The Relation of Lipoprotein[a] Concentrations and Apolipoprotein[a] Phenotypes With Asymptomatic Atherosclerosis in Subjects of the Atherosclerosis Risk in Communities (ARIC) Study

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Plasma levels of lipoprotein[a] (Lp[a]) are associated with increased risk of coronary artery disease and show an inverse correlation with apolipoprotein[a] (apo[a]) molecular weight. We determined Lp[a] levels and apo[a] phenotypes in 171 cases with preclinical extracranial carotid atherosclerosis as ascertained by B-mode ultrasound and in 274 control subjects free of carotid atherosclerosis. Lp[a] protein levels measured by enzyme-linked immunosorbent assay ranged from 4 to 361 μg/mL in cases and from 2 to 392 μg/mL in controls, but median levels of Lp[a] were higher in cases than in controls (51 μg/mL versus 33 μg/mL, $P<.003$). In both groups, all 11 apo[a] polymorphs that are resolved by the procedure used were present, resulting in 43 and 39 different apo[a] phenotypes in cases and controls, respectively. An inverse relation between apo[a] polymorph size and Lp[a] level was observed in both cases ($r=-0.49$, $P<.001$) and controls ($r=-0.34$, $P<.001$). Apo[a] phenotype distributions were similar in cases and controls. However, in 17 phenotypes with three or more subjects per group, the difference of mean Lp[a] concentrations between cases and controls was 32±36 μg/mL (mean±SD). Thus, the higher Lp[a] levels in cases were not associated with a greater prevalence of small apo[a] polymorphs. Stepwise logistic regression analyses of known risk factors for coronary heart disease showed that plasma Lp[a] concentration was an independent predictor of case-control status, while Lp[a] phenotype was not, irrespective of the presence or absence of Lp[a] concentration in the model. These findings confirm the inverse correlation of apo[a] size with plasma Lp[a] levels but imply that sequences at the apo[a] gene locus other than those determining the number of apo[a] kringle type 2 units are involved in the increased expression of apo[a] in subjects with early atherosclerosis. (*Arterioscler Thromb.* 1993;13:1558-1566.)

KEY WORDS • carotid atherosclerosis • lipoprotein[a] • apolipoprotein[a] phenotype

Associations between elevated plasma concentrations of lipoprotein[a] (Lp[a]) and symptomatic atherosclerosis such as coronary artery disease (CAD) and cerebrovascular disease have been established in numerous studies employing various designs and analytic approaches.[1-5] Cross-sectional studies have established the relation between plasma Lp[a] levels and their relevance for various atherosclerotic diseases (reviewed in References 6 and 7). That elevated plasma levels of Lp[a] confer an increased risk for CAD has also been demonstrated in one prospective study.[8] However, no association of Lp[a] with cardiac end points has been found in the Helsinki Heart Study cohort.[9] Thus, the association of Lp[a] levels with incident CAD is currently equivocal.

Received June 10, 1993; revision accepted July 27, 1993.

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Lp[a] comprises a unique class of plasma lipoproteins. Its most abundant proteins are two high-molecular-weight glycoproteins, apolipoprotein (apo) B-100 and apo[a]; the latter is unique to Lp[a]. Apo[a] is covalently linked to apoB-100 by a disulfide bridge[10,11] and exists in polymorphs distinguished by their molecular weights.[10,12-14] The molecular basis for the observed size variation of apo[a] is primarily due to multiple apo[a] alleles that differ in the number of kringle type 2 (plasminogen kringle type 4) repeats.[15-18] In addition, minor variability in apo[a] size might be due to differences in glycosylation, since carbohydrates make up 25% to 40% of the weight of apo[a].[19]

While a number of exogenous factors such as hormones and drugs can affect levels of Lp[a],[20-22] a wealth of data indicates that genetic factors play a dominant role in determining plasma concentrations of Lp[a]. More than 90% of the variability of Lp[a] plasma levels in the general population is determined by size and sequence variations within the apo[a] gene.[23] An inverse relation exists between plasma Lp[a] levels and apo[a] size.[12,13] Estimates of the variance of Lp[a] plasma levels that can be explained by apo[a] size range from 19% to 70% among different populations.[24] Thus, the se-

quences that encode the number of apo[a] kringle 2 units as well as additional sequence variation at the apo[a] gene locus are responsible for the control of plasma Lp[a] levels.

