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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

Sharon L. Reilly,* Robert E. Ferrell,† Bruce A. Kottke,‡ Mohammad I. Kamboh,† 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 influences of the apolipoprotein E (Apo E) polymorphism and of gender on the distributions of plasma levels of total cholesterol (Total-C), ln triglycerides (lnTrig), HDL cholesterol (HDL-C), and apolipoproteins AI (Apo AI), AII (Apo AII), ln E (lnApo E), B (Apo B), CII (Apo CII), and ln CIII (lnApo CIII) were studied in 507 unrelated individuals representative of the adult population of Rochester, MN. Apo E genotypes influenced both phenotypic level and intragenotype phenotypic variability. The mean levels of six of the nine traits were influenced significantly by Apo E genotype. Intragenotype variability in eight of the nine traits was significantly different among Apo E genotypes. These effects were estimated separately in males and females. The contribution of allelic variation in the Apo E gene to the definition of the multivariate mean and variance of the lipid and apolipoprotein hyperspace was evaluated. These findings were used to demonstrate how heterogeneity of risk-factor-trait variance among genotype/gender-specific subgroups of the population at large may influence the evaluation of risk of coronary artery disease.

Introduction

In the majority of cases, an individual's risk of developing coronary artery disease (CAD) is the consequence of complex interactions among the effects of many genetic and environmental factors (Davignon et al. 1983). Interindividual variation in quantitative levels of plasma lipid metabolites (and thrombolytic factors) which are associated with risk of CAD are determined by these gene and environmental effects. With the current ability to identify polymorphic genotypes at the DNA or gene-product level, measurements of the genotype have become a powerful tool for subdividing a population in order to make predictive and/or etiological inferences about risk of disease. Most investigations of measured genetic causes of variation in quantitative traits have focused on the averages of risk-factor levels (Sing and Orr 1976; Sing and Davignon 1985; Boerwinkle et al. 1987; Boerwinkle and Utermann 1988; Lusis 1988; Kaprio et al., in press).

Since many of the biochemical traits commonly associated with risk of CAD, such as plasma levels of cholesterol, triglycerides, and the apolipoproteins, are interrelated metabolically, we should expect that genotypes determining protein polymorphisms will have pleiotropic effects on these risk-factor traits. Pleiotropy is a natural consequence of metabolic systems catalyzed by gene products (Waddington 1957, pp 206–210). Consequently, just as the hyperlipidemias are defined by distinct relationships between measures of lipid metabolism, we hypothesize that genotype-dependent profiles of means, variances, and covariances between metabolically related traits in the normal range of variability may provide better etiological and predictive risk information than that obtained from the effects of a measured genotype on a single trait. In addition, it has been established that males

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Address for correspondence and reprints: Sharon L. Reilly, Department of Human Genetics, M4708 Medical Science II, University of Michigan Medical School, Ann Arbor, MI 48109–0618.

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and females have different natural histories of disease, including characteristics of life-style that predispose to disease (Truett et al. 1967; Gordon et al. 1977; Anderson et al. 1987; Higgins and Thom 1989; Thom 1989). Epidemiological studies have also shown that males and females have different frequency distributions for lipids, apolipoproteins, and concomitants (Gordon et al. 1977; Lipid Research Clinics Program Epidemiology Committee 1979; Abbott et al. 1983; Wilson et al. 1983; Leibel et al. 1989; Reilly et al. 1990). These studies provide strong evidence that the genotype-dependent profiles should be investigated in a gender-specific manner.

Most genetic analyses have not included the additional information about risk-factor-trait distributions that has been gained by investigating heterogeneity of phenotypic variance among genotypes. Characterizing differences in interindividual trait variance among genetically defined subgroups of the population can increase the accuracy of predicting the fraction of each subgroup that is at risk of developing CAD. Such differences may be the consequence of gene × environment interaction, gene × gene interaction, gene-environment frequency correlations, or linkage disequilibrium. In the field of CAD research, Berg (1988, 1990) has used the term "variability genes" to describe allelic variation that influences intragenotype trait variability. In the present paper we evaluate the impact that common allelic variation in the apolipoprotein E (Apo E) gene has on intragenotype phenotypic variance of lipid and apolipoprotein traits in a sample of unrelated individuals.

The three major isoforms of Apo E, referred to as Apo E2, Apo E3, and Apo E4, are the products of three alleles (ε2, ε3, and ε4) of a single gene on chromosome 19. The most common allele, ε3, has a cysteine at residue 112 and an arginine at residue 158. The ε2 allele has cysteines at both the 112 and 158 residues, and the ε4 allele has arginines at both residues. The Apo E molecule is a protein component of circulating plasma chylomicrons, very-low-density lipoproteins, and high-density cholesterol-rich lipoproteins. It facilitates the catabolism of these lipoproteins by acting as a ligand for the E- and B,E-receptors of the liver and peripheral tissues. Binding studies have shown that the E4 isoform has the same receptor binding capability as the E3 isoform but that the E2 isoform has <2% of the normal binding capability (Mahley 1988). However, the E4 isoform may lack the ability to form disulfide bonds with apolipoprotein AII (Apo AII) and would therefore more rapidly associate with triglyceride-rich lipoproteins than would the E3 or E2 isoforms (Weintraub et al. 1987). Given this knowledge of the biochemical impact of allelic variation in the Apo E gene, we hypothesized that each Apo E genotype has different pleiotropic effects on lipid and apolipoprotein means and variances in the population at large and that these effects are gender specific. The study reported here supports this hypothesis.

