Heritabilities of Apolipoprotein and Lipid Levels in Three Countries

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This study investigated the influence of genes and environment on the variation of apolipoprotein and lipid levels, which are important intermediate phenotypes in the pathways toward cardiovascular disease. Heritability estimates are presented, including those for apolipoprotein E and AII levels which have rarely been reported before. We studied twin samples from the Netherlands (two cohorts; *n* = 160 pairs, aged 13–22 and *n* = 204 pairs, aged 34–62), Australia (*n* = 1362 pairs, aged 28–92) and Sweden (*n* = 302 pairs, aged 42–88). The variation of apolipoprotein and lipid levels depended largely on the influences of additive genetic factors in each twin sample. There was no significant evidence for the influence of common environment. No sex differences in heritability estimates for any phenotype in any of the samples were observed. Heritabilities ranged from 0.48–0.87, with most heritabilities exceeding 0.60. The heritability estimates in the Dutch samples were significantly higher than in the Australian sample. The heritabilities for the Swedish were intermediate to the Dutch and the Australian samples and not significantly different from the heritabilities in these other two samples. Although sample specific effects are present, we have shown that genes play a major role in determining the variance of apolipoprotein and lipid levels in four independent twin samples from three different countries.

Apolipoprotein and lipid profiles are important determinants of cardiovascular risk (Rifai et al., 1988). Knowledge of the environmental and genetic influences on these levels is relevant for an understanding of the process of cardiovascular disease. Genes described to date that promote atherosclerosis have mainly been identified in familial syndromes of lipid disorders, many of which are monogenic diseases. For example, specific defects in the genes encoding the low-density-lipoprotein-receptor and apolipoprotein B are found to be responsible for the most common forms of familial hypercholesterolemia (Hansen, 1998; Hobbs et al., 1992; Ludwig et al., 1997). Furthermore, mutations in the ABC1 gene, encoding the cholesterol efflux regulatory protein, induce familial HDL cholesterol deficiency (Brooks-Wilson et al., 1999). However, while these specific mutations have a profound implication for persons with monogenic familial syndromes, they explain only a minor proportion of the population variation in serum apolipoprotein and lipid levels (Goldstein et al., 1973). Hence, it is relevant to estimate the importance of genetic influences in the intermediate phenotypes in the general population. Given significant genetic influences, efforts can be undertaken to identify the genes influencing the variation of these intermediate phenotypes of cardiovascular disease in the population.

In general the heritability, defined as the proportion of the population variation attributable to genetic variation, of total cholesterol, low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol and triglyceride levels is larger than 0.50. Genetic factors also explained more than 50% of the total variance in apolipoprotein AI and B. No obvious age trend in heritability estimates could be detected and none of the previously reported studies found much support for a considerable influence of common environment on the variance of apolipoprotein and lipid levels (Hamsten et al., 1986; Snieder et al., 1999). Many previously reported heritability studies on apolipoprotein and lipid levels focus mainly on lipid levels and lack heritability estimates on apolipoprotein E and AII levels.

In this paper we report the variance components attributable to intermediate phenotypes of cardiovascular disease, including apolipoprotein E and AII levels, compared in

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four twin samples with different mean age and geographical origin. We studied a young Dutch sample (aged 13–22) of 160 twin pairs (Boomsma et al., 1996) and a middle-aged Dutch sample (aged 34–62) of 204 twin pairs (Snieder et al., 1997). A Swedish twin sample (aged 42–88) provided data on 146 twin pairs reared apart and 156 twin pairs reared together (Heller et al., 1993). Heritability estimates for some lipid and apolipoprotein levels have been reported before for the Dutch and Swedish samples. This paper reports for the first time on the heritability of apolipoprotein E level in the middle-aged Dutch sample. The largest sample we studied was an Australian twin sample (aged 28–92) of 1362 twin pairs with data on apolipoprotein and lipid levels, including apolipoprotein E and AII level. Heritabilities for apolipoprotein and lipid levels in these Australian twins have not been reported previously. Each complete sample was investigated independently using the same model in variance components analyses, testing whether genes, common environment, unique environment and sex have influences on the variation of apolipoprotein and lipid levels.

Subjects and Methods

Subjects

Since different sampling methods have been used in the Netherlands, Australia and Sweden all within different time and age ranges, the subjects and methods used will be described for each twin sample.

Young Dutch Twin Sample

The young Dutch twin sample is part of a larger study in which cardiovascular risk factors were determined in adolescent twin pairs and their parents (Boomsma et al., 1993; Boomsma et al., 1996). The data reported here were collected between 1988 and 1992 in a subgroup of 160 pairs of twins between 13 and 22 years of age. Addresses of twins living in Amsterdam and neighbouring 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 if the twin 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. Three triplets were included by discarding the data of the second-born subject. None of the twin pairs reported taking lipid lowering medication.

Zygosity was determined by typing 11 blood group polymorphisms. Zygosity in the dizygotic pairs was confirmed using 103 microsatellite markers on 10 chromosomes typed as part of a genome wide search planned to comprise 229 markers. In total, there were 35 monozygotic male twin pairs (MZM), 31 dizygotic male twin pairs (DZM), 35 monozygotic female twin pairs (MZF), 30 dizygotic female twin pairs (DZF) and 29 dizygotic twin pairs of opposite sex (DOS).

