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The Gender-specific Apolipoprotein E Genotype Influence on the Distribution of Plasma Lipids and Apolipoproteins in the Population of Rochester, Minnesota. II. Regression Relationships with Concomitants

Sharon L. Reilly,* Robert E. Ferrell,† Bruce A. Kottke,‡ and Charles F. Sing*

*Department of Human Genetics, University of Michigan Medical School, Ann Arbor; †Department of Human Genetics, University of Pittsburgh, Pittsburgh; and ‡Division of Cardiology, Mayo Clinic, Rochester, MN

Summary

The influence of the apolipoprotein E (Apo E) polymorphism and gender on the regression relationships between each of nine plasma lipid and apolipoprotein traits (total cholesterol; ln triglycerides; high-density-lipoprotein cholesterol; apolipoproteins AI, AII, B, and CII; ln CIII; and ln E) and four concomitants (age, weight, waist-to-hip ratio, and smoking) was studied in 507 unrelated individuals representative of the adult population of Rochester, MN. Analyses are presented separately for females and males. Each lipid and apolipoprotein trait exhibited at least one Apo E genotype–specific regression relationship with the concomitants investigated in this study. In most cases the heterogeneity of regression was associated with differences between the ε32 and ε33 genotype. This study documents that the influence of Apo E genotype on average levels of plasma lipids and apolipoproteins varies among subdivisions of the population defined by age, body size, and smoking status.

Introduction

The ability to measure variation in genes at the DNA or protein-product level provides an opportunity to stratify a population to investigate the nature of the mapping function between genetic variation and phenotypic variation. For common diseases, such as coronary artery disease (CAD), knowledge about the relationship between genetic variation and phenotypic variation in risk-factor traits can be important for identifying particular subgroups in the population at large that are at high risk. Genotypes at loci that are hypothesized to contribute to risk of CAD, such as the one coding for apolipoprotein E (Apo E), may be better indicators of risk, because each gene may have effects on multiple traits, some of which may be difficult to measure or inaccessible in vivo. The effects of the Apo E polymorphism on multiple risk-factor traits is well established (Menzel et al. 1983; Enholm et al. 1986; Boerwinkle et al. 1987; Ordovas et al. 1987; Boerwinkle and Utermann 1988; Havekes et al. 1988; Kaprio et al. 1991; Xhignesse et al. 1991). In general, CAD-associated genotypes may also have the potential to be presymptomatic measures of how individuals will respond to alteration of the environment, such as drug therapy (Nestruck et al. 1987; Manttari et al. 1991) or future biological changes associated with aging (Davignon et al. 1987).

Estimation of the influence of variation in CAD-associated genes on the average levels of risk-factor traits has been the focus of many studies (Davignon et al. 1988; Lusis 1988; Ferrell 1992). However, the influence of genetic variation on the mean level of a trait measures only one type of genetic influence on phenotypic variation. Knowledge about the influence of genetic variation on intragenotypic variances and covariances between risk-factor traits and on the intragenotypic relationships of these traits with concomi-

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Address for correspondence and reprints: Dr. Sharon L. Reilly, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109–0618.

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tants (e.g., age) may provide additional disease-risk information that is not obtained by examining the trait means alone (Waddington 1957; Murphy 1979; Murphy and Trojak 1986). For example, genotypes that have the same mean risk-factor level but different intragenotypic variances have different proportions of their distributions that exceed a threshold for high risk (Reilly et al. 1991).

Population stratification based on measured genotypes also provides an opportunity to test important assumptions intrinsic to many biometrical genetic analyses; these assumptions include (1) homogeneity of intragenotype variance (Morton and MacLean 1974) and (2) homogeneity of the regression of risk-factor traits on concomitants among genotypes. In a previous paper we demonstrated that the Apo E polymorphism was associated with gender-specific variation in the average levels and intragenotype variances of nine lipid and apolipoprotein traits (Reilly et al. 1991). Variation in many of these apolipoprotein traits have been associated with plasma levels of lipids and with the risk of CAD (Miller and Miller 1975; Albers et al. 1976; Ishikawa et al. 1978; Avogaro et al. 1979; Sniderman et al. 1980; Fager et al. 1981; Hamsten et al. 1986; Kottke et al. 1986; Sedlis et al. 1986). In the present paper we investigate the gender-specific influence of the most frequent Apo E genotypes (ε32, ε33, and ε34) on the regression relationships between the same nine lipid and apolipoprotein traits—total plasma cholesterol (total-C); ln triglycerides (lnTrig); high-density-lipoprotein cholesterol (HDL-C); apolipoprotein AI (Apo AI), AII (Apo AII), B (Apo B), and CII (Apo CII); ln CIII (lnApo CIII); and ln E (lnApo E)—and four concomitants that are demographic and anthropometric risk factors for CAD. These concomitants are age, weight, waist-to-hip ratio (WHR), and smoking. Analyses were performed on males and females separately, since there is much evidence that the univariate distributions of lipids, apolipoproteins, and concomitants are significantly different between genders (Lipid Research Clinics Program Epidemiology Committee 1979; Abbott et al. 1983; Leibel et al. 1989; Reilly et al. 1990). There is also evidence that the Apo E effects are gender specific (Kaprio et al. 1991; Xhignesse et al. 1991). In the present study we observe that some of the regressions of lipid and apolipoproteins on four selected concomitants are significantly different ($\alpha = .05$) among Apo E genotypes within females (12/72 tests) and within males (7/72 tests). This heterogeneity of intragenotypic regression relationships is strongly associated with observed heterogeneity of intragenotypic phenotypic variance among Apo E genotypes.

