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Genetic Determination of Plasma Apolipoprotein AI in a Population-based Sample

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Summary

Apolipoprotein AI (apo AI) is the major protein of high-density lipoprotein (HDL). Using radioimmunoassay, we measured plasma apo AI levels in 1,880 individuals in 283 pedigrees randomly selected from the population with respect to disease status and risk factors for coronary artery disease. Apo AI levels were first adjusted for date of assay (6.8% of apo AI variation) and then adjusted for variability in age and body mass index (an additional 6.6%, 20.4%, and 23.0% of apo AI variation for males, females not using exogenous hormones, and females using exogenous hormones, respectively). A mixture of two normal distributions fit the adjusted data better than did a single normal distribution. Genetic and environmental models that could explain the mixture of normal distributions were investigated using complex segregation analysis. Heterogeneous etiologies for individual differences in adjusted apo AI levels were suggested by the data in the 283 pedigrees. In a subset of 126 pedigrees, there is evidence for the major effect of a nontransmitted environmental factor that explains the mixture of distributions as well as polygenic loci that influence apo AI levels within each distribution. The environmental factor and polygenic loci account for 32% and 65% of the adjusted variation, respectively. In the other 157 pedigrees there is strong support for a single locus with a major effect that accounts for 27% of the adjusted variation. The effect of the polygenic loci is not different from zero in these 157 pedigrees. This is the first study to present evidence for the segregation of a single unmeasured locus with a major effect on levels of apo AI in a population-based sample of pedigrees.

Introduction

Apolipoproteins, protein components of the lipoproteins, have a variety of structural and metabolic roles related to the metabolism of lipids (Pownall and Gotto 1983). They solubilize lipids for transport and act as cofactors, activators, and inhibitors of enzymes involved in the synthesis and degradation of lipoprotein particles. Additionally, apolipoproteins are recognized by cell surface receptor sites that specifically promote the catabolism of the various lipoprotein fractions.

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One of these apolipoproteins, apolipoprotein AI (apo AI), is the major protein component of high-density lipoprotein (HDL). Apo AI enters plasma either on chylomicrons and HDL from the intestine or on HDL from the liver. Following entry of chylomicrons into plasma, almost all of their apo AI is rapidly transferred to HDL (Koren et al. 1987). A review of the function and structure of apo AI is given by Zannis and Breslow (1984). One of its important functions is the activation of lecithin:cholesterol acyltransferase, a plasma enzyme that catalyzes the esterification of plasma cholesterol. Another role is as an acceptor protein in reverse cholesterol transport (Stein et al. 1979). Plasma apo AI is a single polypeptide chain of 243 amino acid residues of known sequence (Brewer et al. 1978). The gene coding for apo AI is in a cluster with the genes for apo CIII and apo AIV on the long arm of chromosome 11 (Bruns et al. 1984; Karathanasis 1985).

The association between lower levels of plasma apo AI and higher prevalence of coronary artery disease (CAD) is generally accepted (Brunzell et al. 1984). Plasma levels of apo AI have been shown in males to be a better discriminator of angiographically documented CAD than is the level of HDL cholesterol (Maciejko et al. 1983; Kottke et al. 1986). A prospective study of individuals in the Lipid Research Clinics cohort has shown that males who died of CAD had significantly lower levels of apo AI at entry into the study than did controls (Albers et al. 1984). Recent studies in Bogalusa reported that the levels of apo AI in the children whose fathers had a myocardial infarction (MI) were significantly lower than the levels in the other children (Freedman et al. 1986).

Several previous studies have applied biometrical genetic techniques to establish the role of genetic variation in determining individual differences in quantitative levels of apo AI. A study of males from the Finnish Twin Registry reported no evidence for polygenic loci influencing apo AI levels (Sistonen and Ehnholm 1980). In contrast to that finding, Berg (1984) reported the heritability of apo AI to be .53, on the basis of an estimate of the intraclass correlation coefficient obtained from monozygotic Norwegian twin pairs. The two twin studies differed in the immunodiffusion assays as well as in the statistical procedures. Recently, Kuusi et al. (1987) reported the heritability of apo AI to be .66, on the basis of a sample of male twins. Hamsten et al. (1986) estimated the heritability to be .43 for a sample consisting of nuclear families ascertained through males with premature MI and through males with no history of angina pectoris or signs of CAD.

Only four studies to date have investigated the possible role of a single locus influencing apo AI levels. A study of one large Utah pedigree ascertained through cases of early MI (Hasstedt et al. 1984) and a study of one very large pedigree selected through the Bogalusa Heart Study (Amos et al. 1987) both reported no evidence for a single locus influencing plasma apo AI levels. The third study, which included 97 individuals from 23 pedigrees enriched by individuals at high risk for CAD, presented evidence that a single locus with a major effect is involved in determining interindividual variation in quantitative levels of plasma apo AI (Moll et al. 1986). Recently, Blangero et al. (1987) reported evidence for a single locus influencing serum apo AI levels in baboons.

No studies to date have been performed in a population-based sample. Here we report on the role of genetic factors in determining plasma apo AI variability in 1,880 individuals from 283 pedigrees randomly selected with respect to disease status and risk-factor levels. Heterogeneous etiologies for individual differences in apo AI levels were suggested by the data. In one subset of pedigrees there is evidence that a nontransmitted environmental factor and polygenic loci influence interindividual differences in apo AI levels. In another subset of pedigrees there is strong support for a single-locus effect while the effect of the polygenic loci is not significantly different from zero. This study is the first to present evidence for the segregation of a single unmeasured locus with a major effect on the quantitative levels of apo AI in a population-based sample of pedigrees.

Material and Methods

Sample

In January 1984 we arranged for officials of the Rochester, MN, school system to send letters to 5,270 households having two or more children enrolled in the city's public and parochial schools. In the letter we first described the purpose of the study, which was to evaluate the effects of environment and inherited traits on lipid transport and hypertension, and then requested that each household willing to consider participation in the study return an enclosed questionnaire providing its phone number, address, and the names of all individuals in the household. From a total of 1,812 questionnaires that were returned (response rate 34.4%), 159 households were judged unsuitable for sampling either because they (*a*) did not want to be contacted (n = 105), (*b*) reported that they planned to move from Rochester within the next year (n = 12), (*c*) reported that there were only adopted children in the household (n = 17), or (*d*) gave various other reasons (n = 25) that participation would be unlikely (e.g., unwillingness to visit the clinic or to have blood drawn). Between December 1, 1984, and January 1, 1988, we contacted 436 of the 1,653 households eligible for sampling, and 2,004 individuals identified by 300 households agreed to participate and completed clinic visits.

