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The Age Dependency of Gene Expression for Plasma Lipids, Lipoproteins, and Apolipoproteins

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Summary

The aim of this study was to investigate and disentangle the genetic and nongenetic causes of stability and change in lipids and (apo)lipoproteins that occur during the lifespan. Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), and lipoprotein(a) (Lp[a]) were measured in a group of 160 middle-aged parents and their twin offspring (first project) and in a group of 203 middle-aged twin pairs (second project). Combining the data of both projects enabled the estimation of the extent to which measured lipid parameters are influenced by different genes in adolescence and adulthood. To that end, an extended quantitative genetic model was specified, which allowed the estimation of heritabilities for each sex and generation separately. Heritabilities were similar for both sexes and both generations. Larger variances in the parental generation could be ascribed to proportional increases in both unique environmental and additive genetic variance from childhood to adulthood, which led to similar heritability estimates in adolescent and middle-aged twins. Although the magnitudes of heritabilities were similar across generations, results showed that, for total cholesterol, triglycerides, HDL, and LDL, partly different genes are expressed in adolescence compared to adulthood. For triglycerides, only 46% of the genetic variance was common to both age groups; for total cholesterol this was 80%. Intermediate values were found for HDL (66%) and LDL (76%). For ApoA1, ApoB, and Lp(a), the *same* genes seem to act in both generations.

Introduction

Numerous epidemiological studies have established the association between separate constituents of the lipid system and the risk of coronary heart disease. High levels of total cholesterol, low-density lipoprotein (LDL), and apolipoprotein B (ApoB) and low levels of high-density lipoprotein (HDL) and apolipoprotein A (ApoA1) are all predictors of atherosclerotic coronary heart disease. Apolipoproteins are of great interest in this respect, because several studies indicate that they are better discriminators than plasma lipids for atherosclerosis (Avogaro et al. 1979; Durrington et al. 1988). An independent association between triglyceride levels and cardiovascular risk remains to be established, although triglycerides have prognostic value in combination with LDL and HDL (Schulte et al. 1994) and as one of the components of the insulin-resistance syndrome (Reaven 1988). Another particle of the lipid system, lipoprotein(a) (Lp[a]), is also considered an important risk factor for coronary heart disease (Rhoads et al. 1986; Durrington et al. 1988). Lp(a) was first described by Berg in 1963 and is structurally related to LDL (Utermann 1989).

Developmental Trends in Means and Variances

Plasma lipid and (apo)lipoprotein levels vary considerably with age, and the changes of the different lipid components do not run in parallel. These age trends differ for males and females. Superimposed on the global change during the whole lifespan, four more specific periods are associated with more dramatic changes: the first years after birth (Berenson et al. 1978, 1979), adolescence (Berenson et al. 1981; Twisk et al. 1995), the menopausal period in females (Matthews et al. 1989; Schaefer et al. 1994), and old age (Kromhout et al. 1990; Newschaffer et al. 1992; Kronmal et al. 1993). The age-dependent variation in lipid and (apo)lipoprotein levels may imply that factors determining lipid levels at a certain age partly differ from those involved at another age. Also, these lipid-determining factors may be partly different for males and females. For example, the drop in cholesterol level during puberty may have another origin than the rise during middle age, which in turn may have a different cause than the change in lipid levels due to menopause. These (sex-specific) age trends in mean levels may indicate the involvement of different genetic or environmental factors, or both, at different ages.

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Reilly et al. (1990) investigated effects of sex and generation on variances in lipids and (apo)lipoproteins. To this goal, lipids were measured in grandparents (mean age: 68 years), parents (mean age: 42 years), and children (mean age: 15 years) of both sexes. Significant differences in variances between generations were found for total cholesterol and LDL in both males and females and for triglycerides, HDL, and ApoA1 in males. Differences were attributable to an increase in variance with increasing age. In a study of 160 twin pairs and their parents, Boomsma et al. (1996) largely confirmed results of Reilly et al. (1990). Variances of total cholesterol, triglycerides, HDL, LDL, ApoA1, and ApoB were all significantly higher in the parental generation. Only for Lp(a) was no generational effect observed. Such an increase in variance from adolescence to adulthood in lipids and (apo)lipoproteins may be due to an increase in the amount of genetic variance, nongenetic variance, or both.

Genetic Developmental Trends

Twin and family studies, using quantitative genetic approaches, indicate that a considerable part of the variation in lipids, lipoproteins, and apolipoproteins results from (poly)genetic influences (reviewed in Iselius 1979, 1988; O'Connell et al. 1988; Lamon-Fava et al. 1991; Heller et al. 1993; Rao and Vogler 1994; Brenn 1994). At present, we know very little about developmental trends in the heritability of lipid parameters, and we know even less about causes of stability and change in these variables. As stated, the sex-specific age trends in levels and variances of lipid parameters may indicate that different genetic and environmental factors may be influential in different sex-age cohorts. Changes in variance components with age can imply changes in heritabilities with age as well as differences in correlations among relatives of different ages. Both twin and parent-offspring studies may be informative in this respect.

Twin Studies

If twins within a specific age range are measured in studies estimating the genetic influence on lipids, lipoproteins, and apolipoproteins, heritabilities for this specific age range are obtained. To get an impression of possible age trends in heritability, we ranked 11 recent twin studies (since 1977) in ascending order according to the age of the twin sample (Snieder 1996). No obvious age trend in heritability estimates could be detected. Heritabilities measured before (Bodurtha et al. 1991) and during puberty (Boomsma et al. 1996) were highly similar, and they remained fairly constant during early adulthood and middle age. Only in the elderly did heritabilities seem to decrease (Heller et al. 1993). The same ranking procedure was applied to recent twin studies of ApoA1, ApoB, and Lp(a). Again, a clear age trend could not be detected.

Parent-Offspring Studies

If there is an age-dependent genetic or environmental effect on the phenotype, one would expect the parent-offspring correlation to be lower than the sibling correlation, and the sibling correlation to be lower than the DZ twin correlations, as the age difference within those pairs decreases. This expectation was confirmed for cholesterol, in a review by Iselius (1979) of earlier twin and family studies. For 4,716 parent-offspring pairs, a mean correlation of .26 was found, whereas, for 2,056 sibling pairs and 622 pairs of DZ twins, these values were .34 and .44, respectively. Boomsma et al. (1996) also observed lower parent-offspring than DZ twin correlations for lipids and (apo)lipoproteins. Two types of age-dependent effects could explain these observations. The influence of unique environmental factors could increase with age, or different genes could influence lipids in childhood and adulthood. In the study of Boomsma et al. (1996), higher phenotypic variances in parents, compared to that of their offspring, could best be explained by a genetic model that specified an increase in unique environmental variance with increasing age. This increase in unique environmental variance subsequently led to smaller heritabilities in the parental generation. The parent-offspring model used by Boomsma et al. (1996), however, assumes that a phenotype is influenced by the same genes in parents and their offspring. This assumption may not be valid, and longitudinal studies, in which the same genetically informative subjects are measured repeatedly, are needed to test such an assumption rigorously.

