Dissecting the Genetic Architecture of Lipids, Lipoproteins, and Apolipoproteins

Lessons From Twin Studies

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Abstract—We review the ways in which twin studies have been used to investigate the genetic architecture of lipids, lipoproteins, and apolipoproteins. We focus on the age dependency of genetic effects and the importance of pleiotropy for the lipid system. Finally, consequences are discussed of age dependency and pleiotropy for the design and power of twin studies aimed at detecting the actual quantitative trait loci (QTLs) involved. It is concluded that twin studies have played an important role and will remain highly valuable for the elucidation of the genetic architecture of lipids, lipoproteins, and apolipoproteins. Twins can efficiently be used to identify the location and function of QTLs. Taking account of pleiotropy and age-dependent gene expression in study design and data analysis will improve the power and efficiency to find these QTLs for components of the lipid system. **(*Arterioscler Thromb Vasc Biol*. 1999;19:2826-2834.)**

Key Words: lipids ■ genetics ■ twin studies ■ age ■ pleiotropy

The heritability of a specific trait or disease, defined as the proportion of variance determined by genetic factors, has traditionally been the main outcome parameter of the classic twin study. For most lipids and (apo)lipoproteins, for example, large heritabilities have been shown, providing a compelling argument to start the search for their genes. For several reasons, twin studies have recently moved away from merely calculating heritabilities based on measurements of single traits at single time points. First, heritabilities derived from cross-sectional studies are merely "snapshots" of a specific point in time: they do not give information on the underlying genetic and environmental sources of continuity and change in the development of the disease or disease trait.[1] Second, many traits or risk factors for disease show a certain degree of interrelatedness, which may be due to the pleiotropic action of genes and/or common environmental influences. Extensions of the classic twin study to more complex designs involving multiple time points and multiple variables are needed to shed light on these questions.

Recent progress in molecular genetics and accompanying developments in biostatistics mark a new era in genetics in which the mapping of quantitative trait loci (QTLs) underlying complex nonmendelian traits and diseases has become within reach. In this new molecular-genetic era, twin studies remain highly valuable and can efficiently be used to identify the location and function of QTLs underlying, for example, complex lipid traits.[2]

This review has 2 main aims. The first is to describe the ways in which twin studies have been used to investigate the genetic architecture of lipids, lipoproteins, and apolipoproteins. More specifically, we discuss the age dependency of genetic effects and the importance of the concept of pleiotropy (ie, the same gene or set of genes simultaneously influences multiple traits) for the lipid system. Second, we discuss the consequences of age dependency and pleiotropy for twin studies aimed at detecting the actual genes involved.

Developmental Trends

Trends in Means

Lipids, including cholesterol and triglycerides, are transported in the circulation by a number of different lipoproteins, of which VLDL, LDL, and HDL are the most important. Apolipoproteins are bound to the surface of the lipoprotein particles and play an important role in lipid metabolism: through apolipoproteins, lipoproteins are actively recognized, bound, and absorbed by specific receptors.[3] Apolipoprotein A1 (apoA1) is part of HDL, and apolipoprotein B (apoB) is the sole protein of LDL. Another particle of the lipid system, lipoprotein(a) [Lp(a)], was first described by Berg[4] and is structurally related to LDL.[5] All of these constituents of the lipid sytem are important risk factors for coronary heart disease, as established by numerous epidemiological studies.[6–9]

The most dramatic changes in lipids, lipoproteins, and Lp(a) occur during the first years of life. During the first 6 months, concentrations double or triple.[10,11] Lp(a) concentrations already reach adult levels at 8.5 months.[11] Levels of

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other lipid traits rise further at a slower rate until about 2 to 3 years, when they approach young-adult levels. Cholesterol and HDL increase slightly between 3 and 5 years. No clear sex differences exist yet.[12] In preadolescents (5 to 10 years), cholesterol and triglyceride levels remain fairly constant.[13]

During puberty (12 to 16 years), cholesterol declines by 10% to 20% in boys, after which time it starts to rise, reaching preadolescent levels again by 20 years of age. In girls the picture is less clear: a drop is sometimes reported,[14] but a recent longitudinal study observed no change.[15] HDL levels in boys descend in this period but remain unchanged in girls.[14,15] Triglyceride levels show an increase in both sexes during adolescence, but the relative increase is higher in boys than in girls.[14] By the end of puberty, girls have equal cholesterol and triglyceride levels but higher HDL levels than do boys. The apoA1 and apoB changes during puberty parallel those in HDL and total cholesterol, respectively. Lp(a) shows no changes during this period and does not differ between sexes.[16,17]