The study participants visited the Mayo Clinic between 7:00 and 9:00 A.M. for blood drawing and examination. Subjects were asked to fast overnight prior to the clinic visit, to abstain from alcohol consumption for 24 h before the clinic visit, and to complete a questionnaire regarding the use of prescription and nonprescription medications. At the beginning of the clinic visit, each subject gave consent and then a blood sam-

ple was drawn by a trained phlebotomist. Afterwards, a physician reviewed the medical history with each subject and performed a brief physical examination. For the physical examination, subjects were required to remove their shoes and outer clothing. Height was measured with a wall stadiometer, and weight was determined on a beam balance.

Two individuals were excluded from the present study because apo AI levels were not measured. Of the remaining 2,002 individuals in the study, another 119 who reported that they were taking glucocorticoids (n = 23), thyroid medication (n = 62), insulin (n = 9), oral hypoglycemics (n = 18), or a combination of these medications (n = 7) were also excluded, because individuals using these medications have different apo AI levels compared with individuals not using these medications (B. A. Kottke, P. P. Moll, W. H. Weidman, V. Michels, and T. L. Fuller, unpublished data). Of the remaining 1,883 individuals, another three were excluded because of no information about age (n = 2) or about height and weight (n = 1). The 1,880 individuals included in the present study were distributed among 283 pedigrees. The number of individuals studied per pedigree ranged from one to 24 individuals (mean 6.64). The distribution of the number of individuals studied in the pedigrees is given in figure 1. The pedigrees with only one individual represent one or more adoptees from a household that included biological

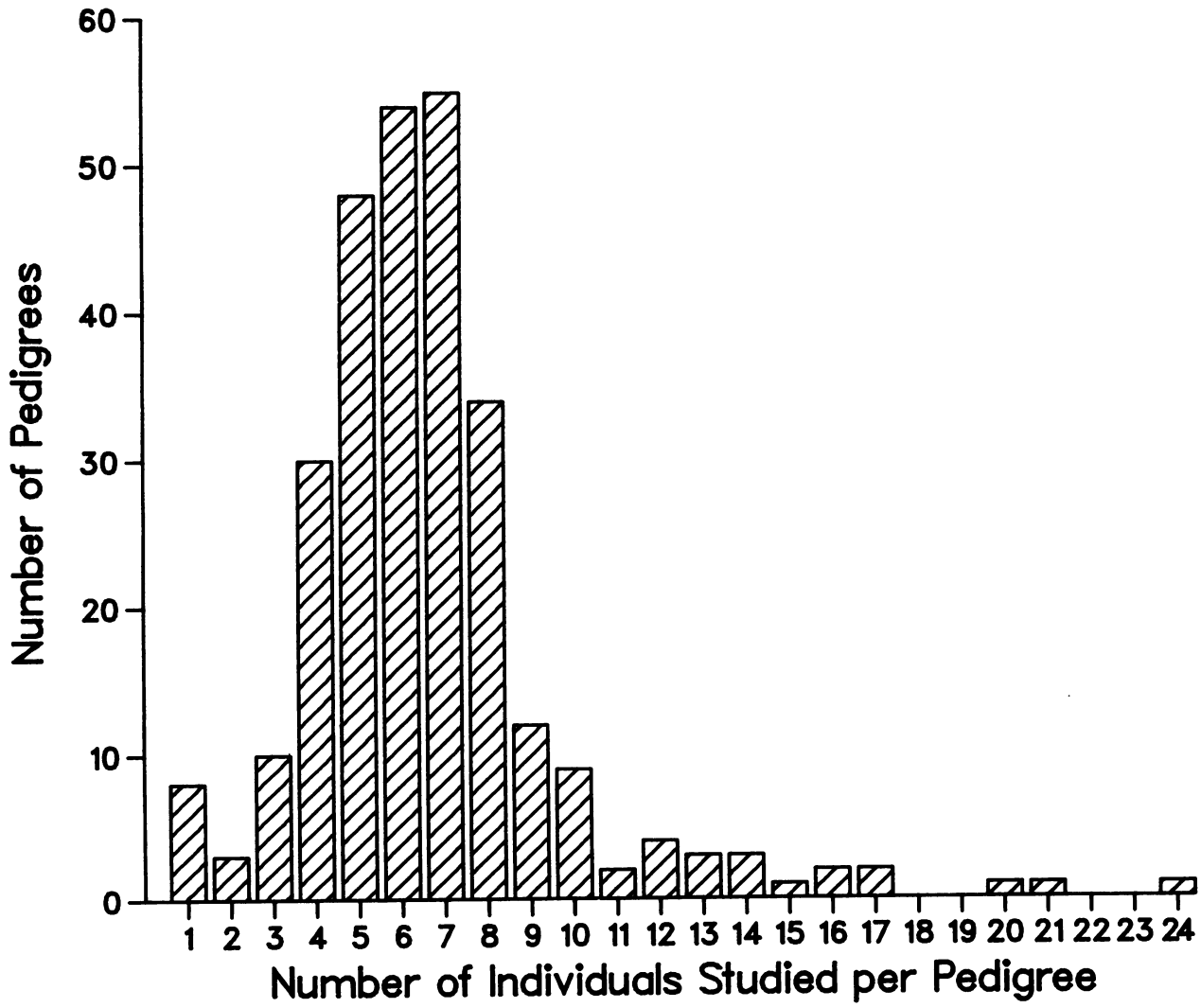

Figure 1 Distribution of the number of individuals studied in the pedigrees.

offspring and parents, while the larger pedigrees represent as many as four different households linked together by genetically related individuals who were measured for apo AI. The number of generations studied ranged from 1 (the adoptees) to 4.

Measurements

The level of apo AI was measured at the Mayo Clinic by a previously described solid-phase radioimmunoassay that uses rabbit polyclonal antibodies (Maciejko and Mao 1982). Purified apo AI was used as the primary standard for quantifying apo AI in plasma, and a pooled plasma (quality-control sample) was used as a secondary standard. All determinations of an individual's apo AI levels were based on six replicate measurements. The interassay coefficient of variation for this assay is 5.6% (Au et al. 1986).