The principal aim of this study was to disentangle the genetic and nongenetic causes of stability and change in lipid, lipoprotein, and apolipoprotein levels that occur during the lifespan. As an alternative to a longitudinal study, a quantitative genetic model was defined in which the regular twin-family design, including parents and their twin offspring, was extended with a group of middle-aged twins of the same age as those parents. This allows the estimation of heritabilities for each generation separately, on the basis of the information available from adolescent and adult twins. If these heritabilities are known, the observed parent-offspring correlation can be used to estimate the correlation between genes that are expressed in adolescence and adulthood (Stallings et al. 1989; Snieder et al. 1995).

Subjects and Methods

Subjects

Data were combined from two different research projects. In the first project, cardiovascular risk factors were measured in a group of 160 adolescent twin pairs and

their parents. Detailed information on recruitment, zygosity determination, and exclusion criteria is given elsewhere (Boomsma et al. 1993, 1996). The adolescent twin sample consisted of 35 pairs of MZ males (MZM), 31 pairs of DZ males (DZM), 35 pairs of MZ females (MZF), 30 pairs of DZ females (DZF), and 29 DZ pairs of opposite sex (DOS). For Lp(a), group sizes were slightly smaller: 33 pairs of MZM, 30 pairs of DZM, 33 pairs of MZF, 30 pairs of DZF, and 26 pairs of DOS.

In a second project, cardiovascular risk factors were studied in a group of 213 middle-aged twins (Snieder et al. 1995). Middle-aged twins were in the same age range (34–63 years) as the parents of the adolescent twin sample (35–65 years). Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Netherlands Twin Registry, and solicitation through the Dutch Twin Club. In addition, a small number of twins who heard from the study in another way volunteered to participate. Informed consent was obtained from all subjects. Data from 10 twins were excluded from the sample. In eight twins, one or both members of the pair used lipid-lowering medication (HMG-CoA reductase inhibitors). In one subject, no blood could be obtained. Data from another subject were discarded because of an extremely high triglyceride value (5.92 mmol/liter). One (MZ) triplet was included in the sample by discarding the data from the second-born subject. In total, 188 males and 218 females were included in the study. HMG-CoA reductase inhibitors and other lipid parameters hardly affect Lp(a) levels (Sundell et al. 1989; Austin et al. 1992; Kostner et al. 1993). Therefore, exclusion criteria for Lp(a) were different: only three twin pairs, in which one or both members had higher values than the upper value of the measuring range of the assay (>128 mg/dl), were excluded.

In 76 same-sex twin pairs, zygosity was determined by DNA fingerprinting (Jeffreys et al. 1985). In the remaining 89 same-sex twin pairs, zygosity was determined by questionnaire items about physical similarity and frequency of confusion by family and strangers during their childhood (Goldsmith 1991). Classification of zygosity in these 89 same-sex twin pairs was based on a discriminant analysis, relating the questionnaire items of the 76 same-sex pairs to their zygosity on the basis of DNA fingerprinting. In that sample, zygosity was correctly classified in 98.7% of the cases. One DZ pair was mistakenly classified as MZ. Grouped according to their zygosity and sex, the sample consisted of 39 pairs of MZM, 36 pairs of DZM, 50 pairs of MZF, 40 pairs of DZF, and 38 pairs of DOS. For Lp(a), group sizes were 42 pairs of MZM, 39 pairs of DZM, 49 pairs of MZF, 40 pairs of DZF, and 39 pairs of DOS.

Blood Sampling and Biochemical Assays

See Boomsma et al. (1993, 1996) for details on blood sampling and lipid determinations in the first project. In the second project, twins arrived at the laboratory at ~10.00 A.M. They were requested to fast and refrain from smoking and the use of alcohol, coffee, and tea after 11.00 P.M. of the preceding night. Blood was collected by venipuncture, using Vacutainer tubes (Becton-Dickinson) containing sodium-EDTA. The tubes were placed on ice and centrifuged promptly (30 min, 2,000 *g*) at 4°C to separate plasma from cells. Aliquots of plasma were snap-frozen using liquid nitrogen and stored at −20°C until processing. Total cholesterol, LDL, HDL, and triglycerides were determined in the same way as in the first project (Boomsma et al. 1996). ApoA1 and ApoB were quantified with the method of Beckman using the Array Protein System (Beckman Instruments) (Maciejko et al. 1987). The Beckman calibrator (standardized to the International Federation for Clinical Chemistry, which is traceable to the World Health Organization International Reference Material for ApoA1 and ApoB no. 1883) was used as standard reference material. Monospecific goat-antihuman ApoA1 and ApoB antisera were used (Beckman). Lp(a) was measured with the same Array Protein System (Beckman). The calibrator from Immuno AG (Vienna GmbH) was used for the calibration curve, which was calculated as a polynomal-5 with the Flexisoft software (Beckman). Goat-antihuman Lp(a) antiserum was used (Immuno AG, Vienna GmbH). The measuring range of the test lies between 2 and 128 mg/dl.

Genetic Analysis of Lipids and (Apo)lipoproteins

As an alternative to a longitudinal study the parent-twin design used by Boomsma et al. (1996) was augmented with twins of the same age as the parents. Parent-offspring correlations, as already estimated by Boomsma et al. (1996), were >0. In the absence of shared environmental influences, such a significant parent-offspring resemblance implies significant heritabilities in both childhood and adulthood and a substantial genetic correlation across time. Preceding the application of the extended model, univariate model fitting to adolescent and middle-aged twin data was performed to estimate heritabilities for each generation separately. Before we describe the univariate twin model and the extended parent-twin model that were used in the data analysis, some general features of the genetic model fitting procedure are summarized.