The development in young males, between 18 and 30 years, shows an increase of cholesterol, LDL, triglycerides, and apoB and no change in HDL. In women, little change[18] or a rise is observed for cholesterol and LDL,[15] a decline in triglycerides, and a rise in HDL and apoA1.[18] This results in the typical sex difference in lipid profile as observed in the middle-aged population. Up to 50 years of age, cholesterol, LDL, and triglycerides continue to rise and HDL decreases slightly in both sexes.[19] In this period, males have higher cholesterol, triglyceride, LDL, and apoB levels but lower HDL and apoA1 levels than do females.[20,21] After the age of 50, female cholesterol levels exceed male levels.[20] Lp(a) increases slightly between 20 and 59 years and has the same level in both sexes in all age groups.[22]

Some lipids are influenced by the menopause. Matthews et al[23] performed a follow-up study of premenopausal women. In some women, menopause occurred during the follow-up period. Menopause led to a significant decrease in HDL. The increase in LDL was twice as large in menopausal compared with premenopausal women of the same age during follow-up. Total cholesterol, triglycerides, apoA1, and apoB changed to the same extent in both groups. Thus, apart from an age effect, no specific influence of menopause on these variables was evident. Lp(a) levels are a bit higher in postmenopausal women, but the effect disappears after age correction.[22]

In elderly people, from ≈65 years on, cholesterol levels drop, especially in males. HDL remains stable.[24,25] The fall in total cholesterol with old age, however, may represent cohort, period, or survivorship effects or a combination of these effects, rather than a true decline.[26]

To summarize, plasma lipid and (apo)lipoprotein levels vary considerably with age; the changes of the different lipid components do not run in parallel, and they differ for males and females. Superimposed on the global change during the whole life span, 4 more specific periods are associated with more dramatic changes: the first years after birth, adolescence, the menopausal period in females, and old age. These (sex-specific) age trends in lipid and (apo)lipoprotein levels indicate that different genetic and/or environmental factors may be involved in males and females and at different ages.

Trends in Variances

Generation and sex influences on the variances of components of the lipid system are less well understood than mean trends. Most studies did not have an explicit interest in age and sex trends in variances. Nevertheless, the large population studies on age and sex trends in mean levels also provide standard deviations. From these, it can be deduced that variances increase in most lipids and (apo)lipoproteins between early adulthood (between 20 and 29 years of age) and middle age (between 40 and 59 years of age) in both males and females.[19,20] During middle age, variances in lipids and (apo)lipoproteins remain relatively stable.[19,20,25,27] Only in old age (70+) are there indications for a decline.[20] These results pertain to both sexes. Except for the drop during old age, variance trends in Lp(a) are similar.[22]

In contrast to the above-mentioned studies, Reilly et al[28] specifically investigated the effects of sex and generation on variances in lipids and (apo)lipoproteins. Lipid variances were compared between grandparents (mean age 68), parents (mean age 42), and children (mean age 15) of both sexes. Significant differences between generations were found for total cholesterol and LDL in both males and females and for triglycerides, HDL, and apoA1 in males only. Differences could be explained by an increase in variance with age. Similar results were found by Boomsma et al[29] in a study of 160 adolescent twin pairs and their parents. Variances were significantly higher in the parental generation for total cholesterol, triglycerides, HDL, LDL, apoA1, and apoB. Only Lp(a) did not show a generation effect.

In summary, most studies report an increase in variance with age for most lipid traits in both sexes. Such an increase in lipid and (apo)lipoprotein variance from adolescence to adulthood may be due to interindividual variation in the rise of lipid levels over time and can only be explained by an increase in 1 or more of the underlying variance components, which can be either genetic or environmental.

Genetic Developmental Trends

Genetic factors have been shown to exert considerable influence on levels of lipids, lipoproteins, and apolipoproteins. A large number of monogenic disorders have been described,[30] but they can account for only a relatively small portion of the population variance in lipid concentrations. Quantitative genetic studies of twins and families indicate that a considerable part of the variation in lipids, lipoproteins, and apolipoproteins results from as-yet-unknown polygenic influences (reviewed in References 31 through 37).