Statistical Analysis

Using analysis of variance, we first estimated the extent to which variability in apo AI was attributable to the specific day on which the measurement was made in the laboratory. Residuals were obtained from the analysis of variance. Then, using multiple regression analysis, we estimated the extent to which variability in residuals for apo AI was attributable to differences in age, age squared, age cubed, and body-mass index (wt/ht^2) for males, females not using exogenous hormones, and females using exogenous hormones, separately. All subsequent genetic analyses were performed on adjusted apo AI levels obtained by adding the sample mean to the residuals from the multiple regression. A maximum likelihood method (Day 1969) was used to determine whether a mixture of normal distributions fit the distribution of adjusted apo AI levels better than did a single normal distribution. A model with a mixture of three normal distributions with equal variances was compared with a model with a mixture of two normal distributions with equal variances. The six parameters of the three-component model include the means of each component (μ_1, μ_2, and μ_3), the relative frequency of the first two components (f_1 and f_2), and the within-component variance (σ^2). The difference between the maximum of the $\log_e$ likelihoods under the two models being compared forms a basis for judging whether a mixture of two distributions fits the data as well as does a mixture of three distributions. Twice the difference between two $\log_e$ likelihoods is distributed approximately as a χ^2 distribution with df equal to the number of parameters restricted by the hypothesis. If the model with a mixture of two distributions could not be rejected, then the model with a mixture of two distributions was compared with a model having one normal distribution.

Several factors, both genetic and environmental, could lead to the rejection of a single normal distribution in favor of a mixture of distributions fitting the data. Complex segregation analysis (Elston and Stewart 1971; Lalouel et al. 1983) was used to test a specific series of models that represent combinations of factors that can influence the distribution of a quantitative trait. Under these models, the variation among individuals for adjusted apo AI levels could be a consequence of the independent and additive contributions of a single genetic or nontransmitted environmental factor with major effects on the apo AI level, small additive allelic effects of a large number of independent polygenic loci, and individual-specific environmental influences. The major factor was modeled as having two alternatives, L (low) and H (high), that may have either a genetic or an environmental origin. These combine to define three classes—or ousiotypes (Cannings et al. 1978)—of individuals, denoted LL, LH, and HH. The relative frequency of L in the population being sampled is denoted p, and the relative frequency of H, denoted q, is equal to $1 - p$. Assuming Hardy-Weinberg proportions, the relative frequencies of LL, LH, and HH individuals are p^2, $2pq$, and q^2, respectively.

Other parameters of this model include the phenotypic mean of each ousiotype (μ_{LL}, μ_{LH}, and μ_{HH}) and the phenotypic variance (σ^2) among individuals with the same ousiotype. The model partitions this variance into a fraction that is attributable to the additive effects of the polygenic loci (h^2) and a fraction ($1 - h^2$) that is attributable to individual-specific environmental effects and measurement error.

The τ_1, τ_2, and τ_3 are the probabilities that individuals of ousiotypes LL, LH, and HH, respectively, transmit the L factor to their offspring. For the general transmission model (Lalouel et al. 1983), these transmission probabilities were each estimated under the constraint that they be between 0 and 1. The single-locus Mendelian model defines the probabilities of transmitting alleles from parents to their offspring as $\tau_1 = 1$, $\tau_2 = .5$, and $\tau_3 = 0$. The nontransmitted environmental effect model, on the other hand, predicts that the probability that an individual is one ousiotype or another is independent both of that person's generation and of the ousiotypes of his or her parents. Under this model, each of the transmission probabilities is taken to be equal to the relative frequency of L, which is p.

Testing hypotheses about the values of parameters

corresponds to restricting one or more parameters to some hypothesized values while estimating the remaining parameters from the data. Twice the difference between the maximum of the $\log_e$ likelihood of a model with unrestricted parameters and the maximum of the $\log_e$ likelihood of a model with restricted parameters is distributed approximately as a χ^2 when the null hypothesis is true. The df for this χ^2 test statistic are equal to the number of parameters restricted to values stated by the hypothesis. Among the models considered, the model with unrestricted parameters is the general transmission model in which the three transmission probabilities as well as p, μ_{LL}, μ_{LH}, μ_{HH}, σ^2, and h^2 are estimated. If another model with restricted parameters could not be rejected when compared with this most general transmission model, then we compared the model having the restricted parameters with other models having further parameter restrictions.

The likelihoods of the models were computed using the Pedigree Analysis Package (PAP) (Hasstedt et al. 1979; Hasstedt and Cartwright 1981), which employs an approximation to the exact likelihood of a model with both a single factor and polygenic loci (Hasstedt 1982). The likelihood for each model considered was maximized using a quasi-Newton method (Lalouel 1979). Parameter estimates associated with a given model were taken to be those that maximized the corresponding likelihood.

Results

The 1,880 individuals in the study ranged in age from 4.8 to 90 years, with a mean of 36.6 years. The sample included 924 males, 898 females not using exogenous hormones, and 58 females using exogenous hormones. Plasma levels of apo AI ranged from 63 mg/dl to 221 mg/dl, with a mean of 131.2, 138.3, and 148.9 mg/dl for males, females not using exogenous hormones, and females using exogenous hormones, respectively. Using analysis of variance, we first adjusted apo AI levels for variability among measurements that was due to the specific day on which the assay was run (6.8%, $P < .01$). For males, the covariates age, age squared, age cubed, and body-mass index explained 6.6% of the variability in apo AI levels after variability for the date of assay was removed. For females not using exogenous hormones, the covariates explained 20.4% of the variability in apo AI levels after variability for the date of assay was removed. For females using exogenous hormones, the covariates explained 23.0% of the variability in apo AI levels after variability for date of assay was removed. The variances of the residuals from the regression were not significantly heterogeneous among males, females not using exogenous hormones, and females using exogenous hormones. These residuals from regression were added to the mean of apo AI in the sample (134.67 mg/dl) to obtain apo AI levels that were adjusted for variability in age, body-mass index, gender, hormone use, and date of the assay.

Among the individuals over age 18, there were no significant differences in the adjusted levels of apo AI among current smokers, former smokers, or individuals who reported that they had never smoked. When the adjusted apo AI levels of individuals using specific groups of medications (e.g., lipid-lowering medications, antidepressant medications, etc.) were compared with the levels of individuals not using medications, no mean differences were observed for any group of medications.

From the commingling analysis, the hypothesis that a mixture of two distributions fits the data as well as does a mixture of three distributions could not be rejected (table 1). However, the hypothesis that a single normal distribution fits the data as well as does a mixture of two distributions was rejected ($\chi^2 = 43.9$, df $= 2$, $P < .001$). On the basis of the maximum likelihood parameter estimates presented in table 1, the sample predicts that 3.0% of the population from which these individuals were drawn falls in the upper-component distribution, with its mean at 179.7 mg/dl, and that 97.0% falls in the lower component, with its mean at 133.3 mg/dl. When males and females were considered separately, the hypothesis that a mixture of two distributions fits the data as well as does a mixture of three distributions could not be rejected in either group (data not shown; $\chi^2 = 0.61$, df $= 2$, $P > .05$ for males and $\chi^2 = 0.97$, df $= 2$, $P > .05$ for females). The hypothesis of a single distribution fitting as well as does a mixture of two distributions was rejected in each group (data not shown; $\chi^2 = 35.35$, df $= 2$, $P < .001$ for males and $\chi^2 = 10.6$, df $= 2$, $P < .01$ for females).

