Atherosclerosis, 99 (1993) 23–33

Printed and Published in Ireland

ATHERO 04971

Lipoprotein(a): relation to other risk factors and genetic heritability. Results from a Dutch parent-twin study

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(Received 8 September, 1992)
(Accepted 16 October, 1992)

Summary

We measured plasma levels of lipoprotein(a) (Lp(a)) in a sample of 152 Dutch adolescent mono- and dizygotic twin pairs and their parents. The distribution of Lp(a) levels was skewed, with the highest frequencies at low levels and was similar for adult men and women and their children. The relationship of Lp(a) concentrations with other lipoprotein and apolipoprotein risk factors for coronary heart disease and with lathosterol, an indicator of whole-body cholesterol synthesis, was studied dependent on sex and generation. In mothers and children there was a small positive correlation between Lp(a) levels and plasma cholesterol and apolipoprotein (apo) B. In mothers and daughters there also was a correlation between Lp(a) and LDL cholesterol levels. No correlation was found between Lp(a) levels and plasma lathosterol, suggesting that there is no relationship between Lp(a) levels and cholesterol synthesis. Associations among family members, i.e. between monozygotic and dizygotic twins and between parents and offspring were used to study familial transmission of Lp(a) levels. Results showed that almost all of the variance in Lp(a) concentrations was accounted for by genetic heritability. A small, but significant, sex difference in heritability was observed, but heritabilities were the same in parents and offspring. Heritability estimates were 93% for females and 98% for males. No evidence was found for assortative mating or for the influence of a shared family environment. These results indicate that nearly all variance in Lp(a) concentrations that is not accounted for by the apo(a) size polymorphism, is also under genetic control.

Key words: Lipoprotein(a); Heritability; Parent-twin study; Cardiovascular risk factors; Cholesterol synthesis

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Introduction

Lipoprotein(a) (Lp(a)) was first described by Berg in 1963 [1] and is considered an important risk factor in the development of premature atherosclerosis. High levels of Lp(a) are associated

with premature coronary heart disease and stroke (for reviews see Refs. 2, 3), especially when plasma levels exceed 0.2–0.3 g/l [4–6]. An even higher risk has been reported when elevated Lp(a) levels are associated with increased levels of total or LDL-cholesterol [7,8]. We studied the association of Lp(a) concentrations with these and other lipid, lipoprotein and apolipoprotein levels (total, LDL and HDL cholesterol, triglycerides, apo A1 and apo B) as well as with plasma levels of lathosterol in a group of healthy men and women, consisting of Dutch adolescent twins and their parents. The determinants of Lp(a) levels remain unclear, but most available evidence suggests that its hepatic production explains most of the observed variation [9]. This justifies posing the question whether there is a relationship between Lp(a) levels and the rate of hepatic cholesterol synthesis. To address this question, we measured plasma lathosterol levels, which have been shown to reflect the rate of hepatic and whole-body cholesterol synthesis [10,11].

Quantitative plasma Lp(a) levels vary greatly between individuals (between 0.01 and 1.5 g/l) and show a continuous distribution that is skewed in most populations [12]. Variation at the apo(a) locus at chromosome 6 contributes significantly to variation in Lp(a) levels [12,13]. Codominant alleles at this locus code for a large number of isoforms that differ in size. In general, there is an inverse relationship between the size of the apo(a) allele and plasma Lp(a) concentrations. Utermann et al. [14] originally identified 6 apo(a) isoforms and a null allele. Gaubatz et al. [15] found 11 different isoforms and Lackner et al. [16] and Kamboh et al. [17] reported methods that detect at least 20 allelic isoforms. Boerwinkle et al. [13], using the measured genotype approach, estimated that in Caucasians 42% of the variance in Lp(a) levels is accounted for by different isoforms of apo(a). Sandholzer et al. [12] measured apo(a) phenotypes and quantitative Lp(a) levels in 7 ethnic groups and found that while the effects of apo(a) types on Lp(a) levels were similar in all groups, the amount of variance explained differed between 19% in Sudanese blacks and >70% in Chinese and Malays. In 3 Caucasian groups, the amount of variance explained by apo(a) type varied between 25% and 37%. The apo(a) size polymorphism thus is a major determinant of Lp(a) levels, but Boerwinkle et al. [13] also showed that there is significant variation within apo(a) types. Sandholzer et al. [12] found that the larger the variance within apo(a) types, the smaller the amount of variance that was attributable to the apo(a) size polymorphism. It is unknown which factors determine variance in Lp(a) concentrations that cannot be attributed to apo(a) phenotype. Part of this variation may be genetically controlled or may be determined by environmental factors shared by family members. To study further the genetic and environmental contributions to individual differences in Lp(a) levels, we measured plasma Lp(a) concentrations in a randomly selected sample of Dutch monozygotic (MZ) and dizygotic (DZ) adolescent twin pairs and their parents. Studies of MZ and DZ twins provide a unique opportunity to separate the influences of shared environmental from genetic factors. By studying both male and female twins sex dependent estimates of genetic and environmental parameters can be obtained. By including parents of twins intergenerational differences in heritabilities and resemblance between spouses in Lp(a) levels can be assessed.