We previously showed that mean plasma Lp[a] levels were significantly higher in subjects with asymptomatic thickening of extracranial carotid arteries when compared with control subjects ("controls") free of carotid atherosclerosis.[25] Because of the relations among apo[a] size, Lp[a] level, and apo[a] phenotype with CAD,[26,27] we hypothesized that the distribution of apo[a] phenotypes might differ between carotid cases and controls. We therefore performed apo[a] phenotyping in 171 carotid cases and 274 controls. While there was an inverse correlation of apo[a] size with Lp[a] levels in both cases and controls, the distribution of apo[a] phenotypes was similar between the two groups, even though median Lp[a] levels were higher in cases than in controls. In fact, in the majority of Lp[a] phenotypes, cases exhibited higher Lp[a] levels than controls. Thus, undetected environmental factors or, more likely, sequence heterogeneity of the apo[a] gene that is unrelated to the number of kringle type 2 repeats may be an important determinant of the elevated plasma Lp[a] levels in the cases with carotid atherosclerosis studied here.

Methods

Study Population

Cases and controls were selected from the Atherosclerosis Risk in Communities (ARIC) Study, a prospective, multicenter investigation of atherosclerosis and its clinical sequelae in women and men aged 45 to 64 years. Approximately 16 000 residents were recruited, 4000 in each of four communities: Forsyth County, NC; the city of Jackson, Miss; the northwestern suburbs of Minneapolis, Minn; and Washington County, Md. The cohort was selected to represent a probability sample of residents living in that community, except for the Jackson sample, which contained only black residents. The general design of the ARIC Study has been described elsewhere.[28]

Carotid wall thickness was measured by B-mode ultrasonography[29] and the technique of Pignoli et al.[30] Subjects were designated as "cases" if the maximum carotid arterial wall thickness was >2.5 mm or if there was bilateral thickening above the ≈90th percentile of the ARIC Study cohort distribution (corresponding to 1.7 mm in the internal carotid, 1.8 mm in the carotid bifurcation, and 1.6 mm in the common carotid). Subjects were designated as controls if the maximum wall thickness was <75th percentile at the near and far walls of each of the carotid segments and popliteal arteries.[31] Controls were group-matched to cases by gender, race, field center, 10-year age group, and time of examination. Cases and controls were required to meet minimum visualization criteria of arterial wall boundaries on ultrasound. Only the data for whites were used in this analysis to avoid confounding influences of race on the relation between Lp[a] levels and apo[a] phenotypes.[32] Other exclusion criteria for case-control selection were (1) evidence of symptomatic cardiovascular or cerebrovascular disease defined by a history of angina on effort, physician-diagnosed heart attack, transient ischemic attack or stroke, or intermittent claudication; (2) use of lipid-altering medication, such as β-blockers, hypolipidemics, or thyroid medication; (3) use of oral contraceptives in premenopausal women; (4) insulin-dependent diabetes mellitus; (5) chronic renal or liver disease; and (6) hypertriglyceridemia defined as fasting triglyceride levels >400 mg/dL. Thus, cases may be considered as a simple random sample of cases (with certain exclusions and assuming random response), while controls are a stratified random sample. Hence, control means and proportions do not estimate population means and proportions but are more like estimates adjusted by the distribution among cases for the levels of the stratifying variables.

Sitting blood pressure was measured after a 5-minute rest, three times, using a random-zero sphygmomanometer. The systolic blood pressure measurement used was the average of the second and third readings. Hypertension was defined by a systolic or diastolic blood pressure >160 or 95, respectively, or by current use of antihypertensive medication. Body mass index (in kilograms per meters squared) was calculated from measurements of weight (to the nearest pound) or height (to the nearest centimeter).

Laboratory Measurements

Venous blood was collected after a 12-hour fast into tubes containing EDTA. Plasma was separated by centrifugation at 4°C. Aliquots were stored at each field center at −70°C and shipped on dry ice at weekly intervals to the ARIC Central Lipid Laboratory. Specimens were stored at −70°C until analyzed. Total plasma cholesterol[33] and triglyceride[34] were measured enzymatically on a Cobas-Fara centrifugal analyzer (Roche Diagnostics, Montclair, NJ) with the respective enzymatic kits (catalog Nos. 236691 and 701912, Boehringer Mannheim Diagnostics, Indianapolis, Ind). High-density lipoprotein (HDL) cholesterol was determined by measuring cholesterol in the supernatant liquid after precipitation of the plasma with magnesium chloride and dextran sulfate.[35] Low-density lipoprotein (LDL) cholesterol levels were calculated according to Friedewald et al.[36] Apolipoprotein A-I and B levels were determined by a radioimmunoassay.[37,38] Plasma levels of Lp[a] were determined by a double-antibody enzyme-linked immunoassay (ELISA) described previously.[39] In brief, the immunoglobulin G (IgG) fraction of goat anti-human apo[a] antisera was bound to the surface of microtiter plates to capture the Lp[a] in plasma samples and in the standard. For detection of bound Lp[a], rabbit anti-human apo[a] serum was used, which was quantified using a goat anti-rabbit IgG peroxidase conjugate. Lp[a], purified as described by Gaubatz et al,[39] was used as a primary standard. Criteria of purity included the absence of all apolipoproteins except apoB and apo[a], as judged by polyacrylamide gel electrophoresis (PAGE) and Western blotting with cognate antibodies, and the absence of LDL as judged by double-decker immunoelectrophoresis. Lp[a] levels are given in micrograms per milliliter and represent the total protein moiety of Lp[a], including apo[a] and associated apoB. With this method, Lp[a] protein was detectable in the plasma of all subjects. For normal Lp[a] protein levels in the range of 1 to 10 mg/dL, the contribution of plasminogen to Lp[a] levels was negligible.[40]

