Am. J. Hum. Genet. 51:829–840, 1992

Lipoprotein(a) in Women Twins: Heritability and Relationship to Apolipoprotein(a) Phenotypes

Melissa A. Austin,* Christoph Sandholzer,† Joseph V. Selby,‡ Beth Newman,§ Ronald M. Krauss,‖ and Gerd Utermann†

*Department of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle; †Institute for Medical Biology and Human Genetics, University of Innsbruck, Austria; ‡Division of Research, Kaiser Permanente Medical Care Program, Oakland; §Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill; and ‖Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley

Summary

Lp(a) is a unique lipoprotein consisting of an LDL-like particle and a characteristic protein, apo(a). Increased levels of Lp(a) constitute a risk factor for coronary heart disease. Variation in the size of the apo(a) protein is a phenotype controlled by the apo(a) gene on chromosome 6 and is related to Lp(a) plasma levels. Based on 169 MZ and 125 DZ adult female twin pairs, this study's purpose was to estimate the proportion of the variation in Lp(a) levels that is due to genetic influences and to determine the extent to which the apo(a) locus explains this heritability. Lp(a) levels were significantly more similar in MZ twins than in DZ twins: mean co-twin differences were 3.9 ± 5.7 mg/dl and 16.0 ± 19.9 mg/dl ($P < .001$), respectively. Intraclass correlations were .94 in MZ twins and .32 in DZ twins, resulting in a heritability estimate of .94 ($P < .001$). Heritability was then calculated using only co-twins with the same apo(a) phenotype: the heritability estimate decreased to .45 but was still highly significant ($P < .001$). Therefore, on the basis of heritability analysis of women twins, Lp(a) levels are almost entirely genetically controlled. Variation at the apo(a) locus contributes to this heritability, although other genetic factors could be involved.

Introduction

Lp(a) is a unique lipoprotein consisting of two components: a particle similar to LDL, including apolipoprotein (apo) B and the apo(a) protein linked by a disulfide bond to the apo B molecule (Utermann 1989). The apo(a) protein is the distinguishing characteristic of Lp(a) and results in a lipoprotein particle that is larger than LDL but more dense. The gene for the apo(a) protein has been mapped to the tip of the long arm of chromosome 6, closely linked to the gene for plasminogen (Drayna et al. 1988; Frank et al. 1988; Weitkamp et al. 1988; Lindahl et al. 1989). These two genes show a high degree of homology, including the protease domain and several sequences that code for protein "kringle" domains (McLean et al. 1987). Because apo(a) contains an amino acid substitution at the corresponding site at which plasminogen is cleaved by its activators to produce active plasmin, it was initially suggested that apo(a) may not be converted to an active serine proteinase (MacLean et al. 1987). However, more recent reports have shown apo(a) to have such activity (Salonen et al. 1989), which has been characterized using synthetic peptide substrates (Jauhiainen et al. 1991*b*).

Recently, Lp(a) has been the focus of intensive research interest, following its original discovery by Berg in 1963 (Berg 1963). Numerous epidemiologic studies have shown that increased levels of Lp(a) in plasma are associated with coronary heart disease (CHD) (Albers et al. 1977; Kostner et al. 1981; Rhoads et al. 1986; Durrington et al. 1988; Rosengren et al. 1990; Sandkamp et al. 1990). The presence of Lp(a) particles in the arterial wall has also been reported to be associ-

Received March 5, 1992; revision received June 1, 1992.

Address for correspondence and reprints: Melissa A. Austin, Ph.D., Department of Epidemiology, SC-36, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195.

0002-9297/92/5104-0017$02.00

ated with atherosclerotic lesions (Walton et al. 1974; Rath et al. 1989; Pepin et al. 1991). In a recent nested case-control analysis of the Helsinki Heart Study, however, no relationship between baseline Lp(a) level and incidence of CHD was seen in either the placebo group or the treated group (Jauhiainen et al. 1991*a*).

Furthermore, several studies have shown Lp(a) to be associated with risk for cerebrovascular disease (Murai et al. 1986; Zenker et al. 1986; Jurgens and Koltringer 1987; Woo et al. 1990). These results, coupled with the homology between the apo(a) and plasminogen genes, have lead to the hypothesis that Lp(a) is genetically controlled and may be involved in both atherosclerosis and thrombosis (Miles et al. 1989; Scanu and Fless 1990; Rouy et al. 1991).

In contrast to most lipoprotein risk factors, Lp(a) levels show little relationship to age, gender, or environmental, behavioral, or other lipid factors (Sundell et al. 1989; Scanu and Fless 1990; Corsetti et al. 1991). Among lipid-lowering drugs, only neomycin and niacin appear to lower Lp(a) levels appreciably (Gurakar et al. 1985; Carlson et al. 1989).

The familial similarity of Lp(a) has been recognized since its discovery (Berg 1963), and familial risk of CHD is also associated with Lp(a) levels (Berg et al. 1979; Durrington et al. 1988; Sandholzer et al., in press). Until recently, however, the mode of genetic inheritance has not been understood (Albers et al. 1974; Sing et al. 1974; Iselius et al. 1981; Hasstedt et al. 1983; Morton et al. 1985). In 1987, Utermann et al. (1987) reported size isoforms of apo(a) that were inherited in families and were associated with Lp(a) concentrations in plasma.

Mean levels of plasma Lp(a) have been consistently associated with the apo(a) size polymorphism in subsequent studies as well (Utermann et al. 1988*b*; Gaubatz et al. 1990; Lackner et al. 1991). Specifically, there is an inverse relationship between apo(a) size and Lp(a) levels. However, there is considerable overlap in Lp(a) distributions among subjects with different isoforms (Utermann et al. 1988*b*). Boerwinkle et al. (1989) have reported that 41.6% of the variance in Lp(a) levels can be attributed to apo(a) phenotypes in Caucasians, while as much as 70% of the variation is accounted for in Asians (Sandholzer et al. 1991). Lp(a) levels are also known to vary dramatically between ethnic groups (Sandholzer et al. 1991). However, this variation cannot be completely attributed to differences in the apo(a) phenotype distribution between these ethnic groups.

Thus, much remains to be learned about the genetics of Lp(a) and the relationship of apo(a) phenotypes to this lipoprotein. Twin studies provide a unique opportunity to examine this relationship further. Analysis of Lp(a) concentration allows estimation of heritability by comparing MZ and DZ twin pairs. Inclusion of apo(a) phenotype data also allows the estimation of the minimal proportion of Lp(a) heritability attributable to the apo(a) locus itself.