For adjusted apo AI, the total sample skewness (g_1) was .325. The hypothesis that the adjusted apo AI levels were sampled from a normally distributed population was rejected using the Lilliefors test ($P < .05$) (Conover 1971, pp. 302–306). For a trait whose distribution is skewed, consideration of a model with a single genetic factor that has a major effect on the trait and of polygenic loci as the model with unrestricted parameters may lead to the false inference that a single locus exists (MacLean et al. 1975). However, normalizing transformations of a biologically skewed trait can lead to a large reduction in the power to detect the pres-

Table 1

Commingling Analysis of Adjusted apo AI Levels in Total Sample of 1,880 Individuals in 283 Pedigrees

Parameters	No. of Components				
	Three		Two		One
μ_1	72.277		133.261		134.670
μ_2	133.243		179.700		...
μ_3	178.491		...		...
σ	17.917		18.300		19.957
f_1	.002		.970		1.000
f_2	.963		.030		...
f_3	.035		...		...
$\log_e$ L	-6,543.041		-6,545.907		-6,567.940
χ^2		1.732[a]		43.866[b,***]	
df		2		2	

[a] Contrast of three components vs. two components.
[b] Contrast of two components vs. one component.
*** Statistically significant at the .001 level of probability.

ence of a single locus with a major effect when one exists (MacLean et al. 1976). The inclusion of a model with a nontransmitted environmental factor having a major effect on the trait as an alternative explanation for the mixture of distributions reduces the possibility that skewness alone will lead to a false inference regarding the presence of a single locus with a major effect (Demenais et al. 1986). Because of these considerations, no normalizing transformation was applied to these data.

Table 2 presents the maximum likelihood estimates of the parameters under eight different models, as well as the associated χ^2 statistics for testing hypotheses about parameters restricted in the different models. In the unrestricted model (model 1 in table 2) all nine of the parameters and their standard errors are estimated. The five reduced models (models 4–8 in table 2) that did not include both a major factor and polygenic loci were each rejected when compared with the unrestricted model. Two models could not be rejected. One model included a nontransmitted environmental factor responsible for the mixture of distributions ($\tau_1 = \tau_2 = \tau_3 = p$) plus the effects of polygenic loci (model 2 in table 2; $\chi^2 = 4.85$, df = 3, $P > .05$). The other model included a single locus ($\tau_1 = 1.0, \tau_2 = .5, \tau_3 = .0$) plus polygenic loci (model 3 in table 2; $\chi^2 = 5.38$, df = 3, $P > .05$).

These two models with a major factor and polygenic loci were then considered to be two different complete models that could explain the data. When the model with a nontransmitted environmental factor plus polygenic loci (model 2) was compared with all the models with further restrictions (models 5, 7, and 8), the χ^2 statistics ranged from approximately 40 to 125 (see table 2). A model with a nontransmitted environmental factor and polygenic loci but with $\mu_{LL} = \mu_{LH}$ fitted the data as well as did model 2 in table 2 (maximum likelihood estimates were $p = \tau_1 = \tau_2 = \tau_3 = .816$, $\mu_{LL} = \mu_{LH} = 133.12$, $\mu_{HH} = 175.09$, $\sigma = 18.42$, $h^2 = .412$, $\log_e$ likelihood = -8,232.94, $\chi^2 = 0.06$, df = 1, $P > .05$). When the model with a single locus plus polygenic loci (model 3) was compared with all the models with further restrictions (models 6–8), the χ^2 statistics ranged from approximately 20 to 125 (see table 2). A model with a single locus and polygenic loci but with $\mu_{LL} = \mu_{LH}$ fitted the data as well as did model 3 in table 2 (maximum likelihood parameter estimates were $p = .829$, $\mu_{LL} = \mu_{LH} = 133.39$, $\mu_{HH} = 178.05$, $\sigma = 18.59$, $h^2 = .369$, $\log_e$ likelihood = -8,234.72, $\chi^2 = 3.08$, df = 1, $P > .05$).

The likelihood ratio criterion cannot be used for comparison between genetic and environmental explanations of the mixture of distributions because neither model represents a restriction of the other model. However, a ratio of two likelihoods can be used to identify the better-supported model without knowing the exact distribution of such a model-choice test statistic (Edwards 1972, p. 235). This approach has been used to search for etiologic heterogeneity by sorting pedigrees into groups that favor one model over another (Beaty

Table 2

Maximum Likelihood Parameter Estimates (±Standard Errors) and χ^2 Statistics for Total Sample of 1,880 Individuals in 283 Pedigrees

	Model							
	1	2	3	4	5	6	7	8
Parameter	General Transmission + Polygenes	Nontransmitted Factor + Polygenes	Single Locus + Polygenes	General Transmission Only	Nontransmitted Factor Only	Single Locus Only	Polygenes Only	No Single Factor, No Polygenes
p	.784 ± .09	.818	.851	.788	.827	.806	1.0	1.0
μ_{LL}	129.65 ± 1.3	134.66	131.06	127.47	133.41	127.97	134.61	134.67
μ_{LH}	143.55 ± 7.8	129.90	140.59	144.12	132.93	143.55	134.61	134.67
μ_{HH}	182.27 ± 10.	175.47	184.48	177.28	179.85	177.27	134.61	134.67
σ	17.46 ± .80	18.31	18.16	16.41	18.31	16.74	19.97	19.96
h^2	.345 ± .08	.418	.292	(.0)	(.0)	(.0)	.367	(.0)
τ_1	.922 ± .05	(p)	(1.0)	.976	p	(1.0)	. . .	. . .
τ_2	.734 ± .12	(p)	(.5)	.561	p	(.5)	. . .	. . .
τ_3	.262 ± .45	(p)	(.0)	.063	p	(.0)	. . .	. . .
Log_e L	−8,230.49	−8,232.91	−8,233.18	−8,242.87	−8,273.51	−8,243.36	−8,253.15	−8,295.54
χ^{2a}		4.85 (NS)	5.38 (NS)	24.76***	86.04***	25.74***	45.32***	130.1***
df		3	3	1	4	4	6	7
χ^{2b}					81.20***		40.48***	125.3***
df					1		3	4
χ^{2c}						20.36***	39.94***	124.7***
df						1	3	4

Note.—Parentheses denote that the value is fixed in the model. NS = not significant.