For internal quality control of lipid and apolipoprotein measurements, control pools were used. The coefficients of laboratory variation were 2.5% for cholesterol, 2.7% for triglyceride, 3.7% for HDL cholesterol, 5.2% for LDL cholesterol, 9% for apoA-I, 9% for apoB, and 9% for Lp[a]. External quality control consisted of participation in the Centers for Disease Control and Prevention's Lipid Standardization Program.[41] In addition, a subset of participant sample aliquots was taken from the blood collection tubes, stored at each field center for an additional week, and sent to the laboratory in the subsequent weekly mailing in a blinded fashion, thereby providing a measure of the overall variability of measurement (ie, variability due to storage, shipping, sample processing, transcription, and analyses). The coefficients of variation in these blinded replicates for total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, apoA-I, and Lp[a] were 3.0%, 6.7%, 4.4%, 5.3%, 11.0%, and 16.9%, respectively.

Lp[a] phenotyping was performed as previously described.[13] Specimens containing 25 μg of Lp[a] protein, based on ELISA measurements, were applied to sodium dodecyl sulfate (SDS)–PAGE gels containing 3.75% acrylamide, 0.10% bisacrylamide, and 0.75% agarose (type C, Behring Diagnostics, La Jolla, Calif). Electrophoresis was performed at 10°C for 18 hours at 25 mA/gel, and electrophoretic transfer of proteins from gel to nitrocellulose was performed for 18 hours at 50 V. Apo[a] polymorphs were localized on the nitrocellulose membrane using IgG purified from rabbit antisera directed against apo[a], followed by detecting the bound IgG with ^{125}I–protein A (ICN Biomedicals, Inc, Irvine, Calif). Radiolabeled bands were visualized after overnight exposure at −70°C to X-omat XAR5 film (Eastman Kodak Co, Rochester, NY) using a Lightening Plus intensifying screen (DuPont, Wilmington, Del). Apo[a] polymorph molecular weights were estimated using apoB-100 (M_r, 512 kD) and cross-linked phosphorylase *b* (97.4 kD) oligomers (Sigma Chemical Co, St Louis, Mo).

Statistical Methods

Standard unmatched *t* tests were used to compare mean levels between cases and controls. Because of the very nonnormal distributions of plasma Lp[a] and triglyceride concentrations, the nonparametric rank-sum test was used[42] to compare median levels of these variables between cases and controls. Because of the large number of apo[a] phenotypes relative to the number of individuals in this sample, traditional χ^2 goodness-of-fit tests and χ^2 tests of independence were inappropriate. A goodness-of-fit test to Hardy-Weinberg expectations was carried out using a computerized permutation algorithm described by Guo and Thompson.[43] Comparisons of apo[a] polymorph and phenotype frequencies between groups (eg, cases versus controls, males versus females) were carried out using a Monte Carlo simulation method to derive probability values for Fisher's Exact Test.[44] The relation between apo[a] polymorph size and plasma Lp[a] concentration was summarized with Spearman's rank correlation.[42]

Conditional stepwise logistic regression[45,46] was used to identify the set of statistically significant predictor variables. The list of potential predictor variables included sex; age; body mass index; pack-years of cigarette smoking; hypertension status; ethanol consumption; and glucose, insulin, total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, apoA-I, apoB, and Lp[a] levels. Because this is a cross-sectional study, the dependent variable in the logistic regression equation is the log of the odds of being a carotid case at the time of baseline examination, rather than the log of the odds of developing symptomatic atherosclerotic heart disease. Logistic regression analysis was used rather than discriminant analysis because the model for logistic regression is less restrictive than the linear discriminant model with respect to assumptions.[47] Variable selection was carried out in an ordered manner using a priori considerations. First, the set of significant demographic and anthropometric variables was identified; to this list the set of biochemical measures, such as plasma lipid concentration (excluding Lp[a]) variables, was added. We next tested whether Lp[a] improved our ability to predict prevalent disease, given that the other recognized predictors were already in the model. And finally, we asked whether information about apo[a] polymorphs and phenotypes contributed to our ability to predict disease beyond that afforded by more traditional risk factors. The latter analysis was carried out with and without plasma Lp[a] concentrations in the model.

