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Genotype-Environment Interaction: Apolipoprotein E (*ApoE*) Gene Effects and Age as an Index of Time and Spatial Context in the Human

Kim E. Zerba*, Robert E. Ferrell† and Charles F. Sing*

**Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109 and †Department of Human Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania 15262*

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ABSTRACT

We analyzed the age-dependence of the estimates of the parameters of the genetic architecture of plasma ApoE levels associated with *ApoE* gene variation. Our study sample included 1988 individuals in multigeneration pedigrees from the Rochester, MN, population. We used a 30-yr sliding window across the age range (5–90 yr) to estimate the age dependency of parameters. Additive *ApoE* allelic variance of transformed plasma ApoE values for both genders, heritabilities for males and phenotypic and residual variance for females peaked in the 20–40-yr age windows and decreased significantly with age ($P < 0.05$). Phenotypic and residual variance for males and dominance variance for both genders did not vary significantly with age. All parameter estimates were significantly different from zero across all age windows for both genders. Most studies of *ApoE* have focused on its functions in the pathophysiology of coronary artery disease (CAD) in middle-aged and older individuals. Our findings suggest the greatest role of this gene is in determining phenotypic differences among younger and middle-aged individuals. These observed genotypic effects on the plasma ApoE levels may contribute to age-dependent differences in physiological health, growth, and risk of disease.

MOST human studies attempt to understand the proximate, mechanistic role of genes in disease. Efforts have concentrated on associations of measured variation in single genes with interindividual phenotypic variation in physiological and biochemistry traits related to the risk of disease. Cross-sectional samples are the norm for these studies, and only a highly restricted age range of individuals is usually considered. Age is routinely considered a nuisance variable in human genotype-phenotype studies. Traditionally phenotypic variation is statistically adjusted for age variation before any genetic analyses. In reality, genotypic and allelic effects are indexed by age because they depend on interactions with environments that vary with the dynamic time and spatial contexts of individuals during ontogeny (ZERBA and SING 1993).

The dynamic features of genotype-environment interaction with ontogeny suggest that an evolutionary perspective may provide a richer understanding of the role of genes in human phenotypic variation. For example, measured genotypic variation associated with phenotypic variation, which in turn is associated with a complex, multifactorial disease of senescence such as CAD, hypertension, cancer, or Alzheimer's disease, could have beneficial physiological associations with health earlier in the ontogeny of the same individuals (WILLIAMS 1957; ROSE 1991; WILLIAMS and NESSE 1991). Such knowledge about antagonistic pleiotropic effects of genes over the ontogeny of individuals could provide critical information for current efforts to use gene therapy to manipulate phenotypic expression or treat human disease.

A first step in evaluating the potential role of antagonistic pleiotropic effects of a gene with age is to examine whether phenotypic variation associated with interindividual variation in the gene is heterogeneous with age. Since many traits vary allometrically with age-related growth, any observed heterogeneity among age groups in the role of gene variation in determining phenotypic variation could be simply due to scaling relationships between the intragenotypic variances and genotypic means. A concern is that the results be presented on a scale where the variance is unrelated to the mean (ATCHLEY 1984; RISKA *et al.* 1984; FALCONER 1989). If a significant relationship is found between phenotypic variances and means among genotypes, a scale transformation applied at the level of the observed data to stabilize intragenotypic variances is necessary.

This paper focuses on an analysis of the relationship between estimates of parameters of genetic architecture of plasma levels of the *ApoE* gene product and age in humans. ApoE is a 34-kD monomeric glycoprotein synthesized in most organs including the liver, brain, gonads, spleen, kidney, muscle, and macrophages (DAVIGNON *et al.* 1988; MAHLEY 1988; DAVIGNON 1993). ApoE plays a key physiological role in lipid metabolism by facilitating the uptake of lipoproteins bound to ApoE by receptor-mediated endocytosis (DAVIGNON *et al.* 1988;

Corresponding author: Kim E. Zerba, Biology Department, Colgate University, 13 Oak Dr., Hamilton, NY 13346.
E-mail: kez@superm.hg.med.umich.edu

MAHLEY 1988). ApoE binds with high affinity to the low density lipoprotein (LDL) receptors of the liver and peripheral tissues, chylomicron remnant receptors of the liver (MAHLEY 1988; MAHLEY *et al.* 1989), and the LDL receptor-related proteins of neuronal cells (ROSES 1995). ApoE is involved in the transport of dietary lipid from the intestine by chylomicron remnants to the liver, lipid from the liver by LDL to peripheral tissues, and of cholesterol from peripheral tissues by high density lipoproteins back to the liver. These lipid transport functions of ApoE are also related to the metabolism of ApoE in neurons that may contribute to the health and maintenance of neuronal cells after adult maturity when neuronal cell division ceases (ROSES 1995).

The structural gene for ApoE is on chromosome *19* and is polymorphic with three common alleles (ϵ_2, ϵ_3, ϵ_4) coding for three plasma isoforms that result in six genotypes in most populations (DAVIGNON *et al.* 1988). It is one of the few important apolipoproteins for which the relationship between polymorphic variation in the population can be directly related to the functional characteristics of the gene product (RALL *et al.* 1982). Many studies have established that allelic variation in the gene coding for ApoE makes an important contribution to predicting interindividual phenotypic differences in traits that are measures of lipid metabolism (SING and DAVIGNON 1985; DAVIGNON *et al.* 1988; KAPRIO *et al.* 1991; XHIGNESSE *et al.* 1991; SING *et al.* 1992). The average effect of the less frequent *ApoE* ϵ_2 allele lowers and the ϵ_4 allele raises plasma LDL-cholesterol levels compared with the most common ϵ_3 allele (SING and DAVIGNON 1985). LDL cholesterol is among the most atherogenic of the lipoproteins and the *ApoE* polymorphism has been associated with the initiation and progression of CAD particularly for individuals carrying the ϵ_4 allele (HIXSON and PDAY RESEARCH GROUP 1991; STENGÅRD *et al.* 1995).

Most human studies have been presented as if the *ApoE* genotype and allelic effects are independent of the effects of age and other concomitants that are CAD risk factor traits. Plasma ApoE levels, however, change with age in the population at large (KOTTKE *et al.* 1991). There is therefore ample, but largely unexplored, potential for the association between *ApoE* genotypic and allelic variation and phenotypic variation to change with age. *ApoE* genotype and allele frequencies, however, have also been observed to vary with age. In particular, several studies have demonstrated a significant decrease in the ϵ_4 allele frequency in samples of older individuals, suggesting the potential for selection against individuals carrying the ϵ_4 allele (DAVIGNON *et al.* 1987; CAULEY *et al.* 1993; EGGERSTEN *et al.* 1993). There is concern as to how genotype frequency variation with age might affect our inferences. Consequently we also present an analysis of the contribution of age variation in the relative frequency and mean value of each genotype to our observed variation in estimates of genetic architecture parameters of plasma ApoE levels with age. Since both genotype frequency and scale are involved in the genetic architecture parameter estimates, we include an analysis of the correlation of the relative frequency with the mean value of each genotype across age.

MATERIALS AND METHODS

Sample: Our study sample included 1988 individuals, 1035 females and 953 males, with an *ApoE* isoform typing, measure of plasma ApoE level and recorded age. These individuals were sampled from three- and four-generation pedigrees ascertained through elementary school children of the Rochester, MN, population as part of the Rochester Family Heart Study (RFHS: described by MOLL *et al.* 1989; Turner *et al.* 1989). This sample was considered to be representative of multigeneration pedigrees in the Rochester, MN, population at large.

Laboratory measures: Blood samples were collected by venipuncture into EDTA. *ApoE* isoforms were determined by isoelectric focusing using the method described by KAMBOH *et al.* (1988). Since the isoforms correspond directly to differences at amino acid residue sites 112 and 158 (RALL *et al.* 1982), we used the isoform typings to infer the *ApoE* genotype for each individual (REILLY *et al.* 1992). Plasma ApoE levels were measured by radioimmunoassay (KOTTKE *et al.* 1991).

Statistical analyses: *Sample adjustment:* Plasma ApoE levels were adjusted for laboratory assay date by linear regression (KAPRIO *et al.* 1991) and the grand mean added back to the residuals. All subsequent analyses were conducted on females and males separately (REILLY *et al.* 1991, 1992).

Scale transformation of observed data: To evaluate whether a transformation was required, we searched for the best fitting regression model of the ith intragenotype variance, σ_i^2, as a power (x) function of the ith intragenotype mean, μ_i:

$$\sigma_i^2 = \beta^x \mu_i^x + \lambda_i, \tag{1}$$

where β^x represents the slope, and λ_i is an independent and additive contribution of random variation to the ith intragenotype variance, and $i = \epsilon_2\epsilon_2, \epsilon_3\epsilon_2, \epsilon_3\epsilon_3, \epsilon_4\epsilon_2, \epsilon_4\epsilon_3, \epsilon_4\epsilon_4$ The best fitting regression was selected according to the model with the largest coefficient of determination, R^2. All regressions were through the origin. We observed highly significant regression relationships for each parameter. Consequently, we transformed the observed plasma ApoE values, Y, to a scale such that the intragenotypic variance was approximately constant among genotypes (BROWNLEE 1984):

$$Y' = \int \frac{dY}{\sqrt{Y^x}}. \tag{2}$$

Sliding window subsampling approach: Our interest is in estimating the continuum of the relationships between the parameters of genetic architecture defined below and age. One possible approach would be to divide the sample into three distinct age classes and test for heterogeneity of parameters among age windows. Three points, however, are not an adequate description of such a relationship. As an alternative approach, we used a fixed-span sliding window to subsample the age range. For each window, we estimated each of the genetic architecture parameters. Each window subsample spanned 30 yr. The midpoints of adjacent windows differed by 1 yr. We considered numerous window span sizes. Span sizes ranging from 20–50 yr provided similar inferences. A span size of 30 yr was chosen to minimize missing *ApoE* genotype strata across the age distribution and maximize the num-

TABLE 1

Descriptive statistics for *ApoE* data

Genotype i	Females			Males		
	N	$\hat{\mu}_i$	$\hat{\sigma}_i^2$	N	$\hat{\mu}_i$	$\hat{\sigma}_i^2$
			Observed data[a]			
$\epsilon_2\epsilon_2$	3	10.16	65.44	3	16.68	126.93
$\epsilon_3\epsilon_2$	113	6.39	6.45	121	6.08	6.57
$\epsilon_3\epsilon_3$	621	5.48	6.78	537	5.25	8.77
$\epsilon_4\epsilon_2$	24	5.07	3.28	26	4.44	2.18
$\epsilon_4\epsilon_3$	252	4.01	2.45	244	4.19	8.15
$\epsilon_4\epsilon_4$	22	3.54	4.10	22	3.19	3.33
			Transformed data[b]			
$\epsilon_2\epsilon_2$		0.2028	0.0474		0.5162	0.0058
$\epsilon_3\epsilon_2$		0.1853	0.0092		0.6534	0.0045
$\epsilon_3\epsilon_3$		0.2200	0.0121		0.6817	0.0057
$\epsilon_4\epsilon_2$		0.2219	0.0063		0.7015	0.0040
$\epsilon_4\epsilon_3$		0.2886	0.0151		0.7255	0.0068
$\epsilon_4\epsilon_4$		0.3836	0.0388		0.7810	0.0112

Data are in milligrams per deciliter.

