A Longitudinal Genetic Study of Plasma Lipids in Adolescent Twins

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Plasma lipids such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol and triglyceride levels contribute to variation in the risk of cardiovascular disease. The early stages of atherosclerosis in childhood have also been associated with changes in triglycerides, LDL and HDL. Heritability estimates for lipids and lipoproteins for adolescents are in the range .71 to .82, but little is known about changes of genetic and environmental influences over time in adolescence. We have investigated the contribution of genetic and environmental influences to variation in lipids in adolescent twins and their nontwin siblings using longitudinal twin and family data. Plasma HDL and LDL cholesterol, total cholesterol and triglycerides data from 965 twin pairs at 12, 14 and 16 years of age and their siblings have been analyzed. Longitudinal genetic models that included effects of age, sex and their interaction were fitted to assess whether the same or different genes influence each trait at different ages. Results suggested that more than one genetic factor influences HDL, LDL, total cholesterol and triglycerides over time at ages 12, 14 and 16 years. There was no evidence of shared environmental effects except for HDL and little evidence of long-term nonshared environmental effects was found. Our study suggested that there are developmental changes in the genes affecting plasma lipid concentrations across adolescence.

Coronary heart disease (CHD) is the one of the most important killers in the developed and developing world, and plasma lipid levels are important risk factors for this and other cardiovascular diseases (Kannel, 2000). The underlying atherosclerotic process has been shown to commence in childhood or adolescence, and has been associated with changes in triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in these age groups (Berenson et al., 1992; McGill, McMahan, Herderick, et al., 2000; McGill, McMahan, Zieske, et al., 2000). A longitudinal genetic analysis of risk factors for CHD, covering the period where male and female levels diverge, can be expected to throw light on the reasons for differing risk between adult men and women and also to provide the basis for a more sophisticated approach to identification of genes and polymorphisms contributing to CHD.

During puberty (12–16 years), plasma total cholesterol levels decrease in boys, whereas a longitudinal study observed no change in girls (Berenson et al., 1981). Conversely, HDL levels decrease during puberty in boys but remain unchanged in girls (Berenson et al., 1981; Twisk et al., 1995). Triglyceride levels increase in both sexes during adolescence but relatively more in boys than in girls (Berenson et al., 1981). Many studies have established the importance of genetic and environmental influences on these risk factors. Cross-sectional twin studies have found substantial heritabilities for plasma lipid levels. Beekman et al. (2002) found heritability estimates in a younger twin sample (aged 13–22) were higher (71%–82%) than in an adult sample (aged 34–92, 48%–77%). Similarly high heritabilities (69%–75%) have been reported in a recent study of young European American twins (mean age 17.9 ± 3.2 years; Iliadou et al., 2005).

Previous studies have examined the stability of genetic effects on lipids in adults and found evidence that there may be partially different genes influencing lipid levels at different ages in adult and adolescent twins (Middelberg et al., 2005, 2006; Snieder et al., 1997). However, little is known about possible changes in genetic and environmental influences over time in adolescence. Repeated-measures data are particularly useful to examine whether the same or different genes influence a trait at different ages. The aim of the present study was to use repeated measures of lipid variables (total cholesterol, HDL, LDL, triglycerides) to investigate (1) whether different genes affect these traits at different ages, (2) whether the same genes affect a trait at each age, but with different magnitude, and (3) whether nongenetic (environmental) effects persist over time. Longitudinal quantitative genetic models were fitted to lipid data

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from 965 Australian twin families. The twins' lipid levels were measured on three occasions (12, 14 and 16 years of age) corresponding roughly to early, middle and late stages of puberty. Teenage siblings of the twins also had their lipid levels measured, but only on one occasion.