Material and Methods

The present paper is the first in a series of three papers that will focus on the influence that the most frequent Apo E genotypes have on lipid and apolipoprotein means, variances, covariances, correlations, and regressions on concomitants. The goal of this series of papers is to provide a consistent and coherent investigation of the second moments of the lipid and apolipoprotein multivariate distribution in the population at large. Therefore, we will use the results from the present study as a precursor to other studies. In order to obtain the best possible sample for all analyses, we have removed some outliers and transformed some distributions such that the lipid and apolipoprotein distributions are approximately normally distributed. Since only nine males and nine females were removed, we still consider our sample to be representative of the population at large while guarding against the potentially high impact that outliers can have on point estimates and hypothesis tests. The details of the sample and statistics are presented below.

Sample

As part of the Rochester Family Heart Study (RFHS), 276 multigeneration pedigrees were ascertained through elementary-school children. The RFHS sample includes a total of 2,002 individuals. Moll et al. (1989) have given details of the sampling methods that were used. A sample of 567 unrelated Caucasian individuals derived from the parental generation of the RFHS had their Apo E genotypes determined. To investigate the heterogeneity in means and variances of lipids and apolipoproteins among the most frequent Apo E genotype/gender-specific subgroups (n = 529), we considered six strata—ε32 males, ε32 females, ε33 males, ε33 females, ε34 males, and ε34 females. Individuals taking drugs which could affect lipid levels (n = 61) and women taking exogenous hormones (n = 9) were not excluded, since the present study focuses on describing the multivariate distributions for subgroups represen-

tative of the population at large. Future studies will examine the effects that exogenous hormones, lipid-lowering drugs, and other environmental factors have on interindividual lipid and apolipoprotein variability and covariability.

Parametric statistical inferences about the variances, covariances, and regressions of lipid traits in the six genotype/gender-specific subgroups rely on the assumption of normality. Four individuals were removed from the sample because they had outlier values for plasma triglycerides and/or Apo E (i.e., triglyceride levels >1,000 mg/dl and/or Apo E levels >25 mg/dl, which are at least 4 SDs from their genotype/gender-specific means). In addition, triglyceride, Apo E, and apolipoprotein CIII (Apo CIII) distributions were transformed, since they were extremely skewed and leptokurtotic. After considering the logarithmic transformation and a variety of power transformations, we decided that to be consistent with the literature the natural logarithm ($\log_e$ = ln) was the most appropriate transformation. The ln triglycerides (lnTrig), ln E (lnApo E), and ln CIII (lnApo CIII) distributions showed a marked reduction in skew and kurtosis. The Lilliefors's test (Conover 1971, pp 302–306) for normality was then performed on all transformed and untransformed lipid and apolipoprotein traits within each of the genotype/gender-specific subgroups after adjustment for date of assay by using a linear-regression model. The null hypothesis of normality was accepted for 32 of the 54 subgroup-specific distributions at the $P > .05$ significance level. The remaining 22 distributions were truncated at 3 SDs on both sides of their subgroup-specific means and then were retested for normality. After truncation, we accepted the null hypothesis of normality ($P > .05$) for 19 of the 22 distributions. Further truncation, at 2.0 and 2.5 SDs, of the remaining three distributions—the ε33 female lnTrig and HDL cholesterol (HDL-C) distributions and the ε34 male HDL-C distribution—did not improve the normality as measured by the Lilliefors's test statistic (i.e., P values were still <.10 but >.05), so we chose to keep those individuals who had values within 3 SDs. A total of 18 individuals—nine males (seven ε33 and two ε34) and nine females (seven ε33 and two ε34)—were excluded as a consequence of the truncation procedure.

After removal of outliers, 507 unrelated individuals remained. There were 30 ε32 males, 30 ε32 females, 156 ε33 males, 154 ε33 females, 61 ε34 males, and 76 ε34 females. The number of women taking hormones (n = 9) was small compared with the number not taking hormones and indicated that our sample was more characteristic of females not taking exogenous hormones. The fraction of smokers in each subgroup—.20 in ε32 males, .33 in ε32 females, .20 in ε33 males, .15 in ε33 females, .20 in ε34 males, and .16 in ε34 females—was independent or genotype and gender (P = .28). However, ε32 females had a significantly greater fraction of smokers than did the other female subgroups ($P < .05$).

Laboratory Methods

All blood samples were collected in EDTA by venipuncture. Total plasma cholesterol (Total-C) and triglyceride levels were measured by standard enzymatic methods (Beckman kits) (Barham et al. 1972; Barr et al. 1981*b*). HDL-C was measured following precipitation of Apo B–containing lipoproteins with polyethylene glycol (Izzo et al. 1981). Aliquots of plasma were frozen at −70°C for apolipoprotein determinations. Plasma levels of Apo AI, Apo AII, Apo CII, Apo CIII, and Apo E were measured (six replicates/sample) using radioimmunoassays, and Apo B levels were measured (four replicates/sample) using an enzyme-linked immunosorbent assay developed and standardized in the Mayo Atherosclerosis Research Laboratory (Barr et al. 1981*a*, 1981*c*; Au et al. 1986*a*, 1986*b*; Kottke et al. 1986). This included the use of quality-control plasmas to correct for day-to-day variability in the assays, as well as the use of primary pure apolipoprotein standards as described elsewhere (Au et al. 1986*a*, 1986*b*). Apo E isoforms used to define Apo E genotypes were determined from frozen plasma samples by narrow-range isoelectric focusing in polyacrylamide gels, followed by immunoblotting using monospecific polyclonal antiserum to human Apo E, as described by Kamboh et al. (1988).