Results

Plasma Lipids, Apolipoproteins, and Lp[a] Concentrations in Study Subjects

Table 1 shows mean values of cardiovascular risk factors in cases and controls. Despite the selection procedure, cases were 1 year older than controls. Cases also had a higher body mass index, a greater pack-year history of cigarette smoking, and a higher prevalence of hypertension. Cases had significantly greater concentrations of total cholesterol (7.8%), triglyceride (30.8%), LDL cholesterol (14.0%), apoB (13.9%), and Lp[a] protein (32.8%) and decreased levels of HDL cholesterol (13%) and apoA-I (5.9%). The differences in triglyceride and Lp[a] protein remained significant after logarithmic transformation of these variables (data not shown).

The range of Lp[a] protein levels was similar in controls (2 to 392 μg/mL) and cases (4 to 361 μg/mL). In both groups, the distribution of Lp[a] protein levels was highly skewed (skewness: 2.1 in controls and 1.6 in cases). Median Lp[a] protein levels were 51 μg/mL in cases and 33 μg/mL in controls ($P<.003$), and the cumulative frequency distribution curve of Lp[a] protein levels in cases was shifted to the right relative to controls (Fig 1).

Apo[a] Phenotype Distributions in Controls and Cases

Control subjects demonstrated 39 of the 66 possible apo[a] phenotypes in the system, based on the resolution of 11 different polymorphs.[13] In these subjects, 60.6% expressed apo[a] phenotypes with only 1 apo[a] polymorph, and 39.4% expressed apo[a] phenotypes with 2 clearly detectable apo[a] polymorphs (Table 2). Cases demonstrated 43 of the 66 possible apo[a] phenotypes; 50.3% exhibited a single apo[a] polymorph and 49.7% exhibited 2 apo[a] polymorphs. The greatest difference was observed for a single-band phenotype, which was 4% more abundant in cases than controls.

TABLE 1. Cardiovascular Risk Factors in Cases With Asymptomatic Atherosclerosis and Controls*

	Controls, n=274	Cases, n=171	P†
Age, y	55.4 (5.3)	56.6 (5.0)	.026
Sex, M/F	174/100	115/56	NS
Body mass index, kg/m^2	26.0 (3.8)	27.2 (4.5)	.0257
Pack-years of cigarette smoking	13.5 (20.3)	32.0 (26.1)	.0001
Prevalence of hypertension‡	9.5	21.9	.0001
Total cholesterol, mg/dL	204 (37)	220 (40)	.0001
Triglyceride, mg/dL	107 (60)	140 (87)	.0001
HDL cholesterol, mg/dL	54 (18)	47 (15)	.0001
LDL cholesterol, mg/dL	128 (36)	146 (36)	.0001
Apolipoprotein A-I, mg/dL	132 (30)	125 (28)	.0008
Apolipoprotein B, mg/dL	86 (26)	98 (25)	.0010
Lp[a] protein, μg/mL	64 (74)	85 (86)	.0096

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and Lp[a], lipoprotein[a].
*Except for sex and hypertension status, data are mean and (SD).
†Probability of the observed differences in means by chance alone. For plasma triglyceride and Lp[a] levels, a nonparametric test was used (see "Methods").
‡Prevalence expressed as a percentage.

This phenotype is characterized by a single apo[a] polymorph with an apparent M_r of 742 kD.[13]

The number of individuals with any one phenotype was typically small. Many types were found in only one case or one control, and none were seen in more than 12% of either group. In both cases and controls, phenotypes defined by single apo[a] isoforms 7 to 10 (M_r of 705 to 796 kD) predominated. Neither in cases nor in controls did gender influence the phenotype distribution (P=.12, modified Fisher's Exact Test). The frequency distributions of apo[a] phenotypes between controls and cases were not significantly different (P=.14, modified Fisher's Exact Test). However, because of the large number of apo[a] phenotype classes relative to our sample size, any global test comparing phenotype frequencies between cases and controls will have low power. Therefore, two separate strategies were used to increase the number of observations per cell analyzed. First, we considered only those phenotype classes that had three or more subjects in both cases and controls. There were 17 such phenotype classes. Considering this reduced subset of common phenotypes, apo[a] phenotypes were not significantly different between cases and controls (χ^2=13.5, P=.14). Second, the number of phenotype classes was reduced by combining multiple polymorphs of increasing M_r (Table 3). Adjoining apo[a] polymorphs were combined in such a way that the frequency of small, medium, and large polymorphs was approximately 0.33 each by gene counting. Again, apo[a] phenotype frequencies were not significantly different between cases and controls (χ^2=6.47, P=.263).