Model-Fitting Procedure

Both univariate and extended models were fitted to variance-covariance matrices by the method of maximum likelihood, using Mx (Neale 1994), a program specifically developed for analysis of data from genetically informative subjects. Mx provides parameter estimates, a χ^2 test of the goodness-of-fit of the model, and Akaike's information criterion (AIC). The overall χ^2

tests the agreement between the observed and predicted variances and covariances in the different zygosity groups. A large χ^2 indicates a poor fit (low probability), while a small χ^2 indicates that the model is consistent with the data (high probability). Submodels were compared by hierarchic χ^2 tests, in which the χ^2 for a reduced model is subtracted from that of the full model. The df for this test are equal to the difference between the df for the full and the reduced model. The eventual purpose of the model-fitting procedure is to explain the pattern of covariances and variances by as few parameters as possible. To evaluate the fit of the models, AIC (calculated as $\chi^2 - 2$ df) was also used. The model with the lowest AIC reflects the best balance of goodness-of-fit and parsimony. Besides AIC, Jöreskog's χ^2/df was used to evaluate the fit of the extended models. This measure can be used when fitting many models with large degrees of freedom. A value of <2 for Jöreskog's χ^2/df indicates an adequate fit of the model to the data (Finkel et al. 1995).

Univariate Analyses

Separate univariate analyses within the adolescent and middle-aged twins groups were executed. Lipid phenotypes (and age) in twin and cotwin of both twin groups, were summarized into 3×3 variance-covariance matrices for each of the five zygosity groups. Age can spuriously introduce a common environmental effect if there is a significant correlation between the phenotype and age, which is the case for many lipid parameters. By incorporating age into the model, the influence of age on the phenotype can be quantified (Neale and Martin 1989).

Sex differences were examined by comparing a full model in which parameter estimates are allowed to differ in magnitude between males and females with a reduced model in which parameter estimates are constrained to be equal across the sexes. In addition to those models, a scalar model was tested. In a scalar model, heritabilities are constrained to be equal across sexes, but total variances may be different. All (non-standardized) variance components for males are constrained to be equal to a scalar multiple, k^2, of the female variance components, such that $h_m^2 = k^2 h_f^2$, $c_m^2 = k^2 c_f^2$, $e_m^2 = k^2 e_f^2$, $d_m^2 = k^2 d_f^2$, and $a_m^2 = k^2 a_f^2$. As a result, the standardized variance components such as heritabilities are equal across sexes, even though the unstandardized components differ (Neale and Cardon 1992). Sex-specific genetic effects can be tested by fixing the correlation between the latent genetic factors (R_G) in the DOS twin group to 0, or estimating it (allowing it to be $<.5$).

The applied univariate model that includes age is presented in figure 1. The path diagram shows an opposite-sex twin pair. Twin 1 is a female, twin 2 a male. The observed phenotypes for twin and cotwin are shown in squares, and latent factors are shown in circles. Correlations between the latent genetic factors (R_G) are 1 in MZ twins and .5 in DZ same-sex twins. R_G can be estimated in DOS twin pairs. Factor loadings of observed variables on the different latent factors are also shown. k is the scalar factor.

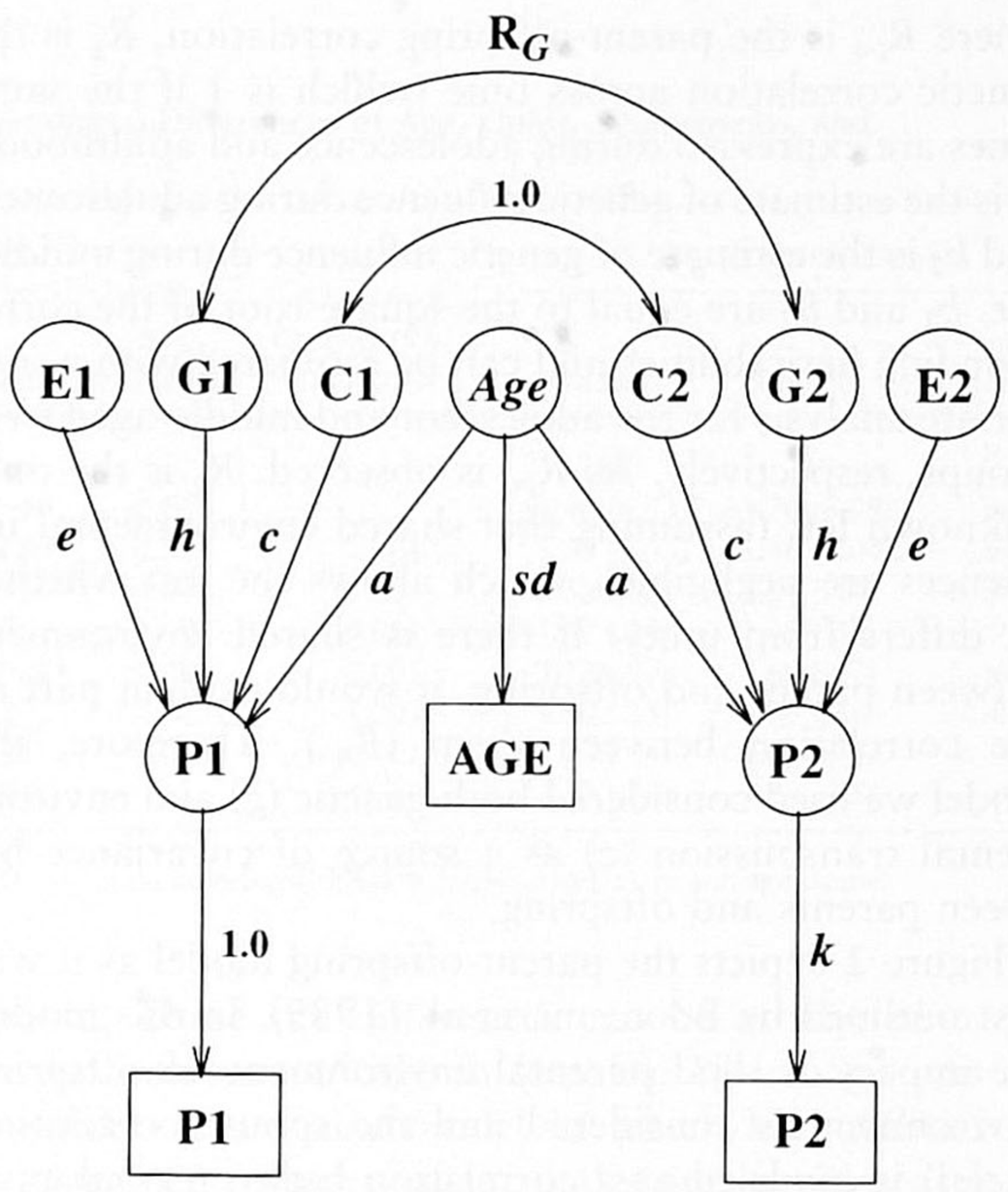

Figure 1 Path diagram for a univariate scalar model including age. An opposite-sex twin pair is shown, twin 1 being female and twin 2 being male. Observed phenotypes (P) for twin 1 and twin 2 are shown in squares, latent factors are shown in circles. Correlations between additive genetic factors (R_G) are 1 in MZ twins and .5 in DZ same-sex twins and can be estimated for DOS twins. Factor loadings of observed variables on the different latent factors are also shown: h = additive genetic effect; c = shared environmental effect; e = unique environmental effect; a = influence attributed to age; sd = standard deviation of age; k = scalar factor.