Statistical Methods

The goal was to estimate and test hypotheses about the homogeneity of genotype means and intragenotypic variance among the most frequent Apo E genotypes (ε32, ε33, and ε34) in males and females separately. Statistical inferences about equality of means based on parametric tests rely on the assumption of homogeneity of variance. To minimize false inferences due to heteroscadasticity of variance, we used the Kruskal-Wallis nonparametric test statistic to evaluate heterogeneity of the means across subgroups (Conover 1971, pp 302–306). In general, the Kruskal-Wallis test is more robust than the analysis of variance, when there is heterogeneity of variance (Tomarken and Ser-

lin 1986). Nonparametric statistical tests for homogeneity of variance are also available—but less powerful than parametric tests—when the trait is approximately normally distributed (e.g., see Ansari and Bradley 1960). As has been described above, we verified that each of the adjusted (for date of assay), truncated, and/or transformed lipid and apolipoprotein traits was approximately normally distributed. Hence, we used Bartlett's univariate test statistic to evaluate heterogeneity of trait variances (Morrison 1971, pp 152–153). We evaluated the impact that genotype has on the means and variances by considering six null hypotheses: ε32 male = ε33 male; ε33 male = ε34 male; ε32 male = ε34 male; ε32 female = ε33 female; ε33 female = ε34 female; and ε32 female = ε34 female. For 54 tests (i.e., nine traits × six tests) at an alpha level of .10 we would expect an experimentwise error rate of approximately five false-positive results from the analyses' mean or variance differences across subgroups. We expect that the truncation procedure used to obtain the sample for these analyses will yield a conservative estimate of the true heterogeneity of means and variances across subgroups in the Rochester, MN, population.

Results

Apo E Genotype Effects on Concomitant Means and Variances

Table 1 presents the descriptive statistics for the age, height, weight, body-mass index (BMI), and waist-to-hip ratio (WHR) distributions in the six Apo E genotype/gender-specific subgroups. The age range of this sample was 26–63 years. The height, weight, and BMI distributions among these subgroups were similar to those of other North American populations (National Heart, Lung and Blood Institute 1983). We used the Kruskal-Wallis test for equality of means and used Bartlett's test for homogeneity of variances among genotypes for each gender (results not shown). The number of statistically significant ($P < .10$) associations is small. In both males and females, the ε32 genotype is associated with significantly higher average WHR than is the ε33 or ε34 genotype. In males only, the variance in BMI is significantly greater in the ε33 genotype than in the ε34 genotype. In females only, the variance in height is significantly greater in the ε33 genotype than in the ε32 genotype. In the next section we show that the Apo E genotype effects on the lipid and apolipoprotein means and variances follow a very different pattern.

Apo E Genotype Influences on Lipid and Apolipoprotein Means

In table 2 we present the point estimates of the mean levels of lipids and apolipoproteins in the genotype/gender-specific subgroups. The results from the Kruskal-Wallis tests for equality of means among genotypes within each gender are presented in figure 1. The genotype-specific means for Total-C are in the range 169.5–195.7 mg/dl and are genotype dependent in females only. The lnTrig means are in the range 4.52–5.13 ln mg/dl and are significantly affected by genotype in males only. The means for HDL-C are in the range 37.3–51.3 mg/dl and are also genotype dependent in males.

The mean levels of Apo AI (range 129.8–148.7 mg/dl), Apo AII (range 33.5–34.8 mg/dl), and Apo B (range 71.7–82.11 mg/dl) are not significantly influenced by Apo E genotype. The lnApo E means are in the range 1.26–1.77 ln mg/dl and are affected by genotype in both males and females. The means for Apo CII are in the range 1.93–2.85 mg/dl and are

Table 1

Concomitant Means for Six Apo E Genotype/Gender-specific Subgroups

	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)
Height (cm)	178.99 (13.82)	177.37 (13.39)	177.54 (11.38)	165.56 (13.02)	164.78 (15.06)	164.62 (13.86)
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)
BMI (kg/cm^2 × 100)	2.79 (.33)	2.72 (.40)	2.66 (.31)	2.58 (.56)	2.55 (.54)	2.53 (.51)
WHR	.92 (.06)	.90 (.06)	.89 (.06)	.80 (.07)	.76 (.05)	.76 (.06)

NOTE.—Data in parentheses are SD.

Table 2

Lipid and Apolipoprotein Means and Variances

	Males			Females		
	ε32	ε33	ε34	ε32	ε33	ε34
Mean:						
Total-C	187.30	193.63	195.66	169.53	180.75	184.97
lnTrig	5.13	4.82	4.83	4.65	4.52	4.54
HDL-C	37.30	41.59	39.96	50.00	51.31	50.50
Apo AI	131.37	132.29	129.80	141.63	141.63	141.68
Apo AII	33.52	34.78	33.48	33.62	34.34	34.62
lnApo E	1.77	1.57	1.32	1.67	1.56	1.26
Apo B	77.36	80.14	82.11	71.73	74.19	77.19
Apo CII	2.85	2.45	2.54	2.30	2.04	1.93
lnApo CIII	2.77	2.64	2.66	2.60	2.55	2.55
Variance:						
Total-C	1,737.50	1,009.61	1,774.82	1,279.00	797.02	1,255.70
lnTrig	.22	.21	.21	.33	.17	.21
HDL-C	55.33	93.96	89.88	221.46	150.61	135.46
Apo AI	210.17	272.05	263.03	333.90	305.20	371.31
Apo AII	19.25	21.40	19.65	21.34	12.21	23.39
lnApo E	.24	.14	.14	.15	.12	.12
Apo B	142.22	187.76	243.93	125.84	141.01	227.90
Apo CII	.73	.66	.54	.80	.45	.58
lnApo CIII	.07	.09	.08	.16	.08	.08

NOTE.—Data are mg/dl.

genotype dependent in males and females. The lnApo CIII means are in the range 2.55–2.77 ln mg/dl and are also genotype dependent in males and females.