Middle-aged Dutch Twin Sample

The middle-aged Dutch twin sample is also part of the larger study (Snieder et al., 1997). The data reported here were collected between 1992 and 1996 in a group of 213 pairs of twins between 34 and 62 years of age. Twins were recruited by a variety of means, including advertisement in the media, advertisement in the information bulletin of the Dutch Twin Registry and solicitation through the Dutch Twin Club. In addition, a small number of twins who heard of the study from other twins volunteered to participate. One triplet was included by discarding the data of the second-born subject. Data of 9 twin pairs were excluded from the sample. In 8 of these twins one or both members of the pair used lipid lowering medication (HMG-CoA reductase inhibitors) and in one subject no blood could be obtained.

Zygosity was decided on the basis of the response to standard questions about physical similarity and the degree to which others confused them. In 76 same-sex twin pairs zygosity was also determined by DNA fingerprinting and in 98.7% of those twin pairs zygosity was correctly classified. Zygosity of the dizygotic twin pairs was confirmed using 103 microsatellite markers in a partial genome scan. In total, there were 41 pairs of MZM, 35 pairs of DZM, 50 pairs of MZF, 40 pairs of DZF and 38 pairs of DOS.

Australian Twin Pair Sample

The Australian twin sample is part of the Semi-Structured Assessment for the Genetics of Alcoholism study (Heath et al., 1997). The data reported here were collected between 1993 and 1996 in a subgroup of 1403 pairs of twins between 28 and 92 years of age. Twins were recruited through the Australian NHMRC Twin Registry. Data of 41 twin pairs were excluded from the sample, since one or both members of the pair used lipid lowering medication.

Zygosity was decided on the basis of the response to standard questions about physical similarity and the degree to which others confused them. Pairs giving inconsistent responses were contacted for clarification. In 329 same-sex twin pairs zygosity was also confirmed by typing 11 highly polymorphic markers and in 98.5% of those twin pairs the zygosity was correctly classified. Of the pairs who were classified as dizygotic, zygosity of 263 pairs was confirmed using 103 microsatellite markers in a partial genome scan. In total, there were 194 pairs MZM, 107 pairs of DZM, 517 pairs of MZF, 272 pairs of DZF and 272 pairs of DOS.

Swedish Twin Pair Sample

The Swedish twin sample is part of the larger Swedish Adoption/Twin Study of Aging sample (Pedersen et al., 1984; Pedersen et al., 1991). The data reported here were collected between 1986 and 1988 in a subgroup of 302 pairs of twins with the same sex between 42 and 88 years of age. There are 146 twin pairs reared apart and 156 twin pairs reared together included in this study. All the older adults in this study reported not taking lipid lowering medication.

Zygosity was determined by typing serologic markers (Pedersen et al., 1984). Of the dizygotic twins reared together, zygosity of 44 twin pairs was confirmed using 103 microsatellite markers in a partial genome scan. In total, there were 22 pairs of MZM reared apart, 31 pairs of DZM reared apart, 24 pairs of MZF reared apart and 69 pairs of DZF reared apart. In addition, there were 27 pairs of

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MZM reared together, 38 pairs of DZM reared together, 42 pairs of MZF reared together and 49 pairs of DZF reared together.

Method

Young Dutch Twin Sample

The subjects were requested to fast from 23:00 PM the preceding night. Blood was taken between 08:30 AM and 10:30 AM by venipuncture using Vacutainer tubes (Becton-Dickinson) containing sodium-EDTA. The tubes were placed on ice and centrifuged promptly (10 min, 3,000 rpm) at 4°C to separate plasma from the cells. Part of plasma was kept on 4°C for lipid determination within the next 5 days. The remainder was frozen using liquid nitrogen and stored at –20°C until processing.

Apolipoprotein E was quantified by enzyme-linked immunosorbent assays (ELISA) (Bury et al., 1986). Apolipoprotein AII, AI and B were quantified by radial immunodiffusion as described by Albers (Albers et al., 1976) and Havekes (Havekes et al., 1981).

Cholesterol and triglycerides were measured using enzymatic methods (Boehringer Mannheim, FRG, CHOD _PAP kit number 236691 and GPO-PAP kit number 701904). High density lipoprotein (HDL) cholesterol was measured after lipoproteins containing apolipoprotein B were precipitated with phosphotungstate-magnesium chloride (Lopes-Virella et al., 1977).

Middle-aged Dutch Twin Sample

The subjects were requested to fast from 23:00 PM the preceding night. Blood was taken at about 10:00 AM 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 the cells. Aliquots of plasma were frozen using liquid nitrogen and stored at –20°C until processing.

Apolipoprotein E was quantified by enzyme-linked immunosorbent assays (ELISA) (Jong et al., 1999). Apolipoprotein AII plasma levels were quantified by radial immunodiffusion as described by Albers (Albers et al., 1976) and Zonderland (Zonderland et al., 1984).

The MZ twin correlation coefficients for apoAII level were about 0.25 as compared to > 0.60 for all other phenotypes in this middle-aged Dutch twin sample. This suggests a measurement problem and therefore, the apolipoprotein AII levels in the middle-aged Dutch twins were not analysed.