Extended Parent-Twin Design

An underlying assumption of the parent-twin design is that the same genes are expressed in parents and their offspring. Stated differently, it is assumed that the correlation between genetic effects during childhood and adulthood equals unity. With the extended parent-twin design, which includes young twins, their parents, and twins of the same age as the parents, the hypothesis can be tested that the correlation between genetic effects during adolescence and adulthood does *not* equal unity. The model can be written as:

$$R_{po} = .5 \times R_g \times h_1 \times h_2 , \qquad (1)$$

where R_{po} is the parent-offspring correlation, R_g is the genetic correlation across time (which is 1 if the same genes are expressed during adolescence and adulthood), h_1 is the estimate of genetic influence during adolescence, and h_2 is the estimate of genetic influence during middle-age. h_1 and h_2 are equal to the square root of the corresponding heritabilities and can be estimated with a univariate analysis for the adolescent and middle-aged twin groups, respectively. As R_{po} is observed, R_g is the only unknown left (assuming that shared environmental influences are negligible), which allows the test whether R_g differs from unity. If there is shared environment between parent and offspring, it would explain part of the correlation between them (R_{po}). Therefore, the model we used considered both genetic (g) and environmental transmission (z) as a source of covariance between parents and offspring.

Figure 2 depicts the parent-offspring model as it was first outlined by Boomsma et al. (1989). In this model, the impact of total parental environment on offspring environment is considered and the spouse correlation ($Re[s]$) is modeled as a correlation between total environments. The total environmental offspring correlation consists of a part that is accounted for by parental influences (through the environmental transmission parameter [z]) and a part independent of the resemblance with their parents ($Re[t]$). Although not shown, age was also incorporated into the model to control for its influence on parameter estimates of the model.

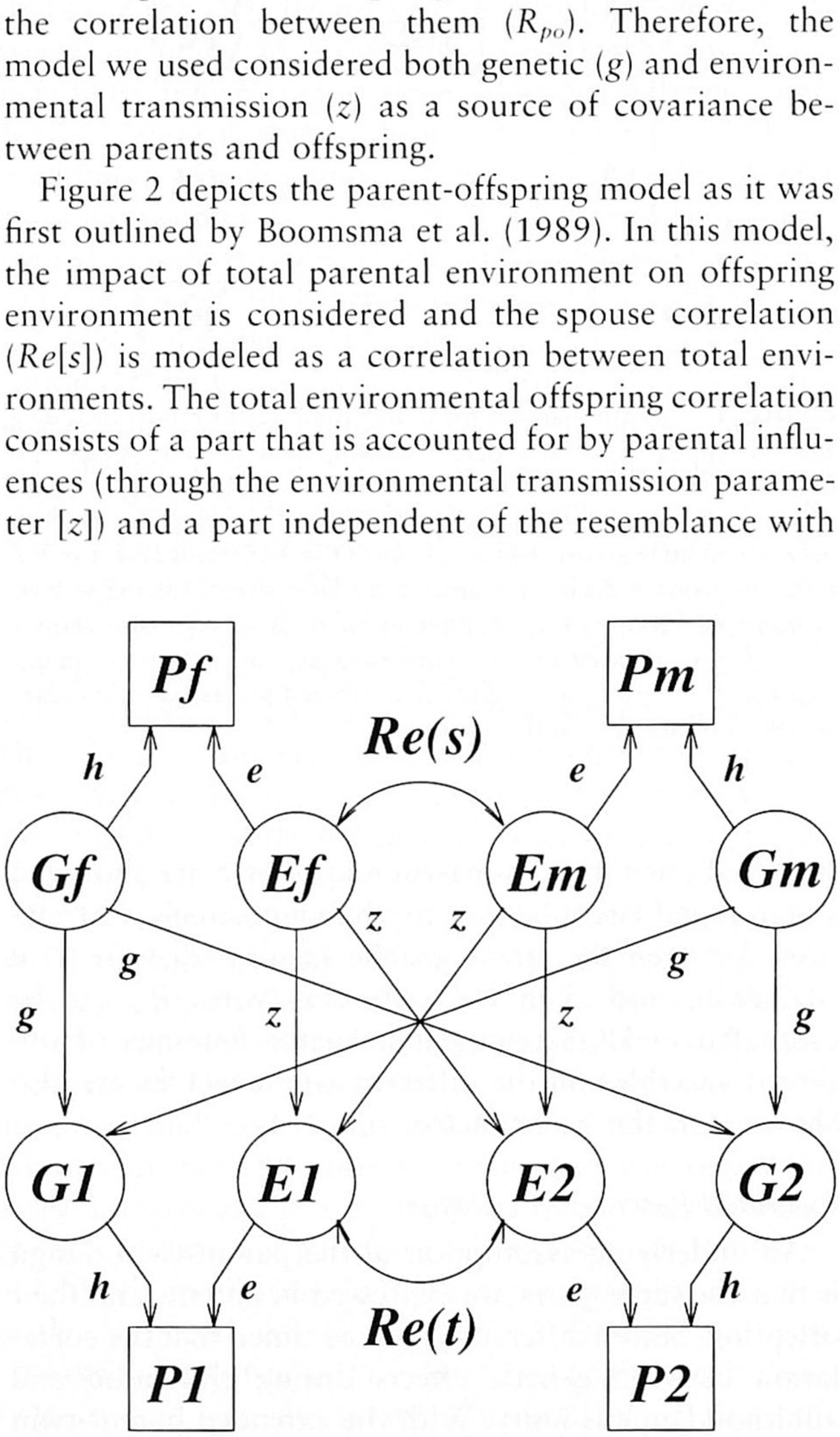

Figure 2 Parent-offspring model. P represents observed phenotypes of father (f), mother (m), twin 1 (1), and twin 2 (2). G and E denote latent genotype and total environment. g is the genetic transmission from parents to offspring. z is the nongenetic transmission from total parental environment to offspring environment, in this case modeled to be equal for fathers and mothers. The correlation between total environments of spouses is represented by $Re(s)$, and the correlation between residual environments of twins by $Re(t)$.