Subjects and Methods

Subjects

The sample for the present analysis was drawn from the 10-year follow-up of adult female twins that was conducted at Kaiser Permanente in Oakland. The original sample consisted of 434 pairs of female twins who were born in 1960 or earlier and who agreed to participate in the initial study during 1978–79 (Austin et al. 1987). At that time, zygosity determination was based on 20 polymorphic loci, with a probability of <.001 of a DZ pair being identical for all loci.

Each twin pair was recontacted during 1989–90 and invited to return for the follow-up study. Response rates were 81% and 82%, respectively for MZ and DZ pairs. At the time of the follow-up study, the average age of participants was 51 years. Each participant completed an extensive health history questionnaire, provided a fasting blood sample, and had a physical examination. The majority of participants (90%, 316 pairs) were Caucasian. Because of the marked differences in Lp(a) distributions among ethnic groups (Sandholzer et al. 1991), only the Caucasian participants were included in the present analysis.

Laboratory Measurements

Fasting blood samples were drawn into EDTA vacutainer tubes for each subject. Samples were immediately centrifuged, and plasma was separated for analysis. An aliquot of each sample was frozen at −70°C, and batches were shipped on dry ice to Innsbruck every few months. Lp(a) concentrations in plasma were determined by a sandwich-ELISA using a polyclonal affinity-purified rabbit anti-Lp(a) antibody for coating and the peroxidase-conjugated monoclonal anti-apo(a) antibody 1A2 as the second antibody (Menzel et al. 1990). Apo(a) phenotyping was performed by SDS-PAGE of plasma under reducing conditions, followed by immunoblotting according to a method described elsewhere (Sandholzer et al. 1991). Immu-

noblotting was performed using the monoclonal anti-apo(a) antibody 1A2, which does not cross-react with plasminogen (Menzel et al. 1990). A goat anti-mouse peroxidase conjugate (Dako) at a 1:500 dilution was used as the second antibody. These analyses were performed blinded to both the identity of co-twins and the zygosity of pairs.

Of these samples, there were 20 pairs in which one or both co-twins were missing either Lp(a) level or apo(a) isoform. These pairs and the individual co-twins in the pairs were excluded from all analyses. Initially, there were 16 pairs of MZ co-twins in which the bands were very faint and in which apo(a) isoforms did not appear identical. In each case, one co-twin had one or two bands that were not detected in the other co-twin. In 14 of the 16 pairs, sufficient plasma was available to reanalyze samples, resulting in identical bands for all co-twins in the same pair. A total of 169 MZ pairs and 125 DZ pairs were included in the analyses after these considerations, for a total of 588 individual women.

Statistical Methods

Skewness was calculated as the third central moment (Snedecor and Cochran 1967), so that values >0 indicate a tail to the right in the frequency distribution, and values <0 indicate a tail to the left. Because of the considerable skewing in the frequency distribution of Lp(a) levels (table 1), all statistical tests were performed using natural log transformations, and antilog mean values and median values are reported.

Comparisons of mean values for MZ and DZ twins were made using Student's *t*-test. When more than two groups of subjects were compared, one-way analysis of variance (ANOVA) was performed (Winer et al. 1971) using orthogonal contrasts for multiple comparisons (Berenson 1983, pp. 102–104). These computations were performed using the Statistical Analysis System (SAS Institute 1985). Intraclass correlations were used to compare Lp(a) values among co-twins, by zygosity (Cavalli-Sforza and Bodmer 1971). As described elsewhere (Boerwinkle and Sing 1968), the contribution of apo(a) phenotypes to the variance of Lp(a) levels was estimated as $\Sigma f_j(\overline{X}_j - \overline{X})^2$, where $\overline{X}_j$ is the average Lp(a) value for the jth apo(a) phenotype, $\overline{X}$ is the grand mean of Lp(a), and f_j is the frequency of the jth apo(a) phenotype. This calculation was performed for all twins and was repeated for one co-twin from each pair.

Heritability analyses were performed using ANOVA-based and maximum likelihood–based estimation. Under the assumptions of each of these methods, heritability estimates can be interpreted as the proportion of variance in Lp(a) values that is attributable to genetic influences, and they range from 0 to 1. The ANOVA calculations were performed with the modifications proposed by Christian et al. (1974), and the classical heritability estimate was calculated as twice the difference of the intraclass correlation coefficients: $h^2 = 2(r_{MZ} - r_{DZ})$. However, as a result of the large difference in the intraclass correlation between MZ and DZ twins, all estimates based on the ANOVA model were >1.0, violating the assumptions of the twin model (Christian et al. 1987).

To overcome this difficulty, maximum-likelihood analysis of the twin data was also performed using the computer program TWINAN90 (Williams et al., in press). Estimation of parameters by this method is performed on the basis of sample covariance matrices for each zygosity, as described by Heath et al. (1989), and is also available via the computer program LISREL (Joreskog and Sorbom 1986). The following models are fit to the data: the ADE model, in which additive genetic variance (σ_a^2), dominant genetic variance (σ_d^2), and residual environmental variance (σ_e^2), are estimated; the ACE model, in which σ_d^2 in the ADE model is replaced by variation due to common twin environment (σ_c^2); and the AE model involving only σ_a^2 and σ_e^2. The most appropriate model is selected on the basis of intraclass correlations and likelihood-ratio

Table 1

Distribution of Lp(a) Plasma Levels in Caucasian Women Twins

	No. of Individuals	Mean Lp(a) Plasma Level ± SD (mg/dl)	Percentile			Skewness	
			10	50	90	Lp(a)	lnLp(a)
MZ twins.....	338	17.3 ± 20.8	1.5	8.5	50.3	1.80	−.09
DZ twins.....	250	19.2 ± 23.3	1.5	10.0	59.8	1.85	−.16
All twins	588	18.1 ± 21.9	1.5	8.9	55.1	1.84	−.12

statistics (Williams et al., in press). Specifically, the ACE model is preferred over the ADE model if $r_{DZ} > r_{MZ}/2$, reflecting effects due to shared environment. Otherwise—i.e., if $r_{DZ} \leq r_{MZ}/2$—the ADE model is preferred, to reflect a dominance effect. The improvement in fit of either the ADE model or the ACE model over the AE model is determined by a likelihood-ratio statistic. As suggested by Christian et al. (1974), an *F*-test of equality of total variances between MZ and DZ twins is used as a test of the appropriateness of the twin model, using a conservative significance level of .2. For the appropriate model, heritability estimates are then calculated as the proportion of genetic variance over the total variance.