Apolipoprotein AI and B were quantified by the method of Beckman using the Array Protein System (Beckman Instruments) (Maciejko et al., 1987). The Beckman calibrator (standardised to the International Federation for Clinical Chemistry, which is traceable to the World Health Organisation International Reference Material for ApoAI and ApoB no. 1883) was used as standard reference material. Monospecific goat-antihuman ApoAI and ApoB antibodies were used (Beckman). Total cholesterol, triglyceride and HDL cholesterol levels were determined in the same way as for the young Dutch twin sample.

Australian Twin Sample

Blood was taken from the subjects throughout the day, depending on their availability. The time since the last meal was recorded. Aliquots of plasma were frozen and stored at –70°C until analysis.

Plasma levels of apolipoprotein E, AII, AI, B and E were quantified on a Behring nephelometer with Behring reagents. Total cholesterol and triglyceride levels were measured on a Hitachi 747 analyser with Boehringer reagents by standard enzymatic methods (Bucolo & David, 1973). HDL cholesterol was measured after lipoproteins containing apolipoprotein B were precipitated with dextran sulphate magnesium chloride (Warnick et al., 1982).

Swedish Twin Sample

The subjects were requested to fast for 12 hours before blood was taken. Blood samples were frozen at –70°C, transported in dry ice, and thawed and analysed when received by the laboratory.

Apolipoprotein AI and B levels were quantified using commercial radioimmunoassay kits (RIA 100, Pharmacia Diagnostics, Uppsala, Sweden). Total cholesterol and triglyceride levels were measured with an enzymatic colorimetric assay (Boehringer Mannheim automated analysis for Hitachi systems 717, Diagnostica, Mannheim, Germany). HDL cholesterol was measured after lipoproteins containing apolipoprotein B were precipitated with phosphotungstate-magnesium chloride (Seigler & Wu, 1981).

In all samples, concentrations of low density lipoprotein (LDL) cholesterol were estimated according to Friedewald (Friedewald et al., 1972), when triglyceride concentrations did not exceed 4.52 mmol/L (Rifai et al., 1992). LDL cholesterol level was considered missing, when the triglyceride concentration did exceed 4.52 mmol/L, which was the case for 1 individual of the middle-aged Dutch sample, for 82 individuals of the Australian sample and for 11 individuals of the Swedish sample.

Statistical Analyses

To study the contribution of genetic and environmental factors to apolipoprotein and lipid levels variability, a structural modelling approach was used. Univariate models were fitted to the data by the method of maximum likelihood. A univariate model was tested in which the phenotypic variance is divided into an additive genetic, a common environment (including family environment) and a unique environment component. The additive genetic component reflects the heritability of a trait and the common environment reflects the shared-family environment. Since the Swedish twin sample consists of twins reared together and apart, an additional correlated environment component, which may reflect, for example, prenatal influence or post-rearing contact, was initially present in the Swedish model. However, correlated environment did not significantly influence the total variance of any of the phenotypes and is therefore not shown in this paper. Model fitting was carried out directly on the raw data. This allows us to model the covariance structure between family members simultaneously with any covariate effects on the means. In all analyses, means were adjusted for sex and age differences between pairs.

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To study sex differences in genetic influences, a model with different parameter estimates for men and women for additive genetic and both environmental components was fitted to the data first, followed by a model in which parameter estimates for the different variance components were constrained to be equal across sexes. We also fitted a sex limitation scalar model, in which the heritabilities are constrained to be equal across sexes, but total variances are allowed to be different; variance components for males are constrained to be equal to a scalar multiple of the female variance components. As a result, the standardised variance components such as heritability are equal across sexes, even though the non-standardised components differ (Neale & Cardon, 1992).

The correlation between the additive genetic factors in dizygotic twins of opposite sex was free to be estimated between 0 and 0.5. When this correlation is estimated significantly smaller than 0.5, different genes in men and women influence the same phenotype, even though the proportion of the variance attributed to genetic factors may be the same.

By the principle of parsimony, the pattern of variances and covariances should be explained by as few parameters as possible. Therefore, the most extensive models were reduced by excluding respectively the genetic or common environmental component from the model for men and women separately. When the best fitting model was the same for men and women, common parameters were equated between sexes. Sub-models were compared to the most extensive model by hierarchic χ^2 tests. The difference in –2ln(likelihood) of the goodness-of-fit of each model is approximated by a chi-square distribution, with degrees of freedom equal to the difference in number of parameters estimated in the two models. A significant increase in the –2ln(likelihood) after a parameter has been excluded from the model indicates that the reduced model fits the data less well than the most extensive model.

Because the distribution of the values for triglyceride level was skewed in all samples, these values were transformed by natural logarithm. All samples were analysed separately. Model fitting was performed using Mx version 1.50 (Neale et al., 1999). Confidence intervals around standardised variance components (e.g. heritabilities) were also obtained from Mx.

Results

Descriptives

Table 1 shows means and standard deviations for levels of apolipoprotein E and AII, levels of other apolipoproteins and lipids in each sample for men and women separately. In all samples women had higher HDL cholesterol and apolipoprotein AI levels than men. Middle-aged Dutch and Australian men had higher mean values than middle-aged Dutch and Australian women for LDL cholesterol, apolipoprotein B and triglyceride level. In younger Dutch and older Swedish subjects this sex difference seemed to be absent.