Subjects and Methods

Subject Sample

Moll et al. (1989) and Turner et al. (1989) provide details of the methods used in the RFHS to sample multigeneration pedigrees representative of the Rochester, MN, population. A sample of 567 unrelated Caucasian adults from these pedigrees were studied. To investigate the heterogeneity of regression slopes, we considered only the most frequent Apo E genotype-gender specific subgroups ($n = 529$)—ε32 females, ε32 males, ε33 females, ε33 males, ε34 females, and ε34 males. Triglyceride, Apo E, and Apo CIII levels were transformed using the natural logarithm ($\log_e$ = ln), since their distributions were extremely skewed and leptokurtotic. Then, to reduce the impact of outliers on the analyses, we removed a total of 22 individuals with lipid or apolipoprotein values that were greater than three SDs from their genotype-gender-specific means, if the trait distribution was not approximately normally distributed originally. Details of the tests for normality and truncation procedure have been presented by Reilly et al. (1991). Women taking exogenous hormones ($n = 9$) were not removed, since exogenous hormones represent only one of a large subset of environments that are affecting lipid and apolipoprotein levels. The fraction of smokers in each subgroup was independent of genotype-gender subgroup ($P = .28$). However, ε32 females had a significantly greater fraction of smokers than did the other female subgroups ($P < .05$). Future studies will examine the effects that exogenous hormones, lipid-lowering drugs, and other environmental factors have on lipid and apolipoprotein regressions on concomitants. The sample of 507 individuals that was used here consisted of 30 ε32 females, 30 ε32 males, 154 ε33 females, 156 ε33 males, 76 ε34 females, and 61 ε34 males.

Laboratory Methods

All blood samples were collected in EDTA by venipuncture. Total-C and triglyceride levels were measured by standard enzymatic methods. HDL-C was measured according to the method of Izzo et al. (1981). Plasma levels of Apo AI, Apo AII, Apo B, Apo CII, Apo CIII, and Apo E were measured using radioimmunoassays (Kottke et al. 1991). The Apo E

isoforms or "phenotypes" were determined from frozen plasma samples by isoelectric focusing according to a method described by Kamboh et al. (1988). The association between Apo E isoforms and Apo E alleles (i.e., DNA changes in the Apo E gene) was essentially one-to-one in a sample of unrelated individuals from the Rochester Family Heart Study (RFHS) (Mailly et al., submitted). Therefore, the Apo E isoforms were used to infer the Apo E genotypes referred to in the present study. Use of the term "Apo E genotype" also avoided confusion when referring to plasma levels of Apo E, which is also an Apo E phenotype.

Statistics

For each Apo E genotype-gender group, we (1) determined the best-fitting polynomial regression of each lipid and apolipoprotein trait on each continuously distributed concomitant and (2) estimated the linear regression of each lipid and apolipoprotein on smoking status (1 = current smoker; and 2 = current nonsmoker). We then (3) tested the null hypothesis of homogeneity of the regression relationships among the most frequent Apo E genotypes in males and females separately and (4) determined whether significant heterogeneity of regression among genotype strata explained the heterogeneity of intragenotypic phenotypic variance reported in a previous study. Each of the adjusted (for date of assay) lipid and apolipoprotein distributions was approximately normally distributed either originally or after transformation or removal of outliers.

To determine the best-fitting polynomial regression model of each lipid or apolipoprotein trait on each continuously distributed concomitant, three regression lines—first order (linear), second order (quadratic), and third order (cubic)—were estimated. Using the *F*-ratio (1-df contrast) = [SSR (complete model) − SSR (reduced model)]/MSE (complete model), where SSR and MSE are sums of squares due to regression and mean square error, respectively, we tested the hypothesis that the third-order (complete model) and second-order (reduced model) polynomial regressions fit the data equally well (Neter et al. 1985). If this null hypothesis was not rejected, the null hypothesis that the second-order polynomial fit the data as well as the first-order polynomial was tested. If this null hypothesis was not rejected, we tested whether the regression coefficient for the first-order polynomial was significantly different from zero. In the hypothesis-testing strategy listed above, the MSE of the third-order polynomial was used in each *F*-test, since it was the most complete model examined.

To detect heterogeneity of regression equations among genotypes, we applied the analysis of covariance (ANOCOV), using the strategy described by Brownlee (1984). For each combination of a particular lipid or apolipoprotein trait with a particular concomitant, we evaluated four null hypotheses: ε32 female = ε33 female, ε33 female = ε34 female, ε32 male = ε33 male, and ε33 male = ε34 male. These contrasts test whether the regression relationships estimated for the ε32 and ε34 genotypes deviate significantly from the regression relationship estimated for the ε33 genotype that is often considered the "normal" or "wild type." Since the ANOCOV test for homogeneity of regression coefficient requires that the regression equations be of the same order, the ANOCOV was performed using the highest-order, best-fitting regression model for the particular pair of genotypes being considered. For example, if the best-fitting polynomial regression was second order for ε32 females and third order for ε33 females, then the ANOCOV to test the null hypothesis that ε32 females = ε33 females was conducted using the third-order regression estimated for both genotypes.