Apo E Genotype Influences on Lipid and Apolipoprotein Variances

In table 2 we also present the point estimates of interindividual variability of lipids and apolipoproteins in the genotype/gender-specific subgroups. The results from Bartlett's test for homogeneity of variance among genotypes within each gender are presented in figure 1. Most of the lipid and apolipoprotein trait variances are either Apo E genotype specific or genotype/gender specific. The intragenotype variances in Total-C are 797–1,737 and are genotype dependent in both males and females. lnTrig variances are 0.17–

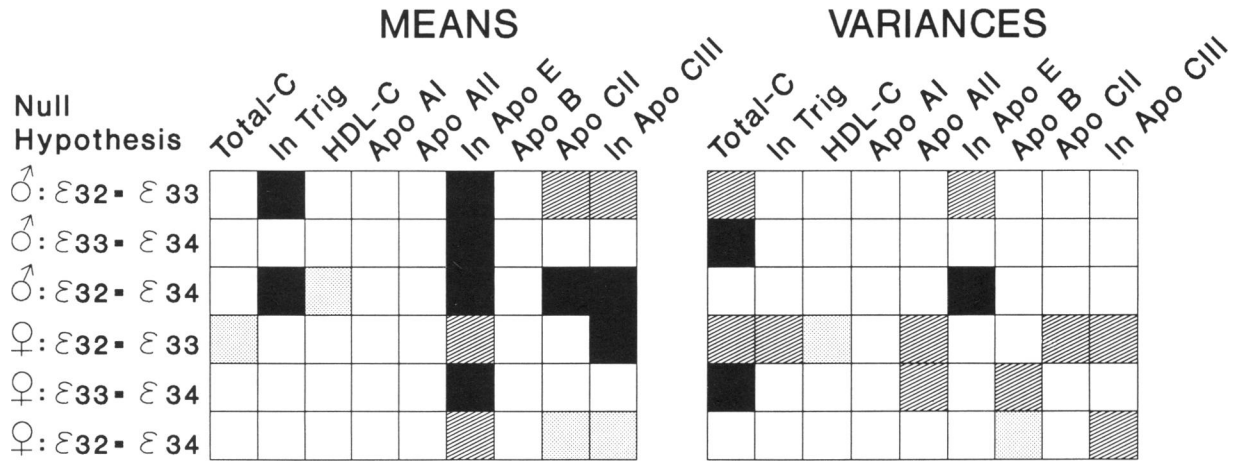

Figure 1 Gender-specific Apo E genotype differences in means and variances of lipids and apolipoproteins. □ = $P > .10$; ▨ = $.05 < P < .10$; ▨ = $.01 < P < .05$; ■ = $P < .01$.

0.33 and are genotype dependent in females only. The intragenotype variances in HDL-C are 55.33–150.6 and are also genotype dependent in females.

For all but Apo AI, the intragenotype variances in apolipoprotein levels show genotype dependence in either males or females. Apo AII variances are in the range 12.2–21.4 and are genotype specific in females. The variances in lnApo E levels are in the range 0.12–0.24 and are genotype specific in males. Apo B variances are in the range 125.8–243.9 and are genotype specific in females. The variances in Apo CII levels are in the range 0.45–0.80 and are also genotype specific in females. Likewise, lnApo CIII variances are in the range 0.07–0.16 and are genotype dependent in females.

These analyses suggest that the distribution of trait values in the nine-dimensional hyperspace is dependent on genotype and gender. In the Discussion we present a simplified example of how information about variance may be used to increase the accuracy of prediction of CAD in specific subgroups of the population. We next turn to combining information about genotype/gender-specific means and variances.

The Lipid and Apolipoprotein Hyperspace

We employ a graphical scheme to integrate the information gleaned from examining the first and second moments of the univariate distributions of the lipids and apolipoproteins. The gender-specific impact that Apo E genotype has on the distance of the mean vector from the origin of the nine-dimensional hyperspace and on a measure of the combined trait variability is given in figures 2 and 3. These representations of the hyperspace are designed to combine multivariate measures of mean levels and trait variance, along with each trait's contribution to these measures. In order to remove the scale differences across lipid and apolipoprotein traits, we standardized the gender-specific distribution of each trait to approximate an $N(0,1)$ distribution. The Euclidian distance ($||\mu||_i$) from the origin of the gender-specific hyperspace pro-

