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Heritability of Longitudinal Changes in Coronary-Heart-Disease Risk Factors in Women Twins

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Summary

Numerous studies have demonstrated genetic influences on levels of coronary heart disease (CHD) risk factors, but there also may be genetic effects on the intraindividual variation in these risk factors over time. Changes in risk factors are likely to reflect genetic-environmental interactions and may have important implications for understanding CHD risk. The present study examines the heritability of changes in CHD risk factors, using data from the two examinations by the Kaiser Permanente Women Twins Study, performed a decade apart. The sample consisted of 348 pairs of women twins who participated in both examinations, including 203 MZ pairs and 145 DZ pairs. Average ages at the two examinations were 41 and 51 years, respectively. By means of three different statistical analytic approaches, moderate heritability estimates were demonstrated for changes in LDL cholesterol (h^2 = .25–.36) and in HDL cholesterol (h^2 = .23–.58), some of which were statistically significant. Although small to moderate heritability estimates were found for systolic blood pressure (.18–.37; $P < .05$ for some estimates), no genetic influence on changes in diastolic blood pressure was detected. Based on longitudinal twin data in women, this study demonstrates a genetic influence on changes in both lipoprotein risk factors and systolic blood pressure over a decade. In addition to environmental factors, which clearly are operating, the effect of various "variability genes" may be acting independently of the genetic influences on the absolute levels of these risk factors. Both mapping the gene(s) underlying intraindividual variations in these CHD risk factors and understanding their function(s) could lead to targeted intervention strategies to reduce CHD risk among genetically susceptible individuals.

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Introduction

Numerous studies have demonstrated genetic influences on levels of coronary heart disease (CHD) risk factors, including blood pressure, lipoproteins, and body mass (Feinleib et al. 1977; Stunkard et al. 1986; Austin et al. 1987; Hunt et al. 1989; Heller et al. 1993; Rice et al. 1993). There may also be genetic effects on the intraindividual variation in these risk factors over time. Changes in risk factors are likely to reflect genetic-environmental interactions, and may have important implications for understanding the effectiveness of therapeutic interventions to reduce CHD risk (Zerba and Sing 1993). It has been proposed that genetic-environmental interactions could reflect the presence of "variability genes" that determine the fluctuation in risk-factor levels in an individual over time (Berg 1994). For example, this concept has been supported by a study of the apolipoprotein (apo) B locus and the apo AI-CIII-AIV gene cluster in relation to variation in serum cholesterol levels over 5–10 years in a group of men with peripheral vascular disease (Monsalve et al. 1991). Similarly, a small study has shown that variation at the β-fibrinogen locus is related to intraindividual variations in plasma fibrinogen levels over 3–4 years (Cook et al. 1988).

Longitudinal twin studies provide a unique opportunity to better understand potential genetic influences on disease and risk factors (e.g., see Carmelli et al. 1994; Marenberg et al. 1994). Prospective twin studies also can be used to estimate the proportion of variance of changes in risk factors, over time, that is attributable to genetic influences. The purpose of the present study is to investigate genetic influences on intraindividual variation in lipoprotein and blood pressure–related CHD risk factors, by performing heritability analysis on changes in risk-factor levels over the course of a decade. The study makes use of a sample of nearly 700 adult women twins who participated in two examinations, a decade apart, by the Kaiser Permanente Women Twins Study.

Subjects and Methods

Study Subjects

The women twins included in this analysis participated in both the first and second examinations by the

Kaiser Permanente Women Twins Study in Oakland, conducted during 1978–79 and 1989–90, respectively. At the first examination (exam 1), 434 pairs of female twins born in 1960 or earlier participated (Austin et al. 1987). At that time, zygosity was determined on the basis of 20 polymorphic loci, such that the probability of misclassification of a pair concordant on all these markers as MZ was $<.001$. The mean age of the women was 41 years, and 90% were Caucasian. Eighty-one percent of the original sample returned for the second examination (exam 2), for a total of 696 women in 348 twin pairs who underwent both examinations, of whom 203 pairs were monozygotic (MZ) and 145 pairs were dizygotic (DZ) (Austin et al. 1992). At both visits, each woman had a physical examination and completed an extensive medical history questionnaire, including demographic information and information on a variety of CHD risk factors and on medication use and menopausal status (Austin et al. 1992; Selby et al. 1993).

Laboratory Measurements and Blood-Pressure Determinations

At both examinations, lipid and lipoprotein determinations were based on blood samples drawn after a 12-h fast. At exam 1, serum total cholesterol (TC), triglycerides (TG), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) were determined by use of the methods of the Lipid Research Clinics Program in a Centers for Disease Control (CDC)–standardized laboratory (Austin et al. 1987). At exam 2, lipid measurements were performed by use of plasma samples in the CDC-standardized Donner Laboratory at the University of California, Berkeley (Edwards et al. 1994). Subjects and their co-twins were excluded from the lipid analyses if, at either exam, either one of each pair was not fasting, was taking lipid-altering medications, or had missing or extreme values (TC >350 mg/dl or TG >400 mg/dl). As a result of these exclusions, data from 604 women in 302 pairs, including 179 MZ pairs and 123 DZ pairs, were available for the lipid analyses.

At both examinations, blood pressure was measured by a trained individual after the patient had been seated for 5 min, by use of a standard mercury sphygmomanometer. At exam 1 a single blood-pressure measurement was obtained, whereas at exam 2 two recordings, 1 min apart, were obtained on the right arm; the results of these two recordings were averaged for the present analysis. For blood-pressure measurements, subjects and their co-twins were excluded if either one was taking antihypertensive medications or had missing values at either examination. As a result, 416 women in 208 pairs, including 133 MZ and 75 DZ pairs, were included in the blood-pressure analysis.

Height (in m) and weight (in kg) were measured at both examinations while subjects were dressed in lightweight clothes with shoes removed. Body-mass index (BMI), used for adjustment of risk factors, was calculated as weight(kg)/height(m)^2.

Statistical and Genetic Analyses

Descriptive statistics of the risk factors, including skewness, were calculated separately for individual MZ and DZ women at exam 1 and at exam 2. Because the frequency distributions of most risk factors at both exams were skewed, these variables were transformed by use of a natural log transformation. However, antilog mean values are reported, for ease of interpretation. Comparisons of mean values for changes (Δ) in risk-factor values, from exam 1 to exam 2, were performed by use of paired *t*-tests based on nontransformed values. These computations were performed by use of the Statistical Analysis System (SAS Institute 1985).

