Longitudinal Genetic Analysis of Plasma Lipids

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The consensus from published studies is that plasma lipids are each influenced by genetic factors, and that this contributes to genetic variation in risk of cardiovascular disease. Heritability estimates for lipids and lipoproteins are in the range .48 to .87, when measured once per study participant. However, this ignores the confounding effects of biological variation measurement error and ageing, and a truer assessment of genetic effects on cardiovascular risk may be obtained from analysis of longitudinal twin or family data. We have analyzed information on plasma high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglycerides, from 415 adult twins who provided blood on two to five occasions over 10 to 17 years. Multivariate modeling of genetic and environmental contributions to variation within and across occasions was used to assess the extent to which genetic and environmental factors have long-term effects on plasma lipids. Results indicated that more than one genetic factor influenced HDL and LDL components of cholesterol, and triglycerides over time in all studies. Nonshared environmental factors did not have significant long-term effects except for HDL. We conclude that when heritability of lipid risk factors is estimated on only one occasion, the existence of biological variation and measurement errors leads to underestimation of the importance of genetic factors as a cause of variation in long-term risk within the population. In addition our data suggest that different genes may affect the risk profile at different ages.

Together with age, smoking and blood pressure, plasma lipid levels are considered key risk factors for cardiovascular disease (Kannel, 2000). High levels of plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, apolipoprotein B (ApoB) and triglycerides, and low levels of high-density lipoprotein (HDL) cholesterol and apolipoprotein A1 (ApoA1), are all predictors of atherosclerotic coronary heart disease. Many studies have established the importance of genetic and environmental influences on these risk factors. Heritabilities for lipids found in twin studies have ranged between 48% and 83% (Beekman et al., 2002).

Lipid levels vary over the lifespan and the phenotypic variance — a measure of the range of values observed in a sample or population — generally shows an increase with age (Boomsma et al., 1996; Reilly et al., 1990; Snieder et al., 1997; Snieder et al., 1999). This increase in lipid variance could be due to greater genetic (or nongenetic) interindividual variations, or increasing variation within an individual. It has been claimed that the increase in cholesterol and apolipoprotein B variance with age is due mainly to an increase in unique environmental variance, probably due to the accumulation of environmental experiences throughout life (Iliadou et al., 2001).

To examine the changes of genetic and environmental influences over time, repeated measurements are better than cross-sectional data. They can help to determine if the same (or different) genes influence a trait at different ages. Snieder et al. (1997) examined age differences in heritability estimates for lipids and suggested that there may be partially different genes influencing lipid levels at different ages. In addition, our recent study (Middelberg et al., 2005) showed evidence that the long-term mean of an individual's plasma total cholesterol is strongly genetically determined and some of the relevant genes are age-specific in their effects.

The aim of the current analysis was to extend this repeated-measures twin study design to other lipid variables (HDL, LDL, triglycerides). This allows us to determine firstly, whether these traits are influenced by the same genes across time or whether there are age-specific genetic effects, and secondly, whether nongenetic (environmental) effects persist across time. Multivariate quantitative genetic modeling was used to analyze lipid data from 415 Australian adult male and female twins who have had their lipid levels measured on more than one occasion.

Received 25 January, 2006; accepted 23 March, 2006.

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https://doi.org/10.1375/twin.9.4.550 Published online by Cambridge University Press

Materials and Methods

Subjects

This analysis uses data collected on five occasions from four different studies with overlapping samples: Alcohol Challenge (two occasions, AC and ACR; Martin et al., 1985; Whitfield & Martin, 1983); Vitamin C (VC; Carr et al., 1981); Sydney (SY; Whitfield et al., 1998), and SSAGA Blood (SB; Heath et al., 1997). Twins were recruited through the Australian Twin Registry or, for some subjects in the earlier AC and VC studies, by advertising and word-of-mouth. The first study (AC) was conducted during 1979 to 1981 to assess genetic contributions to variation in alcohol pharmacokinetics and in susceptibility to intoxication (Martin et al., 1985). Lipid results from a prealcohol blood sample were obtained from 412 young twins (206 pairs who were born 1944–1963). Lipid data were also collected from 80 twin individuals who returned to repeat the protocol between 1 and 17 months after the initial visit (Alcohol Challenge Repeat Study, ACR). The second study (VC) contained 106 MZ twin pairs (mainly recruited from the AC study) who participated in a co-twin control study of the effects of Vitamin C (Carr et al., 1981). Lipid levels analyzed here are from blood samples taken before commencement of Vitamin C supplementation. A follow-up study on twins from the alcohol challenge study was conducted in 1990 to 1992 (SY; Whitfield et al., 1998) in which 334 of the original 412 twins had blood collected. The final study (SSAGA blood — SB) which started in 1993, included blood collection from twins who took part in the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) study to assess genetic, psychological and social risk factors for alcoholism and related disorders (Heath et al., 1997). A total of 3347 twins provided blood samples for the SB study, but most had not participated in the other studies listed. For each of these studies, participants gave informed consent to the questionnaire and blood collection. All studies were approved by appropriate ethics committees.