As stated, sex-specific age trends in the levels and variances of lipid traits may indicate that different genetic and environmental factors may be influential in different sex-age cohorts. This age dependency can take 2 different forms. First, the magnitude of the genetic influence (ie, the heritability) of the lipid trait can differ with age. Second, different genes may affect the trait at different ages; ie, the expression of genes may depend on age. To investigate these 2 possibilities we (1) review evidence from cross-sectional studies of twins in different age ranges, (2) compare correlations between familial pairings that differ in their intrapair age difference: parent-offspring versus sib-pairs versus dizygotic (DZ) twin pairs, and (3) discuss the limited number of (quasi) longitudinal twin studies.

TABLE 1. Twin Studies Estimating Heritability (h^2) in Lipids and Lipoproteins, in Ascending Order According to Age

		Age, y				h^2			
Investigator	Pairs of Twins	Mean (SD)	Range	Sex	Adjustment	TC	LDL	HDL	TRG
Bodurtha et al[39]	65 MZM, 68 MZF	11.0 (?)	?	Male		0.71	0.85	0.76	0.68
	23 DZM, 27 DZF, 50 DOS			Female		0.80	0.85	0.76	0.68
Boomsma et al[29]	35 MZM, 35 MZF	16.7 (2.0)	13.0–22.0	Male		0.80	0.82	0.71	0.60
	31 DZM, 30 DZF, 29 DOS			Female		0.80	0.82	0.71	0.60
Whitfield and Martin[40]	42 MZM, 42 MZF	23.1 (4.6)	18.0–34.0	Male		0.54		0.24	0.53
	38 DZM, 44 DZF, 39 DOS			Female		0.54		0.24	0.51
Knoblauch et al[41]	100 MZ, 72 DZ	33.0 (14.0)	15.0–69.0		Sex	0.58	0.59	0.61	0.66
Hunt et al[42]	73 MZM, 81 DZM	34.5 (9.5)	21.0–61.0		Age	0.61		0.74	0.81
					Age, env	0.65		0.51	0.75
O'Connell et al[33]	39 MZM, 67 MZF	36.3 (12.4)	17.0–66.0		Sex	0.58	0.89	0.60	
	25 DZM, 69 DZF				Sex, env	0.79	1.00	0.63	
Williams et al[43]	44 MZF, 31 DZF	37.6 (?)	17.0–64.0			0.64	0.67	0.71	
Austin et al[44]	233 MZF, 170 DZF	42.0 (?)	?		Age		0.98	0.68	0.80
					Age, env		0.92	0.82	0.63
Berg[45]	35 MZM, 43 MZF	? (?)	33.0–61.0	Male		0.40			0.00
	33 DZM, 47 DZF			Female		0.40			0.72
Snieder et al[46]	39 MZM, 50 MZF	44.1 (6.7)	34.0–63.0	Male		0.68	0.69	0.71	0.59
	36 DZM, 40 DZF, 38 DOS			Female		0.68	0.69	0.71	0.59
Kervinen et al[100]	16 MZA	45.6 (11.8)	?			0.33	0.30	0.73	0.73
					Age, sex, wt, ht	0.28	0.22	0.59	0.32
Feinleib et al[47]	250 MZM, 264 DZM	48.0 (?)	42.0–56.0			0.43	0.57	0.46	0.56
Heller et al[35]	57 MZ, 94 DZ	? (?)	52.0–65.0		Age, sex	0.63		0.76	0.72
	56 MZ, 95 DZ	? (?)	66.0–86.0		Age, sex	0.32		0.55	0.28

TC indicates total cholesterol; TRG, triglyceride; MZM, monozygotic males; MZF, monozygotic females; DZM, dizygotic males; DZF, dizygotic females; DOS, dizygotic opposite sex; env, environment; and MZA, monozygotic twins reared apart.