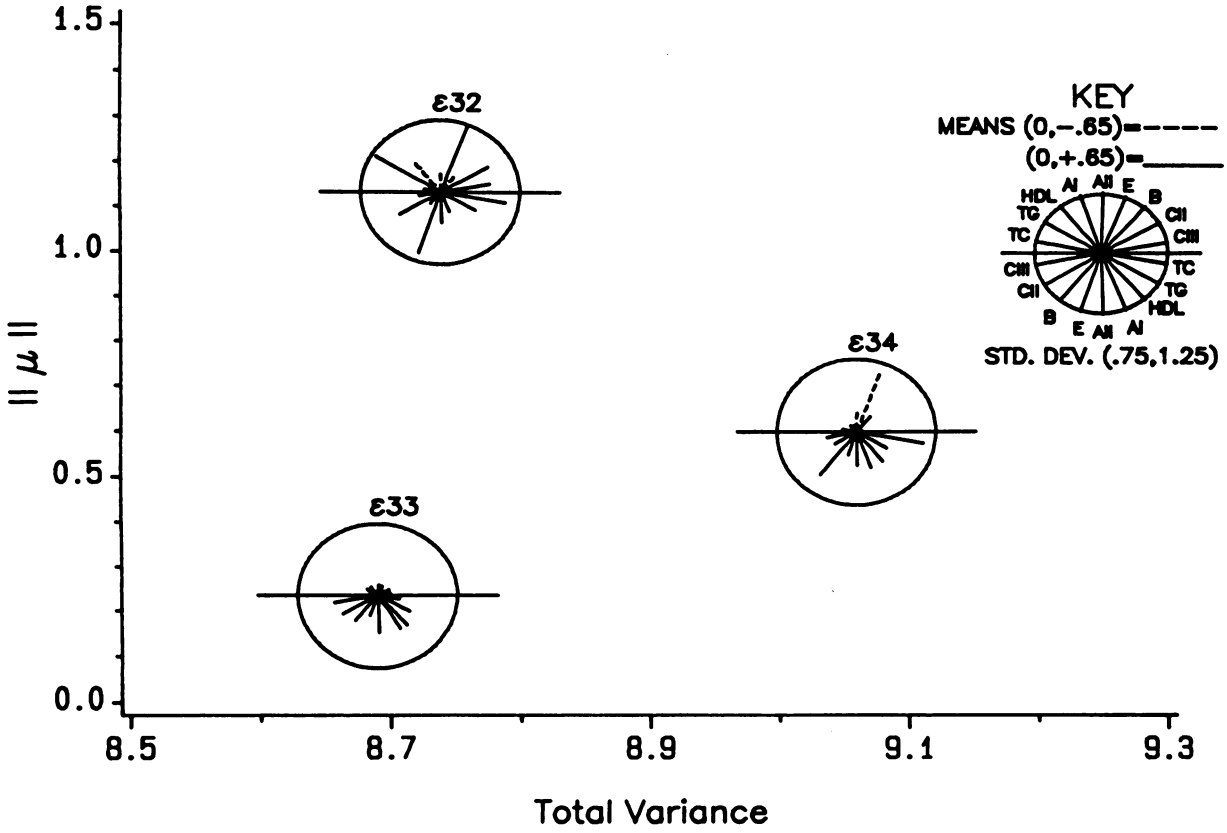

Figure 2 Lipid and apolipoprotein hyperspace for males

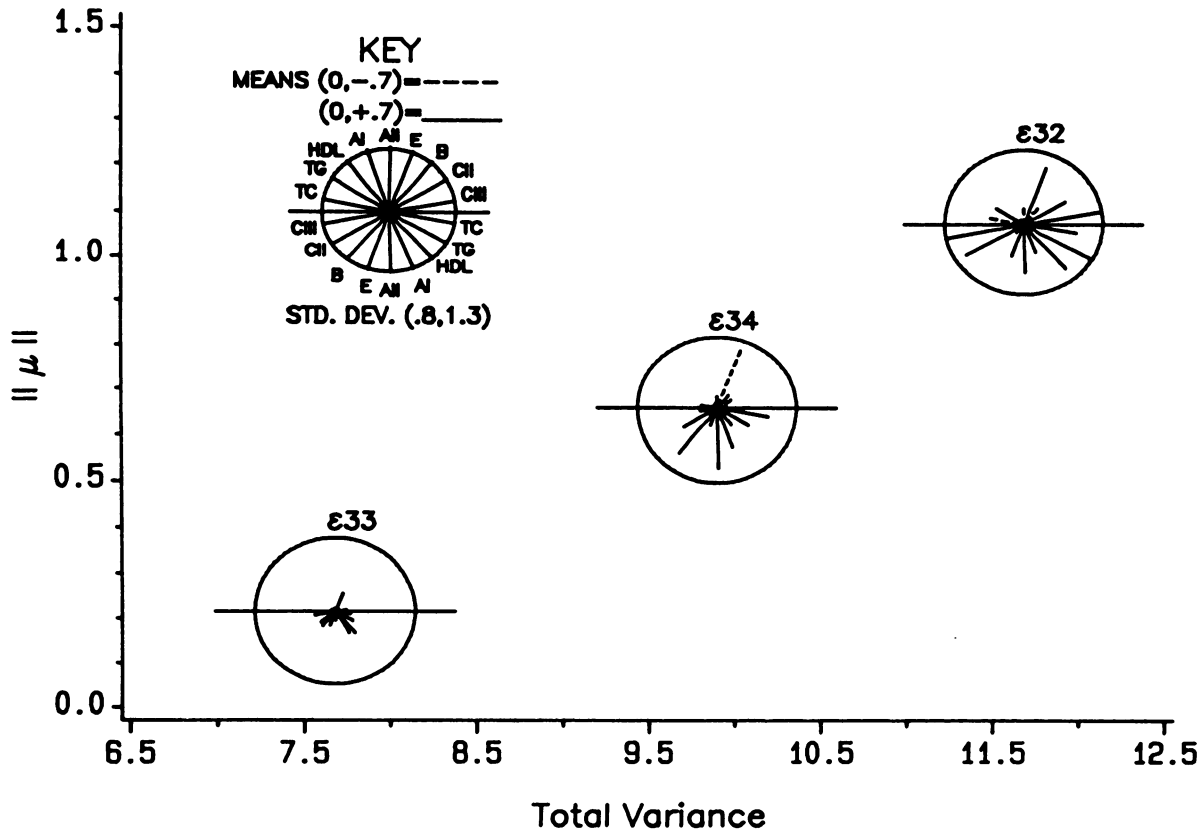

Figure 3 Lipid and apolipoprotein hyperspace for females

vides a composite measure of the shift in the mean levels associated with the *i*th genotype, where