Subjects

This study is part of a larger project in which cardiovascular risk factors are studied in 160 twin pairs and their parents. Addresses of twins (between 14–20 years of age) living in Amsterdam and neighboring cities were obtained from City Council population registries. Twins still living with both their biological parents were contacted by letter. A family was included in the study only if the twins and both parents were willing to comply. In addition, a small number of families who heard of the study from other twins also volunteered to participate.

Zygosity was determinated by analyzing the following bloodgroup antigens: ABO, MNS, P, Rhesus, Lutheran, Kell, Duffy, Kidd; and immunoglobulin antigens Gm, Am and Km. In addition, 36 twin pairs were typed by DNA fingerprinting [18]. Three triplets were included by discarding the data from the middle child.

There were 35 families with monozygotic boys (MZM), 35 families with monozygotic girls

(MZF), 31 families with dizygotic boys (DZM), 30 families with dizygotic females (DZF) and 29 families with dizygotic opposite-sex twin pairs (DOS).

Three fathers of twins were born in the south of Europe and Turkey and 11 in former Dutch colonies (Indonesia, Surinam and the Dutch Antilles); 8 mothers of twins and 4 twin pairs were also born in former colonies. However, most of these subjects are in fact ethnic Dutch. Five families were omitted from the analyses because one or both parents were non-Caucasian and 3 additional families were removed because of very high Lp(a) (>1 g/l) levels in the parents (2 families) or in the children (1 family). This left 152 twin families for analyses (33 MZM, 33 MZF, 30 DZM, 30 DZF and 26 DOS). In these families, plasma for Lp(a) measurement was not available for 2 fathers, 1 mother and 2 offspring. Fasting blood samples were taken between 08:30 and 10:30 h by venipuncture, using Becton-Dickinson Vacutainers containing sodium-EDTA. Plasma was immediately separated from the cells and kept at 4°C for lipid determination within the next 5 days. The remainder was stored in small portions at -20°C until further use.