Twin Correlation Coefficients

Twin correlation coefficients for the traits for each twin sample are shown by zygosity groups for men and women in table 2a–d. Correlation coefficients for apolipoprotein E levels were available in the Dutch young and middle-aged twins and in the Australian sample. In monozygotic (MZ) twins the correlations for apolipoprotein E levels were higher than in dizygotic (DZ) twins, which is a strong indication that genetic factors influence these levels. Correlation coefficients for apolipoprotein AII levels were only available in the young Dutch and Australian twin sample, and these correlations also suggested the influence of genetic factors. This was also the case for the other apolipoprotein and lipid levels.

In the young Dutch twin pairs (Table 2a), the correlation coefficients for total cholesterol and LDL cholesterol in DZ men, but not in women, are relatively low. However, it is unlikely that this low correlation reflects an error in the LDL cholesterol measurements of the young Dutch DZ men, since the phenotypic measurements were performed randomly over the young Dutch sample.

Table 1

Descriptives of the Twin Samples, Means (and Standard Deviations) for Each Sample by Sex, for Levels of Apolipoprotein E, AII, AI and B and for Levels of Total, Low-density-lipoprotein, High-density-lipoprotein Cholesterol and the Natural Log of Triglyceride Levels

Twin sample	Young Dutch twins		Middle-aged Dutch twins		Australian twins		Swedish twins	
Mean (SD)	Men	Women	Men	Women	Men	Women	Men	Women
Number of individuals	161	159	192	218	874	1850	234	350
Age (yr)	16.77 (1.78)	16.71 (2.20)	43.55 (6.47)	44.70 (6.79)	44.10 (10.41)	45.88 (11.69)	65.02 (7.51)	66.09 (8.99)
Apolipoprotein E (mg/dL)	6.03 (2.06)	7.58 (2.42)	2.50 (1.08)	2.57 (0.97)	4.28 (1.89)	3.87 (1.62)	— —	— —
Apolipoprotein AII (g/L)	0.58 (0.07)	0.58 (0.08)	— —	— —	0.35 (0.06)	0.36 (0.07)	— —	— —
Apolipoprotein AI (g/L)	1.34 (0.15)	1.44 (0.23)	1.50 (0.35)	1.93 (0.38)	1.32 (0.22)	1.51 (0.29)	1.27 (0.24)	1.48 (0.28)
Apolipoprotein B (g/L)	0.78 (0.15)	0.80 (0.19)	1.27 (0.34)	1.19 (0.34)	1.05 (0.25)	0.94 (0.25)	1.07 (0.21)	1.12 (0.26)
Total cholesterol (mmol/L)	4.05 (0.65)	4.47 (0.86)	5.39 (1.03)	5.46 (1.04)	5.71 (1.05)	5.60 (1.08)	6.35 (1.10)	7.20 (1.46)
LDL cholesterol (mmol/L)	2.52 (0.63)	2.76 (0.76)	3.71 (0.97)	3.60 (0.97)	3.55 (0.91)	3.37 (0.99)	4.28 (1.01)	4.87 (1.30)
HDL cholesterol (mmol/L)	1.23 (0.22)	1.38 (0.29)	1.07 (0.29)	1.39 (0.33)	1.23 (0.29)	1.56 (0.39)	1.32 (0.36)	1.60 (0.44)
Ln(Triglycerides) (mmol/L)	–0.49 (0.39)	–0.41 (0.36)	0.16 (0.52)	–0.04 (0.40)	0.62 (0.59)	0.29 (0.54)	0.40 (0.51)	0.35 (0.46)

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The MZ correlations in the middle-aged Dutch twins (Table 2b) are higher than the DZ correlations for all phenotypes, indicating that genetic factors influence these traits.

In the Australian sample (Table 2c), the correlation coefficients in DZ twins of opposite sex for apolipoprotein B, total cholesterol, LDL, HDL and triglyceride level seem to be lower than the correlation coefficients of DZ twins of the same sex. This may indicate that different genes affect these traits in men and women.

In the Swedish men (Table 2d), the DZ correlations are more than twice as low as the MZ correlations, which could indicate that dominant genetic factors might influence these traits.

Structural Modelling

Figure 1 shows the proportion of the variances estimated under the conditions of the most extensive model, including a separate additive genetic, common and unique

Table 2a

Twin Correlation Coefficients in the Young Dutch Twin Sample

Young Dutch twins Sex	Men		Women		DOS
Zygosity (number of pairs)	MZ (35)	DZ (31)	MZ (35)	DZ (30)	DOS (29)
ApoE	0.87	0.38	0.86	0.17	0.41
ApoAII	0.90	0.46	0.81	0.60	0.30
ApoAI	0.86	0.34	0.77	0.50	0.53
ApoB	0.83	0.55	0.82	0.60	0.71
Total cholesterol	0.90	0.11	0.81	0.63	0.41
LDL cholesterol	0.89	0.22	0.83	0.56	0.44
HDL cholesterol	0.73	0.48	0.81	0.47	0.57
Triglycerides	0.59	0.59	0.71	0.32	0.17