Materials and Methods

Subjects

The dataset comes from overlapping studies of adolescent twins and their nontwin siblings living in south-east Queensland, Australia, originally focusing on melanoma risk factors (McGregor et al., 1999; Zhu et al., 1999) and cognition (Wright et al., 2001; Wright & Martin, 2004). Adolescent twins have been recruited since 1992, with additional nontwin siblings added subsequently. The sample consists of monozygotic (MZ) and dizygotic (DZ) twin pairs and their siblings, and most (98%) are of mixed European ancestry (mainly British Isles). In total, 2488 subjects were recruited and most twins participated in more than one study. They were recruited from schools in Brisbane and the surrounding regions of south-east Queensland, by contacting the school principals and through media appeals. For the melanoma risk factors study, twins were invited to participate as close as possible to their 12th and 14th birthdays, together with their nontwin siblings (aged 10–18). For the cognition study, twins and their siblings were tested as close to their 16th birthday as possible. Blood samples (nonfasting) were collected at the end of testing sessions from participants and their parents. Blood samples were collected from additional twins and their siblings who were already in the database. For each of these studies, participants gave informed consent to the questionnaire and blood collection, and all studies were approved by appropriate ethics committees.

Zygosity was initially determined from twins' responses to questions about similarity and the degree to which others were unable to distinguish them. Photos of the twins were taken at the testing session and they were also available to resolve any inconsistencies in responses by individual twins or between co-twins. For all same-sex pairs, where DNA samples from blood (or in some cases buccal swabs) were available, zygosity was also determined by typing nine independent highly polymorphic DNA microsatellite markers and the Amelogenin locus (ABI Profiler Plus Kit). This yields a probability of marker concordance given the twins are DZ of less than 10^{-4} (Nyholt, 2006).

Laboratory Measurements

Blood samples from all participants were collected and serum was separated from the blood and stored at –70 °C until analyzed. Total cholesterol, HDL-cholesterol (HDL), and triglycerides were measured using Roche methods on a Hitachi 917 Analyzer. LDL-cholesterol (LDL) was calculated from the total cholesterol, HDL, and triglyceride values by the Friedewald formula (Friedewald et al., 1972) if triglycerides were less than or equal to 4.52 mmol/L. If the serum triglyceride concentration was above this limit, LDL was treated as missing. These biochemical analyses were performed on blood samples from the adolescent twins and their siblings, but not on those from the parents.

Statistical Analysis

Distributions of the variables were examined. If frequency distributions were skewed, an appropriate transformation was used before further analysis. Table 1 gives a summary of the 965 twin families who participated across all three measurement occasions. Data were screened for outliers. A total of 29 twin families were excluded from the analysis due to the family having *z* scores in excess of 3.5 for one or more variables.

Table 1

Breakdown of Number of Twin Families Who Were Tested at Each Age Range

Measurement occasion								
12	14	16	MZF	MZM	DZF	DZM	DZOS	Total
•			23	30	30	31	42	156
	•		6	15	1	9	9	40
		•	67	73	24	20	56	240
•	•		39	34	33	28	53	187
•		•	1	2	1	7	5	16
	•	•	22	20	11	11	26	90
•	•	•	38	27	45	48	78	236
Total at any age			**196**	**201**	**145**	**154**	**269**	**965**
Total at 12			101	93	109	114	178	595
Total at 14			105	96	90	96	166	553
Total at 16			128	122	81	86	165	582

Note: Total number siblings at 12 = 303; Total number siblings at 14 = 86; Total number siblings at 16 = 128.

MZF = monozygotic female, MZM = monozygotic male, DZF = dizygotic female, DZM = dizygotic male, DZOS = dizygotic opposite-sex.

Standard descriptive statistics and genetic model fitting were performed (Neale & Cardon, 1992). Prior to genetic modeling, tests of distributional assumptions (i.e., that all twins and siblings have the same mean and variance) were performed. The variables were also adjusted for the effects of age, squared age (age^2), sex, sex × age and sex × age^2. Preliminary analysis (Middelberg & Whitfield, 2006) showed that consecutive measurement occasions were relatively highly correlated, generally in the range of *r* equaling .4 or greater, indicating that sources of covariation between consecutive measurement occasions could usefully be modeled using multivariate genetic analysis.