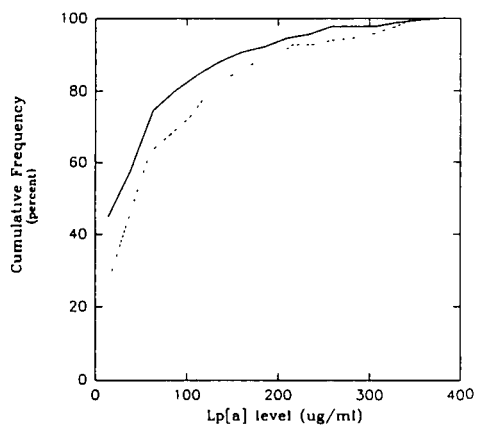

FIG 1. Cumulative frequency distributions of lipoprotein[a] (Lp[a]) concentrations in control subjects (solid line) and cases (dashed line).

The observed phenotype frequencies were significantly different from those expected under Hardy-Weinberg equilibrium (P<.0001 in cases and P<.0001 in controls). This result agrees with our previous studies[13] and is consistent with the notion that the expression levels of some apo[a] alleles are below the limit of detection. Apo[a] polymorph distributions in cases and controls are shown in Fig 2. The polymorph distributions did not differ between cases and controls, irrespective of whether 1 or 2 polymorphs were counted in those phenotypes defined by a single apo[a] species (P=.35 for counting 1 polymorph and P=.35 for counting 2 polymorphs).

Correlation of Apo[a] Phenotype With Lp[a] Protein Levels in Cases and Controls

An inverse relation between apo[a] size and Lp[a] protein level was observed in both cases (r=−0.49, P<.001) and controls (r=−0.34, P<.001). Thus, smaller apo[a] polymorphs were associated with higher plasma Lp[a] protein levels in both groups. Comparison of apo[a] protein levels between cases and controls was possible in 36 phenotype classes. In 24 of them, cases had higher Lp[a] protein levels. Differences in Lp[a] protein levels between cases and controls were also examined in individual apo[a] phenotypes containing

TABLE 2. Frequencies and Lp[a] Levels for Each Apo[a] Phenotype in Cases and Controls

Polymorph		Frequency		Lp[a] Level, μg/mL		
1	2	Controls, n (%)	Cases, n (%)	Controls	Cases	Lp[a] Level, Case−Control
1	...	1 (.36)	1 (.58)	184	195	...
1	2	...	1 (.58)	...	135	...
2	...	3 (1.09)	2 (1.17)	149 (66)	240	...
2	3	1 (.36)	1 (.58)	115	214	...
2	8	2 (.73)	1 (.58)	226	332	...
2	9	...	1 (.58)	...	202	...
2	11	...	1 (.58)	...	7	...
3	...	8 (2.92)	8 (4.68)	155 (76)	178 (102)	23
3	4	1 (.36)	...	438	...	...
3	5	...	1 (.58)	...	111	...
3	6	...	1 (.58)	...	352	...
3	8	...	1 (.58)	...	128	...
3	9	...	1 (.58)	...	253	...
4	...	18 (6.57)	5 (2.92)	125 (114)	116 (101)	−9
4	5	1 (.36)	1 (.58)	49	126	...
4	6	2 (.73)	3 (1.75)	127	154 (42)	...
4	7	4 (1.46)	4 (2.34)	109 (160)	151 (150)	42
4	8	6 (2.19)	7 (4.09)	89 (66)	199 (128)	110
4	9	4 (1.46)	3 (1.75)	86 (108)	111 (94)	25
4	10	6 (2.19)	3 (1.75)	98 (88)	75 (46)	−23
5	...	18 (6.57)	5 (2.92)	58 (61)	106 (55)	48
5	7	6 (2.19)	4 (2.34)	76 (125)	109 (137)	33
5	8	8 (2.92)	2 (1.17)	129 (113)	70	...
5	9	5 (1.82)	2 (1.17)	52 (57)	65	...
5	10	2 (.73)	...	139	...	...
5	11	3 (1.09)	1 (0.58)	17 (6)	38	...
6	...	14 (5.11)	9 (5.26)	57 (62)	39 (37)	−18
6	7	2 (.73)	2 (1.17)	18	79	...
6	8	3 (1.09)	3 (1.75)	61 (36)	57 (24)	−4
6	9	2 (.73)	7 (4.09)	69 (17)	100 (66)	...
6	10	7 (2.55)	2 (1.17)	24 (13)	42 (9)	...
6	11	1 (.36)	3 (1.75)	29	27 (19)	...
7	...	22 (8.03)	11 (6.43)	49 (29)	52 (43)	3
7	8	6 (2.19)	3 (1.75)	57 (58)	104 (31)	47
7	9	5 (1.82)	6 (3.51)	64 (37)	49 (43)	−15
7	10	1 (.36)	4 (1.75)	69	70 (65)	...
7	11	3 (1.09)	1 (0.58)	62 (34)	13	...
8	...	23 (8.39)	21 (12.28)	41 (29)	63 (79)	22
8	9	3 (1.09)	3 (1.75)	74 (52)	99 (41)	25
8	10	9 (3.28)	2 (1.17)	60 (19)	45	...
8	11	1 (.36)	1 (.58)	42	8	...
9	...	30 (10.95)	20 (11.70)	22 (15)	31 (14)	9
9	10	1 (.36)	1 (.58)	54	50	...
9	11	4 (1.46)	1 (.58)	58 (14)	98	...
10	...	31 (11.31)	11 (6.43)	29 (21)	29 (23)	0
11	11	7 (2.55)	...	9 (4)	...	...