Table 2b

Twin Correlation Coefficients in the Middle-aged Dutch Twin Sample

Middle-aged Dutch twins Sex	Men		Women		DOS
Zygosity (number of pairs)	MZ (42)	DZ (35)	MZ (50)	DZ (40)	DOS (38)
ApoE	0.82	0.48	0.92	0.35	0.46
ApoAII	—	—	—	—	—
ApoAI	0.73	0.56	0.67	0.49	0.47
ApoB	0.80	0.67	0.78	0.30	0.35
Total cholesterol	0.75	0.55	0.79	0.41	0.46
LDL cholesterol	0.77	0.65	0.80	0.27	0.37
HDL cholesterol	0.61	0.34	0.70	0.37	0.17
Triglycerides	0.62	0.47	0.60	0.40	0.43

Table 2c

Twin Correlation Coefficients in the Australian Twin Sample

Australian twins Sex	Men		Women		DOS
Zygosity (number of pairs)	MZ (194)	DZ (107)	MZ (517)	DZ (272)	DOS (272)
ApoE	0.51	0.17	0.65	0.33	0.28
ApoAII	0.54	0.17	0.44	0.24	0.23
ApoAI	0.34	0.22	0.56	0.22	0.18
ApoB	0.61	0.48	0.68	0.39	0.28
Total cholesterol	0.63	0.41	0.60	0.43	0.22
LDL cholesterol	0.61	0.39	0.63	0.44	0.22
HDL cholesterol	0.57	0.41	0.64	0.29	0.22
Triglycerides	0.52	0.40	0.55	0.41	0.25

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Table 2d

Twin Correlation Coefficients in the Swedish Twin Sample

Swedish twins Sex	Men		Women		DOS
Zygosity (number of pairs)	MZ (49)	DZ (69)	MZ (66)	DZ (118)	DOS
ApoE	—	—	—	—	—
ApoAII	—	—	—	—	—
ApoAI	0.70	0.15	0.57	0.31	—
ApoB	0.60	0.16	0.77	0.39	—
Total cholesterol	0.54	0.11	0.61	0.41	—
LDL cholesterol	0.53	0.20	0.56	0.42	—
HDL cholesterol	0.71	0.21	0.61	0.32	—
Triglycerides	0.42	0.15	0.63	0.34	—

environmental variance component for men and women in each sample. In the Australian sample, genetic factors explain 46–60% of the variance of apolipoprotein E levels and in the Dutch young and middle-aged samples 85–89%. Common environment explains at most 4% of the total variance of apolipoprotein E level.

Under the full model, genetic factors explain 36–40% of the total variance of apolipoprotein AII level in women and 58–84% in men. Common environment may play a larger role in women than in men. The proportions of the variance explained by common environment are, respectively, 0.10–0.35 and 0.0–0.02, under the full model.

For apolipoprotein AI and B levels, 21–70% of the variance is determined by genetic influences and 0–48% of the variance can be explained by common environment. Between 21–85% of the variance of lipid levels is determined by genetic factors and 0–43% by common environmental factors.

Overall, genetic factors seem to play a larger role than common environment in explaining inter-individual differences in levels of apolipoproteins and lipids.

After fitting the most extensive model, sub-models were compared to the full model by hierarchic χ^2 tests to find a model to explain the data that is as simple as possible. A model in which the variance was only explained by common and unique environment showed in all analyses a significant worse fit than the full model. A model in which the variance was only influenced by a genetic and a unique environmental factor (AE model) did not show a significantly worse fit than the full model in any of the analyses. Therefore, common environment does not play a significant role in determining the levels of apolipoproteins and lipids in any of the samples. In table 3, the genetic variance versus the total variance is listed for men and women separately. Also the heritability estimates and the 95% confidence interval for all traits are listed for the most parsimonious models separately in each sample. All heritabilities were equal across sexes in every sample. In some analyses the scalar model was the best fitting model (Table 3, indicated with [b]), in which the proportion of the variance attributable to genetic factors is equal across sexes, even though the non-standardised estimates for the genetic effect differ.

Since samples are all collected at different times and centres and since the measurements on these samples were all at different laboratories using slightly different methods, it is not surprising that absolute values of the variances differ over the samples. Unfortunately, we are unable to tell which of these factors is responsible for the differences in variance components, but the 95% confidence intervals of the heritability permit comparisons between the four samples.

The heritability estimates for apolipoprotein E level range from 0.57 in the Australian sample to 0.87 in the middle-aged Dutch sample. The heritability estimates in the Dutch samples are significantly higher than the estimate in the Australian sample. Also the heritability estimates for apolipoprotein AII level in Australian sample (0.48) is significantly lower than in the young Dutch sample (0.82).

For apolipoprotein AI and B levels, the heritability ranges from 0.50 in the Australian twins to 0.82 in young Dutch twins. The heritability estimates in the Swedish sample lie between the estimates for the Dutch and Australian samples, which differ significantly from each other. Common environment seems not to play a significant role in the total variance of apolipoprotein AI levels in all the samples. However, its role in the variance of apolipoprotein B levels is unclear. In the young Dutch and Australian twins, models including additive genetic as wel as common environment variance had fits that were almost significantly better than the AE model (Table 3; indicated with [a]). However, in the middle-aged Dutch and Swedish samples, there is no suggestion that common environment might play a role in the inter-individual differences of apolipoprotein B levels.