Serum Lipid Measurements

Blood samples were collected and serum was separated from the blood, and either stored at 4º C until analysis on the next working day or stored at –70º C until analyzed. For AC and ACR samples, subjects were fasting. Total cholesterol and triglycerides were measured enzymatically by Technicon methods on a SMAC analyzer (for the AC, ACR and VC studies) and by Boehringer Mannheim methods on a Hitachi 747 analyzer (for the SY and SB studies). HDL-cholesterol (HDL-C) was measured by precipitation of non-HDL lipoproteins with polyethylene glycol 6000 (for the AC, ACR and VC studies) or dextran/$MgSO_4$, followed by enzymatic cholesterol assay. LDL-cholesterol (LDL-C) was calculated from the total cholesterol, HDL-C, and triglyceride values by the Friedewald equation if triglycerides were less than or equal to 4.5 mmol/L. If the serum triglyceride concentration was above this limit, LDL-C was treated as missing.

Statistical Analysis

The main aim of our analyses was to determine the extent to which the genetic and environmental influences on lipids measured at later times overlap with those affecting risk factor levels at earlier times. In other words, we are interested in determining whether the same genes affect levels across time; and whether environmental effects are unique to each occasion (as would be expected if they reflect only short-term biological variation and measurement error) or whether there are long-term environmental effects. Therefore, only those subjects who participated in more than one study contributed to the data analysis. Participants were included if they participated in either the AC or VC studies and were aged between 18 and 35 years at that time. Within that group, 20 individuals who were taking lipid-lowering drugs and 10 twins considered to be outliers (extreme high or low values HDL and LDL, more than three times the standard deviation from the mean) were excluded from the analysis. Information on the studies is provided elsewhere (Middelberg et al., 2005).

Analysis of the lipid values included descriptive statistics and structural equation modeling. Prior to genetic modeling analyses, tests of the distribution of phenotypes and twin assumptions (i.e., all twins have the same mean and variance) were performed. The distribution of triglyceride levels was skewed and values were log-transformed before further analysis. Initially, intraclass correlations for monozygotic (MZ) and dizygotic (DZ) twins for each study after adjusting for age and sex effects were examined. Phenotypic correlations between studies were also examined. As there was significant correlation between studies, sources of covariation between them were modeled using multivariate analysis. In addition, the intraclass correlation for within-subject repeatability at repeat visits ($S_b^2/(S_b^2 + S_w^2)$) was obtained using a conventional analysis of variance. This measure sets a theoretical upper limit for heritability.

Quantitative genetic analyses were used to identify and quantify genetic and environmental influences on variation in lipids. To investigate the contribution of genetic and environmental factors across the five time points, a model, shown in Figure 1a, was fitted. Preliminary analysis showed that shared environmental factors could be dropped from the model. The same structure was specified for additive genetic and unique environmental sources of covariation and the same factor structure was specified for both sources. Figures 1a and 1b show the genetic and environmental latent factors for the phenotypes included in the analysis. The first latent factor (A1/E1) loads on all five occasions (time points). The second factor (A2/E2) loads on the first, second and third occasions (early). Finally, the third latent factor (A3/E3) loads on the fourth and fifth occasions (late). To ensure identification of the model, the five standardized loadings on the