Results

The frequency distributions of plasma Lp(a) levels are shown in figure 1, and descriptive statistics are presented in table 1. For all 588 individual subjects, the mean and median values for plasma Lp(a) were 18.1 and 8.9 mg/dl, respectively. These values were very similar when stratified by zygosity. As has been reported in a number of studies of Caucasians (Albers et al. 1977; Utermann et al. 1987; Sandholzer et al. 1991), the frequency distributions were highly skewed (overall skewness 1.84), resulting in considerable difference between mean and median values. Natural logarithm transformation, however, largely removed the skewing (overall skewness −0.12), and thus statistical tests were performed using this transformation. SDs, percentiles, and skewness values of Lp(a) plasma levels were also similar for MZ and DZ twins (table 1 and fig. 1).

The frequency distribution of apo(a) phenotypes for all study subjects is given in table 2A. In this sample, the single-band S_4 apo(a) phenotype was most common (32.5%), with S_2 and S_3 phenotypes being the next most common and having equal frequency (16.5%). The B and S_1 phenotypes were least frequent (0.5% and 1.2%, respectively), and, in general, double-band phenotypes were less frequent than single-band phenotypes. If the locus is assumed to be in Hardy-Weinberg equilibrium, then the frequencies

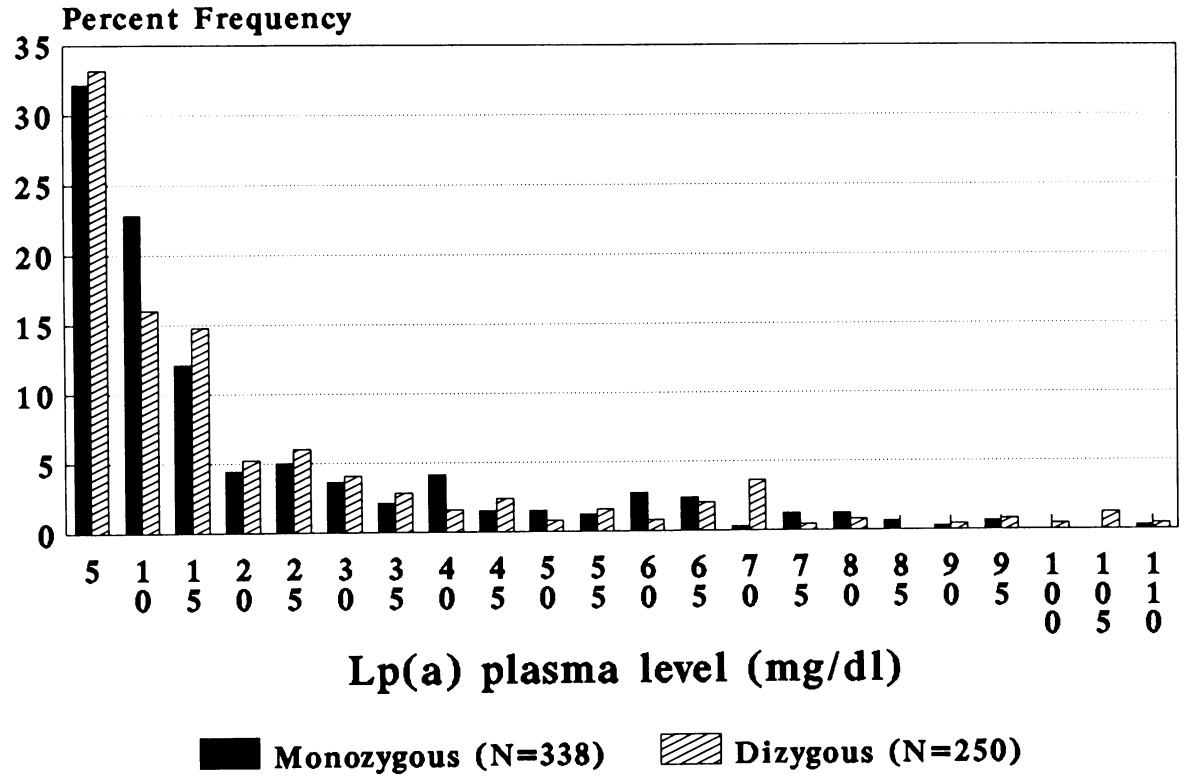


Figure 1 Frequency distribution of Lp(a) plasma levels (in mg/dl) in MZ twins and DZ twins. The distributions for both types of twins are skewed but similar. Median values are 8.9 and 8.5, respectively.

of the double-band phenotypes in this sample are lower than would be expected: .046 versus .065, .049 versus .113, and .095 versus .113 for observed versus expected frequencies, for S_2S_3, S_2S_4, and S_3S_4, respectively. These results are similar to previous reports (Utermann et al. 1987; Sandholzer et al. 1991). More than 14% of subjects had the null phenotype (denoted "O" in table 2). Note that these subjects had a very low mean plasma Lp(a) level (2.0 mg/dl), probably resulting in an inability to detect bands. In addition, because study subjects are twins, observations in table 2A are not independent. However, frequency distributions were similar for MZ twins, DZ twins, and when one co-twin was randomly selected from each pair (data not shown).

Mean and median values of plasma Lp(a), by apo(a) phenotype, are also reported in table 2A, and *P* values for comparisons are in table 2B. As seen in other studies (Utermann et al. 1988*b*; Boerwinkle et al. 1989; Gaubatz et al. 1990; Sandholzer et al. 1991), Lp(a) levels were inversely related to apo(a) size among the single-band phenotypes. These differences were significant for S_2 versus S_3 phenotypes and for S_2 versus S_4 phenotypes. However, because the SDs of Lp(a) levels are large, the distributions for these apo(a) phenotypes actually overlap considerably.

Although the Lp(a) mean value for double-band S_2S_3 phenotype was intermediate between the mean values for corresponding single-band S_2 and S_3 phenotypes, a similar relationship was not seen for S_2S_4 and S_3S_4 phenotypes. These findings are probably due to the fact that apo(a) size isoforms are phenotypes, not genotypes. In particular, the presence of the null phenotype when this method is used does not allow, e.g., an S_2S_2 genotype to be distinguished from an S_2O genotype in the absence of data on parents.