$$||\mu||_i = (\mu^2_{\text{Total-C}_i} + \mu^2_{\ln\text{Trig}_i} + \mu^2_{\text{HDL-C}_i} + \mu^2_{\text{Apo AI}_i} + \mu^2_{\text{Apo AII}_i} + \mu^2_{\ln\text{Apo E}_i} + \mu^2_{\text{Apo CII}_i} + \mu^2_{\ln\text{Apo CIII}_i})^{1/2};$$
$$i = \varepsilon32,\varepsilon33,\varepsilon34 .$$

The sum of the genotype-specific variances, or the Total Variance, provides a measure of the joint variability associated with the *i*th genotype (Van Valen 1974), where

$$\text{Total Variance}_i = \sigma^2_{\text{Total-C}_i} + \sigma^2_{\ln\text{Trig}_i} + \sigma^2_{\text{HDL-C}_i} + \sigma^2_{\text{Apo AI}_i} + \sigma^2_{\text{Apo AII}_i} + \sigma^2_{\ln\text{Apo E}_i} + \sigma^2_{\text{Apo CII}_i} + \sigma^2_{\ln\text{Apo CIII}_i};$$
$$i = \varepsilon32,\varepsilon33,\varepsilon34 .$$

In order to examine the contribution of each trait's mean and variance to the genotype-specific $||\mu||$ and Total Variance, two semicircles which each contain nine rays (one for each trait) are drawn at the intersection of the $||\mu||$ and Total Variance values for each genotype. In figures 2 and 3 the length of a ray represents the magnitude of either the contribution of the trait mean to $||\mu||$ (upper semicircle) or the contribution of the trait variance to Total Variance (lower semicircle).

The male lipid and apolipoprotein hyperspace is represented in figure 2. The Euclidian distance of the genotype-specific centroids from the hyperspace origin ($||\mu|| = 0$) shows that ε33 males have the closest centroid at $||\mu|| = 0.24$, that ε34 males have a mid-range centroid at $||\mu|| = 0.60$, and that the centroid of ε32 males, at $||\mu|| = 1.17$, deviates farthest from the origin. The Total Variance measure shows that ε33 males have the smallest total variability (8.69), that ε32 males have intermediate total variability (8.74), and that ε34 males have the highest total variability (9.06). In addition, the contributions of individual traits to these multivariate measures are different among genotypes.

In figure 3, the representation of the lipid and apolipoprotein hyperspace for females shows a different

relationship between $||\mu||$ and Total Variance across the three Apo E genotypes than is observed for males. Females with the ε33 genotype are closest to the origin, at $||\mu|| = 0.22$, whereas ε34 females are at a midrange distance from the origin, at $||\mu|| = 0.66$, and ε32 females are the farthest from the origin, at $||\mu|| = 1.08$. However, the rank of the genotypes with respect to Total Variance and to the range of Total Variation is different between males and females. Females with the ε33 genotype have the smallest level of total variability (7.68), ε34 females have midrange total variability (9.90), and ε32 females have the greatest total variability (11.69).

In summary, there are three important multivariate results which these graphical representations (figs. 2 and 3) of the hyperspace highlight. First, both the range of $||\mu||$ and the rank of the genotypes with respect to $||\mu||$ are the same between genders. Second, both the range of Total Variance and the rank of the genotypes with respect to Total Variance are different between genders. Although no formal analyses were conducted, this result may suggest that there is nonadditivity of genotype and gender effects on Total Variance. Third, the contribution of each trait to the multivariate measures of means and variances is different across genotype and gender.

Discussion

During the course of our analyses many questions about how to characterize and interpret these multivariate results became apparent: Was there a pattern to the observed pleiotropic effects on means and intragenotype variances? What could be the underlying determinants of the observed patterns? What risk information is gained by the study of the second moments of multivariate distributions? What caveats or insights can the present study provide for future epidemiological and biometrical genetic analyses? In the following discussion, we address some of these issues.

Were there patterns associated with the observed pleiotropic effects on mean levels? First, in the majority of cases, the pleiotropic effects on average levels of lipids and apolipoproteins were localized in the portion of lipid metabolism commonly associated with VLDL catabolism. Specifically, the ε32 genotype was associated with significantly higher mean levels of lnTrig, lnApo E, Apo CII, and lnApo CIII. This phenotypic profile resembles some of the attributes of type III hyperlipidemia and hypertriglyceridemia (Brown et al. 1983). Second, the ε32 genotype was associated with most of the Apo E effects on the lipid and apolipoprotein levels. Many other studies have found similar Apo E effects on Total-C, triglycerides, and HDL-C (Menzel et al. 1983; Enholm et al. 1986; Boerwinkle et al. 1987; Ordovas et al. 1987; Boerwinkle and Utermann 1988; Havekes et al. 1988). Fewer studies have considered the Apo E genotype effects on apolipoproteins (Enholm et al. 1986; Boerwinkle and Utermann 1988; Havekes et al. 1988; Kaprio et al., in press). In general, our results are consistent with these studies, which show that the Apo E genotype has the strongest effect on the level of its own gene product. Third, we observed that Apo E genotype differences in mean levels of lipids and apolipoproteins were more common in males than in females. However, no statistical tests for interaction between genotype and gender were conducted.