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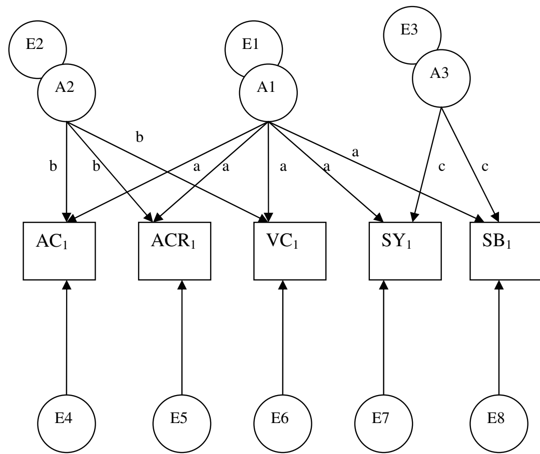

Figure 1a

Path diagram of hypothesized model showing the genetic (A1–A3) and environmental (E1–E8) factors on an observed phenotype measured at five occasions for one twin pair.

Note: The observed phenotypes are shown in squares, and latent factors are shown in circles. Arrows indicate the factor loadings of observed variables on different latent factors.

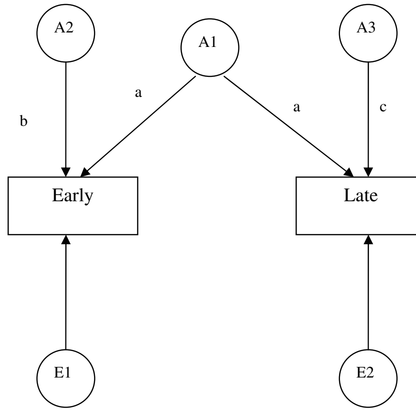

Figure 1b

Path diagram of simplified model showing the genetic (A1–A3) and environmental (E1–E2) factors on an observed phenotype measured at early and late occasions for one twin pair.

Note: The observed phenotypes are shown in squares, and latent factors are shown in circles. Arrows indicate the factor loadings of observed variables on different latent factors.

first factor are constrained equal, as are the three loadings on the second factor and the two loadings on the third factor. Nonshared environment-specific factors (E4–E8) corresponding to specific time points were also fitted. This model provides a general description of the contribution of genetic and environmental factors to the variances and covariance (Neale & Cardon, 1992). Only one twin of a pair is shown in Figures 1a and 1b. Ignoring replicates within the early and late periods, a simplified representation of the model is shown in Figure 1b, and it is this that we shall use for presentation of our results, though we emphasize that the detailed model fitted is as shown in Figure 1a.

A series of submodels nested within an AE model were fitted to the multivariate variance-covariance matrices (using raw data). To allow for differences in total variance between studies, the standardized covariance matrix was pre and post multiplied by the diagonal matrix of standard deviations. The significance of parameters (path coefficients) was tested by removing each sequentially in specific submodels. If a path coefficient did not give a significant contribution (i.e., the change in goodness-of-fit between the data and the model showed $p > .05$), that path coefficient was dropped from the model. To compare the nested models, the likelihood ratio chi-square test (i.e., a difference between –2 log likelihood of the full model from that of a restricted model) was used. For each lipid, a model that included age and sex effects on means was fitted. Effects of age and sex were included as covariates and were treated as fixed effects in the genetic models. We tested whether the same set of genes influenced lipid measurements across all five occasions by dropping the common factor (A1). Since all five loadings are constrained equal, this is a 1 degree-of-freedom (*df*) test. Next we tested the existence of age-specific factors by dropping in turn the younger group factor A2 (the three loadings are constrained equal so this is a 1 *df* test) and the older group factor A3 (similarly, a 1 *df* test). We also tested whether the nonshared environmental effects are long-term or unique to each occasion by dropping each factor and testing if it was significant at 5% significance level. Estimates of the path coefficients and their 95% confidence intervals of the final model were obtained (Neale, 2004).

Data processing and preliminary analyses were done with STATA version 7.0 (StataCorp, 1997). Quantitative genetic and environmental model fitting was performed using the program Mx version 1.57 (Neale, 2004).

