allele at the apo (a) locus. *J Clin Invest* 1994; **93**: 2526-2534.
- Maher VMG, Brown RB. Lipoprotein (a) and coronary heart disease. *Curr Opin Lipidol* 1995; **6**: 229-235.
- Smith EB, Cochran S. Factors influencing the accumulation in fibrous plaques of lipid derived from low-density lipoprotein. Preferential immobilisation of lipoprotein (a) (Lp (a)). *Atherosclerosis* 1990; **84**: 173-181.
- Grainger DJ, Kirschenlohr HL, Metcalfe JC, Weissberg PL, Wade DP, Lawn RM. Proliferation of human smooth muscle cells promoted by lipoprotein (a). *Science* 1993; **260**: 1655-1658.
- Bottalico LA, Keesler GA, Fless GM, Tabas I. Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein (a) and apolipoprotein (a) internalization and degradation. *J Biol Chem* 1993; **268**: 8569-8573.
- McLean JW, Tomlinson JE, Kuang W-J, et al. cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. *Nature* 1987; **330**: 132-137.
- Oelofse A, Jooste PL, Steyn K, et al. The lipid and lipoprotein profile of the urban black South African population of the Cape Peninsula — the BRISK study. *S Afr Med J* 1996; **86**: 162-166.
- Kronenberg F, Lobentanz EM, König P, Utermann G, Dieplinger H. Effect of sample storage on the measurement of lipoprotein (a), apolipoproteins B and A-IV, total and high density lipoprotein cholesterol and triglycerides. *J Lipid Res* 1994; **35**: 1318-1328.
- Utermann G, Kraft HG, Menzel HJ, Hopferwieser T, Seitz C. Genetics of the quantitative Lp (a) trait. I. Relation of Lp (a) glycoprotein phenotypes to Lp (a) lipoprotein concentrations in plasma. *Hum Genet* 1988; **78**: 41-46.
- Vilim V, Krajickova J. Electrophoretic separation of large proteoglycans in large-pore polyacrylamide gradient gels (1.32 - 10.0%) and a one-step procedure for simultaneous staining of proteins and proteoglycans. *Anal Biochem* 1991; **197**: 34-39.
- Lauriere M. A semidry electroblotting system efficiently transfers both high- and low-molecular-weight proteins separated by SDS-PAGE. *Anal Biochem* 1993; **212**: 206-211.
- Huang CM, Kraft HG, Gregg RE. Modified immunoblotting technique for phenotyping lipoprotein (a). *Clin Chem* 1991; **37**: 576-578.
- Kraft HG, Köchl S, Menzel HJ, Sandholzer C, Utermann G. The apolipoprotein (a) gene: a transcribed hypervariable locus controlling plasma lipoprotein (a) concentration. *Hum Genet* 1992; **90**: 220-230.
- Grainger DJ, Metcalfe JC. Transforming growth factor-beta: the key to understanding lipoprotein (a)? *Curr Opin Lipidol* 1995; **6**: 81-85.
- Davies MJ, Woolf N. Atherosclerosis: what is it and why does it occur? *Br Heart J* 1993; **69**: suppl, S3-S11.
- Mollentze WF, Moore AJ, Steyn AF, et al. Coronary heart disease risk factors in a rural and urban Orange Free State black population. *S Afr Med J* 1995; **85**: 90-96.

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