In order to determine whether heterogeneity of regression explained the observed heterogeneity of intragenotypic phenotypic variance, we tested for heterogeneity of the residual variances among Apo E genotypes by using Bartlett's test statistic (Morrison 1971). The regression equations used in the ANOCOV test of homogeneity of regression were used to obtain the estimates of the genotype-specific residual variances. Tests of homogeneity of the residual intragenotypic phenotypic variance were carried out only when there was significant evidence of heterogeneity of regression.

The large number of statistical tests for homogeneity of regression raises the issue of whether to adjust for multiple testing. A uniform adjustment of the alpha level, such as the Dunn-Sidak method (Sokal and Rolf 1981, p. 242), seems inappropriate, since (1) the null hypotheses have different probabilities of type I and type II error, (2) the lipids and apolipoproteins are correlated (e.g., see Reilly et al. 1990), and (3) some of the concomitants are correlated. For instance, the Pearson product-moment correlation between weight and WHR was significantly different from zero for each of the subgroups, except the ε32 males (data not shown), but age was not correlated with weight or WHR in this sample. In addition we call attention

to the arguments presented by Rothman (1990)—i.e., that in observational studies there should be no adjustment for multiple tests. Therefore, instead of adjusting for multiple tests, we emphasize that type I errors are random errors and, hence, that patterns in the results should be given more weight than isolated significant results. In the present paper the results from the statistical analyses are reported in the tables by using three significance intervals—$.10 < P < .05$, $.05 < P < .001$, and $P < .001$— to allow a continuum of liberal and conservative assessment of the results by the reader. However, in the text, only those results that were significant at an alpha level of .05 are discussed directly, since this is the more standard practice.

Results

Univariate Distribution of Lipids, Apolipoproteins, and Concomitants

Table 1 presents descriptive statistics for the lipids, apolipoproteins, and concomitants considered in this study. The age range for this sample of unrelated individuals is 26–63 years. Reilly et al. (1991) present the gender-specific tests of homogeneity of means and variances among the three common Apo E genotypes for the lipids, apolipoproteins, and concomitants. Briefly we summarize the significant findings ($P < .05$). In females, the mean levels of WHR, lnApo E, and lnApo CIII were Apo E genotype dependent. The intragenotype variances of Total-C, lnTrig, Apo AII, Apo B, Apo CII and lnApo CIII were also significantly different among Apo E genotypes in females. In males, the mean levels of lnTrig, lnApo E, Apo CII, and lnApo CIII were Apo E genotype dependent. The intragenotype variances of Total-C and lnApo E were also significantly influenced by Apo E genotype in males. There were no significant differences among genotypes in the variance of any one of the three continuously distributed concomitants.

Best-fitting Polynomial Regressions

In the Appendix the regression coefficients and $R^2 \times 100$ values are listed for the best-fitting polynomial regressions of each lipid and apolipoprotein on each concomitant. To summarize the extent to which variation in the nine lipid and apoliprotein traits was associated with variation in the four concomitants, (i.e., 36 regressions), the number of significant regression models and the polynomial order of the regression are listed below for each Apo E genotype-gender subgroup. In the female ε32 subgroup, 12 (1 linear and 11 cubic) of 36 regressions were significant ($P < .05$). For three of the traits—Apo AI, Apo AII, and lnApo E—there was no evidence for significant regression relationships with concomitants. In the male ε32 subgroup, there were only three significant regressions (three linear) distributed among three traits—Apo AI, Apo AII, and Apo B. In the female ε33 subgroup, there

Table 1

Lipid, Apolipoprotein, and Concomitant Means and SDs for the Six Apo E Genotype–Gender-specific Subgroups

	Mean (SD)					
	Males			Females		
	ε32	ε33	ε34	ε32	ε33	ε34
N	30	156	61	30	154	76
Age (years)	43.71 (6.52)	44.22 (7.15)	43.41 (7.28)	41.79 (5.07)	41.81 (6.36)	41.21 (6.63)
Weight (kg)	89.67 (6.10)	85.68 (6.51)	84.07 (6.02)	70.08 (7.04)	69.20 (5.19)	68.58 (5.71)
WHR	.92 (.06)	.90 (.06)	.89 (.06)	.80 (.07)	.76 (.05)	.76 (.06)
Total-C (mg/dl)	187.30 (41.7)	193.63 (31.8)	195.66 (42.1)	169.53 (35.8)	180.75 (28.2)	184.97 (35.4)
lnTrig (ln mg/dl)	5.13 (.47)	4.82 (.46)	4.83 (.45)	4.65 (.57)	4.52 (.45)	4.54 (.46)
HDL-C (mg/dl)	37.30 (7.44)	41.59 (9.69)	39.96 (9.48)	50.00 (14.9)	51.31 (12.3)	50.50 (11.6)
Apo AI (mg/dl)	131.37 (14.5)	132.29 (16.5)	129.80 (16.2)	141.63 (18.3)	141.63 (17.5)	141.68 (19.3)
Apo AII (mg/dl)	33.52 (4.39)	34.78 (4.63)	33.48 (4.43)	33.62 (4.62)	34.34 (3.50)	34.62 (4.84)
Apo B (mg/dl)	77.36 (11.9)	80.14 (13.7)	82.11 (15.6)	71.73 (11.2)	74.19 (11.9)	77.19 (15.1)
Apo CII (mg/dl)	2.85 (.85)	2.45 (.82)	2.54 (.73)	2.30 (.89)	2.04 (.67)	1.93 (.76)
lnApo CIII (ln mg/dl)	2.77 (.26)	2.64 (.30)	2.66 (.28)	2.60 (.40)	2.55 (.28)	2.55 (.28)
lnApo E (ln mg/dl)	1.77 (.49)	1.57 (.37)	1.32 (.37)	1.67 (.39)	1.56 (.34)	1.26 (.35)