Results

General Characteristics

The means and standard deviations of lipid traits by sex and occasion are shown in Table 1. Men had lower mean levels of HDL and higher triglycerides than women. No consistent pattern for the differences in LDL across the sexes was apparent. The twin correlations for the age- and sex-adjusted mean HDL, LDL and triglycerides for each study are presented in Table 2. In general, the intraclass twin correlations were higher in MZ than in DZ twins, indicating genetic influences (Table 2). Overall, the point estimates of the MZ and DZ correlations vary between studies but the 95% confidence intervals of the estimates are fairly wide. The within-person variance was slightly greater at older ages for all variables (Table 3). The repeatabilities within the three earlier studies and two later studies at repeat studies (r_i) ranged from .78 to .88 for HDL and LDL, but were lower (.56–.73) for triglycerides.

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Table 1

Means and Standard Deviations of Lipids for Each Study by Sex

Variable		AC 1979–1981 Mean ± *SD* (*n*)	ACR 1979–1981 Mean ± *SD* (*n*)	VC 1980 Mean ± *SD* (*n*)	SY 1990–1992 Mean ± *SD* (*n*)	SB 1993–1996 Mean ± *SD* (*n*)
Age	Female	22.2 ± 3.3 (187)	23.3 ± 3.0 (34)	23.6 ± 4.0 (42)	34.5 ± 4.5 (180)	37.6 ± 4.5 (135)
	Male	22.2 ± 3.5 (167)	20.7 ± 2.5 (36)	23.0 ± 3.2 (28)	34.3 ± 4.8 (156)	38.1 ± 4.9 (104)
HDL	Female	1.29 ± 0.31 (180)	1.27 ± 0.31 (33)	1.36 ± 0.32 (38)	1.46 ± 0.34 (172)	1.49 ± 0.32 (126)
	Male	1.06 ± 0.27 (157)	1.00 ± 0.17 (34)	1.05 ± 0.28 (28)	1.22 ± 0.25 (151)	1.21 ± 0.23 (99)
LDL	Female	3.72 ± 0.95 (182)	3.73 ± 0.93 (34)	3.71 ± 0.85 (40)	3.20 ± 0.83 (178)	2.97 ± 0.76 (131)
	Male	3.84 ± 0.95 (155)	3.64 ± 0.93 (35)	3.61 ± 0.74 (28)	3.50 ± 0.88 (151)	3.55 ± 0.86 (93)
Trig (log)	Female	–0.23 ± 0.42 (187)	–0.28 ± 0.35 (34)	–0.13 ± 0.48 (42)	–0.10 ± 0.51 (180)	0.11 ± 0.49 (135)
	Male	–0.06 ± 0.54 (167)	–0.20 ± 0.47 (35)	0.47 ± 0.62 (28)	0.20 ± 0.58 (156)	0.51 ± 0.57 (104)

Note: HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; Trig = triglycerides; *SD* = standard deviation; *n* = number of subjects.

Phenotypic correlations across studies are shown in Table 4. For all measures, the correlations among the group of earlier studies (AC, ACR, VC) were highest and the correlations between earlier and later (SY, SB) studies were lowest.

Structural Modeling

For HDL, dropping the common factor (A1, model 2) causes a major reduction in fit (χ_1^2 = 61.45) indicating that there are common genetic factors influencing HDL in all five studies (Table 5). Dropping A2 (model 3) also worsens the fit by χ_1^2 = 20.39, indicating that there are additional genetic influences unique to younger ages. However, dropping the older group factor, A3, produces no change in fit, suggesting no genetic influence specific to older ages. Repeating this procedure for the unique environmental factors, we find significant evidence for environmental influence common to all studies (E1; giving χ_1^2 = 21.12 for dropping E1), but not for environmental influences unique to younger (E2 — χ_1^2 = 2.28) or older (E3 — χ_1^2 = 2.52) groups.

For LDL, dropping either A1 (model 2) or A2 (model 3) or A3 (model 4) worsens the fits, giving χ_1^2 = 51.37, χ_1^2 = 39.54 and χ_1^2 = 14.00 respectively, suggesting there are common genetic factors influencing LDL in all five studies and there are additional genetic influences specific to younger and older ages. For the unique environmental factors, there is no evidence for environmental influence common to all studies or environmental influences unique to younger or older groups (E1 — χ_1^2 = 3.13; E2 — χ_1^2 = 0.00; E3 — χ_1^2 = 0.00). Environmental influences are specific to each occasion.