Lp[a] indicates lipoprotein[a] and Apo[a], apolipoprotein[a].

TABLE 3. Frequencies of Reduced Apo[a] Phenotypes in Cases and Controls

	Number of Subjects*		
Apo[a] Phenotype	**Control**	**Case**	**Total**
LL†	73 (0.266)	33 (0.193)	106
ML†	32 (0.117)	29 (0.170)	61
MM	70 (0.256)	49 (0.286)	119
SL†	18 (0.066)	12 (0.070)	30
SM	30 (0.110)	23 (0.134)	53
SS	51 (0.186)	25 (0.146)	76
Total	274 (1.00)	171 (1.00)	445

Apo[a] indicates apolipoprotein[a].
*Numbers in parentheses are relative frequencies within each case-control group.
†indicates L, large-size apo[a] polymorph; M, medium-size apo[a] polymorph; and S, small-size apo[a] polymorph.
Pearson χ^2=6.47, P=.263.

three or more subjects per group. In 11 of the 17 comparisons, cases had higher Lp[a] protein levels than controls. The greater Lp[a] protein levels in cases were associated with phenotypes defined by either single or double apo[a] polymorphs. In 5 of the 17 comparisons, Lp[a] protein levels were higher in controls than cases. In all 17 phenotypes compared, the difference between mean Lp[a] protein levels in cases and controls was 32±36 μg/mL (mean±SD). As determined by inspection, no specific apo[a] phenotypes or apo[a] polymorphs were associated with Lp[a] protein level differences between cases and controls.

Apo[a] Phenotypes as Predictors of Case-Control Status

Conditional logistic regression analysis using a stepwise approach based on a priori considerations (see "Methods") revealed that age, body mass index, prevalence of hypertension, pack-years of cigarette smoking, plasma HDL cholesterol, plasma apoB, and Lp[a] protein concentrations were predictors of prevalent case-control status in our study population (Table 4). As expected, the direction of the relation between each of the predictor variables and the probability of having prevalent disease in this cross-sectional study was positive except for HDL cholesterol, which was negative. The addition of apo[a] phenotype data did not increase the ability of the model to predict disease status. It should be noted, however, that without plasma Lp[a] protein concentration in the logistic prediction equation, apo[a] phenotype was marginally significant (P=.07). The inclusion of plasma Lp[a] protein concentration in the model rendered apo[a] phenotype a nonsignificant predictor of case-control status.

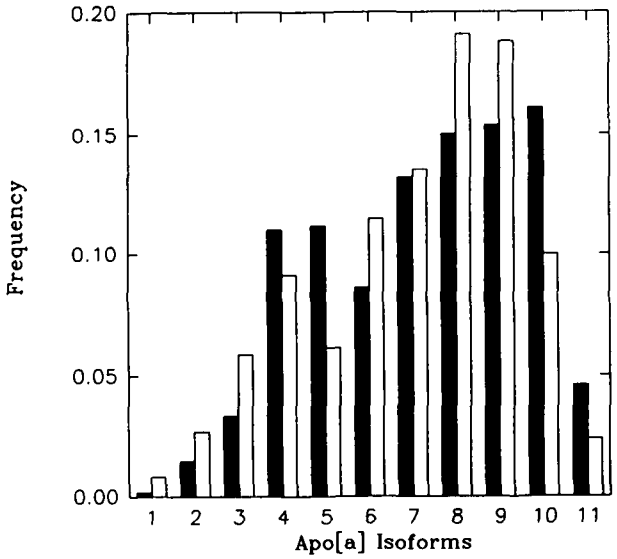

FIG 2. Frequency distribution of apolipoprotein[a] (apo[a]) polymorphs as determined by apo[a] isoform size in cases (filled bars) and control subjects (open bars). In phenotypes with only one apo[a] isoform, the respective isoform was counted only once for computation of frequency distribution.