Twin Studies

When twins within a specific age range are measured in studies estimating the genetic influence on lipids, lipoproteins, and apolipoproteins, heritabilities for this specific age range are obtained. To obtain an impression of possible age trends in heritability, we have listed recent twin studies (since 1977) in ascending order according to the age of the twin sample. Twin studies before 1977 were reviewed by Iselius.[31] Estimates for males and females are given separately. Heritability, estimated as $2(r_{MZ}-r_{DZ})$,[38] was listed for all studies that did not use model-fitting techniques. Some components of the lipid system that have been shown to be heritable and may confer a risk for coronary heart disease, such as LDL subclass phenotypes[34,101] and apoAII and apoE levels,[29] were not included in the Tables, as they were measured in only 1 or 2 twin studies. Results for lipids and lipoproteins are shown in Table 1. Although these studies used different methods to estimate heritability, results were relatively consistent. Most studies found that in both males and females, >50% of the total variance in total cholesterol, LDL, HDL, and triglycerides could be explained by genetic factors. Furthermore, the studies listed did not find much support for a considerable influence of the shared-family environment. No obvious age trend in heritability estimates can be detected from Table 1. Heritabilities measured before[39] and during[29] puberty are highly similar, and they remain fairly constant during early adulthood and middle age. Only in the elderly do heritabilities seem to decrease.[35]

Table 2 shows the results for apolipoproteins and Lp(a). Because fewer studies with mostly smaller twin samples were done for apolipoproteins, estimates were somewhat more variable compared with those in Table 1. Observed heritabilities were roughly within the same range as for lipids and lipoproteins. High heritabilities for Lp(a) are in accordance with findings that the apo(a) gene accounts for >90% of the variation in Lp(a) concentration.[48,49] Again, a clear age trend cannot be detected, although Heller et al[35] found a significantly smaller heritability for apoB in their older compared with their younger twin sample.

Comparison of Different Familial Correlations

Another approach to investigate the age dependency of genetic and environmental effects is to compare correlations between familial pairings that differ in their intrapair age difference.[56] An age-dependent genetic or environmental effect would predict an increase in the familial correlation with decreasing age difference: r(parent-offspring) < r(sib-pair) < r(DZ). Such a pattern was confirmed for cholesterol, as shown in a review by Iselius[31] of earlier twin and family studies. For 4716 parent-offspring pairs, a mean correlation of 0.26 was found, whereas for 2056 sibling pairs and 622 pairs of DZ twins, these values were 0.34 and 0.44, respectively. An alternative explanation for the lower parent-offspring correlation compared with the sibling or DZ twin correlation could be the influence of genetic dominance. This, however, cannot explain the difference between the sibling

TABLE 2. Twin Studies Estimating Heritability (h^2) in Apolipoproteins and Lp(a), in Ascending Order According to Age

		Age, y				h^2		
Investigator	Pairs of Twins	Mean (SD)	Range	Sex	Adjustment	ApoA1	ApoB	Lp(a)
Boomsma et al[16,29]	35 MZM, 35 MZF	16.7 (2.0)	13.0–22.0	Male		0.78	0.48	0.98*
	31 DZM, 30 DZF, 29 DOS			Female		0.78	0.48	0.93*
Sistonen and Ehnholm[50]	65 MZ, 70 DZ	36.0 (?)	20.0–69.0		Sex	0.36		
Berg[45,51]	44 MZM, 54 MZF	? (?)	33.0–61.0	Male		0.53	0.52†	
	46 DZM, 54 DZF			Female		0.53	0.74†	
Snieder et al[46]	39 MZM, 50 MZF	44.1 (6.7)	34.0–63.0	Male		0.58	0.73	0.87
	36 DZM, 40 DZF, 38 DOS			Female		0.58	0.73	0.87
Austin et al[52]	338 MZF, 250 DZF	51.0 (?)	?					0.94
Kuusi et al[53]	17 MZM, 18 DZM	55.0 (?)	48.0–63.0			0.66		
Hong et al[54]	63 MZ, 108 DZ	57.6 (5.3)	50.0–64.0		Age, sex			0.94
Heller et al[35]	57 MZ, 94 DZ	? (?)	52.0–65.0		Age, sex	0.69	0.78	
Hayakawa et al[55]	42 MZM, 22 MZF	? (?)	50.0–74.0	Both		0.48	0.80	
	17 DZM, 2 DZF							
Lamon-Fava et al[34]	109 MZM, 113 DZM	63.0 (?)	60.0–70.0			0.38	0.91	0.67
					Env	0.38	0.85	
Hong et al[54]	55 MZ, 78 DZ	71.1 (4.4)	65.0–86.0		Age, sex			0.94
Heller et al[35]	56 MZ, 95 DZ	? (?)	66.0–86.0		Age, sex	0.52	0.51	

For explanation of abbreviations, see the footnote to Table 1.

*Estimates are the same for parents and their twin offspring (152 families; see Reference 16).

