Am. J. Hum. Genet. 47:414–428, 1990

Genetic Analysis of Apolipoprotein A-I in Two Dietary Environments

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Summary

Although of great clinical and biological importance, the role of genotype-diet interaction in lipoprotein metabolism and atherosclerosis is still poorly understood. We analyzed serum apolipoprotein A-I (apo A-I) concentrations of approximately 600 pedigreed baboons that were fed two dietary regimens: (1) a basal diet and (2) an atherogenic (high-cholesterol, saturated-fat) diet. Complex segregation analysis was performed separately for apo A-I concentrations in each dietary environment. A major locus model with a recessive allele for high levels of apo A-I and a polygenic component best fit the family data for both diets. Using bivariate segregation analysis, we showed that the major genes detected in the univariate analyses represent two distinct loci that act additively to determine apo A-I concentrations. These two loci accounted for approximately 40% of the total phenotypic variance in apo A-I levels in each dietary environment and were also responsible for 33% of the variation in apo A-I response to the atherogenic diet. Both major loci were influenced by genotype-diet interaction in which the two-locus genotypes exhibited heterogeneous responses to the atherogenic diet. Most genotypes responded to the atherogenic diet with an increase in apo A-I, but two genotypes showed a decrease that can be traced to the effect of one of the major loci. The presence of two major loci and genotype-diet interaction may be responsible for the equivocal results obtained in human pedigree studies of apo A-I.

Introduction

Apolipoprotein A-I (apo A-I) is the primary protein component of high-density lipoprotein (HDL). Like HDL cholesterol (HDL-C) levels, serum concentration of apo A-I is inversely related to atherosclerosis in man (Maciejko et al. 1983; Kottke et al. 1986). Several studies have suggested that apo A-I is a better predictor of coronary artery disease than is HDL-C (Avogaro et al. 1979; DeBacker et al. 1982), although this finding is not universally accepted (Schmidt et al. 1985). However, the genetic mediation of apo A-I levels is less well characterized than is that of the major lipoproteins.

Several investigators, using quantitative genetic methods, have attempted to detect genetic effects on serum levels of apo A-I in humans. Sistonen and Ehnholm (1980) found no evidence for a genetic basis of apo A-I in a sample of twins. However, several subsequent studies have obtained results consistent with a moderate heritability ($h^2 \sim .4–.6$) for apo A-I serum levels (Berg 1984; Hamsten et al. 1986; Kuusi et al. 1987).

Evidence for a major locus affecting apo A-I in humans is equivocal. Two studies, using large pedigrees selected because of histories of myocardial infarctions, found no evidence for a major gene influencing apo A-I levels (Hasstedt et al. 1984; Amos et al. 1987). However, Moll et al. (1986) detected a major locus with two codominant alleles and a polygenic component in 23 small pedigrees ascertained through cases of hypertension or early coronary artery disease. More recently, Borecki et al. (1988) presented evidence for a major locus for apo A-I in a single 51-member pedigree ascertained on the basis of a proband with low HDL-C and early myocardial infarction.

Received April 21, 1989; revision received March 15, 1990.

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0002-9297/90/4703-0006$02.00

In a comprehensive study, Moll et al. (1989) found evidence of heterogeneity in the determination of apo A-I levels in 283 randomly selected pedigrees. They found a subset of 157 pedigrees that provided clear evidence for a single major locus accounting for 27% of the total variation in apo A-I levels. A second group of 126 pedigrees exhibited a significant nontransmitted environmental factor and a large polygenic component.

As evidenced by the conflicting results described above, genetic analyses of apo A-I concentration in human populations are difficult to interpret, for several reasons. Statistical characteristics of apo A-I genotypes may depend partially on the environment. For example, Kondo et al. (1989) showed that the effect of a cholesteryl ester transfer protein (CETP) *Taq*I RFLP on apo A-I serum levels was apparent only in nonsmokers. Such genotype-environment (G×E) interaction would obscure genetic effects on apo A-I phenotypes in populations that vary in the environmental risk factor. Although difficult to study in humans, interindividual dietary variability and genotype-specific responses to diet are likely to be important in determining apo A-I serum levels. Concomitant environmental risk factors, such as smoking, obesity, alcohol intake, and contraceptive use, may also interact with genotype. An animal model permits more rigorous experimental control over such confounding exogenous variables and allows unambiguous examination of the extent of G×E interaction.

The baboon is a well-established animal model for atherosclerosis in humans (McGill et al. 1960, 1981). Incipient atherogenesis has been documented in feral baboons without dietary manipulation (McGill et al. 1960). As in human populations, HDL-C concentration is negatively associated with arterial fatty streaks (McGill et al. 1981). Baboons also respond to an atherogenic diet in a way similar to humans (McGill et al. 1981).

A number of studies using polygenic models of baboon HDL-C levels have revealed significant genetic effects (Flow et al. 1981, 1982; Flow and Mott 1984; Kammerer et al. 1984). Preliminary quantitative genetic analysis also revealed a high positive genetic correlation of apo A-I with HDL-C ($\hat{\rho}_G$ = .68) and strong negative genetic correlations of apo A-I and HDL-C with the rate of cholesterol turnover ($\hat{\rho}_G$ = −.95 and −.68, respectively) (Flow and Mott 1984). Similar strong genetic relationships of HDL-C with other parameters of cholesterol metabolism are indicated by significant rank correlations among sire-progeny group means (Flow and Mott 1984). More recently, complex segregation analyses of baboon HDL-C indicate that major genes play a role in the expression of baboon HDL-C phenotypes (MacCluer et al. 1988). In this report, we present the results of genetic analyses of apo A-I concentrations in pedigreed baboons assayed in each of two dietary environments to identify underlying major loci and genotype-diet interaction.

Material and Methods

Description of the Baboon Population

The study population consists of 711 pedigreed baboons resident at the Southwest Foundation for Biomedical Research. The founders of this colony are of East African origin and include a mixture of two subspecies (*Papio hamadryas anubis* and *P. h. cynocephalus*). These subspecies are closely related to each other and interbreed in the wild and in captivity.

Paternal half-sibships constitute the predominant pedigree structure. The pedigrees used in this study include 23 sires, 225 dams, and nearly 500 offspring. Individual sires have 3–31 mates. Full-sibship size range is 1–7, and that of half-sibships is 4–62. Some selective breeding on a subset of the population was arranged on the basis of total cholesterol level in earlier years (Kammerer et al. 1984); however, subsequent analysis of HDL-C and apo A-I concentrations has revealed that the mating structure for these traits is effectively random in our population (MacCluer et al. 1988).