Among the 152 postmenopausal women, 29 used hormones. Median Lp[a] protein levels did not differ between women using hormones and those who were not (31 versus 49 μg/mL, P=.301, Kruskal-Wallis test). There was also no significant difference in the frequency distribution of apo[a] phenotypes between the two groups (P=.768, Fisher's Exact Test). When the 29 women using hormones were excluded from the analyses, nearly identical results were obtained and none of the conclusions changed. The identical set of predictor variables for prevalent case-control status was identified; apo[a] phenotype did not improve the predictive ability of the logistic model.

Discussion

This study confirms our recent findings in an overlapping but different ARIC Study population that the plasma level of Lp[a] protein is an independent risk factor for intima-media thickening of the carotid arteries in white subjects who are free of symptomatic cardiovascular and cerebrovascular disease.[25] The principal new findings of the present study are (1) that the distribution of apo[a] phenotypes is similar among cases and controls, (2) that Lp[a] protein levels are higher in cases than controls with the same apo[a] phenotypes, and (3) that plasma Lp[a] protein concentrations are inversely related to apo[a] polymorph size in both cases and controls. Our case-control selection procedure excluded a number of factors known to affect Lp[a] plasma levels, such as uncontrolled diabetes mellitus,[48,49] chronic renal disease,[50] and lipid-altering drugs.[20] Furthermore, exclusion of a small number of postmenopausal women using hormones that might have altered plasma Lp[a] levels[21,22,51] had no effect on any of the conclusions that were based on analyses of the entire sample. Thus, the higher Lp[a] protein levels in cases are most likely the result of differences at the apo[a] gene locus, which controls more than 90% of the interindividual variability of apo[a] plasma levels.[23] Since polymorph size was similar between cases and controls, DNA sequences other than those coding for the number of kringle type 2 repeats may explain the higher Lp[a] protein levels in cases.

Since previous studies have shown a negative relation of apo[a] size with plasma Lp[a] levels[12,13,24] and with angiographically documented coronary disease,[26,27] the similar phenotypic distributions between our cases and controls were unexpected. Since our phenotyping sys-

TABLE 4. Conditional Logistic Regression Analysis of Prevalent Asymptomatic Atherosclerosis

Factor	Coeff	SE	Risk Ratio*	χ^2	P
Age, y	0.156	0.047	1.168	10.9	.001
Body mass index, kg/m^2	0.039	0.035	1.040	1.29	.256
Cigarettes, pack-years	0.002	0.001	1.002	35.14	.0001
Hypertension, yes/no	0.743	0.366	2.102	4.11	.043
HDL cholesterol, mg/dL	−1.994	0.435	0.301	7.61	.006
ApoB, mg/dL	0.001	0.001	1.001	6.43	.011
Lp[a], μg/mL	0.005	0.002	1.005	7.23	.007

Coeff indicates coefficient; HDL, high-density lipoprotein; apo, apolipoprotein; and Lp[a], lipoprotein[a].
*Risk ratios reflect increase in risk of being a case for each unit of measure.

tem differs from that of Utermann et al,[12] in that more apo[a] polymorphs are resolved, many of the possible phenotype combinations are missing or infrequently observed. Therefore, we tried to emulate the phenotyping system of Utermann et al by combining adjacent polymorphs into one group, but we were still unable to detect any significant difference in the distributions of phenotypes and polymorphs between cases and controls. Thus, the different methodologies used for phenotyping appear not to account for the lack of phenotypic differences between cases and controls. Further support for this conclusion comes from the Framingham Offspring Study,[52] in which the phenotyping system used here was employed in that study and a higher prevalence of small apo[a] polymorphs was found in patients with CAD than in controls.

How can these differing results be reconciled? Previous studies showing a relation of apo[a] size with atherosclerotic disease have focused on myocardial infarction or angiographically verified CAD.[26,27,52] Our study design excluded subjects with prevalent CAD. Because of an association of carotid intima-media thickness with CAD, our selection procedure eliminated three times as many potential carotid cases as controls from the study. Thus, individuals with more advanced stenotic or occlusive atherosclerotic disease have been excluded, the effect of Lp[a] protein on carotid wall thickness may have been reduced, and an association of apo[a] isoforms with disease status may have been lost. It is also possible that the presence of other forms of hyperlipoproteinemia in other studies may have affected the metabolism of Lp[a] particles. However, the difference in median Lp[a] protein levels between our cases and controls was similar to that found in studies using prevalent CAD for classification of case status.[53-55] Furthermore, our cases exhibited a profile of lipid values similar to that usually found for CAD (Table 1).