†Estimates for apoB are based on a smaller number of twin pairs, as listed in Table 1.

and DZ twin correlation. In accordance with Iselius,[31] Boomsma et al[29] also observed lower parent-offspring than DZ twin correlations for lipids, lipoproteins, and apolipoproteins. Two types of age effect could offer an explanation for the decrease in familial correlation with increasing intrapair age difference. First, the influence of nonshared environmental factors could increase with age. However, such an effect would mean an accompanying decrease of heritability with age, which is incompatible with the results listed in Tables 1 and 2. A more likely explanation, therefore, is that the expression of genes may vary with age (eg, genes may switch on and off during development), while the magnitude of heritability remains relatively stable. Recent studies on body mass index[57] and blood pressure[58–60] indicate that it may well be possible that the same phenotype is influenced by different genes in different periods of life. This assumption has been tested in a limited number of longitudinal twin studies.

Longitudinal Studies

Nance et al[61] investigated the causes for continuity and change of HDL cholesterol during adolescence. Longitudinal data of monozygotic (MZ) and DZ twins at 11, 12.5, and 14 years of age were analyzed by multivariate model–fitting techniques. The magnitude of the random environmental influence was constant over time and specific for each occasion. Important developmental changes in gene expression were found for this age period. Depending on the different competing models that all fitted the data well, between 19% and 40% of the total variation in HDL consisted of the effects of newly expressed genes. Total heritability estimates, including the effects of both time-stable and newly expressed genes, were ≈80% and remained stable over the 3 measurement occasions.

Friedlander et al[62] analyzed changes in total cholesterol, LDL, HDL, and triglycerides over a 10-year time period using data from 2 examinations of the Kaiser-Permanente women twin study. Average ages at the 2 examinations were 41 and 51 years. Moderate heritability estimates of ≈30% were demonstrated for changes in LDL and HDL cholesterol. No significant genetic effect on change in total cholesterol or triglycerides was found. The genetic influence on changes in LDL and HDL indicates that new genes come to expression over this 10-year time span.

Williams and Wijesiri[63] studied the stability of genetic influences during middle age. They analyzed longitudinal data of male veteran twins on total cholesterol, LDL, HDL, and triglycerides. Between 48 and 63 years of age, subjects were measured 3 times. As evidenced by the large correlations between genetic effects at successive examinations, similar genetic effects on lipids appear to be present throughout this segment of the life span.

As an alternative to a longitudinal study, Snieder et al[46] measured total cholesterol, LDL, HDL, triglycerides, apoA1, apoB, and Lp(a) in a group of 160 middle-aged parents and their twin offspring and in a group of 203 middle-aged twin pairs of the same age as the parents in the first project. Combining the data of both projects enabled the estimation of the extent to which measured lipid traits are influenced by different genes in adolescence and adulthood and at the same time allowed the estimation of heritabilities for each sex and generation separately. Heritabilities were similar for both sexes and both generations. Larger total variances in the parental generation could be ascribed to proportional increases in both unique environmental and additive genetic variance from childhood to adulthood, which led to similar heritability estimates in adolescent and middle-aged twins.

Although the magnitudes of heritabilities were similar across generations, results showed that for total cholesterol, triglycerides, HDL, and LDL, partly different genes are expressed in adolescence compared with adulthood. For triglycerides, only 46% of the genetic variance was common to both age groups; for total cholesterol, this proportion was 80%. Intermediate values were found for HDL (66%) and LDL (76%). The rest of the genetic variance can thus be attributed to age-specific genetic effects. For apoA1, apoB, and Lp(a), no age effects were found, implying that the same genes act in both generations.

Conclusions With Respect to Age Effects

Based on the reviewed cross-sectional and longitudinal twin studies, it can now be concluded that the magnitude of the genetic influence for total cholesterol, LDL, HDL, and triglycerides remains relatively stable with age, with consistently high heritability estimates. However, the limited number of available longitudinal studies point to the existence of age-dependent gene expression in at least some of the lipids and lipoproteins. New genes are expressed during adolescence[61] and during middle age in women,[62] and gene expression is different in childhood and adulthood.[46] These results indicate that lipids and lipoproteins are influenced by a different combination of multiple genes in different periods of life.