Multivariate genetic models were fitted to data by the method of full information maximum-likelihood (FIML) which makes use of every available data point and takes account of missingness (Neale et al., 2004). To investigate the contribution of genetic and environmental factors across the three occasions a full Cholesky (triangular) decomposition model was fitted in which the number of factors equals the number of observed variables. The full model included a scalar sex correction which allows for different means and variances in males and females. The first factor contributes to all three measurement occasions, the second factor loads on the subsequent two measurement occasions and the third factor is specific to the last measurement occasion. To allow for differences in total variance between studies and between sexes, the standardized covariance matrix was pre- and postmultiplied by the diagonal matrix of standard deviations specific to each sex and occasion. Initially a Cholesky decomposition was specified for all three sources — additive genetic (A), common environment (C), and unique environment (E; 'triple Cholesky model'). Then the need for each source of variance (except E, without which expected matrices are not positive definite) was tested by fitting a series of nested submodels, AE, CE and E. The significance of particular parameters (path coefficients) was tested by removing each sequentially in specific submodels. To compare 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 trait, a model that included fixed effects of age, squared age (age^2), sex, sex × age and sex × age^2 on means was fitted. For the final model, 95% confidence intervals for all estimates of the components of residual variance were obtained (Neale et al., 2004).

Data processing and descriptive analyses were done with STATA version 7.0 (StataCorp, 1997). Quantitative genetic and environmental model fitting was performed using the program Mx version 1.60 (Neale et al., 2004).

Results

General Characteristics

Means and standard deviations of lipid traits for males and females are listed in Table 2. The frequency distribution of triglycerides was skewed and so their values were log-transformed. In the females there was little change in means from 12 to 16 whereas in the males the changes were much larger. Overall, there were no significant differences in means between cotwins within same-sex pairs, or between zygosity groups of the same sex. Males and females differed

Table 2

Means and Standard Deviations (*SD*) for Females and Males According to Age

Variables	Females			Males		
	N	Mean	*SD*	*N*	Mean	*SD*
HDL (mmol/l)						
12	746	1.42	0.33	731	1.45	0.34
14	608	1.40	0.31	597	1.32	0.30
16	658	1.43	0.31	643	1.29	0.28
LDL (mmol/l)						
12	740	2.51	0.64	731	2.42	0.72
14	601	2.49	0.65	588	2.33	0.64
16	657	2.50	0.73	637	2.36	0.64
Total cholesterol (mmol/l)						
12	748	4.51	0.69	737	4.47	0.80
14	608	4.40	0.70	596	4.21	0.74
16	660	4.37	0.87	637	4.13	0.74
Triglycerides (mmol/l)						
12	751	1.27	0.62	740	1.29	0.68
14	610	1.13	0.50	597	1.21	0.57
16	659	0.97	0.43	646	1.06	0.52