Methods

Cholesterol and triglyceride concentrations were determined with enzymatic methods (Boehringer CHOD-PAP kit, number 236691 and Triglyceride kit number 701904). High density lipoprotein (HDL)-cholesterol was measured after precipitation of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) [19]. LDL-cholesterol was calculated by the formula of Friedewald [20]. With this formula triglyceride concentrations must not exceed 4.52 mmol/l [21]. There were no subjects with triglycerides above 4.07 mmol/l. LDL cholesterol was also calculated by a modification of the Friedewald formula to account for the fact that Lp(a) contains 30% by weight cholesterol [7,22]. Lathosterol was measured by gas chromatography [11,23]. Apo A1 and apo B were measured by radial immunodiffusion as described by Albers et al. [24] and Havekes et al. [25]. Lp(a) levels were measured with a bi-site sandwich ELISA using polystyrene microtiter plates (Nunc Immuno plates, high binding quality, Intermed, Roshilde, Denmark), essentially as described for our apo A1 and apo B100 ELISA [26]. The microtiter plates were coated with anti-Lp(a) as a catching antibody; 10 μg/ml IgG, 120 μl/well. IgG used was purified by protein G chromatography from rabbit antiserum, raised against a mixture of Lp(a) from different persons (Behringwerke, Marburg, Germany). Rabbit anti-human apo B (raised in our Institute) conjugated to horse radish peroxidase (EC 1.11.1.7) was used as detecting antibody. Incubations with casein, plasma samples and conjugate were performed at 37°C. The peroxidase-labeled conjugate was visualized using 3,3′,5,5′-tetramethylbenzidine and H_2O_2 as the substrate mixture [27]. The Immuno reference serum (Immuno AG, Vienna, Austria) was used as the standard in this procedure. Dose-response curves were parallel for the reference serum, various plasmas with high and low levels of Lp(a) and for Lp(a) isolated by gradient ultracentrifugation according to Redgrave et al. [28]. The working range of the assay is 0.1 to 3 μg/ml. Intra- and inter-assay variation coefficients are 4.5% and 8.5%, respectively. LDL and plasminogen are not detected with this assay and did not cross-react. The procedure correlated perfectly (r = 0.98, comparison of 24 plasmas with low, median and high Lp(a) level) with the Innotest Lp(a) (Innogenetics, Antwerp, Belgium), essentially as described by Labeur et al. [29].

Statistical analyses

Descriptive statistics. Data obtained on family members do not represent independent observations. Parameter estimation and significance testing can be seriously biased if this dependence between observations is not considered. Dependence between variables from family members was accounted for by computing variance-covariance matrices that on the diagonal contain the variances of Father, Mother, Twin 1 and Twin 2 and off-diagonal contain covariances between variables and between family members. Variance-covariance matrices were computed for the 5 sex-by-zygosity groups, i.e. families of MZ males and females, families of DZ same-sex males and females, and families of DZ opposite-sex

twins, and were used to estimate standard deviations of parameters, under the constraint that these standard deviations are equal across groups. Likewise, matrices containing the means of all parameters were constructed for the 5 family groupings and these were used to estimate means for fathers, mothers, boys and girls, while taking into account the dependence between the observations. Before statistical analyses, all variables were logarithmically transformed to remove skewness. The effects of sex and generation on mean scores of parents and children were tested by χ^2 difference tests that compare the fit of a model which constrains means to be equal to one which allows them to vary. The χ^2 statistic is computed by subtracting the χ^2 for the full model (e.g. a model that specifies 4 different means for fathers, mothers, boys and girls but equates them across the 5 sex-by-zygosity groups) from that for the reduced model. The degrees of freedom (d.f.) for this test are equal to the difference between the d.f. for the full and the reduced model [30]. χ^2 tests were also used to assess whether there is heterogeneity in Lp(a) variances between sexes and generations.

Relation of Lp(a) with other parameters. Correlations between Lp(a) scores and other variables were estimated separately for fathers, mothers and their male and female offspring, but under the constraint that correlations are equal across the 5 sex-by-zygosity groups. χ^2 difference tests were used to test whether correlations differed significantly from zero, and whether they were different in males and females and in parents and offspring. All analyses were carried out with LISREL7, a computer program for linear structural equation modeling [31]. Parameters were estimated using the method of maximum likelihood.

Genetic analyses of Lp(a) variation. Resemblances between family members were first summarized in correlations. Correlations were estimated separately for MZ and DZ male and female and DZ opposite-sex twin pairs; for fathers and mothers with their male and female offspring and for spouses (husband-wife pairs). χ^2 tests were used to assess whether correlations were different from zero, and whether they were different in male, female and opposite-sex twin and parent-offspring pairs. Under the model where familial correlations are explained by genetic factors, MZ twins are expected to be at least twice as similar as DZ twins and parents and offspring, because MZ twins have identical genotypes while DZ twins share on average half of the additive gene effects and have a genetic correlation of 0.5. Genetic values of parents and offspring also have a correlation 0.5, based on Mendelian inheritance. Under the shared environmental model MZ and DZ correlations are predicted to be the same, and parent-offspring correlations depend on the extent to which the family environment is shared by parents and their children. Resemblance among MZ twins is attributable to genes or to shared environmental exposure. Dissimilarity must be caused by individual environmental influences that have affected one twin but not the other [32], so that 1 minus the MZ correlation gives an estimate of the importance of environmental factors not shared among family members. Path analysis was used to quantify the contributions of genetic and environmental factors to variation in the observed phenotype, i.e. log Lp(a) levels. The methodology was based upon the standard biometrical model [33]. Figure 1 shows a simple path diagram for a family of DZ opposite-sex twins, in which variation in Lp(a) concentrations is influenced by additive genetic and individual environmental factors. In this diagram, h_m is the path from genotype to phenotype in males and h_f is the same path in females, and e_m and e_f represent the paths from environment to phenotype in males and females. Based on this diagram, the following expectations for correlations between family members can be derived:

r(Father-son) = 0.5 h_m^2,
r(Mother-daughter) = 0.5 h_f^2,
r(Father-daughter) = r(Mother-son) = 0.5 $h_m h_f$
and
r(DOS) = 0.5 $h_f h_m$

For same-sex MZ and DZ twins these expectations are:

r(DZM) = 0.5 h_m^2 and r(DZF) = 0.5 h_f^2
r(MZM) = h_m^2 and r(MZF) = h_f^2

Genetic model fitting was carried out on the

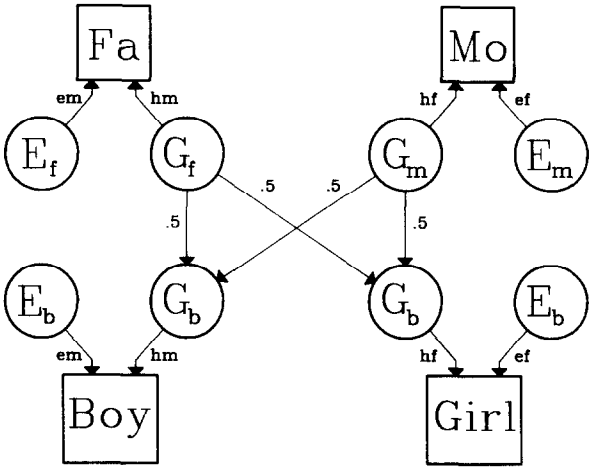

Fig. 1. Path diagram for a family of DZ opposite-sex twins. Squares represent observed variables in fathers, mothers, boys and girls, and circles represent latent variables. G, additive genetic influences; E, environmental factors that are not shared between family members. The influence of G on the phenotype is given by path coefficients h_m (for males) and h_f (for females) and the influence of E on the phenotype is given by e_m and e_f. These path coefficients are equivalent to standardized partial regression coefficients, and the proportion of variance due to genetic and environmental influences is equal to the squared path coefficients. Values of 0.5 represent the contribution of additive genetic values of parents to additive genetic values of offspring based on Mendelian inheritance.

variance-covariance matrices of the 5 family groupings using the maximum likelihood technique for parameter estimation available in LISREL [30,34]. In addition to the additive genetic model presented in Fig. 1, we considered models that included genetic dominance and models in which a shared environmental component was specified, that correlates perfectly between family members. Goodness-of-fit was assessed by χ^2 tests. The overall χ^2 tests the agreement between the observed and the predicted variances and covariances. A large χ^2 indicates a poor fit, while a small value indicates that the data are consistent with the model. Submodels excluding parameters are compared with the full model by hierarchic χ^2 tests.

Results

Descriptive statistics

Table 1 gives means and standard deviations for age, body mass index, total, LDL and HDL cholesterol, triglycerides, apo A1, apo B, lathosterol and Lp(a). The distribution of Lp(a) levels in the population was highly skewed towards the lower levels. Lp(a) levels ranged from 0.003 to 0.81 g/l (median 0.060 g/l) in men and from 0.005 to 0.848 g/l (median 0.062 g/l) in women. For the children the median Lp(a) concentrations were 0.064 g/l and 0.073 g/l for boys and girls, respectively. In this group of healthy Dutch families 21% had Lp(a) levels that were higher than 0.2 g/l. The number of subjects in each group with elevated Lp(a) levels did not differ between generations or sexes (n = 31 for fathers, n = 32 for mothers, n = 38 for boys and n = 30 for girls).