Genotype × gender, genotype × environment, and genotype × genotype interaction are of great interest in explaining the distribution of the common diseases. However, there is no consensus on how to study these effects (Kempthorne 1978). Efforts are in progress in several laboratories, including our own, to develop statistical procedures appropriate for evaluating the contribution of interaction effects to the distribution of quantitative risk-factor traits. In the present study we chose not to use the two-way analysis of variance (ANOVA) to test for statistical significance of an interaction between genotype and gender, for the following reasons: First, this method is not robust to violations of certain assumptions. One important assumption is homogeneity of variance (Glass et al. 1972; Milligan et al. 1987). Eight of nine lipid and apolipoprotein traits considered in the present study displayed heterogeneity of intragenotype variance. In a previous study of the same population we also demonstrated heterogeneity of variance between genders for many of the same traits (Reilly et al. 1990). Second, the two-way ANOVA does not give an orthogonal estimate of interaction effects when cell sizes are not equal. Consequently, significance tests of interaction effects when there is heterogeneity of variance and/or when data are unbalanced could lead to inappropriate inferences. Interstudy inconsistencies with regard to inferences about interaction between genotype and gender could be the consequence of nothing more than either differing patterns of heterogeneity of variance or differing cell size patterns (Milligan et al. 1987).

Was there a pattern associated with the observed effects on intragenotype phenotypic variance? From the standpoint of evolutionary genetics, the pheno-

typic variability of heterozygotes in natural populations is expected to be less than that of homozygotes (Lerner 1954; Bishop et al. 1988). In contrast, we found that heterozygotes in seven of the nine traits had greater phenotypic variance than did the ε33 homozygote. However, both heterozygotes were not always associated with increased intragenotype variance in each of the seven traits. Although no formal test of interaction was conducted, it appears that males and females may have different patterns of association between genotypic variation and levels of intragenotypic phenotypic variance. In general, we observed that Apo E genotype differences in intragenotypic phenotypic variance were more common in females than in males. These results suggest that Lerner's (1954) global generalization about the genetic architecture of quantitative variation in natural populations may be inappropriate for the human traits considered here (Bishop et al. 1988).

Most studies which have investigated the Apo E effect on lipids and apolipoproteins have not examined differences in intragenotype variance. Bradshaw (1965) provides some of the first insights into the origin of differences in phenotypic variance among genotypes represented by plant strains. Murphy (1979) and Berg (1988, 1990) were among the first to recognize the importance of genotype-specific phenotypic variances in human genetics. To increase awareness that genotypes can be associated with variance differences in risk-factor traits, we tested for homogeneity of variance among the three most frequent Apo E genotypes by using data presented on healthy, adult individuals from Germany (Menzel et al. 1983), Framingham (Ordovas et al. 1987), The Netherlands (Havekes et al. 1988), and Finland (Enholm et al. 1986). In figure 4 we show that in the samples from Germany, The Netherlands, and Finland there were significant ($P < .05$) variance differences among Apo E genotypes, in Total-C, triglycerides, and HDL-C. Significant differences in the variability of apolipoproteins among genotypes were also observed in the samples from The Netherlands and Finland. These results suggest that the effects of genes on intragenotype variability may be generalized to other populations.

What characteristics or determinants are associated with the observed pattern of variance effects? The heterogeneity of interindividual variance among Apo E genotype subgroups may be due to gene × environment interaction, gene × gene interaction, linkage disequilibrium, or genotype selection of environments. Gene × environment and gene × gene interaction cause differences in intragenotype variances because the magnitude of the effects associated with the environment or other loci varies among genotypes. For example, individuals with different Apo E genotypes show different responses of their lipids and apolipoproteins to smoking (Kaprio et al. 1989; Sing et al. 1989), lipid-lowering drugs (Nestruck et al. 1987), and hormones (Hanis et al. 1991). Gueguen et al. (1989) have found Apo E genotype-specific longitudinal relationships between triglyceride levels and weight gain. Pedersen and Berg (1989) have shown that gene × gene interaction between the low-density-lipoprotein receptor gene and the Apo E gene contributes to determining variation in cholesterol levels. On the other hand, linkage disequilibrium and genotype- or gender-dependent selection of environments may cause differences in intragenotype variance because the frequency distribution of environments or background genotypes is different across Apo E genotypes. For instance, individuals with genotypes that are associated with higher lipid levels may choose different dietary and exercise regimes as a response to awareness of associated risk of CAD.

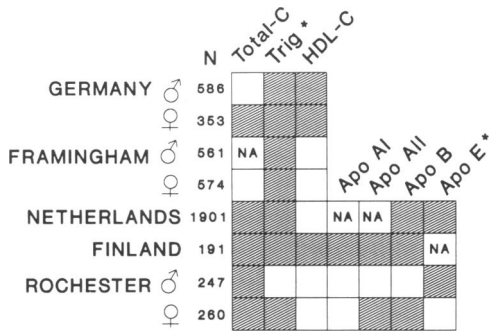

Figure 4 Differences in lipid and apolipoprotein variability among three most frequent Apo E genotypes in several populations. ▨ = At least one pairwise comparison significant at $P < .05$; NA = not available; * = log-transformed in Rochester, MN.