On the basis of data on all individual twins, the contribution of the apo(a) locus to Lp(a) levels as reflected by apo(a) phenotypes was estimated to be 46% (Boerwinkle and Sing 1986). Because co-twins in the

Table 2

Apo(a) Phenotypes and Lp(a) Plasma Levels in Individual Women

A. Apo(a) Phenotype Frequency Distribution and Mean Lp(a) Plasma Levels, by Apo(a) Phenotype

apo(a) Phenotype	No. (%) of Individuals	Lp(a) Level	
		Mean ± SD	Median
B	3 (.5)	66.7 ± 23.1	57.0
S_1	7 (1.2)	28.0 ± 17.7	25.3
S_2	97 (16.5)	33.3 ± 24.0	30.3
S_3	97 (16.5)	16.3 ± 19.4	9.0
S_4	191 (32.5)	10.8 ± 10.2	7.8
O	79 (13.4)	2.0 ± 3.6	1.5
BS_2	0 (0)	. . .	. . .
BS_3	0 (0)	. . .	. . .
BS_4	2 (.3)	9.1 ± 4.4	9.1
S_1S_2	0 (0)	. . .	. . .
S_1S_3	0 (0)	. . .	. . .
S_1S_4	0 (0)	. . .	. . .
S_2S_3	27 (4.6)	28.3 ± 34.6	11.0
S_2S_4	29 (4.9)	50.3 ± 31.8	53.0
S_3S_4	56 (9.5)	16.9 ± 15.3	12.8
All phenotypes	588 (100.0)	18.1 ± 21.9	8.9

B. *P* Values Comparing Mean Values of Lp(a) Plasma Level, by Apo(a) Phenotype[a]

	S_2	S_3	S_4	S_2S_3	S_2S_4	S_3S_4
O	.0001	.0001	.0001	. . .	. . .	. . .
S_2		.0001	.0001	.0043	.0152	. . .
S_3			.3221	.0696	. . .	.2438
S_4				. . .	.0001	.0360
S_2S_3					.0001	.3942
S_2S_4						.0001

[a] Only phenotypes with sample size >10 are included; data are based on analysis of variance using lnLp(a).

same pair are not independent, this calculation was repeated using only one co-twin per pair. A similar estimate, i.e., 49%, was obtained.

The relationship of Lp(a) plasma levels and apo(a) phenotypes was further explored by taking advantage of the twin study design. Co-twin difference in Lp(a) level was calculated for each twin pair, and absolute values were taken. As seen in table 3, mean and median values of these differences were then determined for different types of twin pairs. For the 169 MZ pairs, the mean difference was small (3.9 mg/dl), and the median difference was even smaller (1.8 mg/dl). Since apo(a) phenotype bands are a Mendelian trait (Utermann et al. 1987, 1988*a*), and since MZ co-twins are by definition genetically identical, the co-twins in each MZ pair have the same apo(a) phenotype. For all 125 DZ pairs, the mean difference (16.0 mg/dl) was significantly higher than that for the MZ pairs ($P < .001$).

DZ pairs were then stratified into those pairs in which both co-twins had different apo(a) phenotype ($N = 60$) and those in which co-twins had the same phenotypes ($N = 65$) (table 3). Mean Lp(a) difference in the latter group (7.7 mg/dl) was significantly lower than the mean difference in the former group (25.0 md/dl) ($P < .001$). In addition, the mean difference for the DZ pairs with the same phenotype in co-twins was significantly larger than the difference for MZ twins ($P < .01$). Thus, although co-twins in these two groups had the same apo(a) phenotype, the DZ pairs had larger Lp(a) differences, on average, than did the MZ twins.

To refine this analysis, DZ pairs with the same apo(a) phenotype were further stratified into those pairs with the same double-band phenotype and those with the same single-band phenotype (table 3). The mean Lp(a) co-twin differences in these groups were 9.2 and 7.4 mg/dl, respectively. Thus, although the DZ pairs with the same double-band apo(a) phenotype may be more likely to have the same genotype at the apo(a) locus than are the pairs with the same single-band apo(a) phenotype, the mean co-twin differences for these two groups are not statistically significant.

Intraclass correlations and heritability estimates for plasma Lp(a) values are presented in table 4, for the natural log transformation of Lp(a). The intraclass correlations for MZ co-twins were extremely high ($r_{MZ} = .94$; table 4). The top left panel of figure 2 shows that Lp(a) for co-twins in the same pair were very similar across the entire range of Lp(a) values, although more variability is seen at higher levels. For all DZ pairs, the intraclass correlation was much lower ($r_{DZ} = .32$; table 4). As expected, considerably more variability around the line of identity for co-twins is seen (in the top right panel of fig. 2).

As noted above, because ANOVA-based heritability estimates exceeded 1.0, Lp(a) heritability was estimated using a maximum likelihood–based method (Williams et al., in press). As shown in table 4, r_{DZ} (.317) was *not* greater than $r_{MZ}/2$ (.468). Thus the ADE model was the most appropriate. On the basis of the likelihood-ratio statistic, the ADE model also provided a significantly better fit than did the AE model ($P = .024$), and the test of the twin model was not rejected ($P = .638$). Thus, heritability was calculated on the basis of the ADE model and resulted in an estimate of .935 ($P < .001$). These results indicate that Lp(a) plasma levels in these women twins are virtually completely explained by genetic influences.

Lp(a) heritability estimates were also calculated on

Table 3

Co-Twin Differences in Lp(a) Plasma Levels

	No. of Pairs	Lp(a) Co-Twin Difference (mg/dl)	
		Mean ± SD	Median
MZ pairs	169	3.9 ± 5.7	1.8
DZ pairs:	125	16.0 ± 19.9**	7.0
With different apo(a) phenotype in co-twins	60	25.0 ± 23.5†	15.6
With same apo(a) phenotype in co-twins:	65	7.7 ± 10.7*	3.5
Double-band apo(a) phenotype in co-twins	11	9.2 ± 10.9	4.3
Single-band apo(a) phenotype in co-twins	54	7.4 ± 10.7	3.1

* $P < .01$, compared with MZ pairs.

** $P < .001$, compared with MZ pairs.

† $P < .001$, compared with DZ pairs with the same apo(a) phenotype in co-twins.

Table 4

Intraclass Correlations and Heritability Estimates of Lp(a) Plasma Levels

	No. of Pairs		Intraclass Correlations		Maximum-Likelihood Heritability Analysis	
	MZ	DZ	MZ	DZ	Preferred Model	Heritability Estimate
All twin pairs	169	125	.935 ($P < .001$)	.317 ($P < .001$)	ADE[a]	.935 ($P < .001$)
Twin pairs with same apo(a) phenotype in co-twins	169	65	.935 ($P < .001$)	.708 ($P < .001$)	ACE[b]	.449 ($P < .001$)

Note.—Natural log transformation was used in all calculations of Lp(a) plasma levels.

[a] Includes additive genetic variance, dominant genetic variance, and residual environmental variance components.