Most baboon offspring in this study have undergone a common life cycle. Following weaning at approximately 16 wk, infants were separated from their mothers and placed in a common cage. However, because of maternal rejection or inability to nurse, 64 offspring spent most (at least 12 wk) of the preweaning period in the nursery. The primary difference between nursery-reared offspring and dam-reared offspring lies in their diet (formula feeding versus breast-feeding). Baboon breast milk contains approximately 30 mg cholesterol/dl and has a polyunsaturated/saturated (P/S) fat ratio of 0.46 (Mott et al. 1978), while the infant formula (Similac; Ross Laboratories, Columbus, OH) ingested by nursery-reared infants contains approximately 1 mg cholesterol/dl and has a P/S ratio of 1.6. Because there is strong evidence that infant diet (breast vs. formula feeding) affects subsequent lipoprotein and apolipoprotein levels (Mott et al. 1982, 1990; Lewis et al. 1988), we simultaneously controlled for the possible effect of this preweaning dietary variability in our segregation analyses.

Apolipoprotein and lipoprotein screenings were performed on most offspring at 27 mo of age. However, sires, dams, and some of the offspring were evaluated at various ages. Therefore, we also considered sex-specific age effects on apo A-I levels in our analyses.

Measurement of apo A-I Concentration in Two Dietary Environments

Apo A-I concentrations (mg/dl) were measured in whole serum by the electroimmunoassay method of Laurell (1966) by using polyclonal antisera to purified baboon apo A-I (Mott et al. 1982). Apo A-I serum levels were obtained for approximately 600 animals under two different dietary regimens (Williams et al. 1987): first after being fed a basal diet of commercial monkey chow (0.03 mg cholesterol/kcal and 10% of calories as fat) for an extended period and again after feeding for 7 wk on a high-cholesterol, saturated-fat (HCSF) diet providing 40% of calories as fat, mostly saturated, and with 1.7 mg cholesterol/kcal.

Univariate Complex Segregation Analysis

Univariate complex segregation analysis (Elston and Stewart 1971) was performed using the computer program PAP (Hasstedt and Cartwright 1981). Two traits were analyzed: (1) apo A-I serum concentrations on the basal diet and (2) apo A-I levels on the HCSF diet.

For each trait, we compared a set of restricted models representing various transmission hypotheses with an unrestricted general model allowing a mixture of as many as three normal phenotypic distributions. These three distributions can be related to unobservable genotypes or, more generally, ousiotypes (Cannings et al. 1978) with or without genetic inheritance. Ousiotypes are considered to be the product of two discrete factors, A or a. We use uppercase letters (e.g., A) to denote factors associated with lower levels of apo A-I and lowercase letters to represent higher levels. The three ousiotypes can be denoted as AA, Aa, and aa. To simplify computations, the frequencies of the ousiotypes are assumed to follow Hardy-Weinberg proportions—(p^2:$2p(1-p)$:$(1-p)^2$)—thus requiring only one admixture parameter (p_A) or allele frequency.

Three means (μ_{AA}, μ_{Aa}, and μ_{aa}) were estimated, corresponding to each ousiotype, but we assume a common SD (σ) for the phenotypic distributions. Residual nonindependence among relatives that is due to biological kinship was allowed for by including a polygenic heritability parameter (h^2) which refers to the proportion of phenotypic variance due to additive genetic variance within each ousiotype's phenotypic distribution. The proportion of phenotypic variance attributable to random environmental variation within a phenotypic distribution is given by $1 - h^2$. Three arbitrary transmission parameters (τ_{AA}, τ_{Aa}, and τ_{aa}) denoting the probability that an individual of a given ousiotype transmits factor A to an offspring were also estimated in the most general model. In addition, we simultaneously estimated the effects of covariates including sex, age, age^2, and nursery rearing versus dam rearing on apo AI levels. Sex-specific regressions on age (age^2) were allowed. All regression coefficients (denoted as $\beta(x)$, where x is the covariate of interest) were assumed to be homogeneous across ousiotypes. Covariates were scaled so that observed baseline means (μ_{AA}, μ_{Aa}, and μ_{aa}) refer to dam-reared males at 6.5 years of age (the observed mean age of pedigreed animals).

Several classes of restricted models were tested against the most general model by using the unified approach of Lalouel et al. (1983). The simplest models considered were sporadic models which allow only random environmental effects. In this type of model, all individual trait values are independent of one another. When multiple distributions are considered, the sporadic model is a finite mixture (or commingling) model (Everitt and Hand 1981) and is obtained by forcing equality of the admixture parameter (p_A) and the transmission parameters ($p_A = \tau_{AA} = \tau_{Aa} = \tau_{aa}$). By allowing p_A to vary, this model permits heterogeneity of mixture proportions between generations. A closely related class of model, the environmental transmission model, assumes random environmental effects for major factors but also permits residual polygenic inheritance (i.e., it is a finite mixture model extended to allow nonindependence due to genetic kinship among individuals). A restricted environmental transmission model with only one underlying phenotypic distribution and no between-generation heterogeneity reduces to the classical additive polygenic model of quantitative genetics. The Mendelian models considered incorporate transmission probabilities fixed at their Mendelian expectations ($\tau_{AA} = 1$, $\tau_{Aa} = 1/2$, $\tau_{aa} = 0$). In addition, mixed Mendelian models allow for a residual polygenic background. All parameters were estimated by numerical maximization of the likelihood of the data, given the assumed transmission model, by using a quasi-Newton method implemented in the subroutine GEMINI (Lalouel 1979).

Each restricted model was compared with the unrestricted general model by using likelihood ratio statis-

tics (Λ_i, where i denotes df obtained as twice the difference between the $\log_e$ likelihoods of the unrestricted and restricted models. These test statistics are asymptotically distributed as χ^2 variates with df equal to the difference in the number of parameters between the two competing models. The best model (of those considered) is the one requiring the fewest estimated parameters and exhibiting a likelihood that is not significantly smaller than that for the most general model.

Significant regression parameters for covariates were determined by removing individual regression terms from the most general model. If the likelihood ratio test was significant at the .10 α level, the effect of the covariate was estimated in all subsequent restricted models.

Bivariate Segregation Analysis

Following the univariate segregation analyses, we analyzed the apo A-I serum levels on the two diets simultaneously by using bivariate segregation analysis. Bivariate segregation analysis permits the testing of hypotheses regarding pleiotropic effects of major genes on two quantitative traits. Assessment of the residual additive genetic components is also possible. Our mixed-model bivariate segregation analysis uses a method developed by J. Blangero (unpublished data) that is based on a simplification of the multivariate likelihood via a transformation that simultaneously orthogonalizes the residual additive genetic and environmental covariance matrices. Calculations of the multivariate likelihood are subsequently factored into products of univariate likelihoods. We have implemented this method as a penetrance subroutine in PAP. Because of the size and complexity of our pedigrees, we use a multivariate generalization of the approximate mixed model of Hasstedt (1982) to make likelihood computations feasible. Similar to the univariate approximate mixed model, the multivariate-likelihood calculation is exact when there are no major-gene effects and in the absence of residual additive genetic effects.