Note: HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol

Table 3

Twin Correlations of Lipid Traits at Age 12, 14 and 16

		HDL	LDL	Total cholesterol	Triglycerides (log)
Age	12				
MZF		.87 (.83, .90)	.81 (.74, .86)	.78 (.70, .83)	.80 (.73, .85)
MZM		.87 (.82, .90)	.91 (.88, .93)	.89 (.85, .93)	.81 (.73, .86)
DZF		.61 (.48, .70)	.46 (.28, .59)	.44 (.27, .57)	.44 (.27, .57)
DZM		.49 (.35, .60)	.56 (.42, .66)	.52 (.37, .63)	.56 (.42, .66)
DZOS		.50 (.38, .59)	.45 (.34, .54)	.39 (.28, .49)	.40 (.27, .51)
Tw-Sib (FF)		.45 (.29, .57)	.39 (.23, .52)	.42 (.29, .53)	.50 (.37, .61)
Tw-Sib (MM)		.36 (.18, .50)	.56 (.41, .67)	.52 (.37, .64)	.41 (.23, .55)
Tw-Sib (OS)		.49 (.38, .58)	.42 (.30, .52)	.42 (.30, .51)	.37 (.23, .48)
Pooled					
MZ		.87 (.84, .90)	.86 (.83, .89)	.83 (.79, .87)	.80 (.76, .84)
DZ		.49 (.43, .54)	.47 (.40, .53)	.44 (.39, .50)	.44 (.37, .50)
Age	14				
MZF		.87 (.83, .90)	.81 (.75, .86)	.78 (.70, .83)	.68 (.57, .76)
MZM		.88 (.83, .91)	.83 (.76, .87)	.81 (.75, .86)	.66 (.56, .74)
DZF		.65 (.53, .74)	.38 (.18, .53)	.44 (.24, .59)	.49 (.27, .63)
DZM		.48 (.32, .61)	.41 (.20, .57)	.28 (.02, .47)	.42 (.26, .56)
DZOS		.44 (.32, .55)	.46 (.33, .56)	.39 (.27, .49)	.29 (.15, .41)
Tw-Sib (FF)		.44 (.00, .66)	.46 (.18, .64)	.55 (.26, .71)	.24 (.24, .64)
Tw-Sib (MM)		.67 (.41, .78)	.16 (.00, .48)	.19 (.00, .48)	.10 (.00, .34)
Tw-Sib (OS)		.33 (.04, .53)	.16 (.00, .37)	.51 (.21, .71)	.39 (.12, .56)
Pooled					
MZ		.87 (.84, .90)	.82 (.78, .85)	.79 (.74, .83)	.67 (.60, .73)
DZ		.49 (.42, .56)	.39 (.31, .47)	.39 (.31, .47)	.36 (.27, .44)
Age	16				
MZF		.75 (.68, .80)	.73 (.65, .79)	.70 (.61, .76)	.64 (.54. .72)
MZM		.80 (.74, .85)	.78 (.70, .83)	.68 (.58, .75)	.68 (.58, .76)
DZF		.50 (.34, .63)	.23 (.01, .42)	.37 (.17, .53)	.43 (.23, .57)
DZM		.49 (.31, .62)	.65 (.52, .74)	.62 (.48, .72)	.46 (.28, .60)
DZOS		.40 (.25, .52)	.32 (.19, .43)	.32 (.19, .44)	.17 (.02, .31)
Tw-Sib (FF)		.35 (.10, .53)	.21 (.00, .46)	.07 (.00, .38)	.23 (.00, .43)
Tw-Sib (MM)		.34 (.05, .56)	.00 (.00, .30)	.00 (.00, .26)	.13 (.00, .40)
Tw-Sib (OS)		.36 (.18, .51)	.05 (.00, .28)	.08 (.00, .31)	.18 (.00, .38)
Pooled					
MZ		.78 (.73, .81)	.75 (.70, .79)	.69 (.62, .74)	.66 (.59, .72)
DZ		.42 (.34, .49)	.30 (.21, .38)	.31 (.22, .39)	.26 (.17, .34)

Note: MZF = monozygotic female, MZM = monozygotic male, DZF = dizygotic female, DZM = dizygotic male, DZOS = dizygotic opposite-sex, FF = female–female, MM = male–male, OS = opposite-sex

significantly in their means for total cholesterol at age 12 and HDL at age 12. Variances of males were significantly larger than for females for LDL and triglycerides at age 12 years. However, no significant differences in the variances were detected at other ages or for HDL or total cholesterol.

Twin-pair correlations adjusted for age, age^2, sex, age × sex, and age^2 × sex at each time point are presented in Table 3. Given the young age of our sample, few if any of the females were using oral contraceptives which might affect lipid levels. For all traits and at all ages the correlation between MZ twin pairs was high (greater than .7). The corresponding DZ correlations were lower, suggesting genetic influences at all ages. For HDL and total cholesterol, the correlations between female twins were fairly constant at ages 12 and 14 and then decreased at age 16. The correlations between male twins were fairly constant across ages 12, 14 and 16 for HDL. On the other hand, for LDL and triglycerides, the correlations generally decrease from age 12 to 16 in both sexes. Note that the correlations for triglycerides, in particular, decrease with age in opposite-sex DZ pairs.

Phenotypic correlations of each lipid trait across ages 12, 14 and 16 are shown in Table 4. Correlations between consecutive measurement occasions decrease with age and all correlations are greater than .4.