[a] Best fitting (R^2) power regression of intragenotype variance on mean: Females $\hat{\sigma}^2$ = 6.11E-3 $\hat{\mu}^{4.0}$; $F_{1,5}$ = 818.8, $P < 0.0001$; R^2 = 0.994. Males, $\hat{\sigma}^2$ = 1.12E-1 $\hat{\mu}^{2.5}$; $F_{1,5}$ = 1996.8; $P < 0.0001$, R^2 = 0.998. Data are Y = plasma *ApoE*.

[b] Transformation of observed plasma *ApoE* values, based on above regressions, to stabilize intragenotypic variances. Data are $Y^{-1.00}$ for females and $Y^{-0.25}$ for males.

ber of contiguous nonoverlapping windows. All windows were symmetric about window midpoints such that midpoints of the windows at the ends of the age distribution were 15 yr from the actual limits of the age distribution.

Genetic architecture parameter estimation: For each 30-yr window, the cell means model for the transformed observed plasma ApoE level, Y', for the kth individual in the $\epsilon_i\epsilon_j$, i = 2, 3, 4, and $j \leq i$, genotype class is:

$$Y'_{\epsilon_i\epsilon_j k} = \mu_{\epsilon_i\epsilon_j} + \lambda_{\epsilon_i\epsilon_j k}, \quad (3)$$

where $\mu_{\epsilon_i\epsilon_j}$ is the mean transformed plasma ApoE level of individuals in the $\epsilon_i\epsilon_j$ genotype class. $\lambda_{\epsilon_i\epsilon_j k}$ is an independent and additive contribution of random variation to individual k's transformed plasma ApoE level. Using this model, we estimated the total phenotypic variance, σ_P^2, the unbiased genotypic (BOERWINKLE and SING 1986), σ_G^2, and its two components, additive genetic variance attributable to average effects of the three common *ApoE* alleles and dominance variance, σ_A^2 and σ_D^2, and the residual variance, σ_R^2. We also estimated broad and narrow sense heritabilities, h_B^2 and h_N^2, associated with *ApoE* genotypic and allelic variation.

Partitioning the *ApoE* genotypic variance into additive and dominance variance assumes the *ApoE* genotype frequencies are in Hardy-Weinberg equilibrium (HWE) proportions. In males, the *ApoE* $\epsilon_2\epsilon_2$ genotype was not observed in any age windows >65 yr. We therefore eliminated age windows >65 yr in males from our analyses. To test the HWE assumption for the remaining windows, we first estimated *ApoE* allele relative frequencies by gene counting and *ApoE* genotype expected HWE relative frequencies. We then performed a poorness-of-fit test for deviations of the observed genotype frequencies from expectations under HWE (WEIR 1990) over all genotypic classes for each window. In no case did we find significant deviations from HWE at the 0.05 level of significance.

σ_A^2 and σ_D^2 are biased because the traditional estimate of the genotypic variance is biased (BOERWINKLE and SING 1986). The extent to which this bias is apportioned among the additive and dominance variances is unknown. We follow KAPRIO *et al.* (1991) and assume the bias is apportioned equally among the additive and dominance variances. We therefore estimated approximately unbiased estimates of σ_A^2, σ_D^2 as in KAPRIO *et al.* (1991).

Bootstrap approach to confidence limits and hypothesis testing: The sampling distributions of the estimates of the *ApoE* genotypic and additive variances used here are unknown. For each age window, we therefore used bootstrap methods to estimate the 90 and 95% confidence intervals about the point estimates of all the genetic architecture parameters and to test for heterogeneity of the estimates with age.

The bootstrap procedure for hypothesis testing performs better on a statistic with an asymptotically pivotal distribution, which does not depend on any unknowns such as the variance of the statistic (HALL and WILSON 1991). If the variance of a parameter estimate is not constant but predictable from its mean, the statistic can be transformed to a scale with an approximate constant variance of 1 (BROWNLEE 1984; TIBSHIRANI 1988). This would be the second transformation applied to the analysis of the data of this study. Two such transformations are analogous to the common practice of first transforming observed data for use in correlation analysis to achieve bivariate normality, then subsequently z-transforming the correlation estimate for hypothesis testing.

We tested for constancy of variances of bootstrap estimates of the parameters for the entire sample of each gender separately in the following way. First, each residual, $\lambda_{\epsilon_i\epsilon_j k}$, from (3) was assigned mass $1/N$ for resampling (EFRON 1982). These N residuals were sampled with replacement to form 100 bootstrap samples of an entire gender sample. For each bootstrap sample, the bootstrap estimate of a transformed plasma ApoE level, $Y'^{*b}_{\epsilon_i\epsilon_j k}$, was formed by adding a residual, $\lambda^{*b}_{\epsilon_i\epsilon_j k}$, sampled with replacement from the original vector of N residuals, to

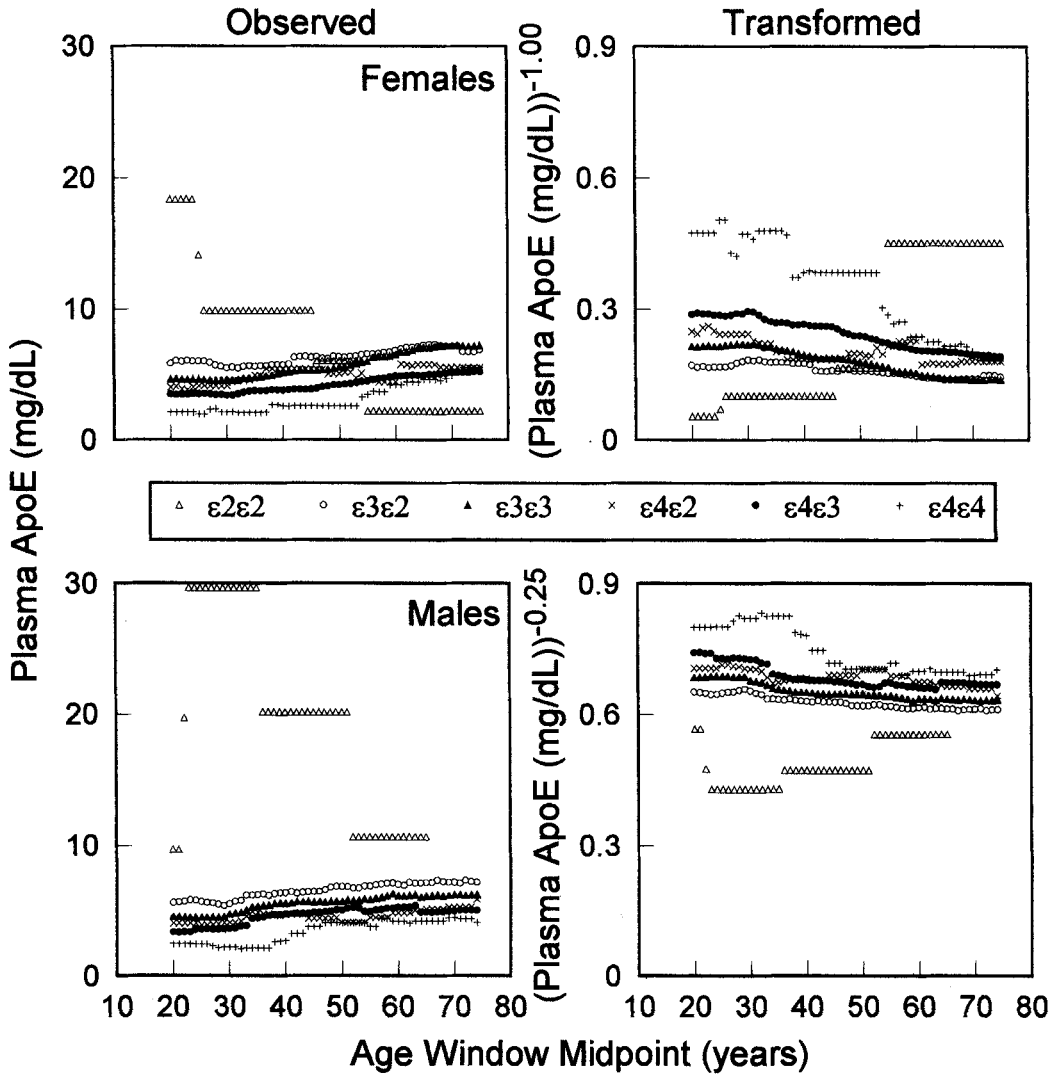

FIGURE 1.—The relationships between the *ApoE* genotype means of plasma ApoE levels and age window midpoint. Observed data: left; transformed data: right. Females: top; males: bottom.

the original point estimate of the plasma ApoE level mean for the $\epsilon_i\epsilon_j$ genotype class, $\hat{\mu}_{\epsilon_i\epsilon_j}$:

$$Y'^{*b}_{\epsilon_i\epsilon_j k} = \hat{\mu}_{\epsilon_i\epsilon_j} + \lambda^{*b}_{\epsilon_i\epsilon_j k} \tag{4}$$