[b] Includes additive genetic variance, variance due to common environment, and residual environmental variance components.

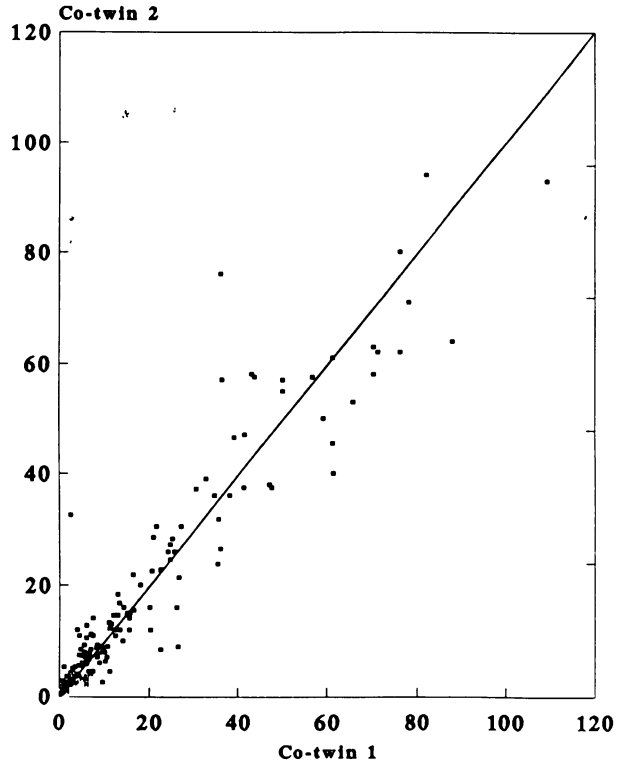
the basis of menopausal status of twins. For twin pairs in which both co-twins were premenopausal (73 MZ pairs and 38 DZ pairs) and for pairs in which both co-twins were postmenopausal (either naturally or surgically) (73 MZ pairs and 61 DZ pairs), near complete heritability was again found (data not shown). Thus among these women twins menopausal status does not appear to influence heritability. Previous analyses have demonstrated that Lp(a) levels in this sample do not vary significantly by menopausal status (Selby et al. 1992).

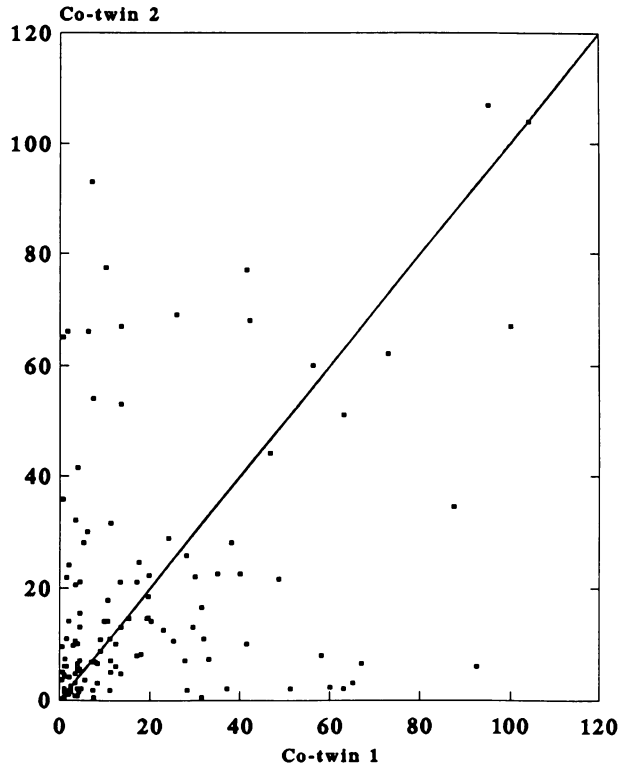
We then investigated the heritability of Lp(a), taking into account apo(a) size phenotypes by using only DZ twins with the same apo(a) phenotype. By matching in this way, the analysis essentially "controls" for apo(a) phenotype as a measure of variation at the apo(a) locus. That is, because apo(a) phenotypes are the same in both the MZ co-twins and DZ co-twins used in this analysis, significant heritability estimates suggest genetic influences not detected by the apo(a) phenotypes. When the 65 DZ pairs with the same apo(a) phenotype were used, the intraclass correlation for DZ twins increased to .71, as seen in table 4 and the bottom panel of figure 2. In this case, because r_{DZ} (.71) did exceed $r_{MZ}/2$ (.468), the ACE model was the most appropriate. Again, the ACE model provided significant improvement over the AE model ($P = .002$), and the test of the twin model was not rejected ($P = .886$). The resulting heritability estimate decreased to .449 but was still highly significant ($P < .001$).

Discussion

On the basis of heritability analysis of this sample of adult women twins, plasma Lp(a) levels appear to be virtually completely genetically controlled. The correlation between MZ co-twins was .94, and the heritability estimate was .935 (table 4). In a previous report (Hewitt et al. 1982), based on 66 MZ and 33 DZ adult twin pairs, the heritability of sinking pre-beta lipoprotein was estimated to be .98. Thus, as with the present study, almost complete heritability was found. Similarly, a recent report based on the NHLBI twin study demonstrated, on the basis of gradient-gel analysis, significantly more concordance for high Lp(a) in MZ twins than in DZ twins (Lamon-Fava et al. 1991). Other reports of Lp(a) in MZ twins have suggested that the variability of Lp(a) levels, as well as the absolute plasma level, are genetically controlled (Berg 1984) but that there are a few exceptional pairs in which the co-twin difference in Lp(a) level is large (Berg 1990).