[a] Compared with unrestricted model (model 1).

[b] Compared with model 2.

[c] Comapred with model 3.

*** Statistically significant at the .001 level of probability.

1980; Beaty and Boughman 1986). Using the maximum likelihood parameter estimates, as obtained under models 2 and 3, from the entire sample, we computed $2\log_e (L_3/L_2)$ for each pedigree to assess support for model 3 relative to model 2. Here the likelihood, L_3, for model 3 is calculated for each individual pedigree on the basis of the observed apo AI levels, the relationships among individuals in the pedigree, and the model 3 maximum likelihood parameter estimates obtained from the entire sample of 283 pedigrees. For L_2, the model 2 maximum likelihood parameter estimates obtained from the entire sample of 283 pedigrees were used to calculate a likelihood for each individual pedigree.

The distribution of $2\log_e (L_3/L_2)$ for the 283 pedigrees is presented in figure 2. A value for $2\log_e (L_3/L_2)$ that is greater than zero for any pedigree indicates that the pedigree gives more support for a single-locus-plus-polygenic-loci etiology (model 3) than for a nontransmitted-environmental-factor-plus-polygenic-loci etiology (model 2). A value less than zero indicates that the pedigree gives more support for a nontransmitted-environmental-factor-plus-polygenic-loci etiology, as the explanation for the mixture of distributions. On the basis of this criterion, 126 pedigrees gave more support for the nontransmitted environmental factor and 157 pedigrees gave more support for the genetic etiology. The mean adjusted apo AI levels in the 1,071 individuals in the 157 pedigrees supporting the single-locus-plus-polygenic-loci etiology was 133.40 mg/dl (SD = 19.86 mg/dl). This was significantly lower ($P < .01$) than the mean adjusted level for the 809 individuals in the 126 pedigrees supporting the nontransmitted-environmental-factor-plus-polygenic-loci etiology (mean = 136.35 mg/dl, SD = 19.99). The variances in adjusted apo AI were not significantly different between these two groups of pedigrees.

From commingling analysis, the hypothesis that a mixture of two distributions fits the data as well as does a mixture of three distributions could not be rejected

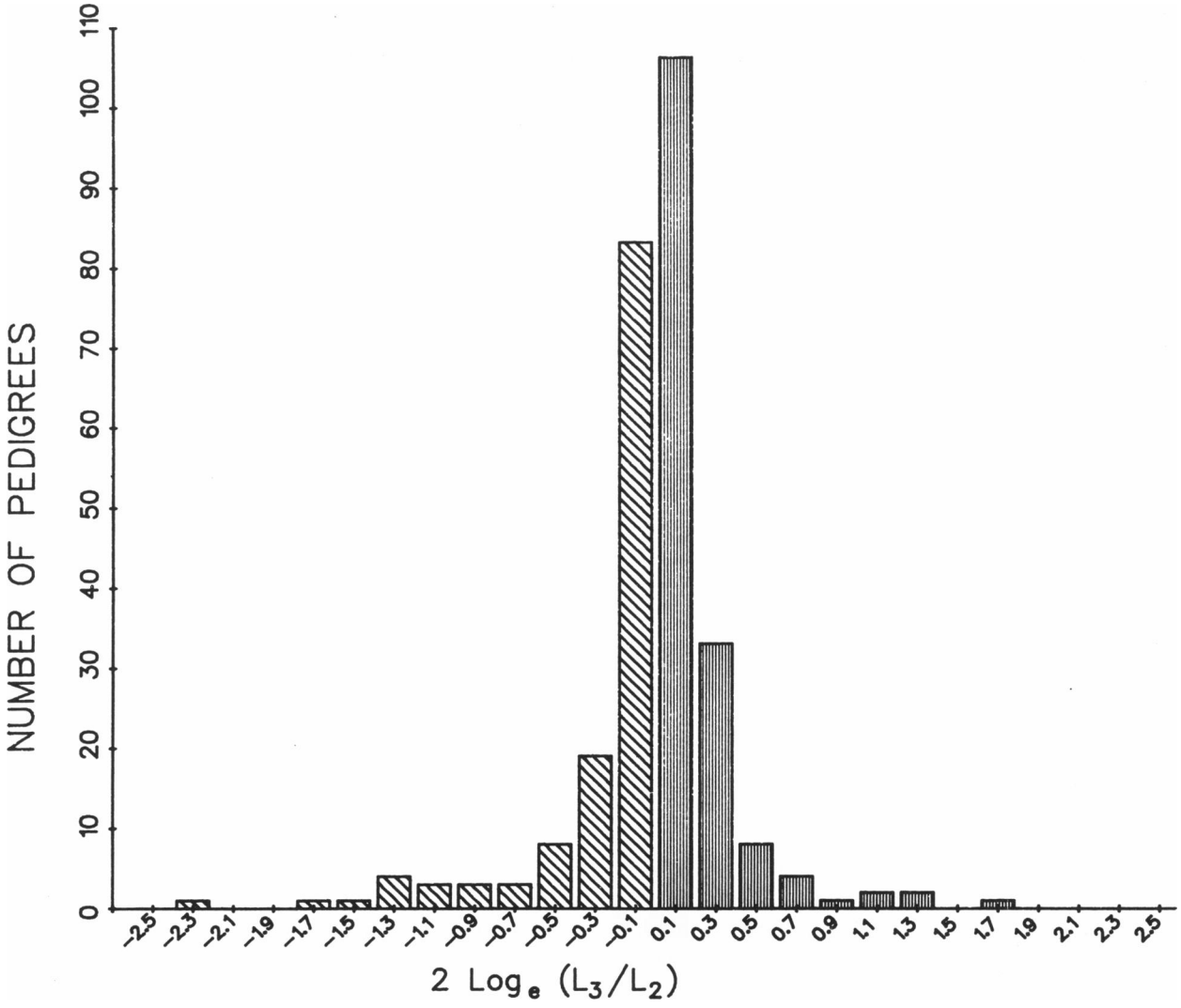

Figure 2 Distribution of $2\log_e(L_3/L_2)$ for the 283 pedigrees. The vertical stripe bars indicate more support for a single-locus-plus-polygenic-loci etiology than for a nontransmitted-environmental-factor-plus-polygenic-loci etiology. The hatched bars indicate more support for a nontransmitted-environmental-factor-plus-polygenic-loci etiology than for a single-locus-plus-polygenic-loci etiology. The likelihoods were computed using the parameter estimates from models 2 and 3 in table 2.