Longitudinal Analysis

To investigate the contribution of genetic and environmental factors across three ages, multivariate Cholesky decomposition models were fitted to the longitudinal data for each trait. Model-fitting results including the saturated models are shown in Table 5 and standardized results of the Cholesky analyses are presented in Figures 1 to 4. For all traits except HDL, an AE Cholesky model provided the most parsimo-

Table 4

Phenotypic Correlations of Lipid Variables Between Ages 12, 14 and 16 According to Sex

HDL	12	14	16
12	–(752, 735)	.61 (421)	.59 (272)
14	.56 (460)	–(608, 597)	.60 (334)
16	.48 (280)	.62 (352)	–(660, 643)
LDL	12	14	16
12	–(745, 736)	.67 (417)	.55 (269)
14	.63 (453)	–(601, 588)	.63 (331)
16	.53 (276)	.64 (350)	–(659, 637)
Total cholesterol	12	14	16
12	–(754, 741)	.72 (422)	.59 (274)
14	.70 (460)	–(608, 596)	.65 (335)
16	.58 (281)	.64 (355)	–(662, 637)
Triglycerides (log)	12	14	16
12	–(757, 744)	.44 (424)	.40 (276)
14	.48 (462)	–(610, 597)	.43 (336)
16	.44 (280)	.41 (353)	–(661, 646)

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

nious fit to the data based on the least significant change in log-likelihood. However, even for HDL the likelihood ratio test in dropping the C matrix from the ACE Cholesky model was only moderately significant ($\Delta\chi^2_6 = 17.14$, $p = .009$) compared with dropping the A matrix ($\Delta\chi^2_6 = 285.3$). Testing of the factor loadings in the C matrix revealed two that were individually significant, on HDL at earlier ages (at 12 and 14), and that accounted for approximately 16% of variance. Consequently, for the other three traits we show only the results for the AE Cholesky model. For all traits, the heritability decreases across ages 12, 14 and 16. The nonshared environmental variance tends to be time specific. Large loadings of all variables on the second and third genetic factors were observed, hence more than one set of genes are responsible for variation across adolescence. If there were only one set of genes, all measurements would have loaded highly on the first genetic factor and the factor loadings on the second and third genetic factors would have been zero. This suggests that new genes influencing these lipid levels are being 'switched on' at age 14 and 16 as puberty progresses. In searching for the particular genes influencing plasma lipid levels during puberty, one might therefore expect to find somewhat different evidence for linkage and association at each age.

Discussion

Our study shows that there is more than one set of genes affecting lipid traits during adolescence. The largest genetic variance was at age 12. The Cholesky AE models fitted the data well for most lipid traits except for HDL. This implies that shared environmental influences have no or little impact on variation in adolescents even though co-twins (and their siblings) will be sharing many dietary and other environmental influences at this time in their lives.

There is a limited number of longitudinal twin adolescent studies for lipid levels. Previous studies indicated existence of age-dependent gene expression in some lipids during adulthood and adolescence (Middelberg et al., 2006; Nance et al., 1998; Snieder et al., 1997, 1999). Our previous studies (Middelberg et al., 2005,

Table 5

Multivariate Analysis: Goodness-of-Fit Parameters of Lipid Data Corrected for Age, Age^2, Sex, Sex × Age and Sex × Age^2

Measurement	Cholesky model	–2LL	*df*	Compared to model	$\Delta\chi^2$	Δdf	*p*
HDL	1. saturated model, ACE	9209.27	3944				
	2. AE	9226.41	3950	1	17.14	6	.009
	3. CE	9494.57	3950	1	285.30	6	.000
	4. E	10,282.86	3956	1	1073.60	12	.000
LDL	1. saturated model, ACE	9062.97	3915				
	2. AE	9074.79	3921	1	11.82	6	.066
	3. CE	9285.99	3921	1	223.03	6	.000
	4. E	9928.89	3927	1	865.92	12	.000
Total cholesterol	1. saturated model, ACE	9129.98	3938				
	2. AE	9135.44	3944	1	5.46	6	.486
	3. CE	9313.13	3944	1	183.15	6	.000
	4. E	9826.22	3950	1	696.24	12	.000
Triglycerides (log)	1. saturated model, ACE	10,129.68	3963				
	2. AE	10,138.14	3969	1	8.46	6	.206
	3. CE	10,277.47	3969	1	147.79	6	.000
	4. E	10,796.59	3975	1	666.92	12	.000