For each bootstrap sample, we estimated each genetic architecture parameter. For each of the 100 bootstrap samples, we also formed an additional 500 nested bootstrap samples. Each nested bootstrap sample estimate of a transformed observed plasma ApoE level, $Y'^{*nb}_{\epsilon_i\epsilon_j k}$, nb = 1, 2, . . ., 500, was formed by adding a nested bootstrap residual, $\lambda^{*nb}_{\epsilon_i\epsilon_j k}$, sampled with replacement from the corresponding higher order bootstrap vector of N residuals, λ^{*b}, to the bootstrap point estimate of the plasma ApoE level mean for the genotype class, $\hat{\mu}^{*b}_{\epsilon_i\epsilon_j k}$:

$$Y'^{*nb}_{\epsilon_i\epsilon_j k} = \hat{\mu}^{*b}_{\epsilon_i\epsilon_j} + \lambda^{*nb}_{\epsilon_i\epsilon_j k}. \tag{5}$$

We then estimated of the mean and variance of the 500 nested bootstrap parameter estimates for each of the 100 bootstrap samples. For each genetic architecture parameter, θ: σ^2_P, σ^2_G, σ^2_A, σ^2_D, σ^2_R, h^2_B and h^2_N, we searched for the best fitting regression model of the bth bootstrap estimate of its variance σ^{2*b}_θ, b = 1, 2, . . ., 100, as a power (x) function of the bth estimate of its mean, $\hat{\mu}^{*b}_\theta$:

$$\sigma^{2*b}_\theta = \beta^x(\hat{\mu}^{*b}_\theta)^x + \lambda^{*b}, \tag{6}$$

where β^x represents the slope, and λ^{*b} is an independent and additive contribution of random variation to the bth bootstrap estimate of the variance of the parameter estimate. The best fitting regression was selected according to the model with the largest coefficient of determination, R^2. All regressions were through the origin. We found a highly significant regression relationship in every case. Consequently, we transformed all parameter estimates used in the sliding window subsampling estimates of the confidence limits and hypothesis testing to a scale such that the variance was ~1 (BROWNLEE 1984):

$$\Theta' = \int \frac{d\Theta}{\sqrt{\beta^x\Theta^x}}. \tag{7}$$

We confirmed that the variance of these transformed estimates was ~1 at the level of the entire sample for each gender.

For each age window, we then estimated the deviations, $\hat{d}^{*b}$, b = 1, 2, . . ., 1000, of the transformed bootstrap estimates about the transformed original point estimate of each parameter:

$$\hat{d}^{*b} = \hat{\Theta}'^{*b} - \hat{\Theta}' \tag{8}$$

TABLE 2

Genetic architecture parameter estimates

	Parameter	Estimate	Regression[a]			Transformation[c]
			$\hat{\beta}^x$	x	R^{2b}	
Females	σ^2_P	1.46E−2	4.25E+1	3.9	0.938	$1.05\ \hat{\sigma}^{2-0.95}_P\hat{\beta}^{x-0.5}$
	σ^2_G	1.56E−3	5.48E−5	1.0	0.971	$2\ \hat{\sigma}^{2\,0.5}_G\hat{\beta}^{x-0.5}$
	σ^2_R	1.30E−2	1.01E+2	4.0	0.926	$\hat{\sigma}^{2-1}_R\hat{\beta}^{x-0.5}$
	σ^2_A	1.51E−3	5.28E−5	1.0	0.978	$2\ \hat{\sigma}^{2\,0.5}_A\hat{\beta}^{x-0.5}$
	σ^2_D	4.83E−5	4.43E−4	1.2	0.918	$2.5\ \hat{\sigma}^{2\,0.4}_D\hat{\beta}^{x-0.5}$
Males	σ^2_P	6.81E−3	4.31E−2	2.5	0.989	$4\ \hat{\sigma}^{2-0.25}_P\hat{\beta}^{x-0.5}$
	σ^2_G	9.06E−4	3.04E−5	1.0	0.990	$2\ \hat{\sigma}^{2\,0.5}_G\hat{\beta}^{x-0.5}$
	σ^2_R	5.90E−3	5.20E−2	2.5	0.988	$4\ \hat{\sigma}^{2-0.25}_R\hat{\beta}^{x-0.5}$
	σ^2_A	8.39E−4	2.65E−5	1.0	0.993	$2\ \hat{\sigma}^{2\,0.5}_A\hat{\beta}^{x-0.5}$
	σ^2_D	6.64E−5	2.07E−4	1.2	0.989	$2.5\ \hat{\sigma}^{2\,0.4}_D\hat{\beta}^{x-0.5}$

[a] Best fitting (R^2) power (x) regression of nested bootstrap variance on mean.
[b] All regressions significant at $P < 0.0001$ for $F_{1,99}$.
[c] Transformation to stabilize nested bootstrap variance at ~1.

These deviations were then sorted and the subsequent 26th and 975th deviations in the ordered array, $\hat{d}$, were used to estimate the lower (L) and upper (U) limits,

$$\hat{L} = \hat{\Theta}' + \hat{d}_{(26)}, \tag{9}$$

and

$$\hat{U} = \hat{\Theta}' + \hat{d}_{(975)}, \tag{10}$$

of the 95% confidence interval for each parameter. In a similar manner, we also estimated the 90% confidence limits.

The null hypotheses were a parameter estimate was not significantly different from zero for a given age window and no heterogeneity in parameter estimates among age windows. Our criterion for rejection of either of these null hypotheses was simply any nonoverlap of the 90 and 95% confidence intervals with zero for a particular age window and among any of the all possible age window pairwise comparisons of confidence limits.

To evaluate the contribution of genotype frequency and mean phenotype variation with age to our estimates of genetic architecture parameter variation with age, we first estimated the simple, unconditional linear regression relationships across age windows of each genetic architecture parameter estimate on each *ApoE* genotype relative frequency and the squared deviation of each genotype plasma ApoE level mean deviation from the age window sample mean. We then considered the correlation of HWE expected relative frequency and mean deviation from the age window sample mean of each genotype across age windows.

RESULTS

Descriptive statistics of the plasma ApoE values for females and males in the Rochester, MN, sample are presented in Table 1. We observed significant regressions of the *ApoE* intragenotypic variance on the genotype mean of plasma ApoE values for both genders. The R^2 values for both regressions were >0.990. These relationships do not appear to result from sample frequency differences among genotypes. Consequently, we transformed the observed plasma ApoE values to an approximately constant intragenotypic variance.

The relationships between the *ApoE* genotype means of observed and transformed plasma ApoE levels and age window midpoint are presented in Figure 1 for females and males, respectively. For the observed data, the pattern of changes in means with age window was similar among genders. The means for all genotypes except ϵ_{22} increased with age, and the differences among means of all genotypes decreased with age. These decreased differences among genotype means with age suggest decreased genotypic variance of observed plasma ApoE levels with age. However, since the genotype means also changed with age, it is unknown the extent to which decreased differences among genotype means with age reflects allometric changes in observed plasma ApoE levels.

On a scale in which the intragenotypic variance in transformed plasma ApoE levels is unrelated to the genotype mean overall all age windows combined, the differences among genotype means decreased with age for males and for females from the 20–54 yr (Figure 1). These decreased differences suggest decreased genotypic variance of transformed plasma ApoE levels with age, independent of allometry.

Genetic architecture parameter estimates for the transformed data are presented in Table 2 for females and males. We observed significant power regressions of nested bootstrap variance of a bootstrap parameter estimate on the nested bootstrap mean for all parameters. The R^2 values for all regressions were >0.910 and most (11/14) were >0.970. These results indicate the nested bootstrap variance of a bootstrap parameter estimate, although variable, was highly predictable from the nested bootstrap mean. Consequently, we transformed all parameter estimates used in determining confidence limits and hypothesis testing to a scale such that the nested bootstrap parameter variance estimate was stable at ~1.