The heritability estimates for lipid levels ranged from 0.48 for triglyceride level in the Swedish sample to 0.83 for LDL cholesterol level in young Dutch twins. In the Australian and Swedish samples, common environment might play a role in the variance of total cholesterol levels, since the models including additive genetic as well as common environmental variance almost fitted better than the simpler models. However, in the Dutch samples no suggestion was found for the influence of common environment on the total cholesterol levels. Furthermore, the models

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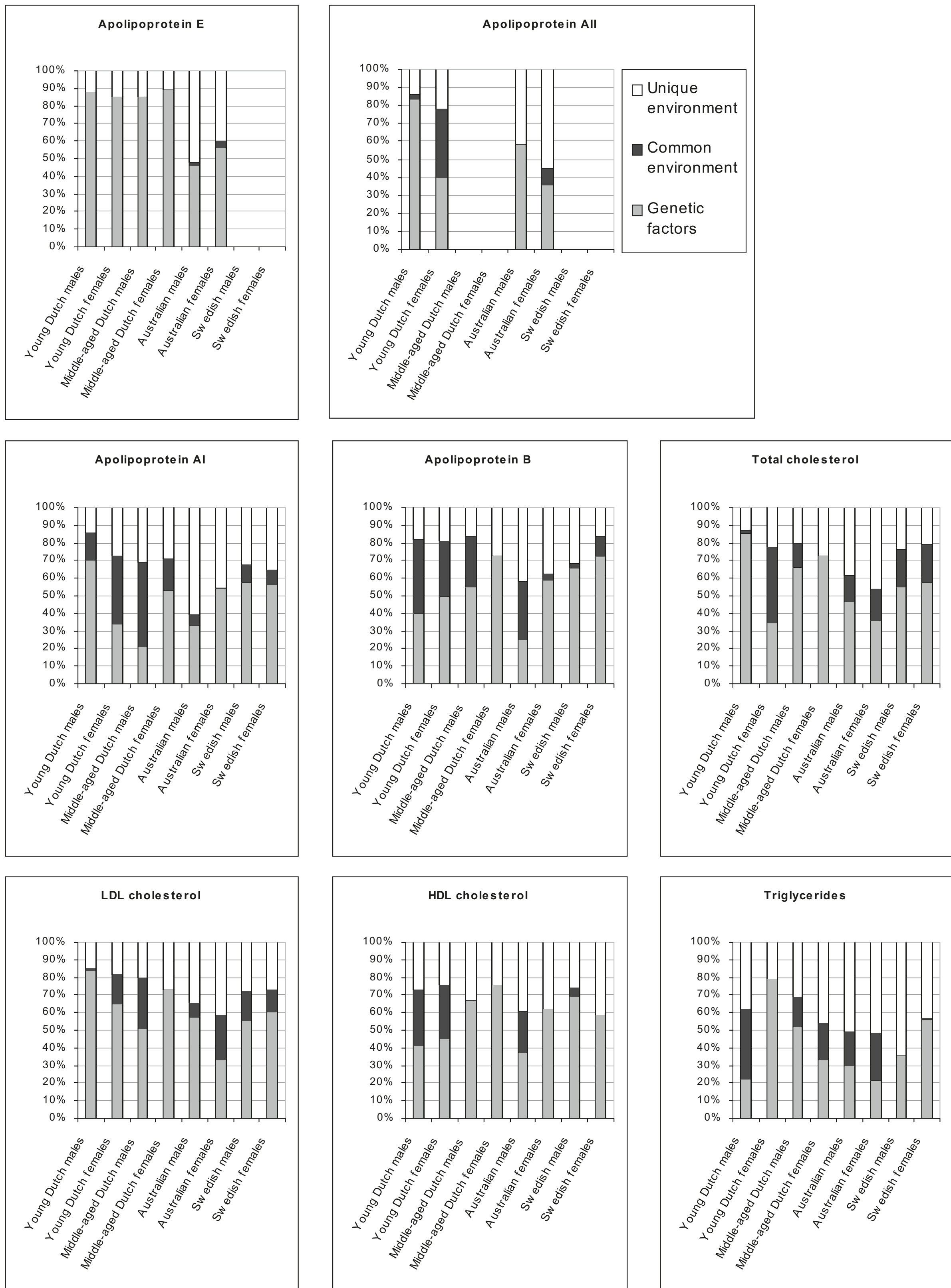

Figure 1

Proportions of variance that can be explained by influence of genes, common environment and unique environment. These proportions are estimated using the full model for each sample estimating parameters for males and females separately.

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Table 3

Genetic Variance and Total Variance for Men and Women Separately and Heritability Estimates Including the 95% Confidence Interval Between Brackets Using the AE Model that Assumes Only Genetic and Unique Environmental Influences. [a]: The Heritability Estimate Might Also Include Common Environmental Influences. [b]: The Scalar Sex Limitation Model Showed the Best Fit. [c]: The Correlation Between the Additive Genetic Factors in DOS is 0.26 for Both Total and LDL Cholesterol