For triglycerides, we find significant evidence of a common genetic factor (A1 — χ_1^2 = 27.79) and additional genetic influences unique to younger ages (A2 — χ_1^2 = 9.24). Dropping E1 or E2 or E3 did not worsen the fit (E1 — χ_1^2 = 0.057; E2 — χ_1^2 = 0.00; E3 — χ_1^2 = 0.00). Table 5 shows the results from the model-fitting analyses. Figures 2 to 4 show the final simplified models for all three variables, showing that for all variables the constant component of lipid levels across time is largely influenced more than one additive genetic factor. This suggests that for each trait, there is a set of genes that influences the trait at all occasions and another independent set of genes influencing the trait early in life. For LDL, an additional independent set of genes also comes into play later in life. Generally, the environmental influences (E) were largely specific to an occasion except for HDL where there is some suggestion that there might be long-term environmental effect.

Table 2

Age and Sex-Adjusted Intrapair Twin Correlations for Each Study

Variable		AC	ACR	VC	SY	SB
HDL	MZ	.71 (.57, .80)	.56 (.20, .77)	.64 (.40, .80)	.47 (.27, .61)	.60 (.33, .75)
	DZ	.53 (.39, .65)	.28 (–.41, .67)	—	.28 (.07, .46)	.28 (.04, .49)
LDL	MZ	.83 (.75, .89)	.79 (.54, .90)	.60 (.34, .77)	.60 (.42, .72)	.80 (.65, .88)
	DZ	.55 (.41, .66)	.55 (.12, .79)	—	.35 (.14, .51)	.35 (.03, .57)
Trig (log)	MZ	.41 (.19, .58)	.73 (.42, .87)	.76 (.58, .87)	.43 (.22, .59)	.52 (.24, .70)
	DZ	.32 (.14, .48)	.53 (.13, .76)	—	.33 (.11, .51)	.34 (.10, .53)

Note: 95% confidence intervals are given in parenthesis.

HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; Trig = triglycerides; MZ = monozygotic twins; DZ = dizygotic twins.

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Table 3

Intraclass Correlation (r_i) and Within-Individual Variance Components (s^2_w) for Males and Females and Both Sexes by Studies

	Earlier studies (AC, ACR, VC)			Later studies (SY, SB)		
	Males	Females	Both sexes	Males	Females	Both sexes
HDL						
Number of results	219	253	472	250	294	544
s^2_w	.012	.018	.015	.019	.045	.032
r_i	.88	.87	.89	.81	.77	.81
LDL						
Number of results	218	256	474	244	309	553
s^2_w	.294	.188	.241	.244	.250	.247
r_i	.78	.85	.81	.81	.78	.81
Triglycerides (log)						
Number of results	230	263	493	260	315	575
s^2_w	.271	.077	.173	.188	.208	.199
r_i	.56	.73	.62	.71	.58	.67

Discussion

Our analysis of these data is based on the concept that the observed value of a quantitative risk factor for an individual at any single time is the result of both long-term factors and short-term variability. The short-term variability, due to biological variation and measurement errors, tends to obscure the long-term effects and leads to phenomena such as regression dilution (Lewington et al., 2003). This well-known effect reduces the apparent association between a risk factor measured on only one occasion, and a clinical outcome. Similarly, the heritability of the long-term mean value of HDL, LDL and triglycerides will be underestimated by single measurements. Longitudinal studies combined with a twin or family design allow assessment of the contributions of genetic, shared environmental, or nonshared environmental components to the long-term mean of plasma lipids concentration and, indirectly, of their contributions to risk. It is of particular interest to determine whether these risk factors show any persistent nonshared environmental effects or whether nonshared environmental effects are unique to each occasion of measurement.