were 19 significant regressions (15 linear, 2 quadratic, and 2 cubic) distributed among all nine traits. In the male ε33 subgroup, there were also 19 significant regressions (17 linear and 2 cubic) distributed, somewhat differently than in the females, among eight of the traits. In the female ε34 subgroup, 21 significant regression (15 linear, 2 quadratic, and 4 cubic) were distributed among eight traits. In the male ε34 subgroup, only six of the regressions (four linear and two cubic) were significant. For three traits—total-C, Apo AI, and lnApo E—there was no evidence for significant regression relationships with concomitants.

Apo E Genotype Influence on Lipid and Apolipoprotein Regressions in Females

Table 2 summarizes the cases where the lipid and apolipoprotein regressions on concomitants were significantly heterogeneous among the three common Apo E genotypes ($P < .10$). Only those tests that are significant at an alpha level of .05 are discussed. For total-C, the regression on weight was significantly different between the ε32 and ε33 genotypes. For lnTrig, third-order polynomial regressions on weight and WHR were also different between the ε32 and ε33 genotypes. Like lnTrig, the HDL-C regressions on weight and WHR were Apo E genotype dependent. On the other hand, none of the Apo AI regressions on concomitants were significantly different among genotypes. For Apo AII, the linear regression on age was significantly different between the ε32 and ε33 genotypes. For Apo B, the regression on age was significantly different between the ε34 and ε33 genotypes. Apo B was the only trait, in females, where a regression relationship was significantly different between the ε34 and ε33 genotypes. For Apo CII, third-order polynomial regressions on both weight and WHR were Apo E genotype dependent. For lnApo CIII, the third-order polynomial regressions on age, weight, and WHR were different between the ε32 and ε33 genotypes.

In order to determine whether heterogeneity of regression explains the observed heterogeneity of intragenotypic phenotypic variance in females, we tested for heterogeneity of the residual variances among Apo E genotypes. These results are presented in table 3. For all but one of the six traits where there was heterogeneity of variance between the ε32 and ε33 genotypes, it was explained by heterogeneity of regression. The exception to this trend was for Apo AII, where the heterogeneity of regression on age did not explain the heterogeneity of intragenotypic variance. In females, Apo B was the only trait where there was heterogeneity of variance between the ε34 and ε33 genotypes. The observed heterogeneity of regression on age or weight did not explain this heterogeneity of intragenotypic variance in Apo B.

Table 2

Genotypes with Lipid and Apolipoprotein Regressions on Concomitants That Were Significantly Different than Those of ε33 Genotype in Females

	Genotypes with Regressions Significantly Different than Those for ε33			
	Age	Weight	WHR	Smoking
Total-C	. . .	$\varepsilon 32_C^{**}$	. . .	$\varepsilon 32_L^{*}$
lnTrig	. . .	$\varepsilon 32_C^{***}$	$\varepsilon 32_C^{***}$	. . .
HDL-C	. . .	$\varepsilon 32_C^{**}$	$\varepsilon 32_C^{**}$	. . .
Apo AI	. . .	. . .	. . .	. . .
Apo AII	$\varepsilon 32_L^{**}$	$\varepsilon 32_L^{*}$	. . .	. . .
Apo B	$\varepsilon 34_L^{**}$	$\varepsilon 34_Q^{*}$	$\varepsilon 32_C^{*}$	. . .
Apo CII	. . .	$\varepsilon 32_C^{***}$	$\varepsilon 32_C^{**}$	. . .
lnApo CIII	$\varepsilon 32_C^{***}$	$\varepsilon 32_C^{**}$	$\varepsilon 32_C^{**}$	. . .
lnApo E	. . .	$\varepsilon 32_C^{*}$	. . .	. . .

[a] The subscript refers to the order of the polynomial used in the ANOCOV (L = linear; Q = quadratic; and C = cubic), and the superscript refers to the P value from the ANOCOV.

* $.05 < P < .10$.

** $.001 < P < .05$.

*** $P < .001$.

Table 3

Heterogeneity of Intragenotypic Phenotypic Variance Associated with Heterogeneity of Regression among Apo E Genotypes

	Genotype with Heterogeneity of Unadjusted Variance[a]	Genotype with Heterogeneity of Residual Variance, after Adjustment for[b]			
		Age	Weight	WHR	Smoking
Females:					
Total-C.....	ε32**		NS		NS
lnTrig.....	ε32**		NS	NS	
Apo AII	ε32**	ε32**	NS		
Apo B.....	ε34**	ε34**	ε34*		
Apo CII	ε32**		NS	NS	
lnApo CIII....	ε32**	NS	NS	NS	
Males:					
Total-C.....	ε32**	NS			
HDL-C	ε32*			NS	
lnApo E	ε32**	ε32*		ε32*	

Note.—Genotypes ε32 and ε34 were tested against genotype ε33.

[a] Results from Reilly et al. (1991).

[b] Adjustment was carried out in a genotype-specific manner by using the same regression equations as were used in the ANOCOV. NS = not significant.

* $.05 < P < .10$.