In the extended model, parameters are estimated by using information from 10 groups: 5 groups of young twins and their parents and 5 middle-aged twin groups, grouped according to the sex and zygosity of the twins. In this multigroup design, path coefficients for the parents equal those of the middle-aged twins. The genetic transmission from parents to offspring is modeled by the g coefficient. This g coefficient equals .5 R_g, and g equals .5 if the same genes are expressed in adolescence and adulthood (since R_g equals 1 in that case [see equation {1}]). Provided that shared environmental influences are absent, equation 1 shows that a significant parent-offspring resemblance implies significant heritabilities in both childhood and adulthood and a substantial genetic correlation across time, i.e., $0 < g \leq .5$. Doubling the estimate of g yields this genetic correlation across time ($R_g = 2 \times g$), which can also be viewed as a measure of the part of the genetic variance common to both age groups.

Statistical Analysis

Because the distributions of triglyceride and Lp(a) levels were skewed, these data were logarithmically transformed to obtain a more normal distribution. Analysis of variance revealed a significant effect of the occasion of measurement (i.e., the batch in which determinations were done) on mean levels of HDL and ApoA1. Prior to further analysis, these variables were corrected for between occasions variation by using the Fisher package (Lange et al. 1988), which enabled quantification of the effect. The effects of sex, generation, and zygosity on the means were tested by likelihood ratio χ^2 tests, using Mx (Neale 1994).

Results

Table 1 shows means and standard deviations of age, lipids, lipoproteins, and apolipoproteins in young twins, their parents, and middle-aged twins. Significance of sex differences in mean values within each of these groups, evaluated by likelihood ratio χ^2 tests, are also shown. Females had a higher HDL and ApoA1 in all three groups. In general, middle-aged males had higher mean values than middle-aged females for risk-enhancing factors like triglycerides, total cholesterol, LDL, and ApoB. The significance of generational differences in mean values between parents and offspring are also listed. Means were significantly higher in parents than in their offspring for most of the variables except Lp(a), which showed the same mean across generations, and HDL, whose mean value was lower in fathers than in their sons. Effects of zygosity on the means were absent.

Table 1

Number of Individuals (*n*), Means, SDs, and Significance of Sex and Generational Differences of Age, Lipids, Lipoproteins, and Apolipoproteins for Males and Females within the Three Subject Groups

	Young Twins			Parents			Parent-Offspring Generational	Middle-Aged Twins		
Data	Males	Females	Sex Difference	Males	Females	Sex Difference	Difference	Males	Females	Sex Difference
n	161	159	...	160	160	...	...	188	218	...
Age (years)	16.77 (1.78)	16.71 (2.20)	ns	48.10 (6.29)	45.60 (5.89)	<.001	...	43.48 (6.53)	44.70 (6.79)	ns
TC (mmol/liter)	4.11 (.63)	4.41 (.77)	<.01	5.83 (1.00)	5.63 (1.09)	<.025	<.001	5.38 (1.03)	5.46 (1.04)	ns
TRG (mmol/liter)	.67 (.30)	.71 (.28)	ns	1.39 (.71)	.95 (.45)	<.001	<.001	1.32 (.69)	1.04 (.45)	<.001
HDL (mmol/liter)	1.24 (.21)	1.37 (.27)	<.001	1.14 (.27)	1.41 (.30)	<.001	<.001	1.07 (.28)	1.39 (.33)	<.001
LDL (mmol/liter)	2.56 (.62)	2.72 (.70)	ns	4.07 (.94)	3.79 (1.02)	<.001	<.001	3.72 (.97)	3.60 (.97)	ns
ApoA1 (g/liter)	1.34 (.14)	1.43 (.21)	<.001	1.40 (.22)	1.56 (.20)	<.001	<.001	1.50 (.35)	1.93 (.38)	<.001
ApoB (g/liter)	.78 (.14)	.80 (.18)	ns	1.12 (.21)	1.02 (.21)	<.001	<.001	1.27 (.34)	1.19 (.34)	<.001
Lp(a) (g/liter)	.14 (.16)	.12 (.13)	ns	.13 (.16)	.13 (.16)	ns	ns	.17 (.24)	.20 (.25)	ns

Note.—Sex and generation differences are tested by hierarchic χ^2 tests (df = 1). TC = total cholesterol; TRG = triglyceride; ns = nonsignificant.

Boomsma et al. (1993, 1996) also reported on generational differences in standard deviations. Except for Lp(a), which had the same standard deviation, parental standard deviations were significantly higher for all lipid parameters.

Familial correlations for twins, spouses, and parents and offspring are presented in table 2. MZ correlations were consistently larger than DZ correlations for all variables in both young and middle-aged twins, which points to the influence of genes. For triglycerides in young twins, the correlation in the DOS group seemed much smaller than in the same-sex DZ groups, which would suggest sex-specific genetic effects. This difference, however, was not significant ($\chi^2 = 3.24$, df = 2). The lack of sex effects was supported by the results from parent-offspring correlations. For all variables, parent-offspring correlations were estimated separately for fathers and mothers with their sons and daughters. χ^2 tests showed that in none of the measured parameters did the correlations depend on the sex of either parent or offspring. Constraining the different parent-offspring correlations to be equal rendered one parent-child correlation, which was significantly different from zero for all variables. Spouse correlations were not significantly different from zero, except for total cholesterol, LDL, and apoB.

Univariate model-fitting results within the adolescent and middle-aged twins groups are presented in tables 3*A* and 3*B*, respectively. For all variables, best-fitting models and standardized parameter estimates are shown. In young twins, best-fitting models included additive genetic influence for all variables, with heritabilities from .47 to .98. Only for ApoB a significant influence of shared environment was found. For triglycerides, LDL, and ApoB, parameter estimates were equal in males and females. For total cholesterol, HDL, and ApoA1, sex differences in parameter estimates were found. These sex differences can be attributed to the larger influence of age in females. When age was not considered, a scalar model in which heritabilities are constrained to be equal for males and females gave the best fit to the data (Boomsma et al. 1996).

In the group of middle-aged twins (see table 3*B*), models without sex differences gave the best account of the data for all variables. A straightforward model including only additive genes (*G*), unique environment (*E*), and age fitted best for total cholesterol, LDL, ApoB, and Lp(a). For triglycerides and ApoA1, a scalar model including *G*, *E*, and age gave the best fit. Also for HDL, a scalar model turned out to be best. For HDL, dominant genetic influences were significant, besides the influence of additive genes and unique environment. In HDL the influence of age was absent. Overall, genetic influence was relatively high with (broad sense) heritabilities ranging from .58 to .73.