Table 4

Phenotypic Correlations of Lipid Variables Between Different Studies According to Sex

HDL	AC	ACR	VC	SY	SB
AC	(157, 180)	.85 (31)	.83 (26)	.51 (153)	.53 (108)
ACR	.62 (32)	(34, 33)	.65 (6)	.52 (33)	.14 (21)
VC	.87 (24)	.90 (8)	(28, 40)	.28 (23)	.64 (16)
SY	.53 (125)	.47 (28)	.54 (23)	(151, 172)	.58 (114)
SB	.60 (81)	−.14 (18)	.54 (18)	.68 (90)	(99, 126)
LDL	**AC**	**ACR**	**VC**	**SY**	**SB**
AC	(155, 182)	.74 (32)	.78 (26)	.40 (154)	.54 (110)
ACR	.73 (32)	(35, 34)	.90 (6)	.39 (33)	.71 (22)
VC	.63 (22)	−.02 (8)	(28, 40)	.54 (23)	.49 (16)
SY	.42 (124)	.66 (29)	.49 (23)	(151, 178)	.62 (118)
SB	.51 (75)	.80 (18)	.36 (17)	.63 (87)	(93, 131)
LDL	**AC**	**ACR**	**VC**	**SY**	**SB**
AC	(167, 187)	.65 (34)	.36 (28)	.34 (158)	.26 (116)
ACR	.40 (35)	(35, 34)	.81 (6)	.19 (33)	.24 (23)
VC	.38 (26)	−.43 (8)	(28, 42)	.35 (25)	−.20 (18)
SY	.37 (133)	.14 (29)	.37 (23)	(156, 180)	.25 (121)
SB	.36 (87)	−.14 (19)	.57 (18)	.48 (96)	(104, 135)

Note: Results for women are shown below the diagonals and for men above. Numbers on which correlations are based are shown in parentheses; numbers on diagonal are *N* for females and males.

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Table 5

Genetic Modeling Analysis: Goodness-of-Fit Parameters of Lipid Data

Measurement	Model	–2LL	*df*	Compared to model	$\Delta\chi^2$	Δdf	*p*
HDL	1. 3 common A factor (AE3)	–85.349	998				
	2. AE3 + drop A1	–23.910	999	1	61.45	1	.000
	3. AE3 + drop A2	–64.956	999	1	20.39	1	.000
	4. AE3 + drop A3	–85.349	999	1	0.000	1	1.000
	5. AE3 + drop E1, E2 & E3	–58.545	1001	1	26.80	1	.000
LDL	1. 3 common A factor (AE3)	2201.64	1005				
	2. AE3 + drop A1	2253.01	1006	1	51.37	1	.000
	3. AE3 + drop A2	2241.18	1006	1	39.54	1	.000
	4. AE3 + drop A3	2215.64	1006	1	14.00	1	.000
	5. AE3 + drop E1, E2 and E3	2205.95	1008	1	4.31	3	.230
Triglycerides(log)	1. 3 common A factor (AE3)	1382.08	1046				
	2. AE3 + drop A1	1409.87	1047	1	27.79	1	.000
	3. AE3 + drop A2	1391.32	1047	1	9.24	1	.002
	4. AE3 + drop A3	1383.64	1047	1	1.56	1	.212
	5. AE3 + drop E1, E2 and E3	1382.14	1049	1	0.06	3	.996

Our previous analysis (Middelberg et al., 2005), which was restricted to plasma total cholesterol, showed that covariation in total cholesterol across time is largely influenced by additive genetic factors and effects of unique environment are largely specific to the different occasions. Our current results on the HDL and LDL components of cholesterol, and triglycerides, confirm the general pattern that genetic effects persist across time. The nonshared environmental effects are generally occasion-specific. This suggests that individuals' long-term lipid levels are genetically determined, and the genetic effects were relatively strong. For example, the genetic variation component contributed up to 48% (HDL), 45% (LDL), and 34% (triglycerides) of the total phenotypic variation. Consistent with our previous findings that suggested there are other genes operating later in life for total cholesterol, the current study also suggested there are genes operating at older ages for LDL. Additional sets of genes operating at earlier ages were also found for HDL, LDL and triglycerides.

In the literature, there are a limited number of longitudinal twin studies for lipid levels. Previous studies indicated existence of age-dependent gene expression in some lipids (Nance et al., 1998; Snieder et al., 1997; Snieder et al., 1999). Results of our current

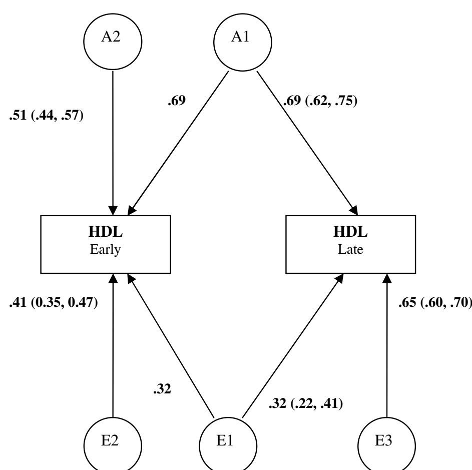

Figure 2

Path diagram of final model showing standardized path coefficients (and 95% confidence intervals) on measured HDL corrected for age and sex.