Although heritability analysis based on twins provides no information about types or number of genes contributing to the heritability, apo(a) phenotype data were incorporated into the present analysis by repeating the heritability analysis by using only DZ twins in which the co-twins in a pair had the same apo(a) phenotype. The heritability estimate decreased to approximately .45. These results imply that the apo(a) locus contributes to the heritability of Lp(a) levels, although this contribution cannot be quantified without assuming Hardy-Weinberg equilibrium and strict polygenic additive inheritance. As seen in table 4, the decrease in the heritability was due primarily to an increase in the intraclass correlation, from .32 in all DZ twins to .71 in the DZ pairs in which co-twins had the same apo(a) phenotype. On the basis of the 11 DZ pairs with the same double band apo(a) phenotype, the intraclass correlation increased even further, to .79. Thus, Lp(a) levels were more similar in DZ





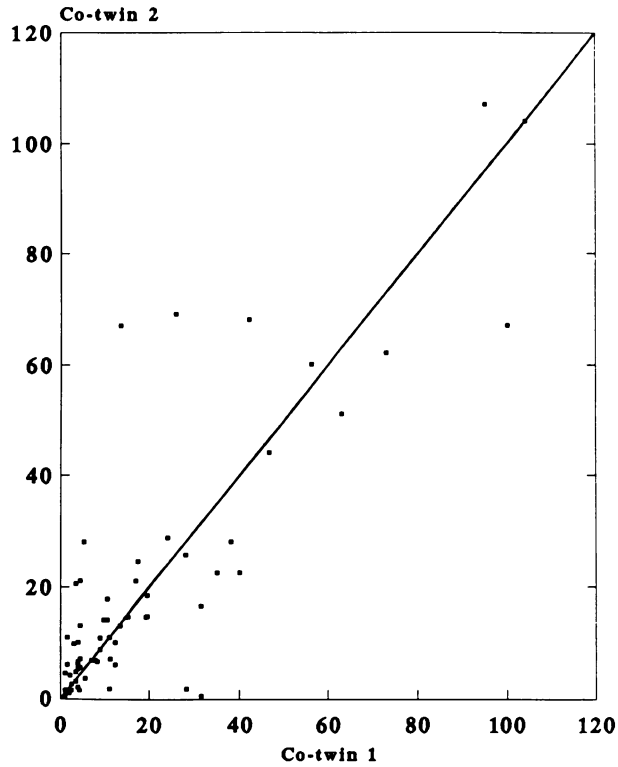


Figure 2 *Top left,* Lp(a) plasma levels (in mg/dl) in MZ co-twins. The Lp(a) level for one co-twin in each pair is shown on the *X*-axis, and the Lp(a) level for the other co-twin is shown on the *Y*-axis. If the levels in two co-twins were identical, they would fall on the line of identity shown at a 45° angle. The intraclass correlation in these 169 pairs is .935. *Top right,* Lp(a) plasma levels in the 125 DZ co-twins. The intraclass correlation is .32. *Bottom,* Lp(a) plasma levels in the 65 pairs of DZ twins in which both co-twins had the same apo(a) phenotype. The intraclass correlation is .71.

pairs with the same apo(a) phenotype in co-twins than among all DZ pairs. Note that, although apo(a) phenotype data are used as markers for variation at the apo(a) locus, the size of the apo(a) protein itself does not influence the results.

However, the heritability estimate of .45 based on DZ pairs with the same apo(a) phenotype (table 4) was still statistically significant. This suggests that other genetic influences may also contribute to Lp(a) levels. A feasible explanation is additional variation *within* the apo(a) locus, variation that is not detected by size variation in the apo(a) protein as reported here. Several studies have recently reported methods that detect

many more alleles at the apo(a) locus (Gaubatz et al. 1990; Kamboh et al. 1991; Lackner et al. 1991). Thus the apo(a) locus is more polymorphic than can be detected by apo(a) size isoforms, and isoforms classified as identical in DZ twin pairs may not represent the same apo(a) allele.

At the apo(a) locus there may also be genetic variation other than the number of kringle IV repeats. For example, it is known that the sequences of the kringle IV domains are not all identical (McLean et al. 1987). It is conceivable that differences in this sequence could contribute to determining Lp(a) plasma levels. Another possibility is variation in the promoter sequence of the apo(a) locus, which could explain the differences in associations of Lp(a) levels and apo(a) alleles seen in different families. Each of these types of intralocus variation, if present, would result in underestimates of the effect of the apo(a) locus on Lp(a) heritability based on isoform data in twins.

Similarly, among DZ twins with the same apo(a) phenotype, the number of parental alleles identical by descent (IBD) cannot be determined from twin data alone. Even if more specific apo(a) genotypes were available on these twins, data on parents would be necessary to determine whether DZ twins are IBD at the apo(a) locus. Also, unlike the few MZ twins who were initially misclassified by apo(a) phenotype because of faint bands on the gels, misclassified DZ twins could not be corrected, since they may not have identical phenotypes. Both of these forms of bias have the effect of underestimating the influence of the apo(a) locus on Lp(a) heritability.

Another possible explanation of these results is that variation at loci other than the apo(a) gene on chromosome 6 are involved in regulating Lp(a) levels. The lower intraclass correlation of Lp(a) in DZ twins with the same apo(a) phenotype, compared with MZ twins (.71 and .94, respectively) could be due to genotypic variation at other loci among the DZ twins—variation that, by definition, is not present in MZ twins. It is interesting that a recent study using a cynomologous monkey model showed that both apo(a) size and hepatic apo(a) mRNA levels independently influence plasma Lp(a) concentration (Azrolan et al. 1991).

It is also important to consider potential bias in the heritability analyses. For example, it has been shown that MZ co-twins are more alike with respect to numerous environmental factors that are known to affect lipid and lipoprotein levels (Austin et al. 1987). Under these circumstances, heritability estimates are biased upward regardless of which statistical estimate is used (Feinlieb et al. 1977). Since very few environmental factors influence Lp(a) levels (Sundell et al. 1989; Scanu and Fless 1990; Corsetti et al. 1991), it seems unlikely that greater environmental covariance among MZ co-twins constitutes an important bias in this analysis. It is possible, however, that environmental factors early in life influence Lp(a) levels, but these influences are difficult to detect in adulthood. In addition, sampling variation can have considerable effects on heritability estimates. Finally, as noted in Subjects and Methods, although the initially nonmatching apo(a) phenotypes in a few MZ pairs were corrected, a similar correction could not be made for DZ twins.

The results for Lp(a) are in contrast to those of twin studies of most other lipoprotein risk factors. In the NHLBI male-twin study, heritability estimates were moderate for lipid levels, ranging from .14 for HDL-C to .68 for plasma triglyceride (Feinleib et al. 1977). When baseline data from the present study were used, heritability values for lipid levels were somewhat higher, before and after adjusting for covariates (Austin et al. 1987), a finding similar to results based on a study of Utah twins (Hunt et al. 1989). Even so, no lipoprotein risk factor other than Lp(a) has shown such high correlations among MZ twins, or such consistently high heritability estimates.

In conclusion, these results based on data on adult women twins demonstrate that plasma levels of Lp(a) are virtually completely heritable and that the apo(a) locus on chromosome 6 makes a major contribution to the heritability of Lp(a) levels. However, the heritability of Lp(a) is not fully accounted for by genetic size variation in the apo(a) protein. Further understanding of Lp(a) genetics will provide important insights into the role of Lp(a) in atherosclerosis risk within families.