The relationships between phenotypic, genotypic

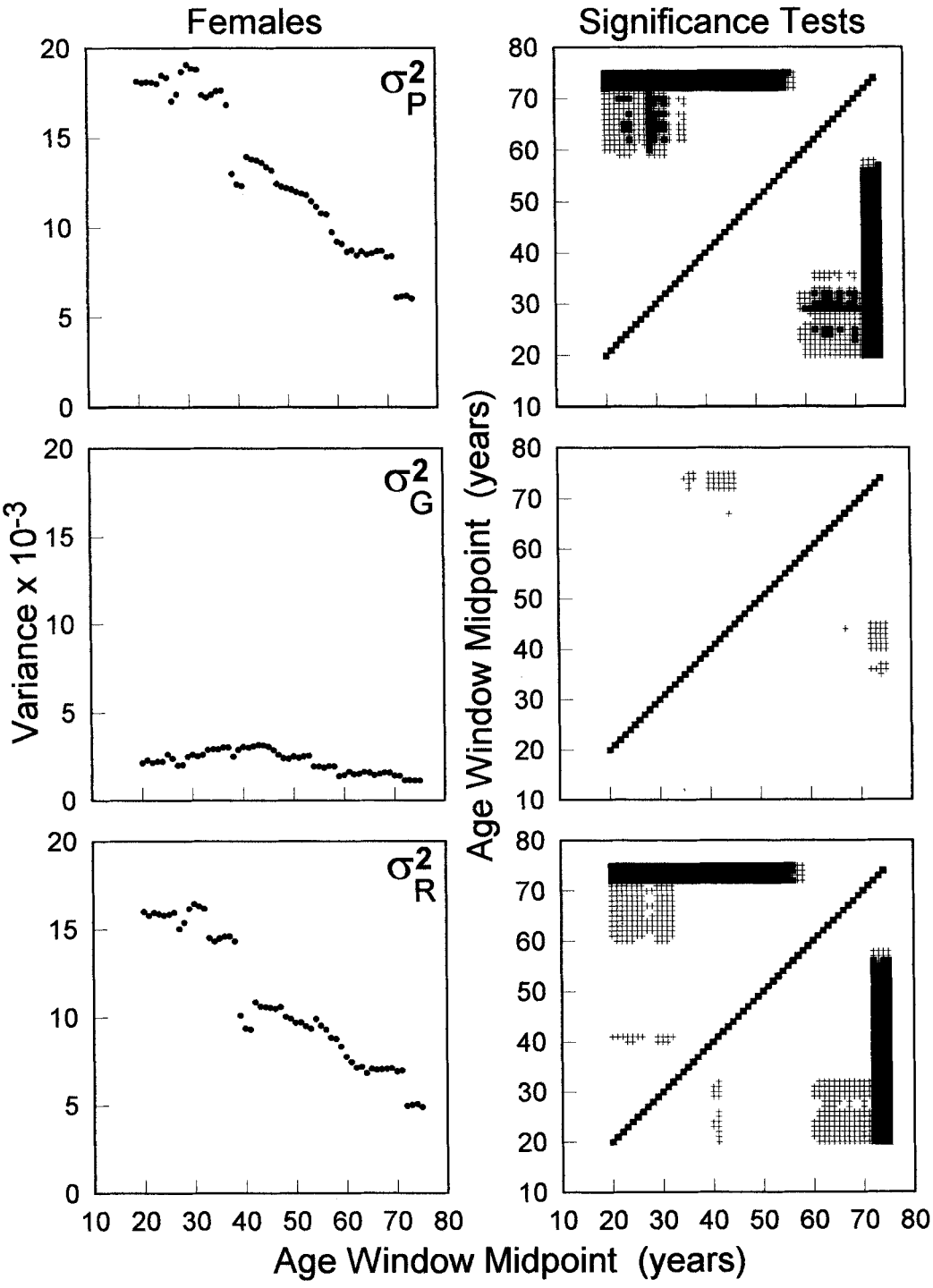

FIGURE 2.—The relationships between phenotypic, genotypic and residual variance of transformed plasma ApoE levels and age window for females (left). The results (right) are presented of the hypothesis tests about parameter estimates. The hypothesis testing strategy is defined in the text. The diagonal coordinates in each of these panels reflect the hypothesis tests that a parameter estimate in a window is significantly different from 0. The off-diagonal coordinates in these panels present the results of hypothesis tests that 90 and 95% confidence limits of parameter estimates for two windows do not overlap. +, $0.10 > P \geq 0.05$; ■, $P < 0.05$. The plots of results of hypothesis tests comparing confidence limit overlap among age windows are presented symmetrically so that either the horizontal or vertical axis can be used as the starting point for a hypothesis test about a particular set of age window comparisons.

and residual variance of transformed plasma ApoE levels and age window are presented in Figures 2 and 3 for females and males, respectively. The magnitudes of the variances for females are about twice those for males. These gender differences in parameter estimates can be attributed to the differences in transformations applied to the observed data to stabilize intragenotypic variances. For females and males, phenotypic, residual

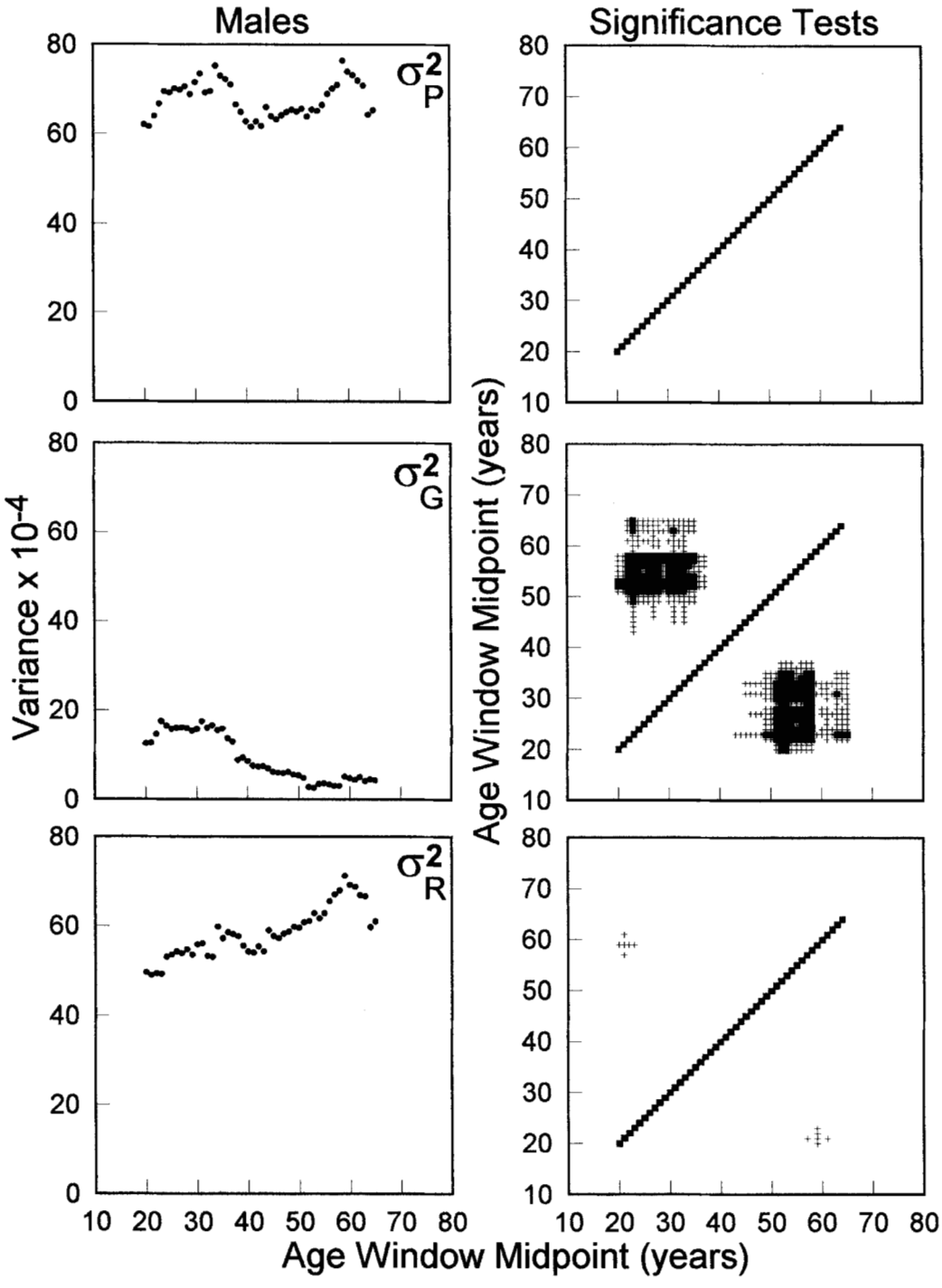

FIGURE 3.—The relationships between phenotypic, genotypic and residual variance of transformed plasma ApoE levels and age window for males (left). The results (right) presented of the hypothesis tests about parameter estimates as described for Figure 2.

and genotypic variances were significantly different from 0 for all windows. For females, the phenotypic and residual variance was highest in the 20–30-yr windows and decreased significantly with age window (Figure 2). In contrast, the genotypic variance was constant with age at the 5% significance level. However, a slight decrease in genotypic variance between the 34–45- and 70–75-yr windows was detected at the 10% significance level. For males, phenotypic and residual variance remained constant with age at the 5% significance level (Figure 3). However, an increase in residual variance between the 20–23- and 58–61-yr windows was detected at the 10% significance level. The genotypic variance peaked in the 20–35-yr windows and decreased significantly with age window.

The relationships between the additive and domi-

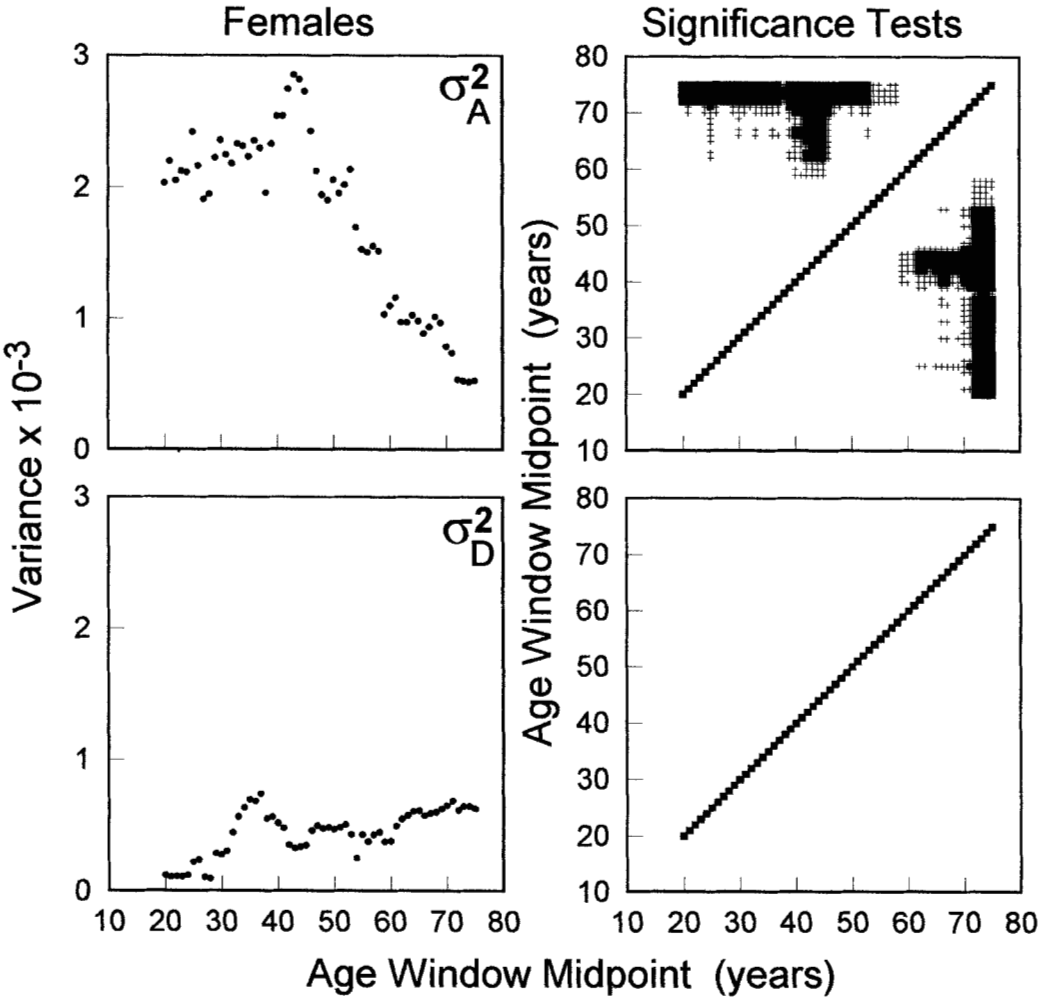

FIGURE 4.—The relationships between the additive and dominance variances, attributable to allelic differences in transformed plasma ApoE levels for females (left). Presented at right are the results of the hypothesis tests about parameter estimates as described for Figure 2.

nance variances, attributable to allelic differences in transformed plasma ApoE levels, with age window are presented in Figures 4 and 5 for females and males, respectively. Again, estimates in females were about twice those for males. All estimates were significantly different from 0 for all windows. For females, additive variance peaked in the 20–50-yr windows and decreased significantly with age (Figure 4). For males, the additive variance peaked in the 20–35-yr windows and decreased significantly with age (Figure 5). In contrast, dominance variance remained constant with age for both genders. These results suggest the impact of the *ApoE* gene, although significant over the entire age range (5–90 yr) is greatest in the younger and middle age windows.