Heritability analysis.—To determine the proportion of variance of the risk factors that is attributable to genetic influences, heritability analyses were performed for risk-factor levels at exam 1, levels at exam 2, and changes (Δ) in levels from exam 1 to exam 2. Heritability estimates near 0 imply that there are no genetic effects, whereas values close to 1 imply strong genetic influence, under the assumption of an underlying polygenic model.

Heritability analyses were performed by use of three different statistical methods: First, the "classical" heritability estimate was calculated as twice the difference of the MZ and DZ intraclass correlations. Second, the analysis of variance (ANOVA) model was employed, with the modifications proposed by Christian et al. (1974). Specifically, if the *F*-test of equality of total variances for MZ and DZ twins was not statistically significant, the "within-pair" (WP) estimate of heritability is reported. If, however, this *F*-test was significant, the less biased, "among-components" (AC) estimate is reported. This AC estimate is a less powerful estimate of genetic variance, because its standard error is larger than that of the WP estimate. Heritability estimates are not reported if the intraclass correlation for DZ twins is not statistically significant at the $P = .1$ level, implying little genetic influence among sibs who, on average, share half their genes. Similarly, heritability estimates are not reported if the DZ intraclass correlation exceeds the MZ intraclass correlation. Third, maximum-likelihood heritability estimates (Christian et al. 1995) were obtained by use of the computer program TWINAN90 (Williams et al. 1992). In this method, parameters are estimated on the basis of the sample covariance matrices for each zygosity, as described by Heath et al. (1989). A series of models are fit to the data, under the assumption that variation in the risk factor is attributable to a combination of nonshared environmental effects (E), common environment variance (C), additive genetic variance (A), and/or dominant genetic variance (D). These models are denoted as follows: E = nonshared environment only; AE = A and E; CE = C and E (no genetic component);

ACE = A, C, and E; and ADE = A, D, and E. The most appropriate model for each risk factor is selected on the basis of likelihood statistics, by use of the recent recommendations proposed by Christian et al. (1995); that is, C is first estimated by means of the ACE model. Its significance is tested by comparison of the likelihood values for the ACE and AE models, by means of a χ^2 statistic. If the null hypothesis is accepted (C = 0), estimates of A are obtained from the AE model, and the statistical significance of A is tested by comparison of the likelihood values of the AE and E models. On the other hand, if the null hypothesis is rejected (i.e., C ≠ 0), then A is estimated on the basis of the ACE model, and the statistical significance of A is tested by comparison of the likelihood values of the ACE and CE models. In general, if the selected model includes a genetic component (A and/or D), the heritability point estimate is calculated as the proportion of the genetic variance divided by the total variance. In the current analysis, the AE model was used for most, but not all, of the heritability estimates calculated by use of this approach.

For the heritability analyses, both the levels of the risk factors at exam 1 and exam 2 and the changes in the risk factors between exams were first adjusted for age, by regression analysis. Regression equations were determined separately for each of these variables, by zygosity (Austin et al. 1987). Intraclass correlation coefficients, reflecting the similarity of co-twins in the same pair, were determined by use of the age-adjusted values of the risk factors.

Adjustment for environmental and behavioral factors.—Because co-twins in the same pair tend to share environment as well as genes, heritability estimates potentially can be spuriously overestimated (Feinleib et al. 1977). Indeed, results based on examination 1 of this study demonstrated that environmental and behavioral factors were more similar in MZ co-twins than in DZ co-twins (Austin et al. 1987). This difference is denoted "differential environmental covariance." To avoid this potential bias, changes in risk-factor levels were adjusted for available environmental and behavioral variables at each of the two examinations, by means of multiple regression analysis, in addition to the age adjustment described above. The environmental and behavioral variables included education, full-time employment outside the home, marital status, medication and oral contraceptive use, menopausal status, parity, exercise, current cigarette smoking, alcohol and coffee consumption, special diet, and degree of current contact with co-twin. For each risk factor–change variable, separate equations were developed for MZ and DZ twins, to allow for differential effects of the environmental variables in the two groups of twins.

On the basis of these equations, a predicted value was calculated for each risk factor–change value for each woman, reflecting primarily the associations between environmental and behavioral variables and risk-factor changes. A residual value was calculated as the difference between the observed and predicted risk-factor values, and these standardized residuals were then used in the heritability analysis.

Furthermore, in order to adjust lipid and blood-pressure changes as completely as possible, the regression analysis was repeated with inclusion of BMI as an additional independent variable. Finally, the risk-factor value at exam 1 also was included in the regression models, to adjust for potential effects of regression to the mean. Heritability analyses were repeated with standardized residuals from these models as well.

Results

Ten-Year Changes in Risk-Factor Levels

The mean values of the risk factors in individual women at exams 1 and 2 are summarized in table 1, by zygosity. Among MZ women (table 1), both TC and LDL-C increased significantly over the 10-year period between exams (mean difference 7.3 mg/dl and 5.3 mg/dl, respectively). Plasma TG also increased significantly (11.0 mg/dl), whereas the HDL-C mean values did not change. Mean values of systolic blood pressure (SBP) increased, with a mean difference of 5.7 mm Hg, but diastolic blood pressure (DBP) did not.

Similar results were seen in DZ twins (table 1). Although both mean TC and mean LDL-C increased (7.3 mg/dl and 2.4 mg/dl, respectively), only the mean difference in TC was statistically significant. Mean TG values also increased in the DZ women (18.7 mg/dl), whereas mean HDL-C increased slightly, but not significantly (1.1 mg/dl). A trend of increased SBP was noticed in DZ women, whereas no such trend for DBP was seen.

Heritability of Risk Factors at Exams 1 and 2

Age-adjusted intraclass correlations and heritability estimates for each of the risk factors, with the three methods of heritability analysis, are summarized in table 2, for exams 1 and 2. At exam 1, the intraclass correlations for MZ pairs were consistently higher than the correlations for DZ pairs, for all risk factors except DBP. Both TC and LDL-C have high heritability estimates, range .65–.92 (all $P \leq .001$), implying that at least two-thirds of the variance in these risk factors may be attributable to polygenic genetic influences. A similar result is seen for HDL-C, with heritability estimates in the range .73–.94 (all $P \leq .001$). The heritability estimates for TG are more moderate, ranging from .29 ($.05 < P < .10$) to .50 ($P < .001$). Moderate genetic influences on SBP are also inferred on the basis of heritability estimates, ranging from .37 ($.05 < P < .10$) to .53 ($P < .05$), but are not inferred for DBP.