Acknowledgments

This work was supported by NIH R-01 grant HL-41830 and NIH First Independent Research Support and Transition Award HL-387b0. The authors gratefully acknowledge excellent technical and data management assistance of Laura Holl, Simon Poon, Tom Fraser, Jerry deLorenze, Danya Zhang, and Elizabeth J. Mayer; helpful comments from Mary-Claire King and Christopher Williams; and Susan Sperline for the preparation of the manuscript.

References

Albers JJ, Adolphson JL, Hazzard WR (1977) Radioimmunoassay of human plasma Lp(a) lipoprotein. J Lipid Res 18:331–338

Albers JJ, Wahl P, Hazzard WR (1974) Quantitative genetic studies of the human plasma Lp(a) lipoprotein. Biochem Genet 11:475–487

Austin MA, King M-C, Bawol RD, Hulley SB, Friedman GD (1987) Risk factor for coronary heart disease in adult female twins: genetic heritability and shared environmental influences. Am J Epidemiol 125:308–318

Azrolan N, Gavish D, Breslow JL (1991) Plasma lipoprotein (a) concentration is controlled by apolipoprotein(a) (apo(a)) protein size and abundance of hepatic apo(a) mRNA in a cynomologus monkey. J Biol Chem 266: 13866–13872

Berenson ML (1983) Intermediate statistical methods and applications. Prentice-Hall, Englewood Cliffs, NJ

Berg K (1963) A new serum type system in man—the Lp system. Acta Pathol 59:369–382

——— (1984) Twin studies of coronary heart disease and its risk factors. Acta Genet Med Gemellal 33:349–361

——— (1990) Lp(a) lipoprotein: an overview. In: Scanu AM (ed) Lipoprotein(a). Academic Press, San Diego, pp 13–15

Berg K, Dahlen G, Borresen A-L (1979) Lp(a) phenotypes, other lipoprotein parameters, and a family history of coronary heart disease in middle-aged males. Clin Genet 16: 347–352

Boerwinkle E, Menzel HJ, Kraft HG, Utermann G (1989) Genetics of the quantitative Lp(a) lipoprotein trait. III. Contribution of Lp(a) glycoprotein phenotypes to normal lipid variation. Hum Genet 82:73–78

Boerwinkle E, Sing CF (1986) Bias of the contribution of single-locus effects in the variance of a quantitative trait. Am J Hum Genet 39:137–144

Carlson LA, Hamsten A, Asplund A (1989) Pronounced lowering of serum levels of lipoprotein(a) in hyperlipidemic subjects treated with nicotinic acid. J Intern Med 226:271–276

Cavalli-Sforza LL, Bodmer WF (1971) The genetics of human populations. WH Freeman, San Francisco

Christian JC, Bochani NO, Castelli WP, Fabsitz R, Norton JA Jr, Reed T, Rosenman R, et al (1987) Plasma cholesterol variation in the National Heart, Lung and Blood Institute Twin Study. Genet Epidemiol 4:443–446

Christian JC, Kang KW, Norton JA (1974) Choice of an estimate of genetic variance from twin data. Am J Hum Genet 26:154–161

Corsetti JP, Sterry JA, Sparks JD, Sparks CE, Weintraub M (1991) Effects of weight loss on serum lipoprotein(a) concentrations in an obese population. Clin Chem 37: 1191–1195

Drayna DT, Hegele RA, Hass PE, Emi M, Wu LL, Eaton DL, Lawn RM, et al (1988) Genetic linkage between lipoprotein(a) phenotype and DNA polymorphism in the plasminogen gene. Genomics 3:230–236

Durrington PN, Ishola M, Hunt L, Arrol S, Bhatnagar D (1988) Apolipoproteins(a), A1, and B and parental history in men with early onset ischaemic heart disease. Lancet 1:1070–1073

Feinleib M, Garrison RJ, Fabsitz R, Christian JC, Hrubec Z, Borhani NO, Kannel WB, et al (1977) The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. Am J Epidemiol 106:234–295

Frank SL, Klisak I, Sparkes RS, Mohandas T, Tomlinson JE, McLean JW, Lawn RM, et al (1988) The apolipoprotein(a) gene resides on human chromosome 6q26-27, in close proximity to the homologous gene for plasminogen. Hum Genet 79:352–356

Gaubatz JW, Ghanem KI, Guevara J Jr, Nava ML, Patsch W, Morrisett JD (1990) Polymorphic forms of human apolipoprotein[a]: inheritance and relationship of their molecular weights to plasma levels of lipoprotein [a]. J Lipid Res 31:603–613

Gurakar A, Hoey J, Kostner G, Papadopoulos NM, Brewer HB Jr (1985) Levels of lipoprotein(a) decline with neomycin and niacin treatment. Atherosclerosis 57:293–301

Hasstedt SJ, Wilson DE, Edwards CQ, Cannon WN, Carmelli D, Williams RR (1983) The genetics of quantitative plasma Lp(a): analysis of a large pedigree. Am J Med Genet 16:179–188

Heath AC, Neale MC, Hewitt JK, Eaves LJ, Fulker DW (1989) Testing structural equation models for twin data using LISREL. Behav Genet 19:9–35

Hewitt D, Milner J, Owen ARG, Breckenridge WC, Maguire GF, Jones GJL, Little JA (1982) The inheritance of sinking pre-beta lipoprotein and its relation to Lp(a) antigen. Clin Genet 21:301–308

Hunt SC, Hasstedt S, Kuida H, Stults BM, Hopkins PN, Williams RR (1989) Genetic heritability and common environmental components of resting and stressed blood pressures, lipids and body mass index in Utah pedigrees and twins. Am J Epidemiol 129:625–638

Iselius L, Dahlen G, de Faire U, Lundman T (1981) Complex segregation analysis of the Lp(a)/pre-B_1-lipoprotein trait. Clin Genet 20:147–151

Jauhiainen M, Koskinen P, Ehnholm C, Frick MH, Manttari M, Manninen V, Huttunen JK (1991*a*) Lipoprotein (a) and coronary heart disease risk: a nested case-control study of the Helsinki Heart Study participants. Atherosclerosis 89:59–67

Jauhiainen M, Metso J, Koskinen P, Ehnholm C (1991*b*) Characterization of the enzyme activity of human plasma lipoprotein (a) using synthetic peptide substrates. Biochem J 274:491–496

Joreskog KG, Sorbom D (1986) LISREL VI. Scientific Software, Mooresville, IN

Jurgens G, Koltringer P (1987) Lipoprotein(a) in ischemic cerebrovascular disease: a new approach to the assessment of risk for stroke. Neurology 37:513–515