** $.001 < P < .05$.

Apo E Genotype Influence on Lipid and Apolipoprotein Regressions in Males

Table 4 summarizes the cases where the lipid and apolipoprotein regressions on concomitants were significantly heterogeneous among the most common Apo E genotypes. For total-C, only the regression on age was significantly different between the ε32 and ε33 genotypes. For lnTrig and HDL-C, the linear regression on WHR was different between the ε32 and ε33 genotypes. For Apo AII, the regression on smoking was significantly different between the ε32 and ε33 genotypes. For Apo CII, the regressions on both WHR and smoking were different between the ε32 and ε33 genotypes. For Apo E, the quadratic polynomial regression on WHR was significantly different between the ε32 and ε33 genotypes. For Apo AI, Apo B, and

Table 4

Genotypes with Lipid and Apolipoprotein Regression on Concomitants That Were Significantly Different than Those of ε33 Genotype in Males

	Genotypes with Regressions Significantly Different than Those of ε33			
	Age	Weight	WHR	Smoking
Total-C	$\varepsilon 32_C^{**}$	. . .	. . .	. . .
lnTrig.....	$\varepsilon 32_C^{*}$	. . .	$\varepsilon 32_L^{**}$	. . .
HDL-C.....	. . .	. . .	$\varepsilon 32_L^{**}$	. . .
Apo AI.....	$\varepsilon 34_L^{*}$	$\varepsilon 34_Q^{*}$	. . .	. . .
Apo AII.....	. . .	. . .	. . .	$\varepsilon 32_L^{**}$
Apo B	. . .	. . .	. . .	. . .
Apo CII.....	. . .	. . .	$\varepsilon 32_C^{**}$	$\varepsilon 32_L^{**}$
lnApo CIII.....	$\varepsilon 32_C^{*}$	. . .	. . .	. . .
lnApo E.....	$\varepsilon 32_L^{*}$	. . .	$\varepsilon 32_Q^{**}$	. . .

Note.—Subscripts and superscripts are as in table 2.

ln Apo CIII, the regressions on concomitants were not Apo E genotype dependent in males.

To determine whether heterogeneity of regression explains the observed heterogeneity of intragenotypic phenotypic variance in males, we tested for heterogeneity of the residual variances among Apo E genotypes (table 3). For total-C, the significant heterogeneity of intragenotypic phenotypic variance between the ε32 and ε33 genotypes was explained by the heterogeneous regressions on age. For HDL-C, the marginally significant heterogeneity of intragenotypic phenotypic variance between ε32 and ε33 genotypes disappeared after adjustment. For lnApo E, the significant heterogeneity of intragenotypic phenotypic variance between the ε32 and ε33 genotypes was reduced to marginal significance after adjustment for the heterogeneous regressions on age or WHR.

Discussion

This study demonstrates that the regression relationships between the plasma lipid and apolipoprotein traits studied and age, body size, and smoking are Apo E genotype specific in the Rochester, MN, population. Each lipid and apolipoprotein trait exhibited at least one Apo E genotype–specific regression relationship with the concomitants investigated. In most cases the heterogeneity of regressions was associated with differences between the ε32 and ε33 genotypes. This finding is consistent with the observation that the ε2 allele is associated with the greatest deviations of lipid metabolism from normality (Davignon et al. 1988). Specifically, the ε32 genotype had the greatest differences from the ε33 genotype in the regressions of lipids and apolipoproteins on age and weight in females and on WHR in both genders. In most cases heterogeneity of intragenotypic regressions explained the previously observed heterogeneity of intragenotypic phenotypic variance.

Lipid and Apolipoprotein Regressions on Measures of Body Size

Our results suggest that Apo E genotype influences the relationship between anthropometric and plasma lipid risk factors in a gender-specific manner. For example, the regressions of total-C, lnTrig, HDL-C, Apo CII, and lnApo CIII on weight were genotype specific in females but not in males. The gender differences in the heterogeneity of regression on weight and WHR among Apo E genotypes suggests that there may be an Apo E genotype × gender × body-size interaction effect on lipid and apolipoprotein levels. Other studies have also shown an Apo E genotype influence on the relationship between plasma lipids and measures of body size. Pouliot et al. (1990) reported that the Apo E polymorphism alters the correlation between measures of body fat distribution and plasma lipoproteins in women. Our results are similar, in that females carrying the ε2 allele show significantly greater association between triglyceride levels and WHR or weight (a component of "fat mass" in Pouliot et al. 1990) than do females carrying the ε4 allele. Geuguen et al. (1989) report an Apo E genotype effect on the longitudinal relationship between triglyceride levels and weight in a pooled sample of males and females.

Since the features of body size are the consequence of both genetic and environmental factors (Bouchard and Pérusse 1988), heterogeneity of regression slopes among Apo E genotypes could be the consequence of either gene × environment interaction or gene × gene interaction. For example, gene × environment interaction may explain the observed heterogeneity of regression (Baker 1988; Jinks and Pooni 1988) on measures of body size if Apo E genotypes affect lipid levels differently in different environments that influence body size. On the other hand, gene × gene interaction may be responsible for the observed heterogeneity of regression (Falconer 1989, pp. 122–123) if variation in a gene associated with body size determines the way Apo E genotype influences lipid and apolipoprotein metabolism. Alternatively, the Apo E polymorphism may be in linkage disequilibrium with another locus that is responsible for the observed results.