Because we found evidence for major genes influencing apo A-I levels on both diets, we used a two-locus version of bivariate segregation analysis. In subsequent description, we will subscript parameters with 1 and 2 to refer to apo A-I on the basal diet and HCSF diet, respectively. Vectors will be denoted by under tildes ($\sim$), and matrices will be represented by boldface capital letters. Parameters for the most general two-locus model included a vector of nine genotypic means ($\underset{\sim}{\mu}_1$ and $\underset{\sim}{\mu}_2$) for each of the two traits, regression coefficients for covariate effects for each trait, frequencies (p_A and p_B) for the alleles associated with low apo A-I serum levels at the two major loci (noted as A and B), within-genotype phenotypic SDs (σ_1 and σ_2) for the two traits, polygenic heritabilities (h_1^2 *and* h_2^2) for each trait, the residual genetic correlation (ρ_G) and environmental correlation (ρ_E) between traits, the gametic disequilibrium between loci, and the recombination rate between loci (θ). Following Lewontin (1964), we parameterized gametic disequilibrium (D') as the proportion of its maximum attainable value. To limit the number of parameters to be estimated, we fixed the regression coefficients of covariates at their maximum-likelihood estimates obtained from the univariate segregation analyses. Therefore, the total number of estimated parameters in the most general two-locus bivariate model was 28.

Hypotheses regarding the genetic mechanism underlying the traits were evaluated using this approach of bivariate segregation analysis. Since we assume that two (or fewer) alleles are present at each locus, our models can be categorized by number of loci (1 vs. 2) and structural relationship among the two loci (linked vs. independent). Using this framework, we tested a general hypothesis in which the two loci are allowed to be linked and in gametic disequilibrium against restricted hypotheses involving two independent loci or a single locus. For the single-locus hypothesis, we set $p_A = p_B$, $D' = 1$, and $\theta = 0$, a procedure which yields only three expected two-locus genotypes (AABB, AaBb, and aabb). The hypothesis of two independent loci assumes that $D' = 0$ and $\theta = 1/2$. These two restricted models were compared with the general two-locus model by using likelihood ratio tests. Rejection of the single-locus hypothesis can be interpreted to mean that (at least) two loci influence the bivariate distribution of apo A-I levels in the two dietary environments. Rejection of the two-independent-loci hypothesis would be evidence for (at least) two nonindependent (and possibly linked) loci.

The two-locus models also allow tests of epistatic effects on genotypic means. We can divide the models under consideration into two categories (epistatic vs. additive) determined by two-locus mean effects, which largely define how genotypes map to phenotypes. To simplify interpretation regarding gene action and interactions, we parameterized two-locus genotypic means in a manner similar to that of Basford and De Lacy (1979). In this schema, the vector of genotype-specific means for a trait is given by

$$\begin{bmatrix} \mu_{AABB} \\ \mu_{AABb} \\ \mu_{AAbb} \\ \mu_{AaBB} \\ \mu_{AaBb} \\ \mu_{Aabb} \\ \mu_{aaBB} \\ \mu_{aaBb} \\ \mu_{aabb} \end{bmatrix} = \begin{bmatrix} 1 & -1 & -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & -1 & 0 & 1 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & 0 & -1 & -1 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 1 & 0 & -1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & -1 & 0 & -1 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} m \\ a_A \\ a_B \\ d_A \\ d_B \\ aa_{AB} \\ ad_{AB} \\ ad_{BA} \\ dd_{AB} \end{bmatrix} , \quad (1)$$

or, in matrix notation, as

$$\underset{\sim}{\mu} = \mathbf{Z} \underset{\sim}{\omega} , \quad (2)$$

where $\underset{\sim}{\mu}$ is the vector of genotypic means, $\mathbf{Z}$ is a design matrix, and $\underset{\sim}{\omega}$ is the vector of two-locus effects. In the bivariate case, each trait will have its own set of means ($\underset{\sim}{\mu}_1$ and $\underset{\sim}{\mu}_2$) and two-locus effects ($\underset{\sim}{\omega}_1$ and $\underset{\sim}{\omega}_2$). In our two-locus bivariate segregation analyses, we estimated $\underset{\sim}{\omega}$ instead of $\underset{\sim}{\mu}$ directly. The components of $\underset{\sim}{\omega}$ have simple interpretations based on gene action. The parameter, m, represents the unweighted mean of the four double homozygotes and serves as a baseline component of all nine two-locus genotypes. Parameters a_A and a_B represent the additive genetic effects of the *A* and *B* loci, respectively. Similarly, d_A and d_B are defined as locus-specific dominance effects. The remaining parameters consist of different forms of two-locus epistatic effects. The aa_{AB} term parameterizes digenic additive × additive interaction, whereas the dd_{AB} represents dominance × dominance interaction. The terms ad_{AB} and ad_{BA} measure the two possible additive × dominance interactions.

This method of defining two-locus effects allows intuitive specification of a number of hypotheses that can be tested using Λ statistics. For example, we tested the null hypothesis of no epistasis (i.e., a model in which effects are additive among loci) by comparing a model in which the four digenic interaction terms were forced to be zero, with the more general model in which the interactions are estimated.

Analysis of G×E Interaction

The analysis of character expression in different environments provides information on G×E interaction. Our bivariate segregation methods constitute a useful framework for detecting the effects of genotype-diet interaction on apo A-I serum levels. In this study, G×E interaction is considered at two levels: (1) the major genes and (2) the polygenic background. We use the term "major genotype-environment interaction" (MG×E) to describe G×E interaction due to the major loci. Similarly, we use the term "polygenotype-environment interaction" (PG×E) to describe G×E interaction due to residual additive genetic effects.

MG×E Interaction

We examined the effects of MG×E interaction on apo A-I serum levels by evaluating how the genotypic means were influenced by diet. In the current context, MG×E interaction is defined as differential mean response to environmental change among genotypes. We modeled the genotypic means in the HCSF dietary environment to be a linear function of the means in the basal environment and of the response to the HCSF diet:

$$\underset{\sim}{\mu}_2 = \underset{\sim}{\mu}_1 + \underset{\sim}{\Delta} , \quad (3)$$

where $\underset{\sim}{\mu}_2$ represents the vector of nine genotypic means of apo A-I on the HCSF diet, $\underset{\sim}{\mu}_1$ is the vector of genotypic means on the basal diet, and $\underset{\sim}{\Delta}$ is the vector of responses to the HCSF diet.