Relation of Lp(a) with other parameters

Lp(a) levels were correlated with age, body mass index and plasma levels of total cholesterol, triglycerides, LDL and HDL cholesterol, apo A1, apo B lathosterol and the ratio lathosterol/cholesterol. In Table 2 the maximum likelihood estimates of these correlations, after logarithmic transformation of all variables, are listed separately for fathers, mothers, boys and girls. The χ^2 for this model in which the correlations for fathers, mothers, boys and girls were allowed to take their own values was compared with the χ^2 of a reduced model in which all 4 correlations were constrained to be zero. Column 5 in Table 2 gives the χ^2 difference between these 2 models. This test of zero correlation between Lp(a) and the other variables showed significance only for age, total cholesterol, LDL cholesterol and apo B. For these variables Table 2 also lists the correlations with Lp(a) from the best fitting model. These best fitting models indicate a small negative, but significant correlation between age and Lp(a) in the offspring, but not in their parents. For total cholesterol, LDL cholesterol and apo B a significant, positive correlation with Lp(a) was observed in mothers and offspring and a zero correlation in fathers. However, when LDL cholesterol was adjusted for cholesterol in Lp(a) the correlation with Lp(a) became smaller and in sons became non-significant. The test of zero correlation between Lp(a) triglyceride levels was close to significance. This was caused by a negative correlation between

TABLE 1

MEANS (S.D.) FOR AGE, BMI, TOTAL CHOLESTEROL, TRIGLYCERIDES, HDL, LDL, LDL CORRECTED FOR Lp(a), Apo A1, Apo B, LATHOSTEROL AND Lp(a) IN PARENTS AND CHILDREN OF TWIN FAMILIES; TESTS OF SEX AND GENERATION DIFFERENCES

Abbreviations: BMI, body mass index (body weight in kilograms/height^2 in meters); TC, total cholesterol; TG, triglyceride; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; LDL-Lp(a), LDL cholesterol corrected for Lp(a) cholesterol; i.e. LDL – 0.7755 · Lp(a); Latho, lathosterol; Latho/TC, lathosterol/cholesterol ratio.

	Fathers (n = 150)	Mothers (n = 151)	Sons (n = 152)	Daughters (n = 150)	Sex differences		Generation differences
					parents	children	
Age (years)	48.0 (6.2)	45.6 (5.8)	16.8 (1.8)	16.7 (2.2)	*	ns	*
BMI (kg/m^2)	25.562 (2.57)	24.783 (3.16)	19.802 (2.06)	20.425 (2.32)	*	*	*
TC (mM)	5.914 (1.06)	5.627 (1.11)	4.063 (0.66)	4.390 (0.82)	ns	*	*
TG (mM)	1.410 (0.73)	0.982 (0.48)	0.664 (0.30)	0.708 (0.29)	*	ns	*
HDLC (mM)	1.131 (0.29)	1.419 (0.31)	1.229 (0.22)	1.354 (0.29)	*	*	*
LDLC (mM)	4.140 (1.00)	3.788 (1.04)	2.541 (0.64)	2.704 (0.74)	*	ns	*
LDL-Lp(a) (mM)	4.044 (1.00)	3.679 (1.04)	2.428 (0.64)	2.608 (0.74)	*	*	*
Apo B (g/l)	1.124 (0.24)	1.033 (0.22)	0.783 (0.15)	0.794 (0.18)	*	ns	*
Apo A1 (g/l)	1.406 (0.22)	1.562 (0.20)	1.344 (0.15)	1.439 (0.24)	*	*	*
Latho (μM)	7.255 (2.78)	6.124 (2.58)	3.870 (1.59)	3.957 (1.62)	*	ns	*
Latho/TC (μM/mM)	1.246 (0.41)	1.082 (0.38)	0.939 (0.35)	0.909 (0.33)	*	ns	*
Lp(a) (g/l)	0.141 (0.17)	0.120 (0.16)	0.137 (0.17)	0.118 (0.13)	ns	ns	ns

*Significant difference between groups ($P < 0.05$).