Note: A1 and A2 are genetic factors, and E1 to E3 are nonshared environmental factors, influencing HDL results in the early and late studies.

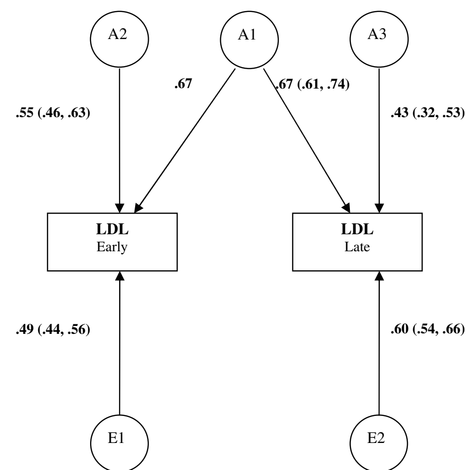

Figure 3

Path diagram of final model showing standardized path coefficients (and 95% confidence intervals) on measured LDL corrected for age and sex.

Note: A1, A2 and A3 are genetic factors, and E1 to E2 are nonshared environmental factors, influencing LDL results in the early and late studies.

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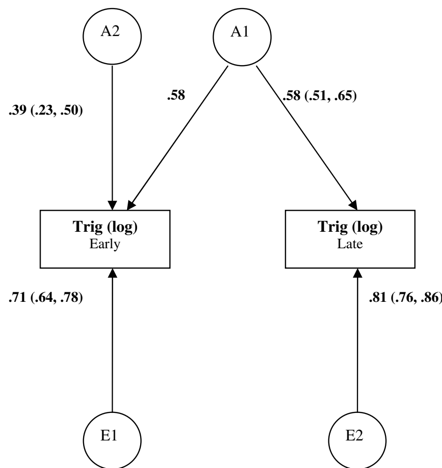

Figure 4

Path diagram of final model showing standardized path coefficients (and 95% confidence intervals) measured log(triglycerides) corrected for age and sex.

Note: A1 and A2 are genetic factors, and E1 to E2 are nonshared environmental factors, influencing log(triglycerides) results in the early and late studies.

analyses suggest that there is evidence for different genes influencing HDL and LDL components of cholesterol, or triglycerides, at earlier age. There is also evidence of different genes influencing LDL at later age. It is difficult to compare our results to the other studies due to different study design or to the different age ranges which were examined. Our results for triglycerides and LDL were similar to those of Friedlander et al. (1997). They analyzed changes in total cholesterol, LDL, HDL and triglycerides in 348 pairs of female twins (average age between 41 and 51 years) who were examined twice. No genetic influence on the changes in triglycerides over this 10-year time span was found, but there were significant genetic influences on the changes in LDL and HDL cholesterol. Our conclusions are drawn from 415 twins, which is a slightly larger sample size compared to the previous study.

Our study design may be used as a guide for future quantitative studies on the genetics of cardiovascular risk. Combination of longitudinal data from multiple studies will allow a more robust estimate of whether age-dependent genetic effects exist, and the use of multiple-occasion data will add power to linkage analyses.

Acknowlegments

We are grateful to the twins and their families for their generous participation in these studies. We would like to thank Pam Saunders, Louise O'Gorman, Dixie Statham and other staff at the Queensland Institute of Medical Research for sample collection; David Smyth and Olivia Zheng for data management; the staff of the Biochemistry Department of Royal Prince Alfred Hospital for lipids analysis; and Michelle Luciano for her valuable advice. Financial support was provided by grants from Australian Brewers Foundation, the National Health and Medical Research Council, and the National Institute on Alcohol Abuse and Alcoholism (AA007535, AA014041) and European Union (QLRT-2001-01254). R.P.S.M. is supported by Dora Lush NHMRC Scholarship (339484).

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