in either group of pedigrees (data not shown; χ^2 = 0.08, df = 2, $P > .05$ in the pedigrees supporting the single-locus etiology and χ^2 = 5.37, df = 2, $P > .05$ in the pedigrees supporting the nontransmitted-environmental-factor etiology). However, the hypothesis that a single normal distribution fits the data as well as does a mixture of two distributions was rejected in each group of pedigrees (data not shown; χ^2 = 41.63, df = 2, $P < .001$ in the pedigrees supporting the single-locus etiology and χ^2 = 10.49, df = 2, $P < .01$ in the pedigrees supporting the nontransmitted-environmental-factor etiology). On the basis of the maximum likelihood parameter estimates, the sample of 157 pedigrees supporting the genetic etiology predicts that 1.6% of the population from which these individuals were drawn falls in the upper-component distribution, with its mean at 193.1 mg/dl, and that 98.4% falls in the lower component, with its mean at 132.4 mg/dl. In the sample of 126 pedigrees supporting the nontransmitted environmental etiology, the maximum likelihood parameter estimates predict that 8% of the population from which these individuals were drawn falls in the

Table 3

Maximum Likelihood Parameter Estimates and χ^2 Statistics for the 809 Individuals in the 126 Pedigrees Supporting a Nontransmitted Environmental Etiology

	Model							
	1	2	3	4	5	6	7	8
Parameter	General Transmission + Polygenes	Nontransmitted Factor + Polygenes	Single Locus + Polygenes	General Transmission Only	Nontransmitted Factor Only	Single Locus Only	Polygenes Only	No Single Factor, No Polygenes
p	.354	.698	.700	.142	.720	.214	1.0	1.0
μ_{LL}	129.75	132.35	135.52	108.22	133.57	109.26	136.13	136.35
θ_{LH}	137.39	132.47	130.40	120.92	133.80	122.70	136.13	136.35
μ_{HH}	173.53	171.82	172.06	144.62	167.69	145.71	136.13	136.35
σ	16.22	16.56	16.82	15.87	17.75	15.63	19.95	19.98
h^2	.984	.952	.897	(.0)	(.0)	(.0)	.553	(.0)
τ_1	.648	(p)	(1.0)	[1.0]	p	(1.0)	. . .	. . .
τ_2	.740	(p)	(.5)	.576	p	(.5)	. . .	. . .
τ_3	.798	(p)	(.0)	.010	p	(.0)	. . .	. . .
Log_e L	−3,480.25	−3,481.12	−3,501.89	−3,530.43	−3,565.37	−3,530.82	−3,529.52	−3,570.61
χ^{2a}		1.74 (NS)	43.28***	100.36***	170.24***	101.1***	98.54***	180.7***
df		3	3	1	4	4	6	7

Note.—Parentheses denote that the value is fixed in the model; brackets denote that the value is at the boundary. NS = not significant.

[a] Compared with unrestricted model (model 1).

*** Statistically significant at the .001 level of probability.

upper-component distribution, with its mean at 167.5 mg/dl, and that 92% falls in the lower component, with its mean at 133.6 mg/dl.

To better define the possible etiologies for the mixture of distributions within each group of pedigrees, all of the models with restrictions on parameters (models 2–8) were compared with the unrestricted model (model 1) in each group separately. Table 3 presents the maximum likelihood parameter estimates and χ^2 statistics to test hypotheses about the restriction of parameters in the different models for the 126 pedigrees supporting the nontransmitted environmental etiology. All restricted models except the model with a nontransmitted environmental factor and polygenic loci (model 2) are rejected for this group of pedigrees. A model with a nontransmitted environmental factor and polygenic loci but with $\mu_{LL} = \mu_{LH} = 132.40$ mg/dl fitted the data as well as did model 2 in table 3 (data not shown; $\chi^2 = 0.25$, df = 1, $P > .05$). The maximum likelihood parameter estimates under model 2 predict that in the subpopulation from which these 126 pedigrees were sampled, the nontransmitted environmental factor explains 31.9%, polygenic loci explain 64.8%, and individual-specific environmental effects explain 3.3% of the adjusted phenotypic variance.

Table 4 presents the maximum likelihood parameter estimates and χ^2 statistics for the 157 pedigrees that support the single-locus etiology for the mixture of distributions. Under the unrestricted model (model 1) the estimate for τ_1 was 1.0, the estimate for τ_3 was .0, and the estimate for h^2 was .0. If we assume that these represent the maximum likelihood parameter estimates, then all of the models with restricted parameters were rejected except for the model with h^2 fixed at .0 (model 4), the model with a single locus (model 6), and the model with a single locus plus polygenic loci (model 3). Since model 6 has more restricted parameters than does either model 3 or model 4, model 6 can be compared with these other two models. Neither model 3 nor model 4 fitted the data significantly better than did model 6. Therefore, the effect of the polygenic loci in this sample of pedigrees is not judged to be different from zero. A model with a single locus but with $\mu_{LL} = \mu_{LH} = 131.38$ mg/dl did not fit the data as well as did the parameter estimates in model 6, in which $\mu_{LL} < \mu_{LH}$, in table 4 (data not shown; $\chi^2 = 18.68$, df = 1, $P < .001$). The parameter estimates for model 6 in table 4 predict that, in the population from which these pedigrees were sampled, 73.4% of the individuals have two copies of the L allele and have a mean adjusted

Table 4

Maximum Likelihood Parameter Estimates and χ^2 Statistics for the 1,071 Individuals in the 157 Pedigrees Supporting a Single-Locus Etiology

	Model							
	1	2	3	4	5	6	7	8
Parameter	General Transmission + Polygenes	Nontransmitted Factor + Polygenes	Single Locus + Polygenes	General Transmission Only	Nontransmitted Factor Only	Single Locus Only	Polygenes Only	No Single Factor, No Polygenes
p	.791	.894	.859	.791	.873	.857	1.0	1.0
μ_{LL}	128.37	132.61	128.59	128.37	132.37	128.47	133.35	133.40
μ_{LH}	144.06	132.62	143.50	144.06	132.61	143.58	133.35	133.40
μ_{HH}	188.96	196.11	189.43	188.96	193.20	189.20	133.35	133.40
σ	16.71	18.60	16.93	16.71	18.32	16.82	19.86	19.85
h^2	[.0]	.117	.003	(.0)	(.0)	(.0)	.196	(.0)
τ_1	[1.0]	(p)	(1.0)	[1.0]	(p)	(1.0)	. . .	. . .
τ_2	.610	(p)	(.5)	.610	(p)	(.5)	. . .	. . .
τ_3	[.0]	(p)	(.0)	[.0]	(p)	(.0)	. . .	. . .
$\log_e$ L	−4,676.80	−4,697.32	−4,676.92	−4,676.80	−4,699.07	−4,678.51	−4,712.51	−4,719.88
χ^{2a}		41.04***	.24 (NS)	.00 (NS)	44.54***	3.42 (NS)	71.42***	86.16***
df		3	3	1	4	4	6	7

NOTE.—Parentheses denote that the value is fixed in the model; brackets denote that the value is at the boundary. NS = not significant.