Lp(a) and triglycerides in fathers. Plasma lathosterol levels, an indicator for the cholesterol synthesis rate, did not correlate with Lp(a), even after correction for total cholesterol levels. To explore the differential relationship of Lp(a) with total cholesterol, LDL cholesterol and apo B in men and women further, mean concentrations of these variables were computed for fathers and mothers with Lp(a) levels above and below 0.2 g/l. In the high Lp(a) group mean cholesterol, LDL, LDL corrected for Lp(a) cholesterol and apo B values were virtually identical for men and women. In the low Lp(a) group these mean concentrations are different, with males having higher mean values than females (P = 0.03, 0.003, 0.003 and 0.000 for total cholesterol, LDL, LDL corrected for Lp(a) and apo B, respectively).

Genetic analyses of Lp(a) variation

Table 3 presents correlations among family members for log Lp(a) concentrations. Familial correlations as estimated by maximum likelihood were computed separately for MZ and DZ male and female and opposite-sex twin pairs, for fathers

TABLE 2

MAXIMUM LIKELIHOOD ESTIMATES OF PHENOTYPIC CORRELATIONS WITH Lp(a), χ^2 TEST OF ZERO CORRELATION AND CORRELATIONS UNDER THE BEST FITTING MODEL

Abbreviations: r(F), r(M), r(B) and r(G) are correlations with Lp(a) for fathers, mothers, sons and daughters, respectively. TC, total cholesterol; LDLC, low-density lipoprotein cholesterol; LDL-Lp(a), LDL cholesterol corrected for Lp(a) cholesterol; BMI, body mass index; TG, triglyceride; HDLC, high-density lipoprotein cholesterol; Latho, lathosterol; Latho/TC, lathosterol/cholesterol ratio.

	χ^2 test						
	r(F)	r(M)	r(S)	r(D)	$r = 0$[a]	Best model	
Age	-0.006	0.088	-0.166	-0.158	8.16	r(F) = r(M) = 0, r(B) = r(G) =	-0.176
TC	-0.038	0.261	0.223	0.284	21.32	r(F) = 0, r(M) = r(B) = r(G) =	0.253
Apo B	0.030	0.240	0.173	0.198	14.43	r(F) = 0, r(M) = r(B) = r(G) =	0.207
LDLC	0.100	0.269	0.253	0.280	23.55	r(F) = 0, r(M) = r(B) = r(G) =	0.259
LDL-Lp(a)	-0.020	0.164	0.065	0.175	7.46	r(F) = r(S) = 0, r(M) = r(D) =	0.169
BMI	-0.072	-0.025	-0.186	-0.026	5.05		
TG	-0.190	-0.029	-0.045	0.073	6.34		
HDLC	0.086	0.083	-0.115	0.029	5.15		
Apo A1	0.083	0.087	-0.052	0.090	3.95		
Latho	-0.105	0.081	0.012	0.133	4.98		
Latho/TC	-0.152	-0.061	-0.101	0.002	5.44		

[a]d.f. = 4, critical value χ^2 test = 6.42 with $\alpha = 0.05$ and $\beta = 0.50$.

and mothers with male and female offspring and for spouses (husband-wife pairs). MZ correlations were above 0.9 for males and females and were about twice as large as the correlations for DZ twins and parents and offspring. Table 4 (A and B) summarize results of testing for the effects of sex and generation on Lp(a) means and variances. No effects of sex and generation on log Lp(a) means and variances were found, as indicated by the non-significant decreases in χ^2 that were obtained when separate parameters for sex and generation effects were included in the model. Table 4 (C) gives results of significance testing of correlations between relatives and tests of the effects of zygosity, sex and generation on the correlations between family members. The test of zero correlation between Lp(a) levels of spouses showed that there was no significant correlation between Lp(a) levels of fathers and mothers (comparing this model with the one labeled 'equal variances' in Table 4 (B), that allowed the 5 spouse correlations to take their own values gave a χ^2 difference of 3.81 with 5 d.f., which is a non-significant increase). The effect of sex on parent-offspring resemblance was tested by first constraining all parent-offspring correlations to be equal. This gave a marked increase in χ^2 (43.32 with 19 d.f.). Estimating different correlations for father-son, father-daughter, mother-son and mother-daughter significantly improved the fit (χ^2 difference is 5.77 with 3 d.f.), indicating that resemblances among parents and children depend both on the sex of the parent and the offspring. The fit for this model was almost identical to one where father-daughter and mother-son correlations are constrained to be equal (χ^2 difference is