Kamboh MI, Ferrell RE, Kottke BA (1991) Expressed hypervariable polymorphism of apolipoprotein (a). Am J Hum Genet 49:1063–1074

Kostner GM, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Quinci GB (1981) Lipoprotein Lp(a) and the risk for myocardial infarction. Atherosclerosis 38:50–61

Lackner C, Boerwinkle E, Leffert CC, Rahmig T, Hobbs HH (1991) Molecular basis of apolipoprotein (a) isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. J Clin Invest 87:2153–2161

Lamon-Fava S, Jimenez D, Christian JC, Fabsitz RR, Reed T, Carmelli D, Castelli WP, et al (1991) The NHLBI Twin Study: heritability of apolipoprotein A-I., B, and concordance for lipoprotein (a). Atherosclerosis 91:97–106

Lindahl G, Gersdorf E, Menzel HJ, Duba C, Cleve H, Humphries S, Utermann G (1989) The gene for the Lp(a)-specific glycoprotein is closely linked to the gene for plasminogen on chromosome 6. Hum Genet 81:149–152

McLean JW, Tomlinson JE, Kuang W-J, Eaton DL, Chen EY, Fless GM, Scanu AM, et al (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 330:132–137

Menzel H-J, Dieplinger H, Lackner C, Hoppichler F, Lloyd JK, Muller DR, Labeur C, et al (1990) Abetalipoproteinemia with an ApoB-100-lipoprotein(a) glycoprotein complex in plasma: indication for an assembly defect. J Biol Chem 265:981–986

Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF (1989) A potential basis for the thrombotic risks associated with lipoprotein (a). Nature 339:301–302

Morton NE, Berg K, Dahlen G, Ferrell RE, Rhoads GG (1985) Genetics of the Lp lipoprotein in Japanese-Americans. Genet Epidemiol 2:113–121

Murai A, Miyahara T, Fujimoto N, Matsuda M, Kameyama M (1986) Lp(a) lipoprotein as a risk factor for coronary heart disease and cerebral infarction. Atherosclerosis 59: 199–204

Pepin JM, O'Neil JA, Hoff HF (1991) Quantification of apo(a) and apo B in human atherosclerotic lesions. J Lipid Res 32:317–327

Rath M, Niendorf A, Reblin T, Dietel M, Krebber H-J, Beiseigel U (1989) Detection and quantification of lipoprotein(a) in the arterial wall of 107 coronary bypass patients. Arterosclerosis 9:579–592

Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg AL (1986) Lp(a) lipoprotein as a risk factor for myocardial infarction. JAMA 256:2540–2544

Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H (1990) Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. Br Med J 301:1248–1251

Rouy D, Grailhe D, Nigon F, Chapman J, Angeles-Cano E (1991) Lipoprotein(a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator: in vitro studies in a plasma milieu. Arterosclerosis Thromb 11: 629–638

Salonen E-M, Jauhiainen M, Zardi L, Vaheri A, Ehnholm C (1989) Lipoprotein(a) binds to fibrinonectin and has serine proteinase activity capable of cleaving it. EMBO J 8:4035–4040

Sandholzer C, Boerwinkle E, Saha N, Tong MC, Utermann G. Apolipoprotein(a) phenotypes, Lp(a) concentrations and lipid levels in relation to coronary heart disease in a Chinese population: evidence for the role of the apo(a) gene in coronary heart disease. J Clin Invest (in press)

Sandholzer C, Hallman DM, Saha N, Sigurdsson G, Lackner C, Csaszar A, Boerwinkle E, et al (1991) Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum Genet 86: 607–614

Sandkamp M, Funke H, Schulte H, Kohler E, Assmann G (1990) Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. Clin Chem 36:20–23

SAS Institute (1985) SAS user's guide: statistics, version 5 ed. SAS Institute, Cary, NC

Scanu AM, Fless GM (1990) Lipoprotein(a): heterogeneity and biological relevence. J Clin Invest 85:1709–1715

Selby JV, Austin MA, Newman B, Mayer EJ, Sandholzer C, Zang D, Utermann G (1992) Little evidence for behavioral influences on lipoprotein(a). Circulation 85:8

Sing CF, Schultz JS, Shreffler DC (1974) The genetics of the Lp antigen. II. A family study and proposed models of genetic control. Ann Hum Genet 38:47–56

Snedecor GW, Cochran WG (1967) Statistical methods, 6th ed. Iowa State University Press, Ames

Sundell IB, Nielson TK, Hallmans G, Hellsten G, Dahlen GH (1989) Interrelationships between plasma levels of plasminogen activator inhibitor, tissue plasminogen activator, lipoprotein(a), and established cardiovascular risk factors in a North Sweden population. Atherosclerosis 80:9–16

Utermann G (1989) The mysteries of lipoprotein (a). Science 246:904–910

Utermann G, Duba C, Menzel HJ (1988*a*) Genetics of the quantitative Lp(a) lipoprotein trait. II. Inheritance of Lp(a) glycoprotein phenotypes. Hum Genet 78:47–50

Utermann G, Kraft HG, Menzel HJ, Hopferwieser T, Seitz C (1988*b*) Genetics of the quantitative Lp(a) lipoprotein trait. I. Relation of Lp(a) glycoprotein phenotypes to Lp(a) lipoprotein concentrations in plasma. Hum Genet 78:41–46

Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmier HG, Seitz C (1987) Lp(a) glycoprotein phenotypes inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. J Clin Invest 80:458–465

Walton KW, Hitchens J, Magnani HN, Kahn M (1974) A study of methods of identification of Lp(a) lipoprotein and of its significance in health, hyperlipidemia and atherosclerosis. Atherosclerosis 20:323–346

Weitkamp LR, Guttormsen SA, Schultz JS (1988) Linkage between the loci for the Lp(a) lipoprotein (LP) and plasminogen (PLG). Hum Genet 79:80–82

Williams CJ, Christian JC, Norton JA Jr. TWINAN90: a FORTRAN program for conducting ANOVA-based and likelihood-based analyses of twin data. Comput Methods Programs Biomed (in press)

Winer BJ (1971) Statistical principles in experimental design. McGraw-Hill, New York

Woo J, Lam CWK, Kay R, Wong HY, Teoh R, Nichols MG (1990) Acute and long-term changes in serum lipids after acute stroke. Stroke 21:1407–1411

Zenker G, Koltringer P, Bone G, Niederkorn K, Pfeiffer K, Jurgens G (1986) Lipoprotein(a) as a strong indicator for cerebrovascular disease. Stroke 17:942–945