[a] Compared with unrestricted model (model 1).

*** Statistically significant at the .001 level of probability.

apo AI of 128.47 mg/dl, 24.5% of the individuals are heterozygotes and have mean levels of 143.58 mg/dl, and 2.1% of the individuals have two copies of the H allele and have mean levels of 189.02 mg/dl. Judged on the basis of the χ^2 statistics, the transmission of the major factor controlling apo AI levels does not appear to be different from the Mendelian expectation. In addition, in both model 1 and model 4 the estimate for τ_2 was .610, with a standard error of .054. The maximum likelihood estimate of τ_2 minus twice the standard error does include the value of $\tau_2 = .50$ given by the Mendelian model for transmission. The maximum likelihood parameter estimates for model 6 predict that, in the subpopulation from which these 157 pedigrees have been sampled, the single major locus explains 27.4% and individual-specific environmental effects explain the remaining 72.6% of the adjusted variance.

The distribution of $2\log_e(L_3/L_2)$ in figure 2 suggests that many of the pedigrees in our sample do not strongly favor one model over the other model, since many of the pedigrees have values close to zero. However, several pedigrees have values that are not close to zero, and these may represent the specific pedigrees with the strongest support for a single factor with a major effect on apo AI levels. Because there are pedigrees with extreme high and low values for $2\log_e(L_3/L_2)$, two explanations for the major effect are likely. The pedigrees with the extreme high values for $2\log_e(L_3/L_2)$ are more likely to be the pedigrees segregating at the single locus, and the pedigrees with the low values are more likely to be the pedigrees with the nontransmitted environmental factor. Since the likelihoods for the two competing models for a single factor were used to define the two subsets, a statistical test of heterogeneity among pedigrees in this sample is not appropriate. The approach used here does, however, identify in the population-based sample a subset of pedigrees in which there is strong evidence for segregation of a single locus with a major effect on apo AI levels.

Discussion

A single factor that could be either genetic or environmental and has a major effect on the phenotype, as well as polygenic loci, is required to explain variation in apo AI levels in this sample of 283 pedigrees randomly selected from the general population with respect to disease status and risk-factor levels. Among these pedigrees heterogeneity for both the transmission

of the major factor and the effect of polygenic loci was suggested by the data. In a subset of 126 pedigrees there is evidence for a nontransmitted environmental factor and polygenic loci, while in the other 157 pedigrees there is strong evidence only for a single-locus effect.

A first question raised by these data is, Why is the estimate of the contribution from polygenic loci different in the two subsets of pedigrees? The estimates of the effects of the polygenic loci are reflected in the estimates of the correlations among relatives. To estimate the correlations we identified a total of 263 spouse pairs among the parents of the children in the sample. For adjusted apo AI levels the product-moment correlation between these spouses was estimated to be .07 ($P > .05$) in the total sample, −.02 ($P > .05$) in the sample of pedigrees supporting the nontransmitted environmental factor, and .13 ($P > .05$) in the sample of pedigrees supporting the single locus. Correlations were also estimated between the parents and the oldest child seen at the clinic with the parents. The estimates of the mother-offspring and father-offspring correlations were .31 ($P < .01$) and .46 ($P < .01$) in the pedigrees supporting the nontransmitted environmental factor and .19 ($P < .05$) and .08 ($P > .05$), respectively, in the pedigrees supporting the single locus. Finally, the estimates of the correlation between the two oldest children seen at the clinic with their parents were .45 ($P < .01$) and .18 ($P < .05$) for the pedigrees supporting the nontransmitted environmental factor and the single-locus etiology, respectively. The father-offspring and sibling correlations were significantly higher ($P < .05$) in the pedigrees supporting the nontransmitted environmental factor than they were in the other pedigrees. Given the low spouse correlation and the significant correlations between parents and offspring and siblings in the group of pedigrees supporting the nontransmitted environmental factor, an estimate of a significant contribution from polygenic loci is not unexpected in this group of pedigrees. In the pedigrees supporting the single genetic locus as the major factor, the similarity in the correlations between spouses, between parents and offspring, and between siblings is consistent with the estimate that the contribution from polygenic loci is not different from zero in this group of pedigrees. The differences between the two groups of pedigrees in the estimates of correlations among relatives, as well as the differences in the contributions from polygenic loci, suggest that the possible heterogeneity in the etiology for the distribution of plasma apo AI within and among pedigrees may extend beyond a single factor with a large effect on apo AI levels.

Other differences between these two groups of pedigrees, in addition to the correlations between relatives, could be associated with the suggested heterogeneity in the single factor (genetic vs. nontransmitted environmental factor). However, there were no differences between these two groups of pedigrees for the distribution of any CAD risk factors—including age, gender, smoking status, body-mass index, blood pressure, and levels of cholesterol, triglycerides, HDL cholesterol, apo AII, and apo E—measured in the present study. The six non-Caucasians in the total sample were distributed equally between the two subsets of pedigrees.

The findings in the present study suggest that in the subset of 126 pedigrees one or more environmental factors influence apo AI levels. At least four environmental factors that influence apo AI levels (smoking, use of exogenous sex steroids, exercise, and alcohol) have been identified in other studies. However, individuals of different age and gender groups would not be expected to have the same opportunity for exposure to these specific four factors, and the model presented for the nontransmitted environmental factor here assumes that the factor has a similar distribution in both genders and in every generation.

Although nonsmokers are reported to have higher mean apo AI levels than do individuals who smoke (Haffner et al. 1985), nonsmoking is not likely to be the factor identified here, since there are no differences in adjusted apo AI levels between smokers and nonsmokers in this group of pedigrees. The factor identified here cannot be hormone use, since the adjustment of apo AI levels included hormone use. The reported frequency of alcohol use has been shown to have a significant positive association with apo AI levels in both males and females (Haffner et al. 1985), but this is an exposure that does not occur with equal frequency in males and females or in all age groups. Finally, endurance-trained young men—but not young women—have significantly higher levels of apo AI than do controls (Berg et al. 1986; Hartung et al. 1986). None of these four factors known to influence apo AI levels in some groups of individuals in the population is the likely explanation for the nontransmitted environmental factor observed here.