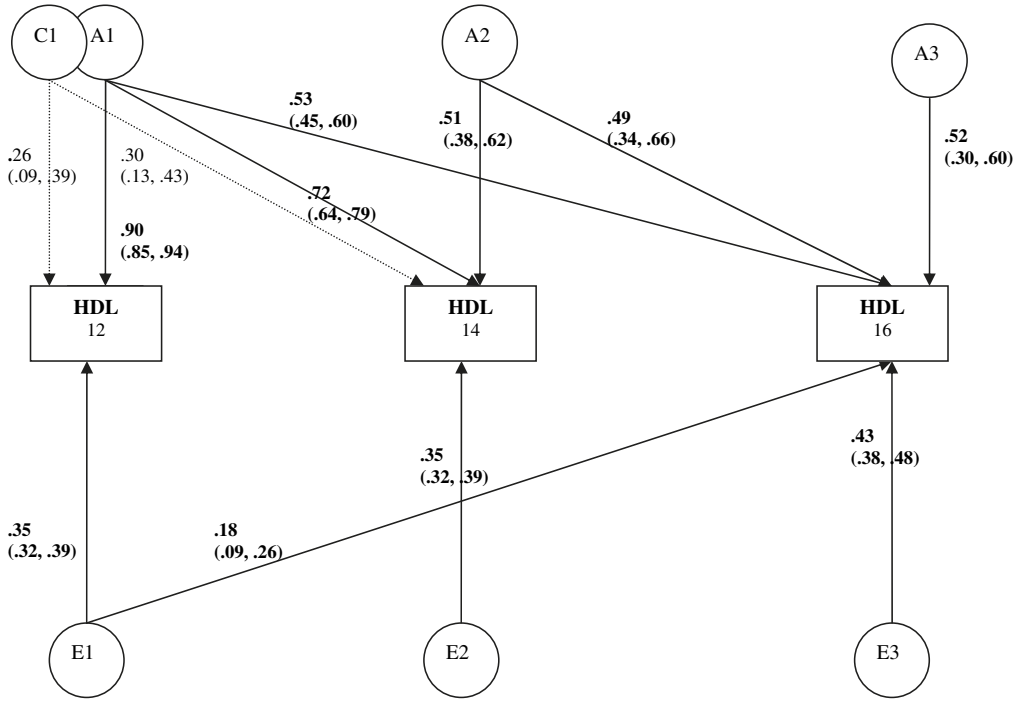

Figure 1

Path diagram showing standardized path coefficients (and 95% confidence intervals) on measured HDL corrected for age, age^2, sex, sex $\times$ age and sex $\times$ age^2.

Note: A1, A2 and A3 are genetic factors, C1, C2 and C3 are shared environmental factors, and E1, E2 and E3 are nonshared environmental factors, influencing HDL results on the three measurement occasions 12, 14 and 16.