In the absence of MG×E interaction, all nine elements of $\underset{\sim}{\Delta}$ will be equal (i.e., there is no genotype-specific response to diet). Using our reparameterization of the mean effects detailed above, we defined equivalent constraints by forcing all elements of $\underset{\sim}{\omega}_2$ to be equal to those of $\underset{\sim}{\omega}_1$, except for the first element, m. The adequacy of this constrained model was tested against the general MG×E interaction model by using a likelihood ratio test. Similarly, we tested for MG×E interaction at each major locus separately. For example, to test the hypothesis that the *A* locus is not influenced by MG×E interaction, we compared the model which allows MG×E interaction at both loci with one that allows MG×E interaction only at the *B* locus. In this case (and on the assumption that there is no epistasis), the elements of $\underset{\sim}{\omega}_2$ were constrained to be equal to those of $\underset{\sim}{\omega}_1$, except for the elements m, a_B, and d_B. Such constraints also lead to equal marginal genotypic means for dietary response at the *A* locus. A similar test was performed for assessing MG×E interaction at the *B* locus. For assessing MG×E interaction, we classified all considered models according to which loci (both, *A* only, *B* only, or none) were involved in genotype-diet interaction.

PG×E Interaction

We also tested for G×E interaction at the level of the residual polygenic determinants of apo A-I concentrations. In the absence of G×E interaction, the same

trait measured in two separate environments should exhibit an additive genetic correlation of 1 (Falconer 1981). Under this condition, all polygenotypes share the same response to the environment, and ranks among polygenotypes remain constant in all environments. The hypothesis of no PG×E interaction ($\rho_G = 1$) was compared against an unrestricted model, in which the ρ_G was estimated, by using a likelihood ratio test. However, because this alternative hypothesis assumes a ρ_G value that is located on the boundary of the parameter space and implies a degenerate multivariate normal distribution for the residual genetic effects, the associated test statistic is distributed not as a χ_1^2 but as a ½:½ mixture of a χ_1^2 distribution and a distribution with all its density at 0 (Hopper and Mathews 1982). Therefore, to maintain the appropriate level of significance, critical values of this test were taken to be those associated with 2α values of the χ_1^2 distribution (Boehnke et al. 1987).

To facilitate comparisons between all the bivariate segregation models (including nonnested ones), we used the Akaike (1974) information criterion, AIC $= -2L + 2k + C$, where L is the $\log_e$ likelihood of the model, k is the number of parameters estimated in the model, and C is a constant. By this criterion, the best-fitting, most parsimonious model is the one with the minimum AIC.

Decomposition of the Phenotypic Covariance Matrix

In the bivariate case, the total phenotypic covariance matrix ($\mathbf{P_T}$) can be decomposed into a number of constituent matrices. J. Blangero (unpublished data) has derived general matrix formulas for obtaining these various covariance matrices. For the present analysis, after selecting our overall best-fitting, most parsimonious bivariate segregation model, we partitioned $\mathbf{P_T}$ into three major components:

$$\mathbf{P_T} = \mathbf{M} + \mathbf{G} + \mathbf{E} , \quad (4)$$

where **M** is the genetic covariance matrix due to major loci, **G** is the residual additive genetic covariance matrix, and **E** is the random environmental covariance matrix. **M** due to major loci was further decomposed as:

$$\mathbf{M} = \mathbf{A_A} + \mathbf{D_A} + \mathbf{A_B} + \mathbf{D_B} + \mathbf{M_E} , \quad (5)$$

where $\mathbf{A_A}$ and $\mathbf{A_B}$ represent the covariance matrices due to additive genetic effects at the *A* and *B* loci, $\mathbf{D_A}$ and $\mathbf{D_B}$ are the analogous matrices due to dominance effects, and $\mathbf{M_E}$ is the covariance matrix due to epistatic effects. After obtaining these covariance matrices, we defined the relative contribution of each component by dividing each matrix element by the analogous element of $\mathbf{P_T}$.

Results

Distribution of apo A-I Levels in Baboons

Table 1 presents summary statistics and sample sizes for subgroups of the pedigreed baboons. Since the sample consists of many related individuals, we include these data only for descriptive purposes. Baboons 7 years of age and older are classified as adults, since physical maturation is complete at this time (Coelho 1985). Animals less than 7 years of age are classified as subadults. All adult baboons in the sample were dam reared, while the subadult group included both dam-

Table 1

Means and SDs of Unadjusted apo A-I Concentrations (mg/dl) for Baboon Subgroups

	Adults (dam reared)		Subadults			
			Dam Reared		Nursery Reared	
Diet	Males	Females	Males	Females	Males	Females
Basal:						
$\hat{\mu}$	127.78	115.74	125.76	124.98	135.83	133.81
$\hat{\sigma}$	19.21	26.85	26.66	28.22	26.35	26.00
N	23	176	163	166	35	21
HCSF:						
$\hat{\mu}$	167.41	152.58	168.10	168.53	160.21	174.05
$\hat{\sigma}$	32.62	30.68	33.77	32.96	31.06	34.52
N	22	177	160	171	33	22

Table 2

Segregation Analysis of apo A-I Levels in Baboons on the Basal Diet: Maximum-Likelihood Estimates and Λ Values

	Model						
Parameter	General	Environmental[a]	Finite Mixture[a]	Recessive Mixed	Recessive	Polygenic[b]	Sporadic[b]
p_A	.649	.736	.689	.697	.420	(1)	(1)
$\mu_{AA} = \mu_{Aa}$	122.64	124.96	121.66	124.14	113.64	125.55	126.10
μ_{aa}	168.09	175.32	172.82	172.96	147.21	125.55	126.10
σ	21.38	22.53	22.14	22.11	21.56	25.81	26.79
h^2	.376	.491	(0)	.378	(0)	.372	(0)
β(sex)	−6.97	−7.21	−6.67	−7.35	−4.85	−5.63	−6.40
$\beta(age_F)$	−1.35	−1.36	−1.02	−1.36	−1.17	−1.23	−1.13
$\beta(age_F^2)$	.089	.088	.072	.089	.092	.097	.090
β(nursery)	8.36	7.99	9.63	7.95	9.08	8.17	9.27
τ_{AA}	1.000	.736	.689	(1)	(1)	(1)	(1)
τ_{Aa}	.417	.736	.689	(½)	(½)	(½)	(½)
τ_{aa}	.343	.736	.689	(0)	(0)	(0)	(0)
Λ	...	10.50	86.66	4.51	39.32	28.86	113.13
df[c]	...	~2	~3	~2	~3	~4	~5
P	...	.0052	<.0001	.1059	<.0001	<.0001	<.0001

Note.—Parentheses indicate that parameter is fixed.
[a] Constraints: $p_A = \tau_{AA} = \tau_{Aa} = \tau_{aa}$.
[b] Constraints: $\mu_{AA} = \mu_{Aa} = \mu_{aa}$.
[c] Values are approximate because τ_{AA} went to bounds.

and nursery-reared animals. A total of 584 baboons were measured on the basal diet and 585 were measured on the HCSF diet, while for 561 there were complete data for both diets. In adult animals, apo A-I concentrations on both diets were higher in males than in females. Nursery-reared subadults had higher apo A-I levels on the basal diet than did dam-reared subadults. This trend was not apparent on the HCSF diet. On both diets, apo A-I levels decreased with age in females but not in males.