For apolipoproteins and Lp(a), less information proved to be available in the literature. Although heritability estimates were, therefore, more variable for these traits also, no clear age trend in the magnitude of heritability estimates could be detected. The only study that investigated whether gene expression was age dependent for these traits found no evidence for such an effect.[46] For Lp(a) this was an expected finding, in accordance with evidence that >90% of the variation in Lp(a) is determined by a single gene, the apo(a) gene,[48,49] which is located on the tip of the long arm of chromosome 6.[5,8] In their study of 5- to 13-month-old babies and their parents, Wang et al[11] concluded, on the basis of the high parent-offspring correlations, that the apo(a) gene is fully expressed before the age of 1 year. The difference in age-specific genetic influence on lipids and lipoproteins compared with the apolipoproteins might point to a simpler genetic architecture for the apolipoproteins. In contrast to the genetic architecture of cholesterol, triglycerides, LDL, and HDL in which, besides some major gene effects, most genetic variation is polygenic,[64–67] genetic variation in apolipoproteins may be largely determined by 1 gene [as in Lp(a)] or a few major genes whose effects remain stable with age.

Pleiotropy in Lipids and (Apo)lipoproteins

The majority of quantitative genetic studies of the lipid system have used univariate analysis to decompose the variation of single lipid variables into its constituent parts. However, lipids, lipoproteins, and apolipoproteins compose a complex system of highly intercorrelated traits[68,69] that most probably act in concert to cause an increased cardiovascular risk. Therefore, it is important to acquire insight into the sources of their covariation. Multivariate genetic modeling of twin (or family) data not only involves a decomposition of phenotypic variances into its various components but also makes it possible to determine to what extent the covariation between multiple measures is due to common genetic and/or common environmental factors.[70,71] Multivariate models can thus be an important aid in unraveling the sources of interrelations between multiple components of the lipid system.

Only a few studies have performed quantitative genetic analyses on multiple components of the lipid system simultaneously. Colletto et al[72] used a bivariate path model to analyze lipid data (cholesterol, triglycerides, VLDL, LDL, and HDL) from 105 pairs of Brazilian twins of both sexes. This approach enabled them to estimate the genetic correlation between the genes for the different lipid components. For all possible pairings, significant genetic correlations were found with the highest values for total cholesterol and LDL (0.76) and for triglycerides and VLDL (0.83). Genetic correlations between HDL and both triglycerides and VLDL were negative (−0.24 and −0.26, respectively). On the basis of the high genetic correlations for all lipid variables, the authors suggested that there may be 1 common genetic factor for all lipid components. Mosteller[73] employed a triangular (Cholesky) decomposition on data from 381 female twin pairs, including body mass index, HDL, LDL, and age. Dominance genetic effects were found to be most important in bringing about the phenotypic correlation between HDL and LDL (−0.15). Heller et al[69] employed multivariate model fitting (a Cholesky decomposition) on data from younger (<65 years) and older (>65 years) twins, reared apart and together, to partition phenotypic correlations between total serum cholesterol, triglycerides, HDL, apoA1, and apoB into their genetic and environmental sources. Both genetic and unique environmental factors were found to be important in mediating the phenotypic correlation, but there was no evidence for a single genetic factor common to all 5 lipids. Using bivariate analysis on data from 100 MZ and 72 DZ twin pairs, Knoblauch et al[41] detected common genetic influences on HDL and triglycerides and on LDL and triglycerides, but not on HDL and LDL. Genetic correlations were not reported.

All of the above studies point to the importance of common genetic effects mediating intercorrelations between lipid traits. However, the results of Heller et al[69] suggest that the underlying genetic architecture is not simple: there does not seem to be 1 gene or set of genes responsible for the covariance between the different lipid components. Several family studies confirm the complexity of the genetic architecture of the lipid system. Vogler et al[74] analyzed family data on VLDL, LDL, and HDL within a multivariate path model. Genetic correlations between HDL and VLDL (−0.22) and between VLDL and LDL (0.35) were found, but phenotypic covariation of all 3 lipoproteins could not be ascribed to a single genotype. Mahaney et al[75] conducted multivariate analysis on data from 569 subjects in 25 pedigrees and found a significant genetic correlation (−0.52) between HDL and triglycerides, indicating pleiotropy. However, the authors found no support for the so-called conjoint hypothesis, which states that combined low HDL and high triglycerides are inherited as a single phenotype. Instead, they concluded that the inverse relation between the 2 traits throughout their normal ranges of variation as well as at the extremes is influenced by shared genes and shared environments. Factor analysis of phenotypic, genetic, and environmental correla-

tion matrices of lipid data from the Tel Aviv–Heidelberg 3-generation offspring study identified a few distinct groups: apoA1-dependent lipids (apoA1 and HDL fractions) and apoB-dependent lipids (apoB, total cholesterol, and LDL). Triglycerides and Lp(a) seemed to be relatively independent of all other lipids.[76]