TABLE 3

MAXIMUM-LIKELIHOOD ESTIMATES OF CORRELATIONS AMONG FAMILY MEMBERS FOR LOG-LP(A) LEVELS

Twins		Parent-offspring		Spouses
MZM	0.979	Father-son	0.563	0.104
MZF	0.933	Mother-son	0.495	
DZM	0.479	Father-daughter	0.515	
DZF	0.430	Mother-daughter	0.371	
DOS	0.634			

TABLE 4

TESTS OF HETEROGENEITY OF LOG-Lp(a) MEANS, VARIANCES AND CORRELATIONS

	χ^2	d.f.	*P*
(A) Means			
Equal means:	43.29	38	0.256
Generation Differences:	42.55	37	0.244
Sex differences:	42.18	37	0.257
Generation and sex differences:	40.04	35	0.256
(B) Variances			
Equal variances:	13.47	19	0.814
Generation differences:	12.76	18	0.805
Sex differences:	12.29	18	0.832
Generation and sex differences:	10.94	16	0.813
(C) Correlations			
Spouse correlation zero	17.28	24	0.836
Equal parent-offspring correlations	60.80	43	0.038
Separate Fa-son, Mo-dau, Fa-dau and Mo-son correlations	55.03	40	0.057
Separate Fa-son, Mo-dau and Fa-dau = Mo-son correlations	55.18	41	0.069
Equal MZ correlations	65.04	42	0.013
DZ = parent-offspring correlation	57.78	44	0.080

0.15). MZ male and MZ female twin correlations also were significantly different from each other, with a higher correlation in males than in females (χ^2 difference of 9.86 with 1 d.f.). Finally, the 3 DZ twin correlations were equated to the corresponding parent-offspring correlations (i.e. DZF equal to mother-daughter, DZM equal to father-son and DOS correlation equal to father-daughter and mother-son correlation) to test if generation has an effect on family resemblances. In the absence of influences of genetic dominance DZ correlations should be equal to parent-offspring correlations if the size of the genetic influences is the same in both generations. The fit of this last model is as good as the fit of the model where the DZ correlations were allowed to take their own values (χ^2 difference is 2.60 with 3 d.f.). Estimates of correlations for parents and offspring and DZ twins based on this last model were: 0.503 for father-son and for DZ males, 0.321 for mother-daughter and DZ females and 0.487 for mother-son and father-daughter and for DZ opposite-sex pairs. This pattern of correlations strongly suggests a sex difference in the heritability of log Lp(a) levels, with higher heritability in males than in females, while there is no evidence for heritability to differ between generations. Also, there does not seem to be an effect of shared environmental influences.

Genetic model fitting to variance-covariance matrices confirmed that models without sex differences in the genetic or environmental parameter estimates did not fit the data (a model including additive genetic and environmental effects without sex differences gave χ^2 = 73.98, d.f. = 48, P = 0.00). A model that allowed for sex differences in genetic and environmental parameter estimates, while constraining total variances to be the same for males and females substantially improved the fit (χ^2 62.93, d.f. = 47, P = 0.06). Allowing for genetic dominance or shared environmental influences did not improve this simple additive genetic model. Parameter estimates for the additive genetic model with sex differences were 0.2775 (S.E. = 0.0182) and 0.006 (S.E. = 0.0015) for genetic and environmental variances in males,

and 0.2626 (S.E. = 0.0192) and 0.0208 (S.E. = 0.0051) for genetic and environmental variances in females. Heritabilities (defined as the genetic variance divided by the total variance) thus are 98% for males and 93% for females.

Discussion

The distribution of Lp(a) levels in the population was highly skewed towards the lower levels, similar to the distribution described in other Caucasian populations [22,35–38]. We did not find differences in the mean and median plasma Lp(a) levels between men and women, or between parents and offspring. This is in agreement with other studies [5,6,29,35], although higher Lp(a) levels in women than in men also have been reported [39]. Elevated Lp(a) levels (exceeding 0.2 g/l), which correlate positively with the development of coronary heart disease, were found for 21% of the subjects in this population of Dutch families. High Lp(a) levels were accompanied by high levels of total cholesterol, LDL cholesterol and apo B in both men and women, but men with low Lp(a) concentrations showed higher mean values of these risk enhancing factors than women.