Lipid and Apolipoprotein Regressions on Age and Smoking

Since age and smoking are measures of an individual's environment, these results provide preliminary evidence for Apo E gene × environment interaction. Moreover, the gene × environment interaction appears to be gender specific. For instance, the influence of age on total-C was Apo E genotype specific in males only. In contrast, the influence of age on Apo AII, Apo B, and lnApo CIII was genotype specific in females only. Smoking also had an Apo E genotype-gender-specific effect on lipid and apolipoprotein levels.

This study contributes to the general inference that Apo E effects are dependent on environment. Other studies have shown that individuals with different Apo E genotypes have different responses, in their lipids and apolipoproteins, to lipid-lowering drugs (Nestruck et al. 1987), hormones (Hanis et al. 1991), and

diet (Xu et al. 1990). The accumulated evidence documents that different Apo E genotypes have different norms of reaction, i.e., penetrance functions.

Impact of Heterogeneity of Regression on Biometrical Genetical Analyses

In most biometrical genetic analyses it is standard practice to remove variation due to concomitants, through multiple regression techniques prior to the estimation of genetic effects. In measured-genotype analyses, if there are genotype-specific regressions, then adjustment for concomitants can affect the estimates of both the measured genetic variance and the fraction of the total phenotypic variance that is attributable to the measured gene. That is, if statistical tests show that the regressions on concomitants are genotype-gender specific, then adjusting for concomitants by using (*a*) no stratification, (*b*) only genotype stratification, or (*c*) only gender stratification will result in adjusted genotype means that will yield biased estimates of both the genetic variance and the fraction of the total adjusted variance attributable to the gene. In cases where there is evidence for genotype-gender-specific regressions on concomitants, one alternative to removing the variation due to the concomitants is to compute estimates of the genetic variance at different levels of the concomitants (Hartl and Clark 1989, pp. 472–476).

In figure 1 we illustrate the consequences of genotype-specific regression on inferences about the Apo E polymorphism, using the HDL-C:WHR relationship in males. We first note that the marginal HDL-C means of the three genotypes, when WHR variation is ignored, are not significantly different. As WHR varies over its range in males (approximately 0.70–1.10), the influence of the Apo E genotype on the expected value of HDL-C changes considerably. The ε32 genotype is associated with the lowest levels of HDL-C when WHR is below approximately 0.90 and is associated with the highest levels when WHR ex-

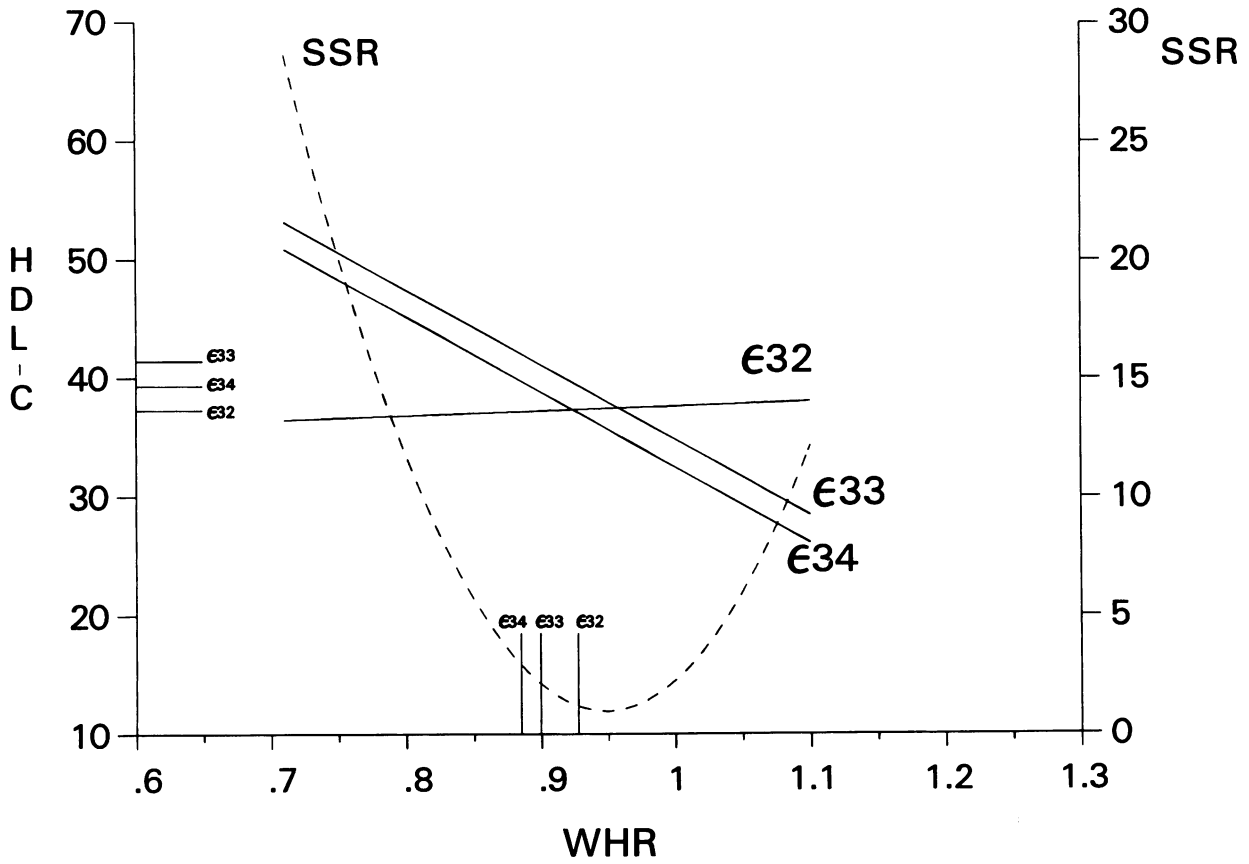

Figure 1 Apo E genotype influence on HDL-C regression on WHR in males. SSR = weighted sum of squared deviations, of the Apo E genotype–specific predicted values, from the average predicted value for a given level of WHR.