What types of risk information are influenced by the results of the present study? Effects on mean levels provide one measure of average risk for a subgroup of the population, while effects on variance can provide additional information on the fraction of individuals in each subgroup who exceed a particular level of risk. In figure 5 we illustrate an example of these two types of risk information by using estimates of means and variances from our study. In this example the ε32 genotype is associated with decreased mean levels of Total-C. Therefore, using information about mean

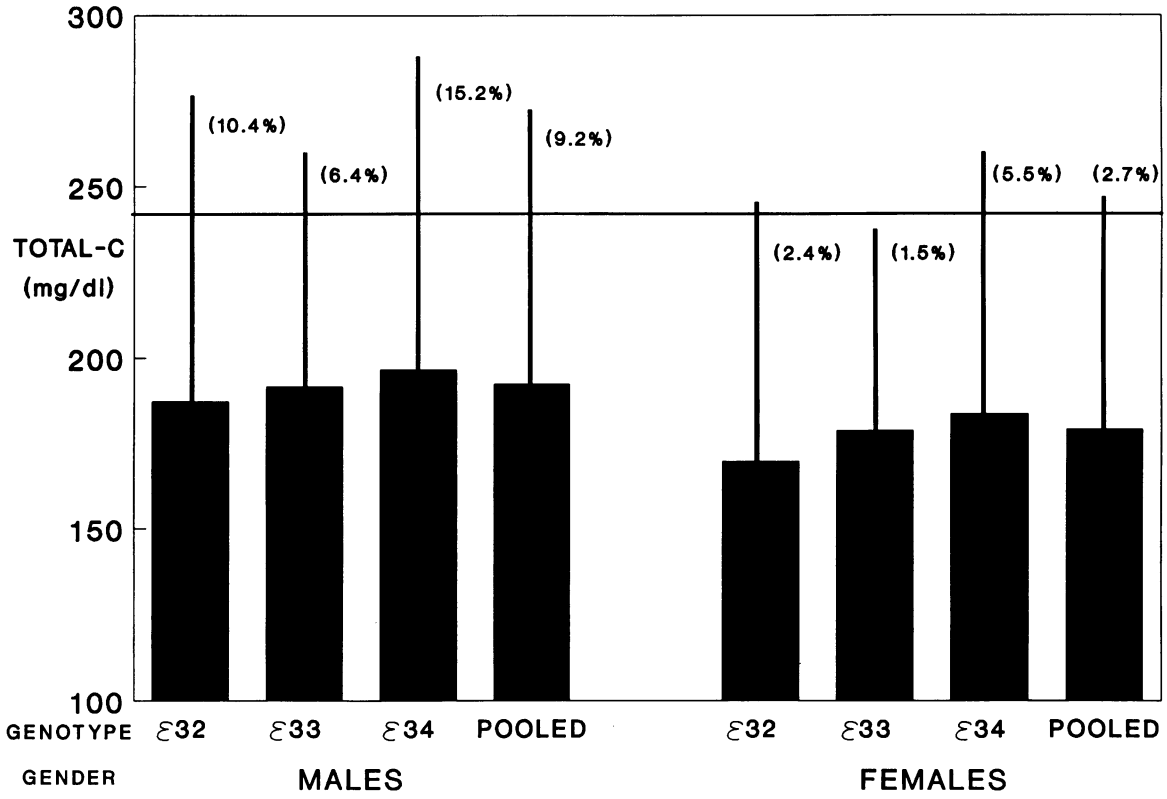

Figure 5 Influence that mean and interindividual variance (2 SD) in total-C levels have on fraction of individuals at high risk

levels only, we might conclude that individuals in the ε32 subgroup have less risk of developing CAD. If a threshold of 240 mg/dl Total-C is imposed as a criterion for high risk, then, because genotype influences intragenotypic variance, different fractions of these subgroups will have increased predisposition to developing CAD. The most striking aspect of this example is the realization that subgroup ε32 has the lowest mean levels of Total-C, while the fraction of individuals with the ε32 genotype who are above the threshold is greater than the fraction of those in the ε33 subgroup who cross the threshold. Such information will play a central role in establishing the extent to which public health strategies to alter disease risk are focused on sick individuals versus sick populations (Rose 1985, 1987).

What caveats or insights can the present study provide for future epidemiological and biometrical genetic analyses? Significant heterogeneity, among genetic subgroups, in the means and variances of the multivariate distribution of lipid and apolipoproteins suggests some guidelines for future studies. First, for reasons given earlier, we argue that males and females should be analyzed separately. Our results further justify separate analyses and suggest that those studies that have pooled males and females may give inappropriate inferences about the impact of genetic variation. Second, the assumption of homogeneity of variance across subgroups within a population should be verified before statistical tests on mean differences across subgroups are performed. Third, the assumption of no gender or genotype effect on intragenotypic phenotypic variability associated with a single gene (measured or unmeasured) with a significant effect on trait levels may be false.

The Apo E gene is both a level and variability gene, with regard to multiple measures of lipid metabolism. These pleiotropic effects on means and variances are gender specific. We suggest that gender-dependent penetrance functions with genotype-specific variance parameters may be necessary to fully understand the relationship between genetic variation and variation in CAD risk determined by intermediate quantitative risk factors.

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