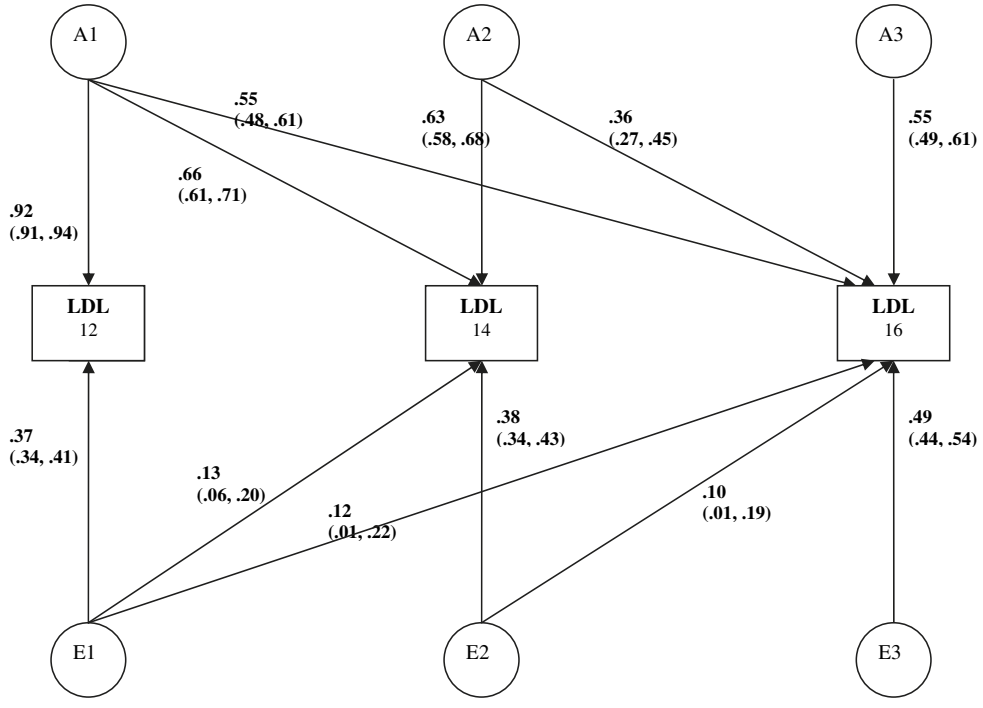

Figure 2

Path diagram showing standardized path coefficients (and 95% confidence intervals) on measured LDL corrected for age, age^2, sex, sex $\times$ age and sex $\times$ age^2.

Note: A1, A2 and A3 are genetic factors, and E1, E2 and E3 are nonshared environmental factors, influencing LDL results on the three measurement occasions 12, 14 and 16.

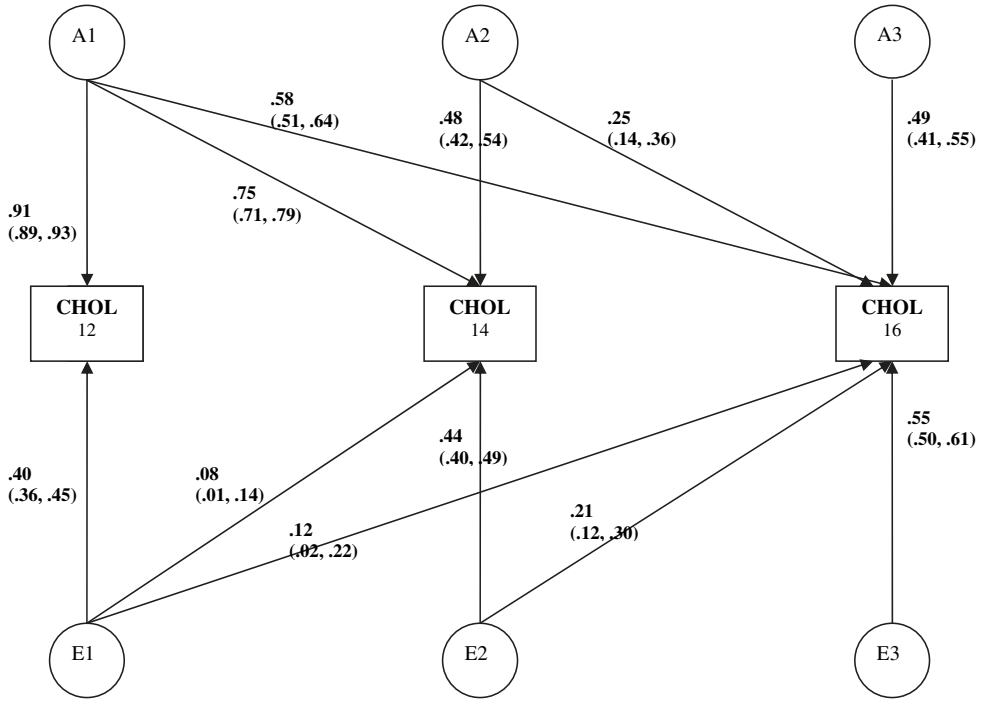

Figure 3

Path diagram showing standardized path coefficients (and 95% confidence intervals) on measured total cholesterol corrected for age, age^2, sex, sex $\times$ age and sex $\times$ age^2.

Note: A1, A2 and A3 are genetic factors, and E1, E2 and E3 are nonshared environmental factors, influencing total cholesterol results on the three measurement occasions 12, 14 and 16.