At exam 2 (table 2), similar intraclass correlation coefficients and high heritability estimates were found for

Table 1

Mean ± SD Values of CHD Risk Factors in Individual Women Twins, by Zygosity

Risk Factor	No. of Women	Mean ± SD		
		Exam 1 (1978–79)	Exam 2 (1989–90)	10-Year Change (Exam 2 − Exam 1)
MZ twins:				
TC (mg/dl)	358	192.4 ± 38.2	199.7 ± 38.8	7.3 ± 29.8*
LDL-C (mg/dl)	358	112.6 ± 32.8	117.9 ± 35.0	5.3 ± 26.7*
TG (mg/dl)	358	83.9 ± 45.3	94.9 ± 54.8	11.0 ± 48.0*
HDL-C (mg/dl)	358	63.0 ± 15.9	62.8 ± 16.4	−.2 ± 12.5
SBP (mm Hg)	266[a]	105.6 ± 13.1	111.5 ± 15.8	5.7 ± 14.5*
DBP (mm Hg)	266[a]	66.7 ± 8.8	66.2 ± 10.4	−.3 ± 10.6
DZ twins:				
TC (mg/dl)	246	195.5 ± 38.8	202.8 ± 33.9	7.3 ± 31.4*
LDL-C (mg/dl)	246	115.4 ± 34.7	117.9 ± 31.4	2.4 ± 27.2
TG (mg/dl)	246	81.4 ± 43.5	100.1 ± 61.4	18.7 ± 51.9*
HDL-C (mg/dl)	246	63.9 ± 16.2	64.9 ± 17.4	1.1 ± 13.8
SBP (mm Hg)	150[a]	106.4 ± 15.0	114.6 ± 18.4	8.0 ± 16.7*
DBP (mm Hg)	150[a]	67.2 ± 9.9	66.1 ± 10.8	−.8 ± 10.9

[a] The smaller sample size for blood pressure is due to the exclusion of women on antihypertensive medications and of their co-twins.

* $P \leq .001$, derived from a paired *t*-test on the natural log transformation of each risk factor.

TC and HDL-C. However, since the DZ correlation was not statistically significant for LDL-C at this exam, no heritability estimate is reported. For TG and for SBP measures, heritability estimates were moderate, but they were not consistent among the different estimation procedures. This may be attributable to a significant difference in the variance of these risk factors for MZ and DZ twins and to the use of the AC estimate from ANOVA analysis. Similarly, heritability estimates for DBP at exam 2 were not conclusive.

Heritability of Changes in Risk Factors over a Decade

Table 3 summarizes the age-adjusted heritability of longitudinal changes of risk factors, by comparing the similarity of changes, in risk-factor levels, from exam 1 to exam 2, among the MZ and the DZ pairs. For ΔTC, the intraclass correlation was slightly higher for MZ pairs than for DZ pairs, but the heritability estimates indicate little, if any, genetic influence. Only moderate genetic influences on ΔLDL-C and ΔHDL-C were seen (heritability range .33–.36 and .23–.27, respectively). For SBP, both the classical and ANOVA heritability estimates were relatively low (.19), yet the maximum-likelihood estimate based on the AE model was higher (.35) and statistically significant. Since the intraclass correlations for both ΔTG and ΔDBP were higher for DZ pairs than for MZ twins, heritability estimates are not reported. These results for ΔTG and ΔDBP are also consistent with the maximum-likelihood analysis, indicating a common environmental component and a nonshared environment component (the CE model) but no genetic component for these variables.

Adjustment of changes for differential environmental covariance.—Perhaps the most serious potential bias in heritability estimates obtained from twin studies is the spurious overestimation of genetic influences because MZ twins may share environments that are more similar than those shared by DZ twins (Austin and Newman 1993). To reduce the possibility of bias attributable to differential environmental covariance, changes in risk-factor levels were adjusted for environmental and behavioral factors, for environmental and behavioral factors and BMI, as previously described, and for environmental and behavioral factors, BMI, and risk-factor levels at exam 1 (Austin et al. 1987; see Subjects and Methods section).

The R^2 values from the regression analyses used for these adjustments are summarized in table 4. R^2 values for age alone were small, range .0–.10, and thus explained only a small proportion of the variance in the risk factors. The R^2 values increased slightly with adjustment for environmental factors but were similar for MZ and DZ twins. For lipids, R^2 values were .09–.29. For blood-pressure variables, the R^2 values were similar, range .07–.14. With the addition of BMI to the adjustment, R^2 values increased for some, but not all, risk factors. Finally, as expected, R^2 values increased additionally with further adjustment for the exam-1 level of each corresponding risk factor.

Adjusted heritability estimates of changes in risk factors.—The heritability analysis of changes in risk-factor levels, after adjustment for additional covariables, are summarized in table 5, for ΔLDL-C, ΔHDL-C, and ΔSBP—that is, those variables found to have a genetic

Table 2

Age-Adjusted Intraclass Correlations and Heritability Estimates of CHD Risk Factors in Women Twins

Risk Factor[a]	No. of Twin Pairs		Intraclass Correlations		h^2[b]		
	MZ	DZ	MZ	DZ	Classical	ANOVA	Maximum Likelihood (Preferred Model)
Exam 1 (1978–79):							
TC	179	123	.67****	.22***	.89****	.92**** (WP)	.66**** (AE)
LDL-C	179	123	.66****	.25***	.81****	.75**** (WP)	.65**** (AE)
TG	179	123	.49****	.31****	.36**	.29* (WP)	.50**** (AE)
HDL-C	179	123	.72****	.29****	.86****	.94**** (WP)	.73**** (AE)
SBP	133	75	.37****	.18*	.37*	.53** (WP)	.38**** (AE)
DBP	133	75	.25***	.28***	...	...	... (CE)
Exam 2 (1989–90):							
TC	179	123	.66****	.24***	.85****	1.07**** (AC)	.63**** (AE)
LDL-C	179	123	.72****	.11	...	...	...
TG	179	123	.62****	.32****	.59****	.35 (AC)	.65**** (AE)
HDL-C	179	123	.73****	.26***	.93****	.97**** (WP)	.73**** (AE)
SBP[c]	133	75	.51****	.31***	.39*	.23 (AC)	.54**** (AE)
DBP[c]	133	75	.48***	.30***	.37*	.19* (WP)	.48**** (AE)

[a] Based on natural logarithm–transformed values.

[b] An ellipsis indicates that calculation of h^2 is inappropriate; i.e., the intraclass correlation for DZ twins either is not statistically significant at the $P = .1$ level or exceeds the MZ correlation.

[c] Based on the average of two determinations (see text).

* $.05 < P \leq .10$.