Although multivariate twin studies have yielded important information on the genetic (and environmental) origin of correlations within the lipid system, only exploratory quantitative genetic models like the triangular (Cholesky) decomposition were applied. These models have limited explanatory power because they do not allow tests of the direction of causation between different phenotypes. Not all associations need be explained in terms of common genetic or environmental factors (see Reference 77 for a range of possible bivariate models). Future studies, therefore, will have to apply models that try to incorporate prior physiological knowledge on the causal relationships between different components of lipid metabolism.[78] One example of such a relation is the basic role that apolipoproteins play in the metabolism of other lipoprotein particles like VLDL, LDL, and HDL, by acting as a structural protein and a ligand for cell-surface receptors.[3]

Implications for Gene Finding

Use of Twins in QTL Detection

High heritabilities of lipids and (apo)lipoproteins provide a strong argument for further research with the ultimate goal to locate the genes and subsequently uncover their function. Identification of these genes and their function will increase our insight into the regulation of levels and the variability of lipids and (apo)lipoproteins, which may eventually enable the development of interventions tailored to subjects with specific genetic predispositions.

Most lipid traits are continuously distributed and influenced by multiple genes [with the notable exception of Lp(a)], each with a relatively small effect. As the transmission of these complex polygenic traits does not follow simple mendelian rules, identification of underlying genes (also called QTLs) is difficult. Recent progress in molecular genetics has enabled the production of a dense marker map of the human genome, which has brought the localization of QTLs within reach. Several methods have been developed to map loci that influence quantitative traits in data from sibling pairs.[79,80] These methods suppose that if a marker is cosegregating with a quantitative trait, then siblings whose trait values are more alike are more likely to receive the same alleles identical by descent (IBD) at a closely linked marker locus than are siblings whose resemblance for the trait is less.

Because DZ twins are genetically full siblings, all sib-pair methodology can be applied equally well to DZ twin data. If measurements of the phenotypes of interest (eg, lipids) are available, all that is additionally needed are DNA marker data. Using DZ twins instead of sib-pairs even has some additional advantages. DZ twins are less likely to have different fathers and, more important, DZ twin pairs are more closely matched for age (or age-dependent gene expression) and environmental influences (eg, cohort effects). This means that differential resemblance among DZ twin pairs will remain strictly a function of differential IBD sharing, whereas differential sib-pair resemblance maybe confounded by environmental and age effects.[2]

The extension of the classic twin study with DNA marker data enables the search for previously unknown QTLs in a genome scan. Alternatively, the effect of known candidate genes may be tested for linkage with the phenotype within the same twin study (See Reference 81 for a list of candidate genes for lipid metabolism and their chromosomal positions). Two recent studies applied the latter approach for lipid data. Knoblauch et al[41] examined linkage between markers close to the LDL receptor gene, the lipoprotein lipase gene, and the macrophage scavenger receptor gene and total cholesterol, LDL, HDL and triglycerides in 72 DZ twin pairs. The data suggested a significant influence of the macrophage scavenger receptor gene on HDL and a weak effect on triglycerides. No other linkages were found. Austin et al[82] tested the effects of 8 different candidate genes on LDL cholesterol, LDL particle size, HDL, triglycerides, and apoB in 126 women twin pairs. Results suggested linkage between markers for the apoB gene and LDL particle size and plasma levels of HDL, triglycerides, and apoB. A further linkage was found between the gene for the microsomal triglyceride-transfer protein and triglycerides.

Another generally more powerful method to investigate the phenotypic effect of a specific locus in twin or sib-pair data is to use tests for association, as opposed to linkage. Such approaches need to take into account the dependency of the observations within sib-pairs.[83] However, these methods may produce spurious results in the presence of population admixture. Therefore, applications of the transmission disequilibrium test for use in sibship data are being developed.[84,85] These transmission disequilibrium tests are insensitive to the effects of population admixture and provide a test for both linkage and association if sibships consist of 2 sibs only (ie, sib-pairs).