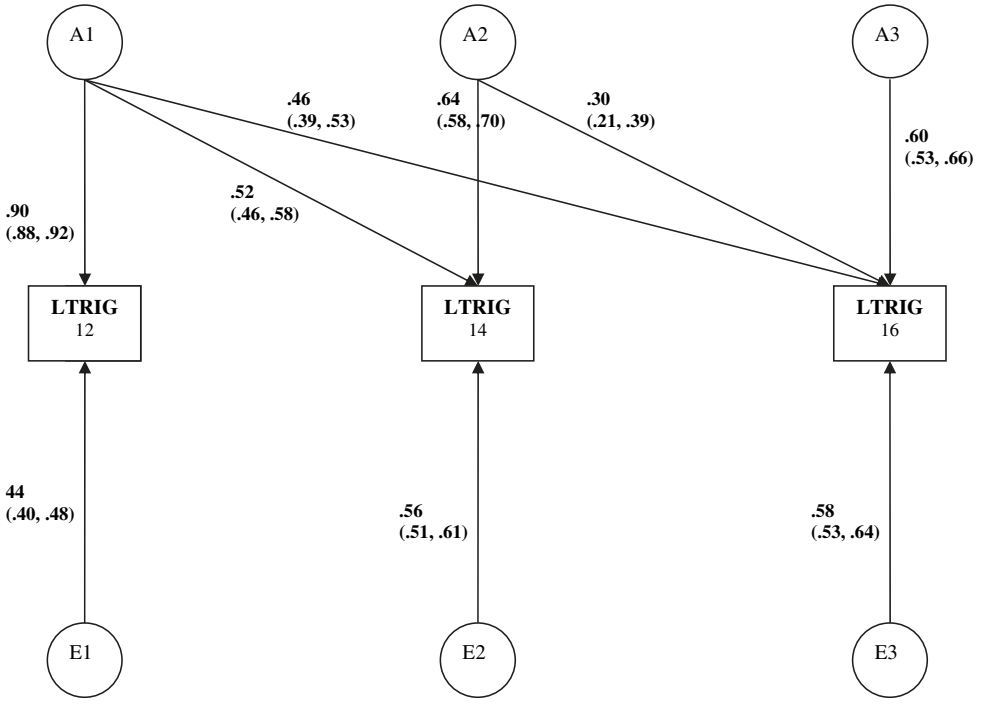

Figure 4

Path diagram showing standardized path coefficients (and 95% confidence intervals) on measured log(triglycerides) corrected for age, age^2, sex, sex $\times$ age and sex $\times$ age^2.

Note: A1, A2 and A3 are genetic factors, and E1, E2 and E3 are nonshared environmental factors, influencing log(triglycerides) results on the three measurement occasions 12, 14 and 16.

2006), which examined younger (< 30 years old) and older (> 30 years old) adult groups, showed that covariation in lipid levels across time is largely influenced by additive genetic factors and effects of unique environment are largely specific to the different occasions. It was suggested that there are to some extent different genes operating at earlier and later ages.

The current study also suggested there are different genes operating across adolescence, from ages 12 to 16. This strengthens the hypothesis that although heritability of these characteristics may be substantial at all ages, there are differing genetic effects at different ages. Our heritability estimate of HDL cholesterol was similar (.80) to Nance et al. (1998) who reported a range of .80 to .83 at ages 11, 12.5 and 14. Similarly, the nonshared environmental influence for ages 11 and 12 was constant and specific for each occasion. We also observed that the heritability decreases with increasing age, which is consistent with the results found by Boomsma et al. (1996) of significantly lower heritabilities in parents compared to twin offspring, suggesting that heritabilities decrease as people grow older. There is some evidence in our results for nonscalar sex limitation, as seen from lower opposite-sex twin-pair correlations compared to the DZ same-sex correlations, in particular for triglyceride at age 16. However, these differences were not quite significant (p = .056). It is unlikely that explicit modeling of nonscalar sex limitation would reveal a picture appreciably different from our model in Figure 4, which assumes the same genes are acting in both sexes.

The shared environmental estimates were zero for all lipids except HDL, indicating no or little impact on the variation in adolescents. Even for HDL, the shared environmental estimate was small. This is consistent with results of Snieder et al. (1997) who found a small significant shared environmental effect at ages 14 to 21 in apolipoprotein B, but not in any of the lipid traits. Our conclusions are drawn from 965 twins and their siblings, which is a much larger sample size compared to previous studies. This study can be used as a guide for future quantitative studies on the genetics of cardiovascular risk, and the multivariate linkage model can be used to detect quantitative trait loci affecting lipid values at differing ages.

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