The relationships between broad and narrow sense heritability estimates and age window are presented in Figures 6 and 7 for females and males, respectively. Despite the roughly twofold differences in total phenotypic and genotypic variance between females and males, the heritability estimates associated with *ApoE* genotypic and allelic variation are in the same range (<0.3). Heritabilities did not vary significantly with age at the 5% significance level for females. A significant decrease in narrow sense heritability between the 40–45- and 73–75-yr windows was detected at the 10% significance level. For males, changes with age in broad and narrow sense heritabilities were nearly identical (Figure 7). Both estimates peaked in the 20–35-yr windows and decreased significantly with age ($P < 0.5$). These changes were also nearly identical to those observed for the additive variance for males. These results also suggest that dominance, although significant throughout the age range, is small compared with the additive variance.

The relationships between number of individuals, *ApoE* genotype and allele relative frequency and age window midpoint are presented in Figure 8. The changes in numbers of individuals with age window reflect the sampling of multigeneration pedigrees. There was variation, but not dramatic changes, in genotype or allele frequencies with age for both genders.

The unconditional contribution of variation in each

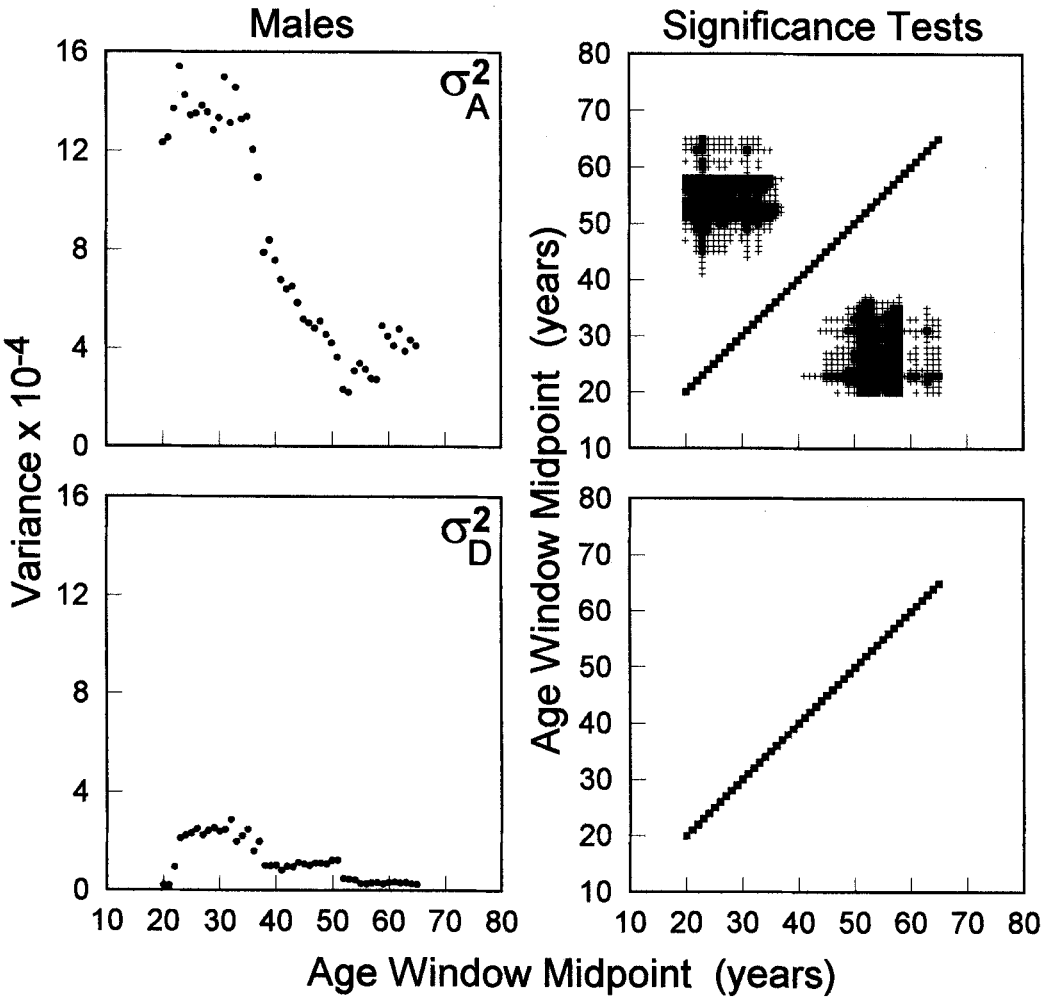

FIGURE 5.—The relationships between the additive and dominance variances, attributable to allelic differences in transformed plasma ApoE levels for males (left). Presented at right are the results of the hypothesis tests about parameter estimates as described for Figure 2.

genotype frequency and squared deviation of genotype mean from the age window sample mean to age variation in genetic parameter estimates is presented in Tables 3 and 4, respectively. Age variation in the frequencies and mean deviations from the age window sample mean of most genotypes contribute significantly to age variation in the genetic architecture parameter estimates. Clearly no one genotype is responsible alone for the patterns of change with age of parameter estimates we observed.

The estimates of correlations among genotype frequencies and phenotype scale deviations across age windows are presented in Table 5. Age variation in genotype frequency and phenotype scale is significantly correlated within and among most genotypes. These results support the observed combined contribution of age variation in frequencies and means of most genotypes to the observed variation in genetic architecture parameter estimates.

DISCUSSION

Our study shows that many of the parameter estimates of the genetic architecture of plasma ApoE levels associated with interindividual variation in the *ApoE* gene are not static, but instead are dynamic throughout the life cycle for females and males from the Rochester, MN, population at large. Age variation in frequencies and phenotypic means of most genotypes contributed to this age variation in genetic architecture parameter estimates. Of greatest interest are the decreases with age observed in genotypic variance and heritabilities for males and additive variance for both genders. Although only significant at the 10% level, a similar trend was observed in the genotypic variance and narrow sense heritability for females over part of the age range. It is significant to note that despite the pattern differences observed in variance heterogeneity with age and the roughly twofold differences in total, genotypic, and additive variances between females and males associated with the scale transformations applied to the observed data, the heritability estimates were in the same range for both genders (<0.3). These results reinforce our confidence in the observed patterns of heterogeneity in variances with age for both genders. The most likely explanation for the decrease in *ApoE* genotypic and

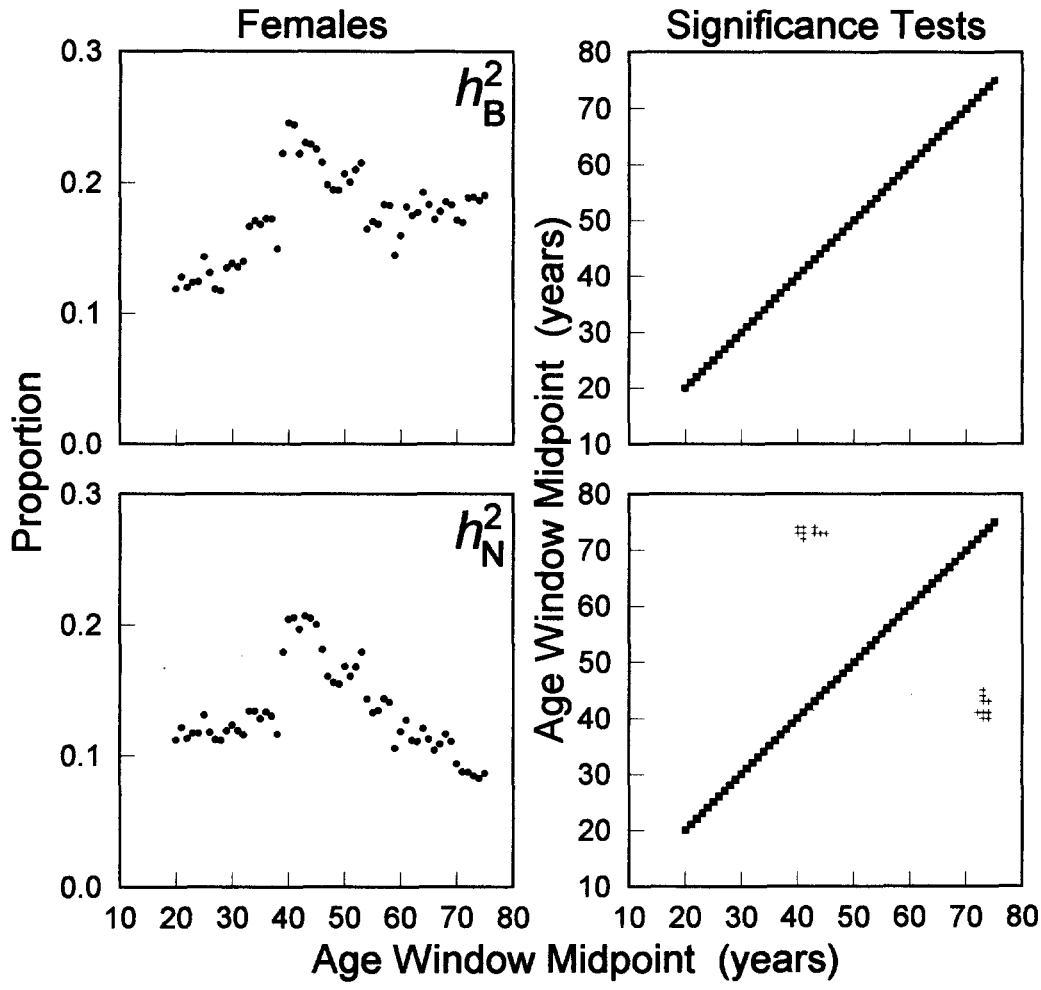

FIGURE 6.—The relationships between broad and narrow sense heritability estimates and age window for females (left). At right the results are presented of the hypothesis tests about parameter estimates as described for Figure 2.

additive variance with age windows is that phenotypic means of the *ApoE* genotypes become more similar with increasing age.