** $P \leq .05$.

*** $P \leq .01$.

**** $P \leq .001$.

component on the age adjustment (table 3). For ΔLDL-C, heritability estimates remained similar after adjustment for environmental factors (range .29–.39) and after additional adjustment for BMI (range .25–.30), indicating moderate genetic influences on the change in LDL-C. After adjustment for the exam-1 level of LDL-C, however, no genetic component was detected. Similar results were seen for ΔHDL-C, except that the heritability estimate actually increased after adjustment for level of HDL-C at exam 1. This increase is attributable to an increase, from .24 to .38, in the ΔHDL-C intraclass correlation for MZs, after adjustment.

Table 3

Age-Adjusted Intraclass Correlations and Heritability Estimates for Changes in CHD Risk Factors in Women Twins

Risk Factor	Intraclass Correlations		h^2[a]		
	MZ	DZ	Classical	ANOVA	Maximum Likelihood
ΔTC	.32****	.27***	.08	.08 (WP)	... (CE)
ΔLDL-C	.33****	.15**	.36*	.35* (WP)	.33**** (AE)
ΔTG	.22***	.28***	...	...	... (CE)
ΔHDL-C	.27****	.15**	.23	.26 (WP)	.27**** (AE)
ΔSBP	.33****	.24**	.19	.19 (WP)	.35**** (AE)
ΔDBP	.16**	.35***	...	...	... (CE)

[a] An ellipsis indicates that calculation of h^2 is inappropriate; i.e., the intraclass correlation for DZ twins either is not statistically significant at the $P = .1$ level or exceeds the correlation for MZ twins.

* $.05 < P \leq .10$.

** $P \leq .05$.

*** $P \leq .01$.

**** $P \leq .001$.

Table 4

R^2 from Multiple Regression of Change in CHD Risk Factors on Age, Environmental and Behavioral Factors, BMI, and Level at Exam 1

Change in Risk Factor	No. of Twin Pairs	R^2 for Regression Model			
		Age	Age and Environmental and Behavioral Factors	Age, Environmental and Behavioral Factors, and BMI	Age, Environmental and Behavioral Factors, BMI, and Level at Exam 1
MZ twins:					
ΔTC	179	.08	.23	.28	.34
ΔLDL-C	179	.09	.16	.22	.28
ΔTG	179	.02	.23	.30	.34
ΔHDL-C	179	.03	.09	.15	.24
ΔSBP	133	.04	.10	.10	.30
ΔDBP	133	.00	.07	.13	.27
DZ twins:					
ΔTC	123	.10	.22	.30	.43
ΔLDL-C	123	.04	.17	.17	.39
ΔTG	123	.04	.23	.29	.33
ΔHDL-C	123	.07	.29	.32	.39
ΔSBP	75	.05	.14	.13	.27
ΔDBP	75	.00	.10	.10	.35

After adjustment for environmental factors and BMI, the DZ intraclass coefficient was .19, not significantly different from 0; hence, heritability for ΔSBP was not calculated. However, after further adjustment for exam-1 SBP level, the DZ correlation was .24 ($P < .05$), and a relatively moderate heritability estimate was maintained (.37), which was statistically significant ($P < .0001$).

Discussion

Consistent with a previous report based on this cohort (Austin et al. 1987), in the present sample of women twins who attended both examinations the heritability estimates at examination 1 were high for TC, LDL-C, and HDL-C and were more moderate for TG levels. Similar results were seen at examination 2, although the DZ intraclass correlation for LDL-C was not statistically significant. Heritability analysis of changes in lipid risk factors in the present study revealed moderate genetic influences on changes in LDL-C and HDL-C but no significant genetic influences on TC or TG (tables 3 and 5). Adjusted estimates showed that approximately one-quarter to one-third of the variances of both ΔLDL-C and ΔHDL-C were attributable to genetic effects under a polygenic model, although evidence for polygenic effects on change independent of effects on level was strongest for HDL-C. Similar genetic effects were much less apparent for changes in blood pressure over 10 years.

Some recent studies have provided evidence that genetic variability at apo gene loci may contribute to the variation in lipid and lipoprotein levels over time (Berg 1988, 1989, 1994; Berg et al. 1989; Monsalve et al. 1991; Humphries et al. 1992), whereas other studies have indicated that genetic factors may control the variation in lipid and lipoprotein response to dietary manipulations in animals (MacCluer et al. 1988; Blangero et al. 1990; Hwa et al. 1992; Kirk et al. 1995; Paigen 1995), as well as in humans (Tikkanen et al. 1990*b;* Xu et al. 1990, 1992; Abbey et al. 1991; Gaddi et al. 1991; Glatz et al. 1991; Gylling et al. 1991; Manttari et al. 1991; Friedlander et al. 1993, 1995*a*). At this time, however, the mechanisms through which genetic factors are involved in determining LDL-C change over time and in response to environmental exposure are not well understood. A variety of plausible mechanisms have been proposed to explain the difference in responsiveness among inbred strains of the same animal species (Lusis et al. 1987; Srivastava et al. 1991; Hwa et al. 1992; Kirk et al. 1995). Recently, it also has been shown that the fractional catabolic rate of LDL is different in both normolipidemic and hyperlipidemic individuals, according to the apo *Xba*I genotypes (Demant et al. 1988; Houlston et al. 1988; Series et al. 1989; Gylling et al. 1991). Although mutations in the LDL-receptor gene indicate that it is a "level" gene, it recently has been shown that variation in the LDL receptor affects the variability of lipid traits and correlations between traits (Roy et al. 1995). However, results observed in a clinical trial conducted on healthy young subjects have indicated that the response levels of LDL-C were not different between the different LDL-receptor genotypes (Friedlander et al. 1995*a*). Thus, it appears unlikely that the LDL receptor plays a major role in the variation of LDL-C over time.