Experimental evidence strongly suggests that cholesterol synthesis is required for the assembly and secretion of VLDL in rats [40,41] and in humans [42]. In order to investigate if Lp(a) levels are related to cholesterol synthesis we looked for an association with plasma lathosterol levels, which has been reported to be a good indicator for hepatic and whole-body cholesterol synthesis in humans [10,11]. No correlation of Lp(a) with lathosterol was found. Earlier, we reported for this same population a positive correlation between lathosterol and cholesterol, LDL and triglyceride levels [23]. These results were ascribed partly to physicochemical partitioning of lathosterol by the lipoproteins carrying these lipids. To account for this, the ratio of lathosterol/cholesterol was also correlated with Lp(a). Again, no significant correlation was found, indicating that Lp(a) levels do not depend on cholesterol synthesis. That cholesterol synthesis does not determine Lp(a) levels is also in accordance with the observation that HMG-CoA reductase inhibitors do not affect Lp(a) levels in plasma [43,44]. Since Lp(a) levels are strongly dependent on hepatic production [9], we suggest that cholesterol synthesis is of less importance for Lp(a) than for VLDL secretion.

The genetic analyses indicate that almost all of the variance in plasma Lp(a) concentrations can be attributed to genetic factors. In this population of family members living together and sharing the same diet, for example, no influence of shared family environment on Lp(a) concentrations was found. Monozygotic twin correlations were above 0.90, and dizygotic twin and parent-offspring correlations were almost exactly half the MZ correlation. Moreover, no resemblance between fathers and mothers of twins was observed. There was a small, but significant difference in heritability estimates for males and females, with heritability estimates for females (93%) being somewhat lower than for males (98%). There were no intergenerational differences in heritability, i.e., the amount of variance explained by genetic factors was the same in parents and in their children. Wang et al. [45] studied apo(a) levels in 1-week- and 5–13-month-old children and their parents. In the 5–13-month-old group they found parent-offspring correlations that already were of the same magnitude as the correlations observed for adolescent children and their parents in this study. Hasstedt and Williams [46] also found evidence for very high heritabilities of Lp(a) levels in Utah pedigrees and estimated that 73% of the variance was due to a three-allele major locus and 26% to a polygenic component. Lamon-Fava et al. [47] found a higher concordance for elevated Lp(a) (above 0.4 g/l) in male MZ than DZ twins aged 59–70 years. Heritability based on concordance rates was 67%. Boerwinkle et al. [13] and Sandholzer et al. [12] estimated that in Caucasians, between 25% and 42% of the variance in Lp(a) levels is accounted for by the apo(a) locus at chromosome 6. Recently, Boerwinkle et al. [48] used the newly developed pulsed-field gel electrophoresis method [16] which distinguishes 19 different apo(a) alleles, and estimated that the apo(a) size polymorphism accounted for 69% of the total variation in Lp(a) concentrations. In these same data a polygenic heritability of 85% was found and based on sibling-pair linkage methods, it was estimated that the remainder of the genetic variance was accounted for by *cis*-acting sequences at the apo(a)

locus. For sibling pairs that shared both apo(a) alleles identical by descent, the correlation of Lp(a) levels was 0.95, which is almost exactly the same as the correlation we observed in identical twins, who share all their alleles identical by descent. We did not measure apo(a) phenotypes, so we do not know exactly in this data set which part of the genetic variance is attributable to variance in apo(a) size polymorphism and which part to other genetic influences. Our results strongly suggest, however, that almost all of the variance that is not accounted for by the apo(a) size polymorphism is also determined by genetic factors.

Acknowledgements

Funded by the Netherlands Heart Foundation (D.I. Boomsma: Grant 88.042 and A. Kaptein: Grant 87.035). We would like to thank Ria Laterveer and Herman van der Voort for skillful technical assistance.

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