These results are consistent with two recent studies. First, in a study of the three common *ApoE* genotypes ($\epsilon_3\epsilon_2$, $\epsilon_3\epsilon_3$, $\epsilon_4\epsilon_3$) from a sample of octogenarian females and males, DAVIGNON *et al.* (1987) found the means of total plasma cholesterol and LDL cholesterol of $\epsilon_3\epsilon_3$ and $\epsilon_4\epsilon_3$ individuals were more similar than those same two genotypes in a healthy sample of younger middle-aged individuals. Second, in a recent longitudinal study of male twins followed over ages 48–63 yr, PAIRITZ JARVIK *et al.* (1994) found an increase in the similarity of the total plasma cholesterol and LDL cholesterol means of the $\epsilon_3\epsilon_3$ and $\epsilon_4\epsilon_3$ individuals from 48 to 58 yr, and then a decreased similarity between 58 and 63 yr. The PAIRITZ JARVIK *et al.* (1994) results are striking because the ranking of the means of the two genotypes changed between the first and last sampling of these individuals. We did not observe any change in the ranking of the transformed plasma ApoE level means of these two common genotypes with age in either gender.

Peaks in genetic variances early in life history followed by general decreases in variance with age have been reported in numerous experimental biometrical genetic studies of growth in mammals (MONTEIRO and FALCONER 1966; ATCHLEY 1984; RISKA *et al.* 1984). These growth studies showed that body size is highly regulated during development. Individuals may start out on different growth trajectories, but the trajectories become more similar with age and the variance of the trait decreases with increasing similarity to final average adult body size (ATCHLEY 1984). The phenomenon has been described as compensatory growth (MONTEIRO and FALCONER 1966). Peaks in genetic variance of growth-related traits early in postnatal development have been shown to correspond the inflection point of maximal growth (ATCHLEY 1984).

Most studies of *ApoE* have focused on its functions in human pathophysiology of mostly middle-aged or older individuals. However, *ApoE* may also have a role in the physiological health and growth of younger individuals. *ApoE* is not only involved in normal lipid metabolism, but also in cell growth and differentiation (MAHLEY 1988). Moreover, cholesterol is essential in the synthesis of steroids by ovarian tissue (GRUMMER and CARROLL

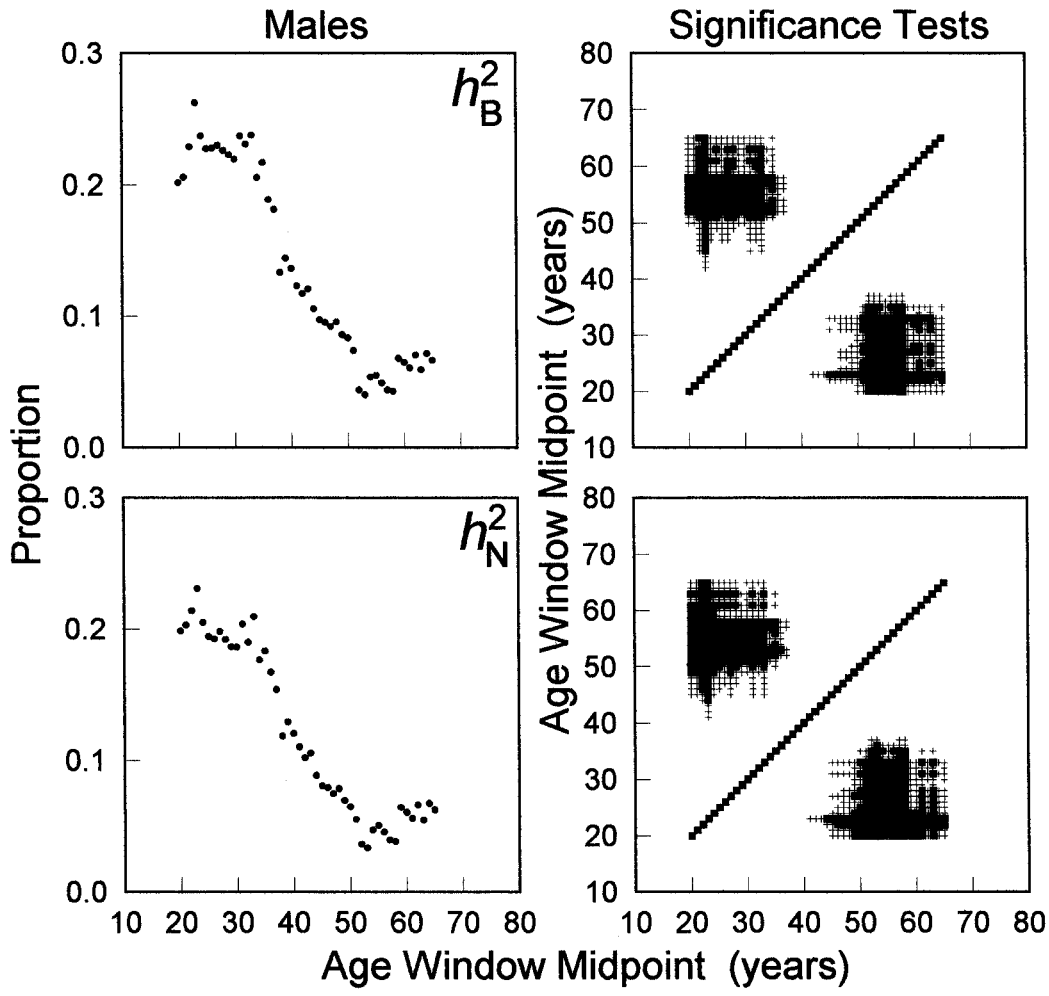

FIGURE 7.—The relationships between broad and narrow sense heritability estimates and age window for males (left). Presented at right are the results of the hypothesis tests about parameter estimates as described for Figure 2.

1988). The role of *ApoE* in the distribution of lipoprotein cholesterol to ovarian tissues is also probably central, but to our knowledge unexplored. The role of the *ApoE* polymorphism in interindividual differences in reproductive fitness associated with its potential function in ovarian steroidogenesis is likely, but unknown. Its role in testicular steroidogenesis is also likely, but unknown. Our results with lipid phenotypes are consistent with *ApoE* being involved in, or responding to, patterns of growth or associated life history changes like puberty, or menopause. In addition, postmenopausal hormone replacement therapy and oral contraceptive use can influence the effects of variation among *ApoE* alleles on LDL cholesterol metabolism in females selected for health (XHIGNESSE *et al.* 1991; but see EICHNER *et al.* 1990). These possibilities need to be explored in greater depth to establish whether the effects of allelic variation in the gene coding for *ApoE* are antagonistically pleiotropic over the life history of an individual. The age-specific relationship between plasma ApoE levels and fitness also needs to be verified.

Our findings have direct implication for the interpretation of measured genotype-phenotype association studies in humans that focus on one highly selected cross-sectional sample of usually middle-aged individuals or a group of individuals who have been adjusted by linear regression to an average age. They are also relevant to current efforts to use gene therapy to manipulate phenotypic expression or the course of human health. Our results suggest the impact of interindividual variation in the *ApoE* gene on plasma ApoE levels is significant throughout the life history of those individuals sampled from the Rochester, MN, population. The greatest role of this gene, however, is in determining average marginal differences among younger and middle-aged individuals. Increased attention to the role of the *ApoE* polymorphism in interindividual differences associated with lipid metabolism, growth, sexual maturity and brain function earlier in human life history could be rewarded in revealing and increased understanding of the underlying complexity in the dynamics of action of this important gene in human disease, health and physiological fitness.