A large number of studies have demonstrated a rela-

Table 5

Adjusted Heritability Estimates for Changes in CHD Risk Factors in Women Twins

	Adjusted for Age and Environmental Factors			Adjusted for Age, Environmental Factors, and BMI			Adjusted for Age, Environmental Factors, BMI, and Level at Exam 1		
Risk Factor	Classical	ANOVA	Maximum Likelihood	Classical	ANOVA	Maximum Likelihood	Classical	ANOVA	Maximum Likelihood
ΔLDL-C	.39**	.38* (WP)	.29**** (AE)	.30*	.29 (WP)	.25**** (AE)	. . .	. . .	. . . (CE)
ΔHDL-C	.23	.23 (WP)	.26**** (AE)	.30	.29 (WP)	.23*** (AE)	.58**	.58** (WP)	.57**** (AE)
ΔSBP	. . .	. . .	. . .	. . .	. . .	. . .	.23	.23 (WP)	.37**** (AE)

Note.—See footnotes to table 3.

tionship between the apo E polymorphism and plasma lipid and lipoprotein levels in healthy subjects (Sing and Davignon 1985). In addition, variation in the apo E gene appeared to have an effect on intragenotypic variances of many lipid traits (Sing and Davignon 1985; Boerwinkle et al. 1987; Reilly et al. 1991; Haviland et al. 1995) and may have a role on cholesterol and LDL-C response to dietary treatment (Tikkanen et al. 1990*a;* Gaddi et al. 1991). Possible mechanisms for this differential effect have been suggested on the basis of in vitro studies (Steinmetz et al. 1989; Demmant et al. 1991). Other studies have provided evidence suggesting that the apo AI (Blangero et al. 1990; Humphries et al. 1992), apo AIV (Mata et al. 1994; McCombs et al. 1994), apo CIII (Lopez-Miranda et al. 1994), and lipoprotein lipase (Ordovas et al. 1995) gene loci also account for some of the variability in LDL response. Our finding that there is no significant genetic contribution to the longitudinal change in LDL-C after adjustment for baseline levels suggest that these candidate genes may have a plieotropic impact on "levels" as well as "variability" of LDL-C.

Because of the age ranges (average ages 41 and 51 years at examinations 1 and 2, respectively) of the women in this sample, it is important to examine whether changes in menopausal status might influence heritability estimates of changes in the risk factors. For example, among the 302 pairs included in the lipid analysis, 109 pairs (36%), including 58 MZ pairs and 51 DZ pairs, had co-twins both of whom changed from premenopausal to postmenopausal. Among these pairs, age-adjusted classical heritability estimates for ΔLDL and ΔHDL were .24 and .27, respectively. These estimates are similar to estimates based on twin pairs who did not change menopausal status (.39 and .26, respectively) and to estimates based on the entire sample (tables 3 and 5). Thus, it appears unlikely that changes in menopausal status have influenced the findings reported here.

Although the observation that we have made may support the "variability gene" concept suggested by Berg (1994), the results also could reflect changes, over a period of time, in penetrance of the underlying "level" genes. The variability-gene effect can be understood as the idea that some genes have been "switched on" in response to specific environmental factors, remain continuously active, and contribute to a consistent change in the phenotype value. The most powerful approach for observing such a continuous effect is controlled intervention trials. The other possibility suggests that at different ages we may observe the expression of different sets of specific genes, all or some of which may be uncorrelated with earlier gene effects. In a longitudinal observation, changes over time probably combine elements of both processes. The possibility that phenotypic expression is a function not only of the underlying genotypes but also of genotype-specific sex and age effects has been shown for various quantitative traits (Moll et al. 1984; Pérusse et al. 1991; Reilly et al. 1991; Tiret et al. 1992; Borecki et al. 1993; Towne et al. 1993; Friedlander et al. 1995*b*). For example, Moll et al. (1984) have shown that a single locus influences LDL levels early in life, LDL variability, and the rate of increase of LDL with age. Towne et al. (1993) have provided evidence that there is a genetic basis for sexual dimorphism exhibited in LDL-C and in small LDL mass. It also has been suggested that the pleiotropic effects of apo E on levels and variabilities of multiple lipid variables are gender specific (Reilly et al. 1991).

In the NHLBI Male Twins Study, three HDL-C measurements were determined within a 16-year period (Christian et al. 1990). Similar to the present study, the first two examinations were performed approximately a decade apart, although the male cohort of twins was somewhat older (42–55 years of age at exam 1) than our cohort of females. The classical heritability estimate, $2(r_{MZ} - r_{DZ})$, based on average values at the three examinations, was .36, considerably lower than our estimates both at exam 1 and exam 2. This estimate may even be biased upward, if the observed higher variance among

DZ twins is due to a larger environmental variance (Christian et al. 1987). In our study the variance in HDL-C change attributable to age and environmental covariables was considerably higher in DZ twins (R^2 = .29) than in MZ twins (R^2 = .09). Although our findings in female twins indicate age-homogeneity in h^2 for HDL-C, the heritability estimates for males were heterogeneous with age (h^2 = .5, .04, and .56 for the total cohort at exams 1, 2, and 3, respectively; and h^2 = .25, .14, and .54, respectively, for male twins who attended all three examinations) (Christian et al. 1990). In another family study, the observed genotype-by-sex interaction for HDL3 and HDL2 mass resulted from significant different additive genetic variances (Towne et al. 1993). It is likely that the hormonal environments of male and females at various ages differ considerably and that the expression of genes controlling HDL-C may be influenced by the sex-age environment encountered.

Several studies have produced results indicating pleiotropic effects on intercorrelated lipid and/or lipoprotein measures and on indicators of adiposity. Two such studies have indicated that phenotypic correlations between HDL-C and adiposity are due to shared environmental factors (Schork et al. 1994; Mahaney et al. 1995), whereas a third study has attributed these correlations to additive genetic effects (Towne et al. 1994). A most striking finding from a previous analysis of our cohort of women twins is the strong genetic influence on change in BMI over the decade between examinations (Austin et al., in press). After being adjusted for age, heritability estimates indicated that ⩾50%—and possibly as much as 85%—of the variance in the change in BMI was attributable to genetic influences. The high heritability in BMI change found in the present twin cohort raises the possibility that the presence of genetic influences on fluctuations in BMI may also influence lipoprotein metabolism. It is also possible that other variables used to adjust for environmental factors may contain a genetic component, thereby resulting in an underestimation of the genetic heritability for the adjusted measures. Yet, present results demonstrate that heritability estimates for change in HDL-C and LDL-C were only slightly modified after adjustments for environmental factors and BMI.

Moderate genetic influences on cross-sectional SBP were seen at both exam 1 and exam 2; moderate genetic influences on cross-sectional DBP were seen at exam 2 only; and little, if any, genetic influence on changes in blood pressure was found (tables 2, 3, and 5). The h^2 estimates of blood-pressure variability at exam 1 and exam 2 are in fair agreement with those reported in other studies (Annest et al. 1979; Krieger et al. 1980; Longini et al. 1984). Interestingly, a recent NHLBI male twin study that examined the genetic influences on blood pressure at three successive points in time (at which the average ages of participants were 48, 57, and 63 years, respectively), has shown that, at the second examination, only 60% of the genetic variance from the first examination was still in evidence and that 40% was new (Colletto et al. 1993). If these different genetic influences are also operating in our sample of female twins, it may explain in part the low heritability of blood-pressure changes that is seen here.