The distribution of apo A-I was examined in a set of 190 unrelated adult founders after adjustment of female apo A-I concentrations to male levels. Using a Kolmogorov-Smirnov (K-S) test, we found no clear evidence of nonnormality in the distributions of apo A-I on the basal diet (P = .20) or the HCSF diet (P = .32). In this subset of independent individuals, the observed phenotypic correlation between apo A-I levels on the two diets was .50, indicating only a moderate overall correlation between environments.

Complex Segregation Analysis of apo A-I

Basal diet.—Results of the complex segregation analysis for apo A-I levels measured on the basal diet are presented in table 2. Only recessive-type models with two ousiotypic means ($\mu_{AA} = \mu_{Aa}$, and μ_{aa}) are presented in the table. All dominant models (μ_{AA}, and $\mu_{Aa} = \mu_{aa}$) were unequivocally rejected ($P < .0001$) when compared with a general model having three arbitrary means (data not shown). Moreover, when the three-distribution general model was compared with the general recessive model, there was no clear evidence for a third distribution ($\Lambda_1 = 2.53$, $P = .11$).

Four covariate effects exhibited Λ_1 values greater than 2.71 (α = .10) when dropped from the general model and were retained in all subsequent models. These included a sex effect (β(sex)), a female-specific age effect ($\beta(age_F)$), a female-specific quadratic effect of age ($\beta(age_F^2)$), and the nursery effect (β(nursery)). The significance of covariate effects was consistent regardless of which general model (the three-distribution general model or the two-distribution recessive general model) was used.

Table 2 shows that all models except the Mendelian recessive mixed model were rejected when compared with the general model. Since the general model exhibits a parameter, τ_{AA}, that is estimated on a boundary (1.00), we have subtracted 1 df for each of these

Table 3

Segregation Analysis of apo A-I Levels in Baboons on the HCSF Diet: Maximum-Likelihood Estimates and Λ Values

PARAMETER	MODEL						
	General	Environmental[a]	Finite Mixture[a]	Recessive Mixed	Recessive	Polygenic[b]	Sporadic[b]
p_A	.567	.652	.559	.565	.518	(1)	(1)
$\mu_{AA} = \mu_{Aa}$	158.98	161.78	156.69	158.93	155.82	166.90	166.82
μ_{aa}	213.89	219.03	212.79	212.53	208.92	166.90	166.82
σ	24.27	26.42	23.70	24.87	24.06	32.26	32.45
h^2	.372	.561	(0)	.315	(0)	.372	(0)
β(sex)	−8.13	−7.89	−7.92	−7.60	−7.34	−5.70	−6.05
$\beta(age_F)$	−2.36	−2.23	−2.22	−2.32	−2.38	−2.01	−1.96
$\beta(age_F^2)$	.151	.148	.149	.147	.158	.120	.120
τ_{AA}	.896	.652	.559	(1)	(1)	(1)	(1)
τ_{Aa}	.483	.652	.559	(½)	(½)	(½)	(½)
τ_{aa}	.265	.652	.559	(0)	(0)	(0)	(0)
Λ	...	8.84	46.00	5.78	20.16	37.71	82.61
df	...	3	4	3	4	5	6
P	...	.0315	<.0001	.1231	.0005	<.0001	<.0001

NOTE.—Parentheses indicate that parameter is fixed.
[a] Constraints: $p_A = \tau_{AA} = \tau_{Aa} = \tau_{aa}$.
[b] Constraints: $\mu_{AA} = \mu_{Aa} = \mu_{aa}$.

comparisons. All models containing only a single phenotypic distribution (the sporadic and polygenic models) were strongly rejected ($P < .0001$), as were two distribution models without a polygenic component (the finite-mixture and Mendelian recessive models). There was sufficient information in the pedigree data to reject the environmental transmission model at a somewhat higher P value (.0052).

The best-fitting recessive mixed model exhibited a frequency of .70 ± .07 for the low–apo A-I allele. The expected frequency for individuals of the low– apo A-I phenotype $\bar{A}$ (i.e., genotypes AA and Aa) is .91, while that for the high–apo A-I phenotype $\bar{a}$ (genotype aa) is .09. There is also evidence of a significant polygenic component for this model ($\hat{h}^2$ = .38 ± .09). The difference between the major-locus phenotypic means is greater than two within-phenotype SDs ($\hat{\mu}_{AA} = \hat{\mu}_{Aa}$ = 124.1 ± 2.8, $\hat{\mu}_{aa}$ = 173.0 ± 8.3, $\hat{\sigma}$ = 22.1 ± 1.2). For this model, 29% of the total phenotypic variance is determined by the major locus. The polygenic background accounts for approximately 27% of the total variance, while 44% can be attributed to the environmental variance component. Nursery rearing increased apo A-I levels by 8.0 ± 3.3 mg/dl over that of dam-reared animals, while females had apo A-I levels 7.4 ± 2.4 mg/dl lower than those of males.

The τ parameters estimated in the general model were not significantly different from Mendelian expectations, with τ_{AA} being estimated at its boundary expectation of 1. τ_{Aa} was estimated at .42 ± .11, which is not significantly different from ½. However, τ_{aa} was estimated at a marginally high value of .34 ± .17 (although it was not significant by a likelihood ratio test).

HCSF diet.—Segregation analyses of apo A-I levels on the HCSF diet also revealed evidence for a recessive major locus with a polygenic background (table 3). As above, only recessive-type models are presented in this table. All dominant models were rejected, while the three-distribution general model was not significantly different from a two-distribution recessive general model (Λ_q = .67, P = .41). Significant covariates included only the β(sex), $\beta(age_F)$, and $\beta(age_F^2)$ effects on apo A-I levels.

The τ transmission parameters estimated in the general model are again consistent with Mendelian transmission ($\hat{\tau}_{AA}$ = .90 ± .08, $\hat{\tau}_{Aa}$ = .48 ± .09, $\hat{\tau}_{aa}$ = .27 ± .13). The two-distribution environmental transmission model with a polygenic component could be rejected (P = .032), which strengthens our inference for a major locus.