The traditional approach to characterizing mea-

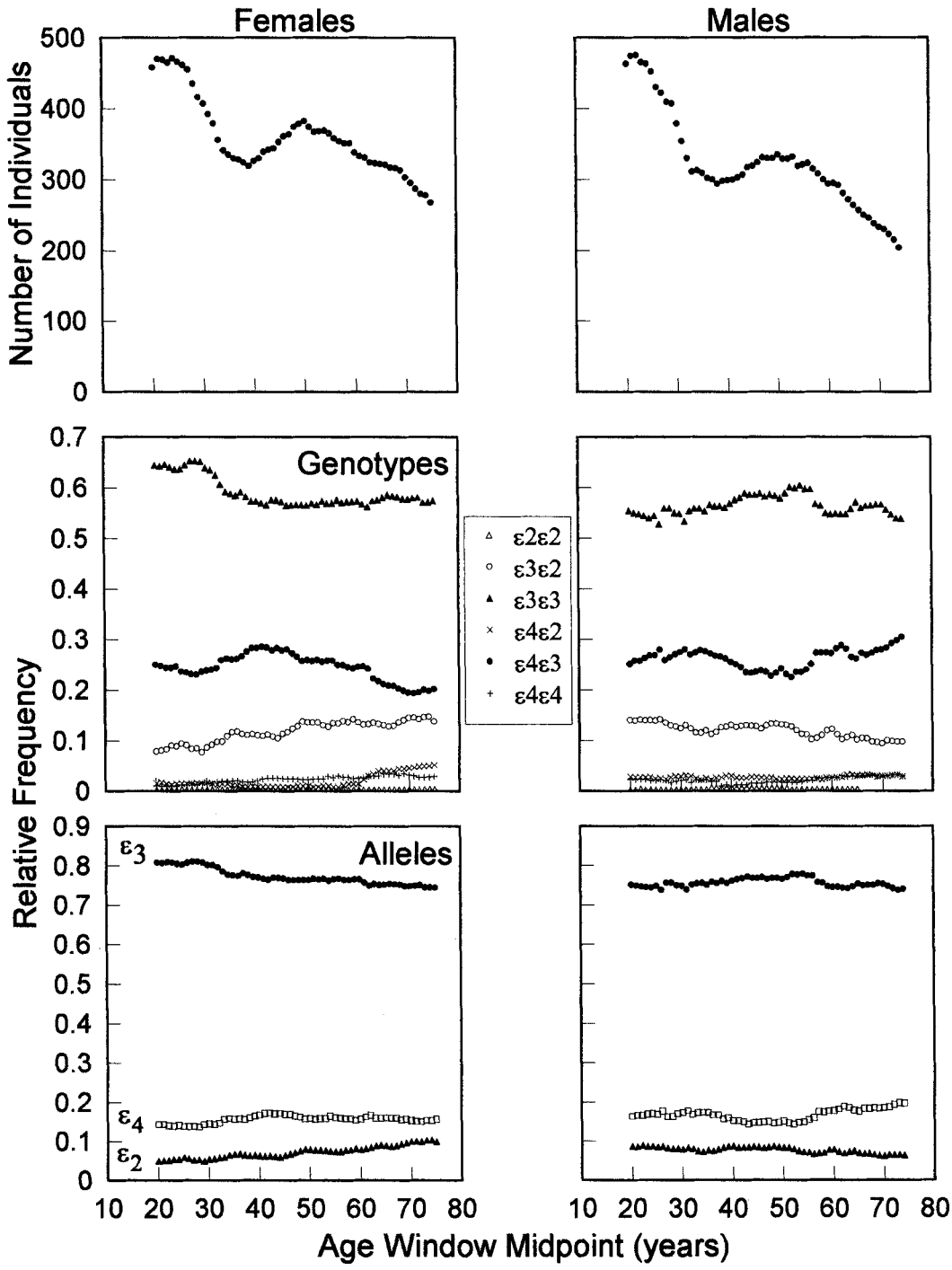

FIGURE 8.—The relationships between number of individuals, *ApoE* genotype and allele relative frequency and age window midpoint. Females: left; Males: right.

sured genotype effects in natural interbreeding populations has focused on marginal effects, ignoring the potential for heterogeneity in genotype effects among important subdivisions within the population. The large body of literature reporting population estimates for the variance in age-adjusted plasma cholesterol levels attributable to variation among *ApoE* genotypes is but one small subset of the evidence of the widespread acceptance of the paradigm. Our results show that adjusting for age variation before estimating variances in such studies is inappropriate and only serves to distort the realities of the dynamics of gene action during ontogeny.

The paradox is striking between the apparent consistency of marginal *ApoE* allele effects among populations and the absurdity of the using such marginal effects to characterize a measured genotype effect within subdivisions of the population at large. The relationship be-

TABLE 3

Associations of genetic architecture parameters with genotype frequency across age windows

Gender	Parameter	Regression	Genotype relative frequency[a] ($f_{\epsilon_i\epsilon_j}$)					
			$\epsilon_2\epsilon_2$	$\epsilon_3\epsilon_2$	$\epsilon_3\epsilon_3$	$\epsilon_4\epsilon_2$	$\epsilon_4\epsilon_3$	$\epsilon_4\epsilon_4$
Females		$\hat{\beta}_0$	2.17E−2	3.26E−2	−5.59E−2	2.96E−2	2.22E−2	2.82E−2
	σ^2_P	$\hat{\beta}_1$	−1.60	−1.76E−1	1.16E−1	−7.37E−1	−3.83E−2	−6.27E−1
		$R^2 \times 100$	84.28***	84.77***	82.93***	89.94***	1.14	20.14**
		$\hat{\beta}_0$	3.30E−3	4.59E−3	−2.76E−3	3.84E−3	−4.74E−3	9.84E−4
	σ^2_G	$\hat{\beta}_1$	−1.98E−1	−2.13E−2	8.33E−3	−7.22E−2	2.90E−2	5.05E−2
		$R^2 \times 100$	57.18***	54.71***	18.97***	37.90***	28.58***	5.74
		$\hat{\beta}_0$	1.85E−2	2.80E−2	−5.31E−2	2.58E−2	2.69E−2	2.72E−2
	σ^2_R	$\hat{\beta}_1$	−1.40	−1.55E−1	1.07E−1	−6.65E−1	−6.73E−2	−6.77E−1
		$R^2 \times 100$	79.80***	80.83***	88.14***	90.35***	4.33	29.03***
		$\hat{\beta}_0$	3.15E−3	4.80E−3	−5.79E−3	3.99E−3	−4.24E−3	1.27E−3
	σ^2_A	$\hat{\beta}_1$	−2.51E−1	−2.73E−2	1.27E−2	−9.81E−2	2.50E−2	2.05E−2
		$R^2 \times 100$	76.56***	74.64***	36.54***	58.37***	17.76*	7.92
		$\hat{\beta}_0$	1.53E−4	−2.19E−4	3.03E−3	−1.42E−4	−5.04E−4	−2.82E−4
	σ^2_D	$\hat{\beta}_1$	5.30E−2	5.96E−3	−4.33E−3	2.59E−2	3.96E−3	2.99E−2
		$R^2 \times 100$	48.26***	50.40***	60.58***	57.76***	6.29	23.83***
		$\hat{\beta}_0$	1.50E−1	1.16E−1	6.02E−1	9.94E−2	−3.70E−1	−6.88E−2
	h^2_B	$\hat{\beta}_1$	4.62	5.35−1	−7.15E−1	3.35	2.27	10.15
		$R^2 \times 100$	10.29*	11.41*	46.37***	27.05***	58.41***	75.44***
		$\hat{\beta}_0$	1.67E−1	2.04E−1	1.41E−1	1.63E−1	−4.36E−1	−4.36E−2
	h^2_N	$\hat{\beta}_1$	−6.11	−6.29E−1	−1.24E−2	−1.31	2.37	7.30
		$R^2 \times 100$	18.27**	15.95**	<0.01	4.19	64.51***	40.40***
Males		$\hat{\beta}_0$	8.10E−3	9.79E−3	1.39E−2	5.69E−3	2.27E−3	4.91E−3
	σ^2_P	$\hat{\beta}_1$	−2.08E−1	−2.50E−2	−1.25E−2	4.14E−2	1.82E−2	6.94E−2
		$R^2 \times 100$	24.62***	34.31***	28.87***	4.81	53.65***	51.24***
		$\hat{\beta}_0$	−4.11E−4	−9.23E−4	1.15E−2	−3.41E−3	−1.01E−3	−2.66E−5
	σ^2_G	$\hat{\beta}_1$	2.10E−1	1.54E−2	−1.67E−2	1.68E−1	7.84E−3	3.58E−2
		$R^2 \times 100$	14.81**	7.62	30.27***	46.46***	5.86	8.03
		$\hat{\beta}_0$	8.51E−3	1.07E−2	3.45E−3	9.11E−3	3.28E−3	4.94E−3
	σ^2_R	$\hat{\beta}_1$	−4.18E−1	−4.04E−2	4.16E−3	−1.26E−1	1.04E−2	3.36E−2
		$R^2 \times 100$	50.25***	45.14***	1.62	22.55***	8.80*	6.08
		$\hat{\beta}_0$	−3.45E−4	−7.45E−4	9.49E−3	−3.06E−3	−1.03E−3	−8.82E−5
	σ^2_A	$\hat{\beta}_1$	1.81E−1	1.29E−2	−1.51E−2	1.50E−1	7.46E−3	3.36E−2
		$R^2 \times 100$	14.64**	7.16	33.15***	49.23***	7.06	9.46*
		$\hat{\beta}_0$	−6.67E−5	−1.78E−4	1.01E−3	−3.49E−4	2.42E−5	6.16E−5
	σ^2_D	$\hat{\beta}_1$	2.91E−2	2.46E−3	−1.55E−3	1.81E−2	3.83E−4	2.14E−3
		$R^2 \times 100$	10.84*	7.47	10.03*	20.66**	0.53	1.10
		$\hat{\beta}_0$	−8.64E−2	−1.91E−1	1.31	−4.91E−1	−7.46E−2	2.58E−2
	h^2_B	$\hat{\beta}_1$	3.49E+1	2.72	−2.07	2.42E+1	8.56E−1	4.14
		$R^2 \times 100$	20.33**	11.86*	30.34***	48.18***	3.47	5.36
		$\hat{\beta}_0$	−7.40E−2	−1.59E−1	5.69E−3	−4.43E−1	−8.80E−2	1.27E−2
	h^2_N	$\hat{\beta}_1$	3.03E+1	2.31	4.14E−2	2.17E+1	8.40E−1	3.99
		$R^2 \times 100$	19.95**	11.17*	4.81	50.54***	4.36	6.47

Simple, unconditional linear regressions of each genetic parameter estimate on genotype relative frequency across age windows for each genotype separately. Degrees of freedom for F tests of statistical significance of each regression were 1,54 for females and 1,44 for males.

[a] Estimated under Hardy-Weinberg equilibrium expectations.

* $0.05 > P \geq 0.01$.