In various studies the estimates of familial correlations for blood pressure (Province et al. 1989; Tambs et al. 1993) and its genetic/environmental determinants (Sims et al. 1987; Tambs et al. 1993; Hong et al. 1994) have been reported to change with age. Results of the Rice et al. (1990) study also found evidence for an age-dependent admixture in the distribution of SBP. These findings are also consistent with recent results reported by Pérusse et al. (1991), who have suggested that variability in SBP may be influenced by a major effect of allelic variation, at a single gene, that is sex and age dependent. Most recently, Cheng et al. (1995) have used complex segregation analysis to detect a recessive major-gene effect on changes in DBP over 7.2 years of follow-up. It should be noted that in their study, as in ours, no polygenic background seems to control the change of DBP over time. Interestingly, we have observed a higher h^2 for change in SBP after the adjustment for baseline levels, which suggests that the genetic factors that affect blood pressure over time may control basal levels of SBP in a different direction.

The heritability results presented in the present study, especially those for lipids, have important implications for understanding and potentially preventing CHD in women. A number of studies have demonstrated significant associations between changes in CHD risk factors and subsequent risk of CHD. For example, Groover et al. (1960) followed a series of individuals over a 5-year period, repeatedly measuring their serum cholesterol, and found that all persons who experienced a myocardial infarction belonged to the group of people who had exhibited the highest variation in serum cholesterol. In a 25-year follow-up of men in the Western Collaborative Group Study, those with the largest weight gain and loss had an increased risk of coronary death compared with the "no change" and "gain only" groups (Hamm et al. 1989). Similarly, a 32-year follow-up of men and women in The Framingham Study showed that subjects with body-weight fluctuations had higher risk of both CHD and total mortality (Lissner et al. 1991). The modest heritability of change in lipids and lipoproteins, as well as the high heritability of ΔBMI recently described among women twins in this study (Austin et al., in press), demonstrates the presence of genetic influences on fluctuations in these risk factors. Therefore, both mapping the gene(s) underlying intraindividual variations in these risk factors and understanding their function(s) could lead to targeted intervention strategies to prevent CHD in genetically susceptible individuals.

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References

Abbey M, Belling B, Clifton P, Nestel P (1991) Apolipoprotein B gene polymorphism associates with plasma cholesterol changes induced by dietary fat and cholesterol. Nutr Metab Cardiovasc Dis 1:10–12

Annest JL, Sing CF, Biron P, Mongeau JG (1979) Familial aggregation of blood pressure and weight in adoptive families. II. Estimation of the relative contributions of genetic and common environmental factors to blood pressure correlations between family members. Am J Epidemiol 110:492–503

Austin MA, Friedlander Y, Newman B, Edwards K, Mayer EJ, King M-C. Genetic influences on changes in body mass index: a longitudinal analysis of women twins. Obesity Res (in press)

Austin MA, King M-C, Bawol RD, Hulley SB, Friedman GD (1987) Risk factors for coronary heart disease in adult female twins: genetic heritability and shared environmental influences. Am J Epidemiol 125:308–317

Austin MA, Newman B (1993) Genetic influence on smoking. N Engl J Med 328:353

Austin MA, Sandholzer C, Selby JV, Newman B, Krauss RM, Utermann G (1992) Lipoprotein(a) in women twins: heritability and relationship to apolipoprotein(a) phenotypes. Am J Hum Genet 51:829–840

Berg K (1988) Variability gene effect on cholesterol at the Kidd blood group locus. Clin Genet 33:102–107

——— (1989) Predictive genetic testing to control coronary heart disease and hyperlipidaemia. Arteriosclerosis 9 Suppl 1:I50–I58

——— (1994) Gene-environment interaction: variability gene concept. In: Goldbourt U, de Faire U, Berg K (eds) Genetic factors in coronary heart disease. Kluwer Academic, Dordrecht, pp 373–383

Berg K, Kondo I, Drayna D, Lawn R (1989) "Variability gene" effect of cholesteryl ester transfer protein (CETP) genes. Clin Genet 35:437–445

Blangero J, MacCluer JW, Kammerer CM, Mott GE, Dyer TD, McGill HC Jr (1990) Genetic analysis of apolipoprotein A-I in two dietary environments. Am J Hum Genet 47:414–428

Boerwinkle E, Visvikis S, Welsh D, Steinmetz J, Hanash SM, Sing CF (1987) The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, beta-lipoprotein, and triglycerides in a sample of unrelated individuals. Am J Med Genet 27:567–582

Borecki IB, Bonney GE, Rice T, Bouchard C, Rao DC (1993) Influence of genotype-dependent effects of covariates on the outcome of segregation analysis of the body mass index. Am J Hum Genet 53:676–687

Carmelli D, Selby JV, Quiroga J, Reed T, Fabsitz RR, Christian JC (1994) 16-Year incidence of ischemic heart disease in the NHLBI twin study: a classification of subjects into high- and low-risk groups. Ann Epidemiol 4:198–204

Cheng LS-U, Carmelli D, Hunt SC, Williams RR (1995) Evidence for a major gene influencing 7-year increases in diastolic blood pressure with age. Am J Hum Genet 57:1169–1177

Christian JC, Borhani NO, Castelli WP, Fabstiz R, Norton JA Jr, Reed T, Rosenman R, et al (1987) Plasma cholesterol variation in the National Heart, Lung and Blood Institute Twins Study. Genet Epidemiol 4:443–446

Christian JC, Carmelli D, Castelli WP, Fabsitz R, Grim CE, Meaney J, Norton JA Jr, et al (1990) High density lipoprotein cholesterol: a 16-year longitudinal study in aging male twins. Arteriosclerosis 10:1020–1025

Christian JC, Kang KW, Norton JA (1974) Choice of an estimate of genetic variance from twin data. Am J Hum Genet 26:154–161

Christian JC, Norton JA Jr, Sorbel J, Williams JC (1995) Comparison of analysis of variance and maximum likelihood based path analysis of twin data: partitioning genetic and environmental sources of covariance. Genet Epidemiol 12: 27–35

Colletto GMDD, Cardon LR, Fulker DW (1993) A genetic and environmental time series analysis of blood pressure in male twins. Genet Epidemiol 10:533–538

Cook M, Godenir N, Green FR, Stirling Y, Meade TW, Humphries SE (1988) Genetic variation at fibrinogen locus is involved in determinig fibrinogen levels: evidence from thirteen individuals sampled on repeated occasions. Biochem Soc Trans 16:541–542

Demant T, Beford D, Packard CJ, Shepherd J (1991) Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metamolism in normolipidaemic subjects. J Clin Invest 88: 1490–1501