The frequency of the allele associated with lower apo A-I levels in the best-fitting recessive mixed model was

Table 4

Bivariate Segregation Analysis of apo A-I levels in Baboons on Two Diets: Comparison of Models

Model	No. of Loci	Structural Relationship between Loci	Two-Locus Mean Effects	MG×E	Tests versus Model 1 Λ	Model 1 df	Model 1 P	Model 2 Λ	Model 2 df	Model 2 P	Model 3 Λ	Model 3 df	Model 3 P	AIC
1	2	Linked	Epistatic	A,B	...	...	...	...	...	...	...	...	...	48.00
2	2	Linked	Additive	A,B	6.77	8	.5616	...	...	...	...	...	...	40.73
3	2	Independent	Additive	A,B	8.33	~9[a]	.5013	1.55	2	.4596	...	...	...	38.29
4	2	Independent	Additive	None	57.52	~11[a]	<.0001	50.75	4	<.0001	49.20	2	<.0001	83.48
5	2	Independent	Additive	A	48.30	~10[a]	<.0001	41.53	3	<.0001	39.98	1	<.0001	76.26
6	2	Independent	Additive	B	28.08	~10[a]	.0032	21.31	3	.0001	19.75	1	<.0001	56.04
7	1	...	...	A	53.78	~12[a]	<.0001	47.00	5	<.0001	...	...	...	77.74
8	1	...	...	B	36.18	~12[a]	.0006	29.40	5	<.0001	...	...	...	60.13

[a] Approximate because of boundary condition for $\hat{\theta}$ in model 1.

estimated as .57 ± .07, which is considerably smaller than that on the basal diet. Approximately twice as many high homozygotes (aa) are expected on the HCSF diet (F(aa) = .18) as on the basal diet (F(aa) = .09), and mean phenotypic values of apo A-I levels are increased 35–40 mg/dl over those on the basal diet. The h^2 on the HCSF diet is .32 ± .16. The major locus accounts for approximately 42% of the total variance, with the polygenic background determining 18% and with the remaining 40% being attributable to the environmental component.

Because the allele frequencies obtained in the two univariate segregation analyses were so different (.70 vs. 57), we hypothesized that these results were due to two distinct loci with possible G×E interaction. To further examine this question, we employed the two-locus bivariate segregation analysis described above.

Bivariate Segregation Analysis of apo A-I: Both Diets

Results from the two-locus bivariate segregation analyses for apo A-I levels measured on both diets are presented in tables 4 and 5. Table 4 defines the eight models considered and presents both the Λ statistics for different model comparisons and the AIC values for each model. Table 5 provides the maximum-likelihood parameter estimates for each model and also defines the parameter constraints. In these models, the *B* locus refers to the locus identified in the univariate analyses of apo A-I on the basal diet, and the *A* locus refers to that identified for the HCSF diet. We present only those models which exhibit the same intralocus dominance relationships as seen in the univariate models (i.e., recessive inheritance of high apo A-I levels). This entails constraining $a_A = d_A$ and $a_B = d_B$ for each trait. To assure that such constraints were reasonable, we compared the most general model given in table 4 with an even more general model in which these constraints were removed (data not shown). The resulting likelihood ratio test was not significant ($\Lambda_4 = 6.23$, $P = .183$), suggesting that the constrained model was acceptable.

The models range in complexity from the general model (model 1) with two linked loci, epistatic mean effects, and MG×E interaction at both loci to models involving single loci (*A* or *B*). The single-locus models were obtained by using as starting values the parameter estimates from the appropriate univariate analysis. The parameter estimates for these two single-locus models (models 7 and 8) represent two distinct local maxima, each of which provides important evidence for the underlying genetic architecture of apo A-I.

The first set of model comparisons given in table 4 consists of tests of each model versus the general model (model 1). Since the maximum-likelihood estimate of θ for this model was obtained on a boundary of the parameter space ($\hat{\theta} = .000$), we have reduced, by 1, the number of df for each test involving the comparison of a model in which θ is constrained to ½. Only models 2 and 3 provide likelihoods that are not significantly worse than the general model. The test for epistasis is given by the comparison of model 2 and model 1, which differ only with regard to parameterization of two-locus mean effects. The likelihood ratio test for this comparison ($\Lambda_8 = 6.77$, $P = .562$) suggests that there are no epistatic interactions between the two loci. Similarly, the comparisons between the

Table 5

Bivariate Segregation Analysis of apo A-I in Baboons on Two Diets: Maximum-Likelihood Estimates

Parameter	Model[a]							
	1	2	3	4[b]	5[c]	6[d]	7[e]	8[e]
p_A	.544	.553	.550	.492	.568	.623	.534	.751
p_B	.741	.749	.749	.760	.816	.760	.534	.751
θ	.000	.001	(½)	(½)	(½)	(½)	(0)	(0)
D'	.190	.109	(0)	(0)	(0)	(0)	(1)	(1)
Basal:								
m_1	156.04	158.32	159.87	151.35	153.95	164.35	165.16	152.77
$a_{A1} = d_{A1}$	9.03	11.82	12.45	15.51	9.98	15.68	13.67	(0)
$a_{B1} = d_{B1}$	27.21	26.33	27.68	18.10	21.54	28.50	(0)	28.29
aa_{AB1}	−4.07	(0)	(0)	(0)	(0)	(0)	(0)	(0)
ad_{AB1}	−7.30	(0)	(0)	(0)	(0)	(0)	(0)	(0)
ad_{BA1}	2.76	(0)	(0)	(0)	(0)	(0)	(0)	(0)
dd_{AB1}	3.40	(0)	(0)	(0)	(0)	(0)	(0)	(0)
h_1^2	.314	.388	.381	.245	.304	.377	.300	.451
σ_1	20.50	20.96	20.72	20.60	23.00	20.44	23.33	22.95
HSCF:								
m_2	177.45	181.20	183.03	193.09	205.67	178.83	183.76	168.61
$a_{A2} = d_{A2}$	23.26	25.65	25.45	15.51	25.36	15.68	24.84	(0)
$a_{B2} = d_{B2}$	−6.73	−4.26	−1.89	18.10	21.54	−1.40	(0)	−.39
aa_{AB2}	1.86	(0)	(0)	(0)	(0)	(0)	(0)	(0)
ad_{AB2}	−9.56	(0)	(0)	(0)	(0)	(0)	(0)	(0)
ad_{BA2}	−4.43	(0)	(0)	(0)	(0)	(0)	(0)	(0)
dd_{AB2}	1.73	(0)	(0)	(0)	(0)	(0)	(0)	(0)
h_2^2	.177	.203	.191	.158	.143	.246	.136	.301
σ_2	23.90	24.61	24.57	27.05	24.08	29.15	24.53	31.65
ρ_G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ρ_E	.190	.161	.152	.043	.122	.187	.101	.270

Note.—Parentheses indicate that parameter is fixed.
[a] As defined in table 4.
[b] Constraints: $a_{A1} = d_{A1} = a_{A2} = d_{A2}$; $a_{B1} = d_{B1} = a_{B2} = d_{B2}$.
[c] Constraints: $a_{B1} = d_{B1} = a_{B2} = d_{B2}$.
[d] Constraints: $a_{A1} = d_{A1} = a_{A2} = d_{A2}$.
[e] Constraints: $p_A = p_B$.

general model and the two single-locus models show that more than one simple major locus is involved in the genetic mediation of apo A-I.