Twin sample	Young Dutch twins			Middle-aged Dutch twins			Australian twins			Swedish twins		
	Genetic/Total variance		Heritability (95% C.I.)	Genetic/Total variance		Heritability (95% C.I.)	Genetic/Total variance		Heritability (95% C.I.)	Genetic/Total variance		Heritability (95% C.I.)
	Men	Women		Men	Women		Men	Women		Men	Women	
ApoE	4.368/5.079	4.368/5.079	**0.86 (0.80–0.91)**	0.884/1.016	0.884/1.016	**0.87 (0.82–0.91)**	2.045/3.588	2.045/3.588	[b]**0.57 (0.52–0.61)**	—	—	—
ApoAII	0.005/0.006	0.005/0.006	**0.82 (0.79–0.82)**	—	—	—	0.002/0.003	0.002/0.003	[b]**0.48 (0.42–0.53)**	—	—	—
ApoAI	0.036/0.045	0.017/0.021	[b]**0.80 (0.71–0.86)**	0.084/0.117	0.084/0.117	**0.72 (0.62–0.79)**	0.026/0.051	0.040/0.080	[b]**0.50 (0.45–0.55)**	0.048/0.079	0.048/0.079	**0.61 (0.49–0.70)**
ApoB	0.023/0.027	0.023/0.027	[a]**0.82 (0.75–0.87)**	0.084/0.106	0.084/0.106	**0.79 (0.71–0.85)**	0.036/0.057	0.036/0.057	[a]**0.63 (0.58–0.66)**	0.048/0.065	0.048/0.065	**0.75 (0.66–0.81)**
Total cholesterol	0.325/0.396	0.578/0.704	[b]**0.82 (0.74–0.87)**	0.740/0.961	0.740/0.961	**0.77 (0.68–0.83)**	0.608/1.067	0.593/1.040	[a, b, c]**0.57 (0.52–0.61)**	1.145/1.709	1.145/1.709	[a]**0.67 (0.56–0.76)**
LDL cholesterol	0.384/0.463	0.384/0.463	**0.83 (0.76–0.88)**	0.656/0.852	0.656/0.852	**0.77 (0.69–0.84)**	0.504/0.826	0.563/0.922	[a, b, c]**0.61 (0.57–0.65)**	1.040/1.576	1.040/1.576	**0.66 (0.55–0.75)**
HDL cholesterol	0.058/0.077	0.036/0.048	[b]**0.75 (0.65–0.82)**	0.073/0.101	0.073/0.101	**0.72 (0.60–0.80)**	0.053/0.085	0.096/0.155	[b]**0.62 (0.57–0.66)**	0.116/0.181	0.116/0.181	**0.64 (0.54–0.72)**
Triglycerides	0.090/0.127	0.109/0.153	**0.71 (0.57–0.80)**	0.096/0.155	0.168/0.271	[b]**0.62 (0.50–0.72)**	0.168/0.336	0.130/0.259	[a, b]**0.50 (0.45–0.55)**	0.102/0.213	0.102/0.213	**0.48 (0.35–0.59)**

including common environment for the Australian LDL cholesterol and triglyceride levels also nearly fitted better than AE models, although no indications for common environmental influences were observed in the other samples.

In general, the four twin samples show high heritability estimates for all apolipoprotein and lipid levels without sex differences. The effect of common environment on the variances was not significant in any of the samples. No significant difference has been observed between the heritability estimates of the two Dutch samples. The young Dutch twins show significantly higher estimates for apolipoprotein AI and LDL cholesterol levels than the Swedish twins do; while in the middle-aged Dutch twins none of the heritability estimates significantly differ from the estimates in the Swedish twins. Roughly, there seems no difference between the Dutch and Swedish twins in heritability estimates, although the Swedish estimates are consistently lower.

The most striking differences in heritability are those between the Dutch and the Australian samples, that would have to be explained by sample specific differences. The heritability estimates in the Dutch twin cohorts are significantly higher than in the Australian twins, except for HDL cholesterol and triglyceride levels.

Analysing the Australian sample for total and LDL cholesterol, models in which the correlations between the additive genetic factors of DZ twins of opposite sex were fixed to 0.5 like in DZ twins of same sex, fitted significantly worse than the models in which these correlations were free to be estimated. The correlation coefficient between the additive genetic factors of DZ twins of opposite sex was then estimated to 0.26 (Table 3, indicated with [c]). Hence, only half of the genes influencing total and LDL cholesterol levels are acting in both Australian men and women indicating that the other half of the genes are sex specific. However, this was not found in the two Dutch samples that are much smaller than the Australian sample and therefore have less power to detect sex-specific genetic effects. The DOS correlations in the Dutch samples, however, do not suggest such an effect. Since no twins of opposite sex were present in the Swedish twin pair sample, no conclusions can be drawn from this sample.

When the correlation in DZ twins was less than half of the correlation in MZ twins, models including a dominance genetic factor were examined. However, none of these models fitted significantly better than the model which only included an additive genetic and unique environmental factor.

Discussion

In this paper, data from three different countries were analysed to obtain heritabilities for intermediate phenotypes of cardiovascular disease. We found that 48 to 87% of the total variances is attributable to genetic factors. The two Dutch samples showed the highest heritabilities ranging from 0.62 to 0.87. The Swedish heritabilities ranged from 0.48 to 0.75 and the Australian heritabilities ranged from 0.48 to 0.63. The differences in heritability estimates between the Dutch samples and the Australian sample were significant. We did not find evidence for

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influences of common environment on the variance of any apolipoprotein or lipid level in any of the samples. No sex differences in heritability estimates were observed in any of the twin samples.