Demant T, Houlston RS, Caslake MJ, Series JJ, Shepherd J, Packard CJ, Humphries SE (1988) Catabolic rate of low density lipoprotein is influenced by variation in the apolipoprotein B gene. J Clin Invest 82:797–802

Edwards KL, Austin MA, Newman B, Mayer E, Krauss RM, Selby JV (1994) Multivariate analysis of the insulin resistance syndrome. Arterioscler Thromb 14:1940–1945

Feinleib M, Garrison RJ, Fabstiz R, Christian JC, Hrubec Z, Borhani NO, Kannel WB, et al (1977) The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. Am J Epidemiol 106:284–295

Friedlander Y, Berry EM, Eisenberg S, Stein Y, Leitersdorf E (1995*a*) Plasma lipids and lipoproteins response to a dietary challenge: analysis of four candidate genes Clin Genet 47: 1–12

Friedlander Y, Elkana Y, Sinnreich R, Kark JD (1995*b*) Genetic and environmental sources of fibrinogen variability in Israeli families: the Kibbutzim Family Study. Am J Hum Genet 56:1194–1206

Friedlander Y, Kaufmann NA, Cedar H, Kark JD (1993) XbaI polymorphism of the apolipoprotein B gene and plasma lipid

and lipoprotein response to dietary fat and cholesterol: a clinical trial. Clin Genet 43:223–231

Gaddi A, Ciarrocchi A, Matteucci A, Rimondi S, Ravaglia G, Descovich GC, Sirtori CR (1991) Dietary treatment for familial hypercholesterolemia—differential effects of dietary soy protein according to the apolipoprotein E phenotypes. Am J Clin Nutr 53:1191–1196

Glatz JFC, Demacker PNM, Turner PR, Katan MB (1991) Response of serum cholesterol to dietary cholesterol in relation to apolipoprotein E phenotype. Nutr Metab Cardiovasc Dis 1:13–17

Groover ME, Jernigan JA, Martin CD (1960) Variations in serum lipid concentration and clinical coronary disease. Am J Med Sci 53:133–139

Gylling H, Aalto-Setälä K, Kontula K, Miettinen TA (1991) Serum low density lipoprotein cholesterol level and cholesterol absorption efficiency are influenced by apolipoprotein B and E polymorphism and by the FH-Helsinki mutation of the low density lipoprotein receptor gene in familial hypercholesterolemia. Arterioscler Thromb 11:1368–1375

Hamm PB, Schekelle RB, Stamler J (1989) Large fluctuation in body weight during young adulthood and twenty-five-year risk of coronary death in men. Am J Epidemiol 129: 312–318

Haviland MB, Lussier-Cacan S, Davignon J, Sing CF (1995) Impact of apolipoprotein E genotype variation on means, variances, and correlations of plasma lipid, lipoprotein, and apolipoprotein traits in octogenarians. Am J Med Genet 58: 315–331

Heath AC, Neale MC, Hewitt JK, Eaves JL, Fulker DW (1989) Testing structural equation models for twin data using LISREL. Behav Genet 19:9–35

Heller DA, de Faire U, Pedersen NL, Dahlen G, McClearn GE (1993) Genetic and environmental influences on serum lipid levels in twins. N Engl J Med 328:1150–1156

Hong Y, de Faire U, Heller DA, McClearn GE, Pedersen N (1994) Genetic and environmental influences on blood pressure in elderly twins. Hypertension 24:663–670

Houlston RS, Turner PR, Revil J, Lewis B, Humphries SE (1988) The fractional catabolic rate of low density lipoprotein in normal individuals is influenced by variation in the apolipoprotein B gene: a preliminary study. Atherosclerosis 71:81–85

Humphries SE, Green FR, Henney AM, Talmud PJ (1992) The variability gene concept and the risk of coronary artery disease. In: Bearn AG (ed) Genetics of coronary heart disease. Institute of Medical Genetics, Oslo, pp 123–142

Hunt SC, Hasstedt SJ, Kuida H, Stults BM, Hopkins PN, Williams RR (1989) Genetic heritability and common environmental components of resting and stressed blood pressure, lipids and body mass index Utah pedigrees and twins. Am J Epidemiol 129:625–638

Hwa JJ, Zollman S, Warden CH, Taylor BA, Edwards PA, Fogelman AM, Lusis AJ (1992) Genetic and dietary interactions in the regulation of HMG-CoA reductase gene expression. J Lipid Res 33:711–725

Kirk EA, Moe GL, Caldwell MT, Lernmark JA, Wilson DL, LeBoeuf RC (1995) Hyper- and hypo-responsiveness to dietary fat and cholesterol among inbred mice: searching for level and variability genes. J Lipid Res 36:1522–1532

Krieger H, Morton NE, Rao DC (1980) Familial determinants of blood pressure in northeastern Brazil. Hum Genet 53: 415–418

Lissner L, Odell PM, D'Agostino RB, Stokes J III, Kreger BE, Belanger AJ, Brownell KD (1991) Variability of body weight and health outcomes in the Framingham population. N Engl J Med 324:1839–1844

Longini IM, Higgins MW, Hinton PC, Moll PP, Keller JB (1984) Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. Am J Epidemiol 120:131–144

Lopez-Miranda J, Ordovas JM, Marin C, Jansen S, Lopez-Segura F, Salas J, Blanco A, et al (1994) The S*st*I polymorphic site at the apolipoprotein C-III gene predicts plasma low density lipoprotein response to changes in dietary fat in young men. Circulation 90:I-74

Lusis AJ, Taylor BA, Quon D, Zollman S, Leboeuf RC (1987) Genetic factors controlling structure and expression of apolipoprotein B and E in mice. J Biol Chem 262:7594–7604

MacCluer JW (1995) Plasma HDL-C, triglycerides, and adiposity: a quantitative genetic test of the conjoint trait hypothesis in the San Antonio Family Heart Study. Circulation 92:3240–3248

MacCluer JW, Kammerer CM, Blangero J, Dyke B, Mott GE, VandeBerg JL, McGill HC Jr (1988) Pedigree analysis of HDL cholesterol concentration in baboons on two diets. Am J Hum Genet 43:401–413

Mahaney MC, Blangero J, Comuzzie AG, VandeBerg JL, Stern MP, Manttari M, Kosninen P, et al (1991) Apolipoprotein E polymorphism influences the serum cholesterol response to dietary intervention. Metabolism 40:217–221

Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U (1994) Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 330:1041–1046

Mata P, Ordovas JM, Lopez-Miranda J, Lichtenstein AH, Clevidence B, Judd JT, Schaefer EF (1994) Apo A-IV phenotype affects diet-induced plasma LDL cholesterol lowering. Arterioscler Thromb 14:884–891