The second set of model comparisons given in table 4 contrasts model 2, which incorporates two linked loci with additive mean effects and MG×E interaction at both loci, with all other models. Again, all models can be rejected except model 3. The comparison of model 3 with model 2 provides the best test of structural relationships between the two loci. The associated likelihood ratio test ($\Lambda_2 = 1.55$, $P = .460$) shows the hypothesis that the two loci are independent cannot be rejected. However, examination of the maximum-likelihood estimates for D' (.109 ± .323) and of $\hat{\theta}$ (.001 ± .354) suggest that there is little power in such a test.

For the tests of MG×E interaction, we compared model 3 with models 4, 5, and 6. The comparison between models 3 and 4 represents a global test of MG×E interaction. The resulting likelihood ratio test ($\Lambda_2 = 49.20$, $P < .0001$) indicates that there is strong evidence for the influence of major genotype-diet interaction on apo A-I levels. Similarly, the remaining tests show that such genotype-diet interaction is detectable at both the A ($\Lambda_1 = 19.75$, $P < .0001$) and B ($\Lambda_1 = 39.98$, $P < .0001$) loci.

As can be seen in table 5, the estimated ρ_G was

Table 6

Genotypic Means ± SEs for apo A-I Serum Levels (mg/dl), Obtained from the Best-fitting Bivariate Segregation Model

Genotype	Frequency	$\hat{\mu}$ Basal	HCSF	Response
Two-locus means:				
AABB..........	.170	119.74 ± 1.60	159.47 ± 2.00	39.73 ± 1.56
AABb..........	.114	119.74 ± 1.60	159.47 ± 2.00	39.73 ± 1.56
AAbb..........	.019	175.10 ± 5.91	155.69 ± 7.20	−19.40 ± 7.67
AaBB..........	.278	119.74 ± 1.60	159.47 ± 2.00	39.73 ± 1.56
AaBb..........	.186	119.74 ± 1.60	159.47 ± 2.00	39.73 ± 1.56
Aabb..........	.031	175.10 ± 5.91	155.69 ± 7.20	−19.40 ± 7.67
aaBB..........	.113	144.64 ± 3.49	210.37 ± 4.40	65.73 ± 4.24
aaBb...........	.076	144.64 ± 3.49	210.37 ± 4.40	65.73 ± 4.24
aabb...........	.013	199.99 ± 6.58	206.59 ± 8.19	6.60 ± 8.65
Marginal means:				
A locus:				
AA..........	.303	123.23 ± 1.64	159.23 ± 2.02	36.00 ± 1.64
Aa..........	.495	123.23 ± 1.64	159.23 ± 2.02	36.00 ± 1.64
aa...........	.202	148.13 ± 3.50	210.13 ± 4.41	62.00 ± 4.28
B locus:				
BB..........	.561	124.78 ± 1.53	169.78 ± 2.17	45.00 ± 1.45
Bb...........	.376	124.78 ± 1.53	169.78 ± 2.17	45.00 ± 1.45
bb...........	.063	180.14 ± 5.87	166.00 ± 7.25	−14.14 ± 7.65

1.000 in all models considered. Therefore, there is no evidence for any PG×E interaction.

On the basis of the likelihood ratio tests, model 3 represents the best-fitting, most parsimonious model. This model also exhibits the minimum AIC, as seen in the last column of table 4. The gene frequency estimates ($\hat{p}_A$ = .550 ± .051, $\hat{p}_B$ = .749 ± .038) obtained for this model are concordant with the univariate analyses. The within-genotype phenotypic SDs in the bivariate analysis were estimated as 20.72 ± 0.91 for apo A-I on the basal diet and as 24.57 ± 1.17 for that on the HCSF diet. Residual additive genetic h^2 values were estimated as .381 ± .099 for apo A-I concentration on the basal diet and as .191 ± .092 for that on the HCSF diet. The ρ_E was very low ($\hat{\rho}_E$ = .152 ± .09), while the ρ_G was extremely high ($\hat{\rho}_G$ = 1.000). Because the ρ_G was estimated on its upper boundary, standard errors (SEs) could not be obtained. However, to assess the precision of this estimate, we performed a grid search on this parameter to find the value from which the observed estimate was significantly different at the .05 α level. For each value of ρ_G taken at regular .05 intervals, we maximized the likelihood over all other parameters. We then used quadratic interpolation to find the expected value of ρ_G at Λ_1 = 2.71, which represents the α = .05 probability level for this case. We obtained .83 as the minimum acceptable ρ_G value that is consistent with the data. This value represents the approximate lower bound of the 95% confidence interval for $\hat{\rho}_G$.

Table 6 presents the genotypic means ± SEs derived from the best-fitting model. Both the two-locus genotypic means and marginal means for each locus are provided. We also calculated the expected responses (apoA-I_{HCSF} − apo A-I_{basal}) to the HCSF diet. Examination of the marginal means for the *B* locus reveals that allelic variation at this locus has essentially no effect in the HCSF environment. Furthermore, the bb genotype appears to determine a potentially maladaptive negative response to the HCSF diet. Figure 1 graphically depicts the two-locus genotypic means and their dietary responses. The differential response among genotypes is apparent. Dependent on the genotypic background at the *A* locus, the bb genotype exhibits either a slight positive response to diet (when paired with an aa genotype) or a large negative response (when paired with an AA or Aa genotype). The observed changes in rank among genotypes in different environments represent a classical form of G×E interaction.

Table 7 presents the proportion of the total phenotypic variance in apo A-I serum levels that is attributable to different genetic and environmental sources. To-

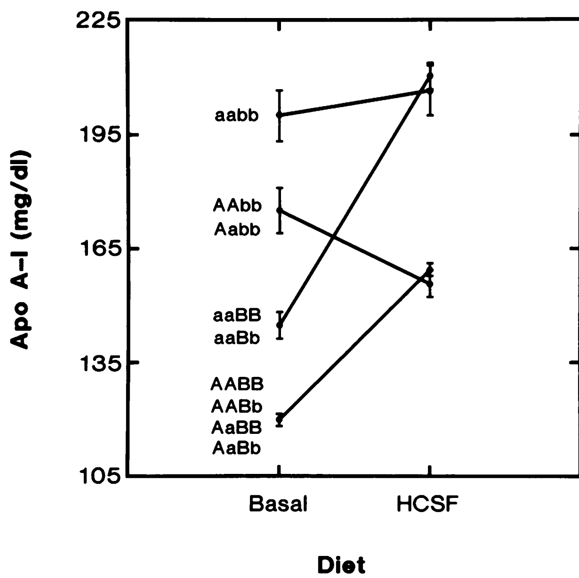

Figure 1 Two-locus genotypic means for apo A-I serum levels in two dietary environments. Error bars represent SEs.

gether, the two major genes account for approximately 40% of the variability in apo A-I levels on both diets. However, there are differences among loci. For example, the *A* locus accounts for 14% (9% additive and 5% dominance) of variation on the basal diet, while the *B* locus accounts for 25% (10% additive and 15% dominance). Similarly, the *A* locus determines 41% (25% additive and 16% dominance) of the variance on the HSCF diet, while the *B* locus contributes virtually nothing, as expected from examination of the mean effects. The *B* locus accounts for nearly twice as much variance in dietary response as does the *A* locus (21% vs. 11%). Therefore, each locus influences both the level of apo A-I and response to dietary challenge. Residual polygenic effects account for an additional 23% and 11% of the variance on the basal and HCSF diets, respectively. Dietary response exhibits little variance due to residual polygenic effects. Although not shown in table 7, major-locus effects account for 50% of the total covariance between apo A-I levels on the two diets, while polygenic factors are responsible for an additional 36% of this covariance.