While the association between elevated levels of Lp[a] and atherosclerotic disease has been clearly established in numerous studies, the mechanism(s) whereby Lp[a] leads to accelerated atherosclerosis and/or precipitation of clinically overt disease is not fully understood. Apo[a] is found in atherosclerotic lesions[56,57] and vein grafts.[58] Apo[a] collocalizes with apoB and fibrin,[59] but the ratio of apo[a] to apoB in aortic tissue is higher than that in plasma, and Lp[a] may account for the majority of apoB found in fibrous plaques.[60] The high abundance of apo[a] in arterial tissues may be explained by its ability to interact with intimal components, such as proteoglycans, glycosaminoglycans, and fibronectin,[61,62] thereby initiating or promoting atherogenesis. Nevertheless, a causal relation between accumulation of Lp[a] in the arterial wall and atherogenesis has not been established. Another atherogenic mechanism relates to the greater oxidizability of Lp[a] than LDL, probably due to the smaller β-carotene content in Lp[a] that serves as an antioxidant. Copper- or endothelial cell–oxidized Lp[a] is more prone to aggregation and is more avidly taken up by monocytes/macrophages than unoxidized Lp[a]. Hence, lipid accumulation and foam cell formation are more likely to occur with oxidized Lp[a].[63,64]

Additional mechanisms that may explain the association of Lp[a] with prevalent coronary or cerebrovascular disease may relate to its thrombogenic role. Because of its structural homology with plasminogen,[65,66] Lp[a] may compete with it for binding to endothelial and mononuclear cells as well as platelets, thereby reducing the conversion of plasminogen to plasmin and inhibiting fibrinolysis.[67-69] Furthermore, the thrombogenic potential of Lp[a] may be enhanced by modulating the expression of plasminogen activator inhibitor in endothelial cells.[70]

Turnover studies suggest that plasma Lp[a] levels are determined by the rate of Lp[a] synthesis rather than its rate of catabolism[71] and that the principal site of Lp[a] synthesis is the liver.[72,73] Carefully controlled family studies indicate that the apo[a] gene locus controls more than 90% of the variability in Lp[a] levels.[23] Since the abundance of apo[a] mRNA varies in the liver of humans and monkey models,[15,74] transcriptional events are likely to account for part of the variability in apo[a] gene expression. Apo[a] size is an established determinant of Lp[a] plasma levels and, depending on the population studied, may account for 19% to 70% of the variability in Lp[a] levels.[24] Since apo[a] size correlates with apo[a] mRNA size[15,16] but not with mRNA abundance,[16] posttranscriptional events must, at least in part, underlie the inverse relation between apo[a] size and Lp[a] plasma levels. The regulatory role of translational or posttranslational events in the biosynthesis of apo[a] is also evident in studies of baboons exhibiting apo[a] mRNA in the liver with no detectable Lp[a] in plasma.[15] Thus, a number of mechanisms may control the hepatic production of large and small apo[a] and their assembly into Lp[a].

The difference in the prevalence of small apo[a] isoforms between our cases with preclinical atherosclerosis and symptomatic cases studied by others may reflect distinct steps in the progression of atherosclerosis. Lp[a] plasma concentration, irrespective of apo[a] size, may be the main determinant of its atherogenicity in early atherosclerosis (characterized by intima-media wall thickening) in the absence of thrombotic events. In contrast, small apo[a] polymorphs, typically present in elevated concentrations, may be the main determinant of the thrombogenic potential of Lp[a] that may be implicated in the precipitation of clinical events.

Acknowledgments

ARIC is carried out as a collaborative study supported by contracts N01-HC-55015, NO1-HC-55016, NO1-HC-55018, NO1-HC-55019, NO1-HC-55020, NO1-HC-55021, and NO1-HC-55022 from the National Heart, Lung, and Blood Institute. Dr Boerwinkle is an Established Investigator of the American Heart Association and is supported by RCDA HL-02453 from the National Institutes of Health. We are indebted to Jeannette Bensen, MSc, Catherine Paton, Amy Haire, Delilah Posey (Forsyth Co Field Center); Bobbie J. Alliston, RN, Faye A. Blackburn, Catherine W. Britt, Barbara L. Davis (Jackson Field Center); John O'Brien, Linda Goldman, Barbara Kuehl, Anne Murrill (Minneapolis Field Center); Carol Christman, Sonny Harrell, Joel Hill, Joan Nelling (Washington Co Field Center); Valerie Stinson, Pam Pfile, Hoang Phan, Teri Trevino (Central Hemostasis Laboratory); John Crouch, Debbie Rubin-Williams, Cynthia Stahoviak, Yi-Hsin Yang (Coordinating Center); and Charles E. Rhodes, Karima Ghanem, Val Creswell, Doris Epps (Central Lipid Laboratory).

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