Thus, significant heritabilities were found for all measured elements of the lipid system in both young and middle-aged twins. Furthermore, parent-offspring correlations for all variables were significantly different from zero. Next, the extended parent-twin model was used to gain insight into the genetic correlation across time.

Results of fitting the extended model are shown in table 4*A* for lipids and lipoproteins and in table 4*B* for apolipoproteins and Lp(a). Nonstandardized parameter estimates of best-fitting models in which the genetic transmission coefficient *g* is fixed at .5 and in which *g* is estimated are listed for all variables. Jöreskog's χ^2/df was <2 for all fitted models, which indicates that all models showed an adequate fit to the data (Finkel et al. 1995). For total cholesterol, triglycerides, HDL, and LDL, there was evidence for *g* to be <.5. For total cholesterol, the improvement in fit for a model in which *g* was estimated instead of fixed to .5, just failed to reach significance. This means that a model with *g* fixed to .5

Table 2

Maximum-Likelihood Estimates of Familial Correlations for Measured Lipids, Lipoproteins, and Apolipoproteins

	N[a]	TC	TRG[b]	HDL	LDL	ApoA1	ApoB	Lp(a)[b]
Young twins:								
MZM	35	.86	.57	.69	.85	.84	.83	.98
DZM	31	.28	.46	.47	.35	.40	.56	.48
MZF	35	.74	.77	.74	.79	.73	.79	.93
DZF	30	.57	.21	.38	.46	.52	.50	.43
DOS	29	.30	.01	.45	.40	.30	.67	.63
Spouses:								
Fa-Mo	160	.21	.07	.07	.21	.12	.31	.10
χ^2 test of zero spouse correlation (df = 1)		6.73*	.66	.76	6.91*	2.40	15.99**	1.51
Parent-Child:								
Fa-So	161	.27	.15	.15	.33	.24	.24	.56
Fa-Da	159	.22	.09	.23	.23	.36	.30	.52
Mo-So	161	.37	.14	.37	.35	.35	.35	.50
Mo-Da	159	.23	.20	.24	.27	.30	.34	.37
Overall Parent-Child	640	.28	.13	.23	.30	.28	.31	.46
χ^2 test of one Pa-Ch correlation (df = 3)		2.43	.89	3.94	1.47	1.54	1.32	5.27
χ^2 test of zero Pa-Ch correlation (df = 1)		28.14**	8.05*	24.58**	30.81**	37.58**	30.16**	113.20**
Middle-aged twins:								
MZM	39	.75	.62	.62	.78	.60	.79	.91
DZM	36	.55	.40	.23	.64	.06	.68	.21
MZF	50	.79	.59	.65	.77	.64	.78	.90
DZF	40	.41	.40	.24	.30	.26	.30	.58
DOS	38	.46	.44	.21	.41	.37	.35	.54

NOTE.—N = number of pairs; Fa-Mo = spouse correlation; Fa-Son = father-son correlation; Fa-Da = father-daughter correlation; Mo-So = mother-son correlation; Mo-Da = mother-daughter correlation; Pa-Ch = parent-child correlation estimated over all pairs.

[a] For Lp(a), number of pairs were different (see text).

[b] For triglyceride and lipoprotein(a), logarithmically transformed values were used.

* $P < .01$.

** $P < .001$.

cannot be rejected, although AIC and Jöreskog's χ^2/df were smaller for the model in which g was estimated. For triglycerides, HDL, and LDL models estimating g fitted significantly better. These results indicate that in measured lipids and lipoproteins partly different genes are expressed in childhood than in adulthood. Computation of the genetic correlation across time ($R_g = 2 \times g$ [see equation {1}]), showed that it ranged from .46 in triglycerides to .80 in total cholesterol, with intermediate values for HDL and LDL.

For apolipoproteins and Lp(a), a different picture emerged. Models for ApoA1, ApoB, and Lp(a), in which g is fixed to .5, show the same (ApoA1 and Lp[a]) or almost the same fit (ApoB) as the model in which g is estimated. In addition, values of AIC and Jöreskog's χ^2/df were lower. A model in which g is fixed to .5 thus gives the most parsimonious account of the data in these variables, i.e., the same genes influence apolipoproteins and Lp(a) in childhood and in adulthood. Estimation of g also yielded a value of .5 for ApoA1 and Lp(a). ApoB is the only variable for which environmental transmission (z) turned out to be significant. This means that, for ApoB, a combination of both genetic and environmental transmission explains the parent-offspring correlation. The small value of g (.12), when it was estimated, went together with a relatively large value of z (.41), whereas z was only .18 in the model in which g was fixed at .5.

As mentioned earlier, variances were higher in the parental generation for all variables except Lp(a). As can be seen from tables 4*A* and 4*B*, the higher variance in the parents could in most cases be attributed to a rise in both additive genetic and unique environmental variance from childhood to adulthood. For total cholesterol, LDL, and ApoB in females, a rise in variance due to age also played a role. For most variables, standardized estimates of heritability did not differ very much across generations (table 5), because increases in additive genetic variance and increases in the sum of environmental and age variance were roughly proportional.

Table 3

Standardized Parameter Estimates of Best-Fitting Univariate Models for Measured Lipids, Lipoproteins, and Apolipoproteins in Young Twins and Middle-Aged Twins

	h^2	c^2	e^2	a^2	k	χ^2	df	P	AIC
Model 1				A. Young Twins					
TC:									
Males	.87	...	.13	...	...	18.20	24	.79	−29.80
Females	.68	...	.27	.05	...				
TRG:[a]									
Males	.63	...	.34	.03	...	35.43	26	.10	−16.57
Females	.63	...	.34	.03	...				
HDL:									
Males	.70	...	.30	...	...	32.66	24	.11	−15.34
Females	.70	...	.26	.04	...				
LDL:									
Males	.82	...	.18	...	...	24.66	27	.59	−29.34
Females	.82	...	.18	...	...				
ApoA1:									
Males	.84	...	.16	...	...	35.87	24	.06	−12.13
Females	.61	...	.31	.08	...				
ApoB:									
Males	.47	.34	.19	...	...	29.21	25	.26	−20.79
Females	.47	.34	.19	...	.82				
Lp(a):									
Males	.98	...	.02	...	...	26.92	25	.36	−23.08
Females	.92	...	.08	...	...				
				B. Middle-Aged Twins					
TC:									
Males	.68	...	.21	.11	...	29.75	26	.28	−22.25
Females	.68	...	.21	.11	...				
TRG:									
Males	.59	...	.39	.02	...	21.93	25	.64	−28.07
Females	.59	...	.39	.02	1.26				
HDL:[b]									
Males	.71	...	.29	...	...	33.00	25	.13	−17.00
Females	.71	...	.29	...	.89				
LDL:									
Males	.69	...	.21	.10	...	31.34	26	.22	−20.66
Females	.69	...	.21	.10	...				
ApoA1:									
Males	.58	...	.41	.01	...	28.63	25	.28	−21.37
Females	.58	...	.41	.01	.83				
ApoB:									
Males	.73	...	.20	.07	...	30.77	26	.24	−21.23
Females	.73	...	.20	.07	...				
Lp(a):									
Males	.87	...	.09	.04	...	27.68	26	.37	−24.32
Females	.87	...	.09	.04	...				