Discussion

Our results indicate that at least two major loci are involved in the determination of apo A-I serum levels in baboons. These loci affect serum levels of apo A-I and also influence the magnitude of response to an atherogenic diet. Since we could find no evidence for epistasis, these two loci appear to act additively. Jointly, they determine large proportions of the total phenotypic variance, both in levels and in dietary response.

The two-locus results indicate the importance of genetic background in determining single-locus effects. Depending on the genotype at the *A* locus, individuals of the bb genotype either are well buffered from environmental challenge and maintain high apo A-I levels or exhibit a drastic decrease in apo A-I levels.

We have documented significant MG×E interaction at both major loci. Recent measured genotype analyses on a number of lipoprotein phenotypes (Berg et al. 1989; Blangero et al. 1989; Kaprio et al. 1989; Kondo et al. 1989; Tikkanen et al. 1989), incorporating genetic variants at candidate loci, have suggested that such G×E interaction is likely. However, in all these studies, the candidate loci considered involve relatively minor genes that determine only a small amount of the total phenotypic variance. Correspondingly, the observed magnitudes of G×E interaction have been small. We have for the first time established an important role for genotype-diet interaction acting at major loci in a normal population. If human apo A-I exhibits a similar genetic architecture, such findings may be useful in identifying individuals likely to be responsive to dietary intervention.

How do these findings based on a nonhuman primate model compare with those from human studies? With regard to previous pedigree analyses in humans,

Table 7

Proportion of Total Phenotypic Variance in apo A-I Serum Levels That Is Accounted for by Genetic and Environmental Components

Component	Basal	HCSF	Response
A locus:			
Additive	.0874	.2538	.0702
Dominance	.0534	.1551	.0429
Total	.1408	.4089	.1131
B locus:			
Additive	.1023	.0003	.0860
Dominance	.1525	.0005	.1282
Total	.2548	.0008	.2142
Total major loci:			
Additive	.1897	.2541	.1562
Dominance	.2059	.1556	.1711
Total	.3956	.4097	.3273
Polygenic	.2305	.1128	.0044
Environmental	.3738	.4775	.6683

our results are most comparable with those of Moll et al. (1989), who examined a large set of randomly selected pedigrees. As mentioned above, our study incorporates an effectively random selection of baboon pedigrees which are not enriched for any particular distributional characteristic of apo A-I. Although Moll et al. separated their pedigrees into two subsets, with one exhibiting only major-locus transmission and the other exhibiting only environmental and polygenic transmission, our parameter estimates for the effects of the *B* locus in baboons on the basal diet are remarkably concordant with their unpartitioned results. Moll et al. could not reject a major-locus model with nonadditive allelic effects in which the homozygotes differed by approximately 53 mg/dl, while in baboons this difference is 55 mg/dl. The gene frequency for a low-apo A-I allele in Moll et al.'s analysis of all their human pedigrees was .85, while that for the *B* locus in baboons was .75. Conceivably, we could be dealing with a very similar allelic variant in baboons.

Other aspects of Moll et al.'s (1989) analysis may represent evidence for a possible second locus in humans that is similar to the *A* locus detected in the present study. In their examination of the 126 pedigrees which support a nontransmitted environmental etiology, they provide parameter estimates for a single-locus model without a polygenic component, a model that appears to represent a second local maximum. An analogous general model is not significantly better than this Mendelian model. For the putative second human locus, with an allele frequency of .21, the mean for the low homozygote was 109.26, that for the heterozygote was 122.70, and that for the high homozygote was 145.71. Again, these estimates are similar to our marginal *A*-locus results.

Such concordance suggests that the two loci detected in baboons may also be present in humans. A puzzling aspect of this homology is that it is the basal diet parameters in the baboon that resemble those in humans. We would expect that humans have diets more similar to the HCSF diet. Regardless, it is possible that the involvement of two loci and the attendant MG×E interaction due to variability of diet may be partly responsible for the somewhat equivocal results found in Moll et al.'s (1989) study. In future human pedigree studies, dietary information may be required on individuals, to decompose adequately the genetic determinants of apo A-I variation.

Additional evidence points to two major loci contributing to the genetic determination of apo A-I levels. Borecki et al. (1988) obtained results from single-locus bivariate segregation analysis to indicate two loci influencing apo A-I. They hypothesized that one locus had effects on both apo A-I and apo A-II levels, while a second locus contributed independently to apo A-I. A more complete analysis allowing for two loci may help to clarify their interesting findings. Similarly, data obtained from inbred mice support the contention that at least two major loci influence both apo A-I levels and response to an atherogenic diet (Paigen et al. 1987, 1989; LeBouef et al. 1989).

After detection of these two apo A-I loci, our next priority is to locate them in the genome. Preliminary analyses by Kammerer et al. (1988*b*) using the sibpair test suggest that one of our loci may be linked to a marker, adenine phosphoribosyltransferase (APRT), found on chromosome 16q in humans, a marker which is in turn linked to two major candidate genes known to be involved in reverse cholesterol transport—i.e., the genes for lecithin:cholesterol acyltransferase (LCAT) and CETP. Subsequent analyses using data on a polymorphic *Pvu*II site at the LCAT locus have provided evidence of an association with apo A-I levels on both diets (Kammerer et al. 1988*a*). A similar analysis failed to find an association with a *Pst*I site at the APOA1 structural locus, which is located in humans on chromosome 11q13-qter (Kammerer et al. 1988*a*). Therefore, we suspect that one of our major apo A-I loci may be LCAT or CETP. In future analyses, we intend to use bivariate quantitative-trait linkage analysis to try to establish the locations of the loci detected in the present study.

Acknowledgments

This research was supported by National Institutes of Health grants HL28972, GM12782, GM31575, and contract HV53030. We would also like to thank the many laboratory technicians, computer programmers, and animal caretakers who helped to make this study possible.

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