The total variance of apolipoprotein E levels depended largely on genetic influences. We have previously reported heritability of apolipoprotein E levels in the young Dutch twin sample (Boomsma et al., 1996). These results are now supplied with heritability estimates in the middle-aged Dutch twins of 0.87 and in the Australian twins of 0.57, which augments the role of genetic factors in determining the level of apolipoprotein E. Also a large part of the variance of apolipoprotein AII levels can be explained by genetic factors, although there are sample specific differences in the heritability estimates. The heritability estimate in the young Dutch twins was 0.82 and in the Australian twins 0.48. Our findings are in line with the few previously reported studies (Berg, 1984; Hasstedt et al., 1984; Sistonen & Ehnholm, 1980).

We found high heritabilities for apolipoprotein AI levels in all samples ranging from 0.50 in the Australian twins to 0.80 in the young Dutch twins. Heritability estimates for levels of apolipoprotein AI previously gave contradicting results. Some studies found no evidence for additive genetic influences (Sistonen & Ehnholm, 1980; Hasstedt et al., 1984) and others found heritabilities of comparable magnitude (Berg, 1984) as we report in this paper for the four samples.

The total variance of apolipoprotein B levels in young Dutch, middle-aged Dutch, Australian and Swedish twins is for 63 to 82% attributable to additive genetic influences. This corresponds to previous findings reviewed by Snieder (Snieder et al., 1999). Since the influences of common environment are almost significantly present in young Dutch and middle-aged Australian twins (Table 3, indicated with [a]), it may well be that these influences play a role in apolipoprotein B levels. However, Boomsma (Boomsma et al., 1996) showed that when the young Dutch twin sample was extended with parental data, the most parsimonious model on the apolipoprotein B levels was the model only including additive genetic and unique environmental factors. And since in the other twin samples no evidence was found for influences of common environment, we conclude that the common environment does not play a large role in the variation of apolipoprotein B levels. Furthermore, the heritability estimates for total cholesterol, LDL, HDL and triglyceride levels ranged from 0.48 to 0.87 without sex differences, which is in correspondence with previously reported heritabilities (Snieder et al., 1999).

In the Australian sample, models for total and LDL cholesterol levels, in which the correlation between the additive genetic factors of DZ twins of opposite sex were fixed to 0.5, fitted significantly worse than the models in which this correlation was free to be estimated. Hence, only half of the genes influencing total and LDL cholesterol levels are acting in both Australian men and women. This could not be confirmed in the two Dutch samples, possibly due to lack of power. Since no twins of opposite sex were present in the Swedish twin pair sample, no conclusions can be made from this sample. From a biological point of view, it seems not very likely that only in the Australian sample different genetic influences act in men and women. It may be, however, that Australian women are exposed to higher levels of hormonal treatment than Dutch women are (Shelley et al., 1995; Barentsen, 1996) that explains part of the variance in their LDL levels (Crook & Seed, 1990) and that part of the response to these hormones is genetically determined (Birley et al., 1997). Since no other study reported such genetic sex differences, we are cautious in drawing a firm conclusion.

From our data can be concluded that none of the lipid traits studied in this paper is significantly influenced by common environment. In literature, contradicting results are found in relation to common environmental factors (Rice et al., 1991; Vogler et al., 1989; Hunt et al., 1989; Ellison et al., 1999). However, since our twin samples are quite large, especially the Australian sample including 1362 twin pairs, it seems unlikely that we have lack of power in this study to obtain significance.

We have shown that large parts of the variances of intermediate phenotypes of cardiovascular disease can be explained by genetic factors. A number of candidate genes are known to influence some of these traits. For example, it is known that the *APOE* $\varepsilon2/\varepsilon3/\varepsilon4$ polymorphism influences apolipoprotein E levels (Reilly et al., 1991; Neale et al., 2000). Additionally, the T allele of the –219GT *APOE* promoter polymorphism is independently associated with lower levels of apolipoprotein E (Lambert et al., 2000). However, only approximately 20% of the genetic variance can be explained by known polymorphisms at the *APOE* locus. None of the heritabilities reported can be totally explained by known polymorphisms in candidate genes.

In this study design, the influence of age on heritability is difficult to assess. Each sample was collected in a different time period and the apolipoprotein and lipid measurements were performed in different laboratories. It is impossible to distinguish between age and geographical origin of the samples. Age effects in the Dutch twin samples have been analysed by Snieder (Snieder et al., 1997) using the data on parents of young twins in addition to the data on twins themselves. It appeared that at different ages partly different genes influence the variance of levels of triglycerides, total, LDL, and HDL cholesterol. But age had no effect on the heritability of intermediate phenotypes of cardiovascular disease in the Dutch samples.

Assuming that age does not influence the heritabilities leads us to conclude that the significant differences between the Dutch and the Australian samples are caused by sample specific factors. Each sample is drawn from populations which are likely to differ in their genetic as well as environmental makeup. The larger influence of environmental factors in the Australian sample might be a genuine effect, explained either by a larger main effect of environment on lipid levels or by an interaction with genetic factors (Birley et al., 1997). Furthermore, the measurements are performed with slightly different methods and in different laboratories, which could result in different measurement errors, which are included in the unique environmental factors. Increases in unique environmental estimates would lead to concomitant decreases in estimates of genetic factors.

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In conclusion, we have shown that apolipoprotein E and AII levels show high heritabilities in four independent samples, comparable with other intermediate phenotypes of cardiovascular disease. Common environment does not influence the variances and there are no sex differences in the heritabilities of these intermediate phenotypes. Given the high heritabilities observed, these four twin cohorts would provide a unique sample for QTL mapping of the genes involved.

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