NOTE.—h^2 = heritability; e^2 = proportion of variance explained by unique environment; a^2 = proportion of variance explained by age; c^2 = proportion of variance explained by shared environment; k = scalar factor. For further abbreviations, see table 1.

[a] For TRG, R_g in the DOS group (see fig. 1) could be set to zero.

[b] For HDL in male and female twins, h^2 reflects broad sense heritability, because the best-fitting model contained dominance variance: $d^2 = .63$.

Table 4

Nonstandardized Variance Components of Best-Fitting Extended Models

	ADULTS			ADOLESCENTS							
	V_G	V_E	V_A	V_G	V_E	V_A	g	χ^2	df	AIC	χ^2/df
MODEL	A. Lipids and Lipoproteins										
TC (g = .5):											
Males	.734	.262	. . .	.363	.054	. . .	.50	237.83	159	−80.17	1.50
Females	.734	.262	.165	.363	.168	.042					
TC (g free):											
Males	.758	.231	. . .	.354	.053	. . .	.40	234.02	158	−81.98	1.48
Females	.758	.231	.159	.354	.157	.039					
TRG (g = .5):											
Males	.029	.016	. . .	.015	.009	.001	.50	223.78	159	−94.22	1.41
Females	.013	.016	.001	.015	.009	.001					
TRG (g free):											
Males	.031	.014	. . .	.015	.008	.001	.23	211.03	158	−106.07	1.34
Females	.015	.014	.001	.015	.008	.001					
HDL (g = .5):											
Males	.051	.029	. . .	.027	.016	.001	.50	258.84	159	−59.17	1.63
Females	.051	.029	. . .	.058	.016	.001					
HDL (g free):											
Males	.055	.024	. . .	.027	.016	.001	.33	251.82	158	−64.18	1.59
Females	.055	.024	. . .	.054	.016	.002					
LDL (g = .5):											
Males	.649	.233	. . .	.359	.082	. . .	.50	245.39	161	−76.61	1.52
Females	.649	.233	.110	.359	.082	. . .					
LDL (g free):											
Males	.677	.197		.344	.079	. . .	.38	239.69	160	−80.31	1.50
Females	.677	.197	.108	.344	.079	. . .					
	B. Apolipoproteins and Lipoprotein(a)										
ApoA1 (g = .5):											
Males	.027	.033	. . .	.021	.003	. . .	.50	287.92	159	−30.08	1.81
Females	.043	.033	.003	.021	.016	.004					
ApoA1 (g free):											
Males	.027	.033	. . .	.021	.003	. . .	.50	287.92	158	−28.08	1.82
Females	.043	.033	.003	.021	.016	.004					
ApoB (g = .5):[a]											
Males	.048	.026	. . .	.013	.011	. . .	.50	298.89	157	−15.11	1.90
Females	.048	.026	.007	.013	.020	.002					
ApoB (g free):											
Males	.048	.026	. . .	.012	.011	. . .	.12	298.41	156	−13.59	1.91
Females	.048	.026	.007	.012	.021	.002					
Lp(a) (g = .5):											
Males	.272	.031	.004	.296	.006	. . .	.50	235.38	160	−84.62	1.47
Females	.272	.031	.004	.253	.023	. . .					
Lp(a) (g free):											
Males	.272	.031	.004	.296	.006	. . .	.50	235.38	159	−82.62	1.48
Females	.272	.031	.004	.253	.023	. . .					

NOTE.—V_G = additive genetic variance; V_E = unique environmental variance; V_A = variance due to age; g = genetic transmission coefficient; χ^2/df = Jöreskog's χ^2/df. The best-fitting model in which g is fixed at .5 and the one in which g is estimated are shown. For further abbreviations see table 1.

[a] For ApoB, z = .18 in the model in which g is fixed at .5, and z = .41 in the model in which g is estimated.

Table 5

Standardized Parameter Estimates of Best-Fitting Extended Models for Lipids, Lipoproteins, Apolipoproteins, and Lp(a)

	Adults		Adolescents	
Model	h^2	a^2	h^2	a^2
	Lipids and Lipoproteins			
TC:				
Males	.77	...	.87	...
Females	.66	.14	.64	.07
TRG:				
Males	.69	...	.61	.04
Females	.51	.03	.61	.04
HDL:				
Males	.69	...	.61	.03
Females	.69	...	.76	.02
LDL:				
Males	.77	...	.81	...
Females	.69	.11	.81	...
	Apolipoproteins and Lp(a)			
ApoA1:				
Males	.46	...	.87	...
Females	.55	.03	.51	.09
ApoB:				
Males	.65	...	.52	...
Females	.59	.08	.37	.05
Lp(a):				
Males	.89	.01	.98	.02
Females	.89	.01	.92	.08

NOTE.—Proportions of variance explained by additive genes (h^2) and age (a^2) are shown. The remaining proportion (not shown) is explained by unique environment (e^2). For lipids and lipoproteins, standardized estimates of models in which g is estimated are shown. For apolipoproteins and Lp(a), standardized estimates of models in which g is fixed at .5 are shown.

Only for ApoA1 in males were heritabilities substantially lower in adults, because of a greater increase in unique environmental variance. For ApoB, heritability was larger in adults, because of the disappearance of the influence of shared environment.