McCombs RJ, Marcadis DE, Ellis J, Weinberg RB (1994) Attenuated hypercholesterolemic response to a high-cholesterol diet in subjects heterozygous for the apolipoprotein A-IV-2 allele. N Engl J Med 331:706–710

Moll PP, Sing CH, Lussier-Cacan S, Davignon J (1984) An application of a model for a genotype-dependent relationship between a concomitant (age) and a quantitative trait (LDL cholesterol) in pedigree data. Genet Epidemiol 1:301–314

Monsalve MV, Robinson D, Woolcock NE, Powell JT, Greenhalgh RM, Humphries SE (1991) Within-individual variation in serum cholesterol levels; association with DNA polymorphisms at the apolipoprotein B and AI-CIII-AIV loci in patients with peripheral arterial disease. Clin Genet 39: 260–273

Ordovas JM, Lopez-Miranda J, Mata P, Perez-Jimenez F, Lichtenstein AH, Schaefer EJ (1995) Gene-diet interaction in determining plasma lipid response to dietary intervention. Atherosclerosis Suppl 118:S11–S27

Paigen B (1995) Genetics of responsiveness to high-fat and high-cholesterol diets in the mouse. Am J Clin Nutr 62: 458S–462S

Pérusse L, Moll PP, Sing CF (1991) Evidence that a single gene with gender- and age-dependent effects influences systolic

blood pressure determination in a population-based sample. Am J Hum Genet 49:94–105

Province MA, Tishler P, Rao DC (1989) Repeated-measures model for the investigation of temporal trends using longitudinal family studies: applicaton to systolic blood pressure. Genet Epidmiol 6:333–347

Reilly SL, Ferrell RE, Kottke BA, Kamboh MI, Sing CF (1991) The gender-specific apolipoprotein E genotype influence on the distribution of lipids and apolipoproteins in the population of Rochester, MN. I. Pleiotropic effects on means and variances. Am J Hum Genet 49:1155–1166

Rice T, Borecki IB, Bouchard C, Rao DC (1993) Segregation analysis of fat mass and other body composition measures derived from underwater weighing. Am J Hum Genet 52: 967–973

Rice T, Bouchard C, Borecki IB, Rao DC (1990) Commingling and segregation analysis of blood pressure in French-Canadian population. Am J Hum Genet 46:37–44

Roy M, Sing CF, Betrad C, Davingnon J (1995) Impact of a common mutation of the LDL receptor gene, in French-Canadian patients with familial hypercholesterolemia, on means, variances and correlations among traits of lipid metabolism. Clin Genet 47:59–67

SAS Institute (1985) SAS user's guide, 5th ed. SAS Institute, Cary, NC

Schork NJ, Weder AB, Trevisan M, Laurenzi M (1994) The contribution of pleiotropy to blood pressure and body-mass index variation: the Gubbio study. Am J Hum Genet 54: 361–373

Selby JV, Austin MA, Newman B, Zhang D, Quesenberry CP, Mayer EJ, Krauss RM (1993) LDL subclass phenotypes and the insulin resistance syndrome. Circulation 88:381–387

Series J, Cameron I, Caslake M, Gaffney D, Packard CJ, Shepherd J (1989) The *Xba*I polymorphism of the apolipoprotein B gene influences the degradation of low density lipoprotein in vitro. Biochim Biophys Acta 1003:183–188

Sims J, Hewitt JK, Kelly KA, Carroll D, Turner JR (1986) Familial and individual influences on blood pressure. Acta Genet Med Gemellol (Roma) 35:7–21

Sing CF, Davignon J (1985) Role of apoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 34:268–285

Srivastava RAK, Jiao S, Tang J, Pfleger BA, Kitchens RT, Schonfeld G (1991) In vivo regulation of low-density lipoprotein receptor and apolipoprotein B gene expression by dietary fat and cholesterol in inbred strains of mice. Biochim Biophys Acta 1086:29–43

Steinmetz A, Jakobs C, Motzny S, Kaffarnik H (1989) Differential distribution of apo-lipoprotein E isoforms in human plasma lipoproteins. Arteriosclerosis 9:405–411

Stunkard AJ, Foch TT, Hrubrec Z (1986) A twin study of human obesity. JAMA 256:51–54

Tambs K, Eaves LJ, Moum T, Holmen J, Neale MC, Naess S, Lund-Larsen PG (1993) Age-specific genetic effects for blood pressure. Hypertension 22:789–795

Tikkanen MJ, Huttunen JK, Enholm C, Pietinen (1990*a*) Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. Arteriosclrosis 10:285–288

Tikkanen MJ, Xu C-F, Hamalainen, Talmud P, Narna S, Huttunen JK, Pietinen P, et al (1990*b*) XbaI polymorphism of the apolipoprotein B gene influences plasma lipid response to diet intervention. Clin Genet 37:327–334

Tiret L, Andre J-L, Ducimetiere P, Herbeth B, Rakotovao R, Guegen R, Spyckerelle Y, et al (1992) Segregation analysis of height-adjusted weight with generation-and age-dependent effects: The Nancy Family Study. Genet Epidemiol 9:389–403

Towne B, Blangero J, Siervogel RM (1993) Genotype by sex interaction in measures of lipids, lipoproteins, and apolipoproteins. Genet Epidemiol 10:611–616

Towne B, Roche AF, Chumlea WC, Guo S, Siervogel RM (1994) Analysis of pleiotropy between body mass, fat pattern, and serum HDL-C and LDL-C concentrations. Am J Physical Anthropol Suppl 18:196–197

Williams CJ, Christian JC, Norton JA Jr (1992) TWINAN90: a FORTRAN program for conducting ANOVA-based and likelihood-based analyses of twin data. Comput Methods Programs Biomed 38:167–176

Xu CF, Angelico F, Del Ben M, Pannozzo F, Mazzarella B, Miller NE, Humphries SE, et al (1992) Polymorphisms at the apolipoprotein loci and response of plasma lipids to dietary change in Italian children. Nutr Metab Cardiovasc Dis 2:26–32

Xu C-F, Boerwinkle E, Tikkanen MJ, Huttunen JK, Humphries SE, Talmud PJ (1990) Genetic variation at the apolipoprotein gene loci contribute to response of plasma lipids to dietary change. Genet Epidemiol 7:261–275

Zerba KE, Sing CF (1993) The role of genome type-environment interaction and time in understanding the impact of genetic polymorphism on lipid metabolism. Curr Opin Lipidol 4:152–162