Implications of Pleiotropy

Sib-pair strategies have several advantages compared with other methods.[86] Trait and genetic-marker data need be obtained from siblings only, rather than from large multigenerational pedigrees. Furthermore, sib-pair methods do not involve any assumptions concerning the mode of transmission, which implies that the intermediate step of segregation analysis between twin/family studies and linkage analysis is no longer necessary. However, 1 major drawback that sharply contrasts with the aforementioned advantages of the sib-pair method is that even with large numbers of highly polymorphic markers that enable determination of the IBD status of siblings, the power to detect a single locus that influences quantitative traits in humans remains low (eg, see References 87 and 88).

One strategy to increase the power to detect QTLs is to make use of the pleiotropic influence of genes on multiple lipid traits and to use multivariate genetic modelling.[89] Ample evidence shows that correlations between lipid traits are at least partly due to common genetic factors. Multivariate analysis can be used to test whether the same genetic factor, or QTL, pleiotropically influences multiple phenotypically correlated lipid traits. If a common genetic factor is found, then scores on this factor can be constructed for an individual by standard methods for the estimation of factor scores.[90,91]

This approach to estimate an individual's genotypic value at a QTL reduces not only environmental variance but also the background genetic variance not associated with the QTL. In several simulation studies, Boomsma and colleagues[92–94] and Martin et al[2] have shown that, with this application of multivariate modeling, the power to detect QTLs in a sib-pair analysis of quantitative traits can be increased substantially.

Once genes responsible for coaggregation between lipid traits have been located and their physiological pathways uncovered, this knowledge can be effectively applied in the development of (pharmacological) therapies and the design of intervention strategies. The most cost-effective use of resources is to focus on intervention that will have the largest impact on overall risk. Pharmacological intervention that affects multiple risk factors will have a greater effect on public health than do interventions focusing on a single risk factor only.[36]

Implications of Age Dependency

Evidence of age-dependent gene expression also has important implications for the design of gene-finding studies. Because genes influencing a lipid trait at 1 age period may be different from genes expressed during another age period, selection of homogeneous age samples will optimize the power to detect those genes. Obviously, selection of such a homogeneous age sample is easier in sib- and DZ twin–pair designs than in family studies.

Alternatively, twin or sib-pair studies can be optimally used to gain a better understanding of several types of age-dependent gene expression. First, gene regulation of lipid changes during a specific phase in development can be investigated. Twins or sib-pairs can be followed up longitudinally through a specific age period like puberty or the menopause, when gene expression is most likely to change, with the aim of pinpointing the specific loci involved in developmental changes in gene expression. Second, age-dependent changes in the penetrance of underlying "level" genes can be examined. Possibly triggered by the cumulative exposure to certain environmental factors, specific genes may be switched on, leading to a gradual change in lipid values with age. Jarvik et al,[95] for example, found that changes with age over a 16-year period (48 to 64 years of age) in total cholesterol and triglycerides were dependent on the specific apoE genotype. Finally, an effort can be made to map so-called "variability" genes. These genes are sensitive to environmental changes that cause large intraindividual variability in lipid traits, which per se may comprise a risk factor for coronary heart disease.[62] Magnus et al[96] and Martin et al[97] found that intrapair variance of cholesterol in MZ pairs (which must be solely due to environmental factors) who had blood group M^- (ie, blood group N) was greater than in pairs who had M^+ (ie, blood groups M and MN), suggesting a higher environmental sensitivity of the M^- genotype. This concept was confirmed in a low-fat dietary intervention study, which showed that individuals with blood group N had the greatest lowering in their cholesterol values, whereas the heterozygous group (MN) showed the least change.[98] Finding environmentally sensitive genes may thus have important practical implications in enabling the targeting of treatment to the most responsive individuals. MZ twin studies could be especially useful in finding these environmentally sensitive genes.[99]

Conclusions

Twin studies are and have been very useful in dissecting the genetic architecture of lipids, lipoproteins, and apolipoproteins. Even in the absence of genotypic data, they have provided a wealth of information on the importance of genetic factors in determining lipid traits, the age-dependent expression of these genetic factors, and their pleiotropic effect on multiple lipid traits. Rapid developments in molecular genetics and biometrics have brought in a new era in which the detection of QTLs that underlie complex lipid traits has come within reach. Twin studies will continue to play an important role in this new era. Taking account of pleiotropy and age-dependent gene expression in study design and data analysis will improve the power and efficiency to find QTLs for components of the lipid system.

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