Discussion

The main aim of this study was to investigate and disentangle the genetic and nongenetic causes of stability and change in lipid, lipoprotein, and apolipoprotein levels that occur during the lifespan. To achieve this goal, data from two different research projects were combined. In the first project, parents with their twin offspring were measured. In the second project, data from middle-aged twins were collected. Combining the data of both projects enabled the specification of an extended quantitative genetic model in which the genetic transmission from parent to offspring could be estimated. Evidence was found for the genetic transmission coefficient (g) to be $<.5$ for total cholesterol, triglycerides, HDL, and LDL, which indicates that partly different genes influence these variables in childhood compared to adulthood. Values for the genetic correlation across time, or the part of the genetic variance that is common to both age groups, ranged from 46% in triglycerides to 80% in total cholesterol, with intermediate values for HDL and LDL. The remaining genetic variance can thus be attributed to age-specific genetic effects. In contrast to the results for lipids and lipoproteins, for apolipoproteins and Lp(a) evidence was found that the *same* genes act in childhood and in adulthood.

Our study compared middle-aged parents (and twins of similar age) with their adolescent offspring. Adolescence in both sexes and the menopausal period in females are associated with substantial changes in lipid concentrations (Berenson et al. 1981; Matthews et al. 1989; Schaefer et al. 1994; Twisk et al. 1995). In these periods, large changes take place in production of sex hormones, which may cause lipid changes through their effect on LDL receptors and important lipid enzymes like hepatic lipase, lipoprotein lipase, and lecithin:cholesterol acyltransferase (Lobo 1990; Krauss 1991; Seed 1991). The effect of female sex hormones on lipid metabolism can be illustrated by two findings from our own data: postmenopausal women in the middle-aged twin group showed significantly higher levels of total cholesterol, LDL, and ApoB; women using contraceptives had higher levels of HDL, ApoA1, and triglycerides. Differences remained significant after correction for age. These effects are in accordance with the literature (Lobo 1990; Seed 1991). It is not unthinkable that timing as well as magnitude of changes in both male and female sex hormones are controlled by specific genes. It might therefore not be too surprising that a substantial part of the variance in lipids and lipoproteins was specific to either adolescence or middle age.

In contrast to our study, which compared children around their puberty with parents in their middle age, Williams and Wijesiri (1993) studied stability of genetic influences during middle age. They analyzed longitudinal data of male veteran twins on total cholesterol, LDL, HDL, and triglycerides. Between 48 and 63 years of age, subjects were measured three times. As evidenced by the large correlations between genetic effects at successive examinations, similar genetic effects on lipids appear to be present throughout this segment of the lifespan.

In accordance with the literature, we found larger variances in the parental generation (Pooling Project Research Group 1978; Reilly et al. 1990; Kronmal et al. 1993; Verschuren et al. 1993; Schaefer et al. 1994). The observed generation effect on the variance of lipid parameters may reflect the weakening of homeostatic

control mechanisms of the lipid system with aging (Reilly et al. 1990). Larger variances in the parental generation could be ascribed to increases in both unique environmental and additive genetic variance from childhood to adulthood. An explanation for the larger unique environmental variance in adults may be that they are exposed to a larger range of environmental variations like variation in diet, exercise, stress, and alcohol or cigarette consumption, each of which may affect lipid metabolism. An increase in additive genetic influence, on the other hand, could have been caused by a less tight gene regulation of homeostasis with increasing age (Reilly et al. 1990). Increases were roughly proportional for both sources of variance, which led to heritabilities of similar magnitude in the parental generation. Furthermore, observed heritabilities for most lipid variables did not differ much between males and females. Both the magnitude of observed heritabilities and the lack of a clear age trend in heritabilities are in accordance with the literature (Snieder 1996). This implicates that the relative influence of additive genes on total cholesterol, LDL, HDL, and triglycerides remains more or less the same with age, although the genes are partly different at different ages.

Boomsma et al. (1996) found a large decrease in heritabilities of parents compared to their offspring for all measured lipid variables. Thus, conclusions based on data from parents and their twin offspring only were quite different from the results of our study that included extra information from middle-aged twins. If only data from parents and offspring are available, heritabilities cannot be estimated for each generation separately, because the middle-aged twin group is absent. Without the extra information from the middle-aged twin group, one has to assume that the same genes are expressed in parents and their offspring. This assumption proved to be incorrect for total cholesterol, LDL, HDL, and triglycerides, which explains observed differences in heritability estimates of the parental generation.

Using the same parent-offspring model, no intergenerational differences in heritability were observed in the same sample for plasma Lp(a) concentrations (Boomsma et al. 1993). Our study showed that the same genes influence Lp(a) in childhood and in adulthood, which means that the assumption of a perfect genetic correlation across time was valid in this case. This finding did not come as surprise, because it is in accordance with evidence that variation in Lp(a) is probably determined by a single gene. Boerwinkle et al. (1992) found that >90% of the variation in Lp(a) concentration could be ascribed to the apo(a) gene (see also DeMeester et al. 1995), which is located on the tip of the long arm of chromosome 6 (Utermann 1989; Schulte et al. 1994). Wang et al. (1992) concluded that the apo(a) gene is fully expressed before the age of 1 year. In their study of 5–13-mo-old babies and their parents, they observed parent-offspring correlations that already were of the same magnitude as the parent-offspring and DZ twin correlations in this study.

For apolipoproteins, there also was no evidence of age-specific genetic influences. ApoB was the only variable for which (a small) environmental transmission proved to be significant. In combination with the significant spouse correlation, the shared environment in young twins, and the lack of its influence in middle-aged twins, these data point to one or more environmental factors that have their influence on family members, but only for as long as they live together.

The difference in age dependency of genes influencing total cholesterol, LDL, HDL, and triglycerides, compared to those that influence ApoA1 and ApoB, is an interesting finding that offers ground for further speculation. Apolipoproteins serve some basic physiological functions in metabolism of other lipoprotein particles, for example by acting as a ligand for cell-surface receptors (Rader and Brewer 1994). Their more basic function is supported by findings that indicate that apolipoproteins better discriminate atherosclerotic patients from controls than plasma lipids and lipoproteins (Avogaro et al. 1979; Durrington et al. 1988). Complex segregation studies found evidence for major gene effects on ApoA1 and ApoB (Hopkins and Williams 1989; Schulte et al. 1993), probably pointing to the influence of the apolipoprotein genes themselves, which have been mapped to chromosome 11 (ApoA1) and chromosome 2 (ApoB) (Weiss 1993). The difference in age-specific genetic influence in lipids and lipoproteins compared to the apolipoproteins might point to a simpler genetic architecture for the apolipoproteins. In contrast to the genetic architecture of cholesterol, triglycerides, LDL, and HDL in which, besides some major gene effects, most genetic variation is polygenic (Sing and Moll 1989, 1990; Hopkins and Williams 1989; Weiss 1993), genetic variation in apolipoproteins may be determined by one (just like in Lp[a]) or a few major genes. Moreover, expression of these genes is not dependent on age, as is apparent from this study.

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