ceeds approximately 0.95. At the tails of the WHR distribution, we observe the greatest differences among genotypes (i.e., greatest genetic variance), in predicted values of HDL-C; and at the center of the WHR distribution, we observe the smallest differences among genotypes (i.e., smallest genetic variance), in the predicted values of HDL-C. This example serves to illustrate that the genetic component of variance attributable to the Apo E polymorphism may be dependent on the level of a second variable. More important, the example given in figure 1 illustrates that Apo E genotype × WHR interaction effects on HDL-C levels are the significant effects of the Apo E gene and that the marginal effects on HDL-C when WHR is ignored are trivial.

In unmeasured genetic analyses, the adjustment for concomitants is intended to reduce the total phenotypic variation, in order to increase the power to detect major-gene effects. This study suggests that this practice may distort inferences about genetic effects embedded within the phenotypic distribution and that it could possibly obscure the discovery of factors with a major-gene effect. That is, if a factor with a major-gene effect also has an influence on the regression relationships with concomitants, then an a priori adjustment (*a*) will adjust the whole sample in a manner that reflects the unmeasured genotype with the highest frequency and hence (*b*) may distort the estimates of parameters that define the other underlying genotypes. One recent example highlights the importance of incorporating concomitants into complex segregation analyses. In the past, there have been several attempts to find a major gene for blood pressure (Kreiger et al. 1980; Morton et al. 1980; Marazita et al. 1987; Carter and Kannel 1990). Pérusse et al. (1991) recently found evidence for a single major gene that influences systolic blood pressure by using a genotype-gender-specific age-dependent penetrance function. The work presented here and elsewhere (MacCluer 1992) also suggests that measures of age, smoking, and body size should be incorporated into biometrical genetic models—rather than removing (by regression prior to genetic analyses) variation due to these concomitants.

Conclusion

In order to understand and utilize information about complex genotype-to-phenotype relationships, we must first become familiar with the different types of mapping functions that can exist. In this paper we have demonstrated that linear and nonlinear relationships between plasma risk factors and anthropometric risk factors for CAD are Apo E genotype dependent. In general, our studies (Kaprio et al. 1989; Haviland et al. 1991; Reilly et al. 1991; Zerba et al. 1991) and studies of animal and plant genetics indicate that gene × environment interaction, gene × gene interaction, and linkage disequilibria are likely to be the rule, rather than the exception. Inferences about norms of reaction would be enriched if studies considered a priori that genetic variation at a single locus can influence interindividual variation in multiple traits and that such genetic variation may also influence the relationships between such traits. Increasing knowledge about the complex mapping function between genes and phenotypes will also increase the potential for modeling these complicated gene effects so that they may be incorporated into more-realistic risk-prediction models for CAD.

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Appendix

Table A1

Regressions on Concomitants

	ε32				ε33				ε34			
	β_L	β_Q	β_C	$R^2 \times 100$	β_L	β_Q	β_C	$R^2 \times 100$	β_L	β_Q	β_C	$R^2 \times 100$
Total-C:												
Females:												
Age	1.014			2.1	−48.69*	1.103*	−.008*	7.2**	1.972***			13.6***
Weight	−90.30**	1.206**	−.005**	22.4*	2.217**	−.014**		3.1*	19.69*	−.225*	.008*	6.2
WHR × 100 ...	1.279			6.1	.464			.8	−365.3*	4.736*	−.020*	6.9
Smoking	−29.68**			15.8**	−8.250			1.1	−16.52			2.9
Males:												
Age	−403.5**	9.283**	−.070**	20.0	6.866*	−.071*		2.8*	.088			.1
Weight	.082			.1	.149			.4	.716			3.7
WHR × 100 ...	52.54*	−.284*		11.4	.367			.4	−677.4*	7.798*	−.030*	9.4
Smoking	−8.773			.7	−8.124			1.1	6.665			.4
ln Trig:												
Females:												
Age	.016			2.0	.011**			2.7**	.018**			6.8**
Weight	−1.679***	.023***	−.000***	59.8***	.011***			16.3***	.008**			5.9**
WHR × 100 ...	−11.71***	.143***	−.001***	62.3***	.022***			7.6***	.027**			12.0**
Smoking	−.321			7.2	−.190**			2.7**	−.299**			5.7**
Males:												
Age	−3.935**	.088**	.001*	18.2	.012**			3.7**	.006			.8
Weight	.005			2.5	.013***			15.1***	.008			3.5
WHR × 100 ...	−.007			.7	.026***			9.5***	.030**			13.9**
Smoking	−.100			.8	−.228**			3.9**	−.049			.2
HDL-C:												
Females:												
Age	−.175			.4	.016			.01	4.914**	−.061**		6.4*
Weight	24.18*	−.358**	.002**	43.0**	−.301***			13.6***	−.250**			8.9**
WHR × 100 ...	249.0**	−3.077**	.012**	42.0**	−.662***			8.2***	−.718***			13.6***
Smoking	.761			.1	6.825**			3.9**	10.23***			10.4***
Males:												
Age	.010			.1	−.162			1.4	−.145			1.2
Weight	−.178*			10.9*	−.305***			17.7***	−.228**			7.5**
WHR × 100 ...	.042			.1	−.633***			13.0***	−.635**			14.5**
Smoking	5.036			7.6	2.074			.7	3.303			1.9
Apo AI:												
Females:												
Age	−.260			.5	.322			1.4	.173			.4
Weight	−.317			5.1	6.486*	−.082*	.0003*	5.8**	−.376**			7.3**
WHR × 100 ...	.159			.4	.466*			2.0*	−.795**			6.1**
Smoking	−7.980			4.4	4.268			.8	11.76**			5.0**