** $0.01 > P \geq 0.001$.

*** $P < 0.001$.

tween allelic variation in *ApoE* and measures of lipid metabolism appears to be a very good example of a complex adaptive system (*sensu* GELL-MANN 1994; SING *et al.* 1996). There is a mix of invariant, marginal allelic effect properties that are combined with context-dependent properties that appear as chaos to those who do not know the contexts. The invariant order properties are the consistently observed *ApoE* allelic effects on measures of lipid metabolism across populations (DAVIGNON *et al.* 1988). In contrast to this apparent among population consistency in average marginal allelic effects is our observed heterogeneity of plasma ApoE variances with age. Gender differences in patterns of such variance heterogeneity represent population subdivi-

TABLE 4

Associations of genetic architecture parameters with genotype scale across age windows

Gender	Parameter	Regression	Genotype mean deviation squared[a] $\epsilon_2\epsilon_2$	$\epsilon_3\epsilon_2$	$\epsilon_3\epsilon_3$	$\epsilon_4\epsilon_2$	$\epsilon_4\epsilon_3$	$\epsilon_4\epsilon_4$
Females		$\hat{\beta}_0$	1.66E−2	8.50E−3	1.97E−2	1.25E−2	3.54E−3	7.80E−3
	σ^2_P	$\hat{\beta}_1$	−8.99E−2	2.10	−1.49E+1	5.10E−1	3.99	1.21E−1
		$R^2 \times 100$	30.80***	83.57***	28.86***	2.56	37.41***	66.80**
		$\hat{\beta}_0$	3.01E−3	1.79E−3	2.22E−3	1.91E−3	1.12E−3	1.32E−3
	σ^2_G	$\hat{\beta}_1$	−2.00E−2	1.95E−1	−1.57E−2	2.90E−1	4.61E−1	2.06E−2
		$R^2 \times 100$	67.35***	31.77***	<0.01	36.25***	21.92***	85.38***
		$\hat{\beta}_0$	1.36E−2	6.70E−3	1.74E−2	1.06E−2	2.42E−3	6.48E−3
	σ^2_R	$\hat{\beta}_1$	−6.98E−2	1.90	−1.49E+1	2.21E−1	3.53	1.00E−1
		$R^2 \times 100$	22.96***	84.87***	35.55***	0.59	36.13***	56.74***
		$\hat{\beta}_0$	2.63E−3	1.20E−3	1.88E−3	1.50E−3	1.36E−4	8.16E−4
	σ^2_A	$\hat{\beta}_1$	−2.18E−2	2.64E−1	−2.55E−1	2.52E−1	6.88E−1	2.21E−2
		$R^2 \times 100$	66.44***	48.37***	0.3	22.95***	40.65***	81.65***
		$\hat{\beta}_0$	3.75E−4	5.92E−4	3.38E−4	4.06E−4	9.81E−4	5.08E−4
	σ^2_D	$\hat{\beta}_1$	1.76E−3	−6.86E−2	2.40E−1	3.72E−2	−2.27E−1	−1.47E−3
		$R^2 \times 100$	6.14	46.34***	3.85	7.07*	62.52***	5.10
		$\hat{\beta}_0$	1.88E−1	1.97E−1	1.03E−1	1.62E−1	2.05E−1	1.75E−1
	h^2_B	$\hat{\beta}_1$	−3.31E−1	−1.01E+1	1.62E+2	1.30E+1	−1.27E+1	1.61E−2
		$R^2 \times 100$	6.09	28.05***	49.42***	24.23***	5.51	<0.01
		$\hat{\beta}_0$	1.72E−1	1.30E−1	7.84E−2	1.18E−1	8.46E−2	1.05E−1
	h^2_N	$\hat{\beta}_1$	−9.57E−1	1.97	1.24E+2	1.48E+1	2.08E+1	6.79E−1
		$R^2 \times 100$	51.60***	1.09	29.53***	31.67***	14.93**	31.10***
Males		$\hat{\beta}_0$	6.60E−3	6.63E−3	6.34E−3	6.81E−3	6.91E−3	6.51E−3
	σ^2_P	$\hat{\beta}_1$	4.22E−3	9.13E−2	5.47	−1.08E−1	−1.36E−1	2.93E−2
		$R^2 \times 100$	7.41	2.85	30.12***	1.53	2.33	22.80***
		$\hat{\beta}_0$	1.88E−4	−4.51E−5	3.52E−4	1.25E−3	−1.18E−4	3.38E−4
	σ^2_G	$\hat{\beta}_1$	1.83E−2	6.5E−1	7.36	−7.63E−1	9.76E−1	6.64E−2
		$R^2 \times 100$	82.58***	85.66***	32.14***	45.46***	70.75***	68.85***
		$\hat{\beta}_0$	6.41E−3	6.68E−3	5.99E−3	5.57E−3	7.03E−3	6.17E−3
	σ^2_R	$\hat{\beta}_1$	−1.41E−2	−5.60E−1	−1.88	6.56E−1	−1.11	−3.70E−2
		$R^2 \times 100$	41.93***	54.23***	1.81	28.72***	78.65***	18.35**
		$\hat{\beta}_0$	1.96E−4	−4.80E−5	2.84E−4	1.10E−3	−1.22E−4	3.01E−4
	σ^2_A	$\hat{\beta}_1$	1.52E−2	5.74E−1	6.70	−6.90E−1	8.69E−1	5.73E−2
		$R^2 \times 100$	75.53***	88.53***	35.53***	49.42***	74.67***	68.21***
		$\hat{\beta}_0$	−8.15E−6	2.85E−6	6.75E−5	1.49E−4	3.92E−6	3.75E−5
	σ^2_D	$\hat{\beta}_1$	3.14E−3	7.75E−2	6.53E−1	−7.35E−2	1.07E−1	9.12E−3
		$R^2 \times 100$	92.61***	46.37***	9.70*	16.15***	32.52***	49.75***
		$\hat{\beta}_0$	3.36E−2	−3.48E−3	5.99E−2	1.83E−1	−1.99E−2	5.61E−2
	h^2_B	$\hat{\beta}_1$	2.55	9.36E+1	9.76E+2	−1.11E+2	1.46E+2	9.03
		$R^2 \times 100$	79.06***	87.84***	28.13***	47.77***	78.35***	63.27***
		$\hat{\beta}_0$	3.41E−2	−4.72E−3	4.87E−2	1.61E−1	−2.08E−2	4.97E−2
	h^2_N	$\hat{\beta}_1$	2.11	8.28E+1	8.98E+2	−1.01E+2	1.30E+2	7.81
		$R^2 \times 100$	70.64***	89.86***	31.11***	51.43***	81.92***	61.76***

Simple, unconditional linear regressions of each genetic parameter estimate on the genotype mean deviation squared. Age window sample mean was estimated assuming Hardy-Weinberg equilibrium. Degrees of freedom for F tests of statistical significance of each regression were 1,54 for females and 1,44 for males.

* $0.05 > P \geq 0.01$.

** $0.01 > P \geq 0.001$.

*** $P < 0.001$.

[a] $(\bar{Y}_{\epsilon_i\epsilon_j} - \bar{Y}_{HWE})^2$

sion, context-dependent, effects of genome type-phenotype relationships.

Our study is one example of a vast potential for such context-dependent genome type-phenotype relationships. Another recent example is the observed dependence of an *ApoE* genotype association with variation in plasma levels of high density lipoprotein cholesterol on waist-to-hip ratio in females but not males (REILLY *et al.* 1992). Other examples of important population subdivision contexts on which *ApoE* effects on lipid levels depend include gender (KAPRIO *et al.* 1991), hormone use (XHIGNESSE *et al.* 1991) and lipid lowering drugs (NESTRUCK *et al.* 1987; CARMENA *et al.* 1993). Much complexity in genome type-phenotype relation-

TABLE 5

Correlations among genotype frequencies and scale across age windows

Variable	Genotype	Genotype frequency ($f_{\epsilon_i\epsilon_j}$)						Genotype mean deviation ($\bar{Y}_{\epsilon_i\epsilon_j} - \bar{Y}_{HWE}$)					
		$\epsilon_2\epsilon_2$	$\epsilon_3\epsilon_2$	$\epsilon_3\epsilon_3$	$\epsilon_4\epsilon_2$	$\epsilon_4\epsilon_3$	$\epsilon_4\epsilon_4$	$\epsilon_2\epsilon_2$	$\epsilon_3\epsilon_2$	$\epsilon_3\epsilon_3$	$\epsilon_4\epsilon_2$	$\epsilon_4\epsilon_3$	$\epsilon_4\epsilon_4$
Genotype frequency ($f_{\epsilon_i\epsilon_j}$)	$\epsilon_2\epsilon_2$		0.996***	−0.863***	0.959***	−0.137	0.234	0.457***	−0.921***	0.322*	−0.138	−0.729***	−0.849***
	$\epsilon_3\epsilon_2$	9.80***		−0.879***	0.969***	−0.109	0.261	0.424**	−0.934***	0.359**	−0.125	−0.733***	−0.823***
	$\epsilon_3\epsilon_3$	−0.038	0.153		−0.969***	−0.378**	−0.689***	−0.192	0.904***	−0.633***	−0.194	0.655***	0.623***
	$\epsilon_4\epsilon_2$	0.529***	0.358*	−0.865***		0.138	0.490***	0.323*	−0.948***	0.511***	0.019	−0.708***	−0.754***
	$\epsilon_4\epsilon_3$	−0.542***	−0.692***	−0.818***	0.423**		0.930***	−0.431***	−0.072	0.637***	0.656***	0.052	0.309*
	$\epsilon_4\epsilon_4$	−0.455**	−0.617***	−0.871***	0.509***	0.994***		−0.256	−0.410**	0.753***	0.579***	−0.201	−0.016
Genotype mean deviation ($\bar{Y}_{\epsilon_i\epsilon_j} - \bar{Y}_{HWE}$)	$\epsilon_2\epsilon_2$	0.462**	0.398**	−0.328*	0.527***	0.009	0.050		−0.393**	−0.081	−0.329*	−0.258	−0.754***
	$\epsilon_3\epsilon_2$	0.429**	0.320*	−0.506***	0.666***	0.184	0.224	0.807***		−0.574***	0.035	0.668***	0.783***
	$\epsilon_3\epsilon_3$	−0.265	−0.397**	−0.730***	0.494***	0.764***	0.769***	0.277	0.420**		0.207	0.049	−0.221
	$\epsilon_4\epsilon_2$	−0.344*	−0.239	0.535***	−0.627***	−0.255	−0.294*	−0.526***	−0.584***	−0.643***		−0.151	0.359**
	$\epsilon_4\epsilon_3$	0.665***	0.593***	−0.309*	0.606***	−0.118	−0.064	0.762***	0.853***	0.355*	−0.717***		4.77***
	$\epsilon_4\epsilon_4$	0.038	−0.067	−0.586***	0.535***	0.469**	0.482***	0.657***	0.707***	0.790***	−0.753***	0.586***	

Estimated under Hardy-Weinberg equilibrium expectations. Females above diagonal ($N = 56$), males below diagonal ($N = 46$).
* $0.05 > P \geq 0.01$.
** $0.01 > P \geq 0.001$.
*** $P < 0.001$.

ships in subdivisions of the population at large remains to be explored.

In conclusion, it is significant to note that our study of the *ApoE* polymorphism adds to a growing body of literature that suggests that, even though gene effects are nonlinear at the molecular level and heterogeneous among subdivisions of the population, allele effects emerge at the population level as average constructs that may be consistent among populations. A general theory for how genetic information is organized and translated across the levels of biological hierarchy from genome type to quantitative phenotype that takes into account these observations is needed.

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