Males:													
Age	.527			5.6	.158			.5	−.439				3.9
Weight	−.410**			15.3**	−.340***			7.6***	−3.333*	.019*			5.0
WHR × 100 ...	.264			1.1	128.8*	−1.426**	.005**	6.1**	−.402				2.0
Smoking	10.94*			9.4*	4.539			1.2	5.6003				1.9
Apo AII:													
Females:													
Age	−.202			4.9	.088**			2.5**	.008				.1
Weight	−.116*			10.6*	−.014			.3	−.072*				4.2*
WHR × 100 ...	.053			.7	.032			.2	−.029				.1
Smoking	1.590			2.7	1.116			1.3	2.124				2.6
Males:													
Age	−35.76**	.852**	−.007**	24.0*	−.116**			3.2**	−7.255	.179	−.002*		16.7**
Weight	−.071			5.0	−.051*			2.2*	−.006				.1
WHR × 100 ...	−.002			.1	−.056			.5	.014				.1
Smoking	5.777**			28.7**	1.120			.9	1.463				1.8
Apo B:													
Females:													
Age	128.3*	−3.05*	.024*	13.4	.105			.3	.679**				8.8**
Weight	−28.22**	.387**	−.002**	32.9**	.137**			3.0**	1.656**	−.008**			12.7**
WHR × 100 ...	−236.9***	2.884***	−.012***	44.6***	.460**			4.2**	−158.9*	2.061*	−.009*		13.5**
Smoking	−8.269*			12.5*	−9.797***			8.7***	−10.06**				6.0**
Males:													
Age	−.318			3.0	.126			.4	.143				.4
Weight	.327**			14.4**	.282***			7.6***	.178				1.7
WHR × 100 ...	.148			.5	.724***			8.5***	−260.8**	3.004**	−.012**		13.1**
Smoking	−2.720			.9	−6.498**			3.6**	−3.238				.7
Apo CII:													
Females:													
Age	.050			8.0	−1.142*	.026*	−.0002*	7.5**	.045***				15.4***
Weight	−3.150***	.042***	−.0002***	57.3***	.012***			7.2***	.501**	−.005**	.0001**		16.0**
WHR × 100 ...	−15.93**	.193**	−.001**	42.9**	.300*	−.002*		6.9**	−9.125**	.117**	−.0005**		15.3**
Smoking	−.639			11.8	−.140			.6	−.212				1.0
Males:													
Age	−.014			1.2	.016*			2.0*	.016				2.5
Weight	−.0005			.1	.016***			7.1***	.003				.2
WHR × 100 ...	18.49*	−.199*	.001*	14.7	−5.87*	.062*	−.0002*	6.7**	−14.12**	.163**	−.001**		16.0**
Smoking	.603			8.3	−.206			1.0	−.073				.2
lnApo CIII:													
Females:													
Age	4.361*	−.100*	.001*	33.7**	.009**			3.8**	.016***				15.7***
Weight	−.906**	.012**	−.0001**	28.0**	.004**			3.3**	.182**	−.002**	.0001**		9.1*
WHR × 100 ...	−5.906**	.070**	.0003**	35.6**	.008*			2.2*	−3.037*	.004*	−.0002		9.2*
Smoking	−.135			2.7	−.060			.6	−.080				1.1

(*continued*)

Table A1 (continued)

	ε32				ε33				ε34			
	β_L	β_Q	β_C	$R^2 \times 100$	β_L	β_Q	β_C	$R^2 \times 100$	β_L	β_Q	β_C	$R^2 \times 100$
Males:												
Age	−2.234**	.051**	−.000**	19.2	.009**			4.1**	.004			1.2
Weight	0			.1	.005**			5.3**	.001			.3
WHR × 100 ...	−.004			.9	.009**			2.9*	.010*			4.6*
Smoking	−.032			.2	−.105*			1.9*	.053			.6
ltApo E:												
Females:												
Age	−.006			.5	−.078*	.001**		4.9**	.015**			8.4**
Weight	−.784*	.010*	−.0001*	20.3	.001			.3	.256**	−.003**	.0001**	12.1**
WHR × 100 ...	.017*			9.6*	−2.022*	.025*	−0*	2.7	−.225	.001*		3.8
Smoking	−.061			.6	.108			1.3	−.032			.1
Males:												
Age	−.016			4.5	.007*			1.8*	−.0004			.1
Weight	.004			1.7	.005**			3.5**	−.0002			.1
WHR × 100 ...	.614*	−.003*		12.9	−.015**			4.7**	.008			1.6
Smoking	7.6			1.6	−2.4			.3	−3.8			.7

* $.05 < P < .10$.
** $.001 < P < .05$.
*** $P < .001$.

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