The individuals in these 126 pedigrees can be stratified into two groups: those individuals with higher levels of apo AI (greater than 175 mg/dl, i.e., 2 SD above the mean of the total sample) and those with lower levels of apo AI (less than 175 mg/dl). In the 126 pedigrees supporting the nontransmitted environmental etiology, except for the mean levels of HDL cholesterol and apo

AII no risk factors measured in the present study could distinguish, within the same pedigrees, those individuals with higher levels of apo AI from those individuals with lower apo AI levels.

In addition to the nontransmitted environmental factor, the present study also identified a segregating locus with a rare allele for high apo AI levels in the sample of 157 pedigrees. Comparisons between individuals with higher apo AI levels and individuals with lower apo AI levels were made to help identify the effect of the genetic factor in these pedigrees. When individuals with higher adjusted apo AI levels (greater than 175 mg/dl) were compared with the other individuals in these same pedigrees, those with the higher apo AI levels did have significantly higher mean levels of total cholesterol, HDL cholesterol, and apo AII (data not shown). This is not unexpected given the metabolic relationships between apo AI, apo AII, and HDL cholesterol.

Among the three previous studies in humans that considered the possibility of a single locus with a major effect on apo AI levels, the two studies that each included only a single large pedigree found no evidence for the effects of such a locus (Hasstedt et al. 1984; Amos et al. 1987). A possible explanation for the discrepancies in findings between our study and the study of others is that the polymorphic locus with a major effect identified in the present study was not segregating in either of the other two pedigrees. Another possible explanation for the conflicting results are the differences in the methods used to unmask the antigenic sites of apo AI. Previous studies have shown that 90% of the sites of apo AI are masked by lipids (Schonfeld and Pfleger 1974). The inclusion of Tween 20 in the assay of Maciejko and Mao (1982) used in the present study allows measurement of masked as well as of surface antigenic sites without altering the secondary structure of apo AI. The segregating locus identified in our study might be associated with such masked antigenic sites, which would not have been detected or might have been altered by the procedures used to measure apo AI in the other studies.

The statistical properties of the locus identified here differ from those estimated in the only other study in humans to report a single locus for apo AI (Moll et al. 1986). It reported a rarer allele for lower apo AI levels. Here we report a rarer allele for higher apo AI levels. While the same polyclonal assay for quantitative levels of plasma apo AI was used in both studies, the criteria for selection of the pedigrees were very different. In the earlier study, the 97 individuals were all adults in high-risk pedigrees who were being evaluated for coronary risk factors. In the present study of 283 pedigrees, the individuals ranged in age from 4.8 to 90 years and were selected at random with respect to risk-factor levels and disease status. Until other studies investigate the genetic basis for apo AI levels in randomly selected multigeneration pedigrees, there are no studies directly comparable to the present one.

A question raised by our finding is, What is the nature of the segregating locus identified in pedigrees selected from the general population? This locus may represent structural variation in the coding or noncoding sequences in the AI-CIII-AIV region on chromosome 11. Information about variation in the amino acid sequence for the structural locus for apo AI on chromosome 11 (Bruns et al. 1984; Cheung et al. 1984) comes from studies of electrophoretic variation. It is unlikely that the rarer allele identified here is one of the structural variants described elsewhere for apo AI (Franceschini et al. 1980; Utermann et al. 1982; Menzel et al. 1984). All are much rarer than the allele for higher apo AI levels identified here, and none are associated with an increase in either apo AI or HDL levels. Information about variability at the apo AI locus also comes from studies of the DNA. The locus identified here is also not the DNA rearrangement detected by Karathanasis et al. (1983, 1984), in which homozygotes had barely detectable levels of apo AI. However, recent studies using RFLPs have suggested the presence of variants that may be consistent with the genetic effects described here.

Several studies have investigated the association between RFLPs in the apo AI-CIII-AIV region and disease status or levels of HDL cholesterol (Deeb et al. 1986; Ordovas et al. 1986; reviews by Hegele and Breslow 1987; Wallace and Anderson 1987; Lusis 1988). One recent study considered RFLPs in this region and plasma levels of apo AI. Using RFLPs detected with the enzymes *Sst*I, *Pst*I, and *Xmn*I, Kessling et al. (1988) studied 109 unrelated, middle-aged men selected to include a wide range of HDL cholesterol concentrations. All three of these polymorphisms arise from sequence changes outside the coding regions of the apo AI, apo CIII, and apo AIV genes and therefore do not, in themselves, alter the amino acid sequence of any of the proteins. Kessling et al. (1988) reported that men with the rare allele of the *Pst*I RFLP had significantly higher apo AI levels than did men without the allele. In their sample, genetic variation at the *Pst*I RFLP site accounted for 6.5% of the interindividual variance in apo AI. When the RFLPs for *Sst*I and *Xmn*I were also considered, genetic variation defined by the five common

haplotypes accounted for 16% of the apo AI variation (Kessling et al. 1988). This measured-genotype strategy, which uses information about DNA markers for loci physiologically involved in the etiology of a quantitative trait of interest (Boerwinkle et al. 1986; Sing et al. 1988), suggests that at least three RFLPs can be used to distinguish gene variants associated with apo AI variability (Kessling et al. 1988). The study by Kessling et al. (1988), as well as other studies (Deeb et al. 1986; Ordovas et al. 1986) and recent reviews (Hegele and Breslow 1987; Wallace and Anderson 1987; Lusis 1988), suggest that common polymorphic genetic variations at several restriction-endonuclease sites in the region of the apo AI gene on chromosome 11 may be associated with variation both in risk for CAD and in quantitative levels of apo AI. The RFLPs in the apo AI-CIII-AIV region can be used to identify haplotypes that might be associated with the segregating single locus in some of the pedigrees in our study or with the polygenic loci segregating in other pedigrees.

The single-locus variability in our study could also be attributable to a mutation in the apo AI-CIII-AIV region that has not been identified. The inherited quantitative variation in these pedigrees could be one of the many mutations (up to one-third of all amino acid substitutions) that are still not detectable (Neel 1984). It is also possible that this locus is either a trans-acting factor in apo AI gene expression or another gene product involved in apo AI function.

Variation in genes involved in lipid metabolism that reside on other chromosomes may influence apo AI levels. The metabolism of the subfractions of HDL is closely related to the function of two endothelial lipolytic enzymes, lipoprotein lipase (LPL) and hepatic endothelial lipase (HL) (Nikkila et al. 1980). The gene for LPL resides on chromosome 8, while the gene for HL resides on chromosome 15 (Lusis 1988). One subfraction of HDL (HDL_2) is formed during catabolism of plasma triglyceride–rich lipoproteins by LPL, whereas its degradation is associated with the function of HL (Patsch et al 1978; Nikkila et al. 1982; Kuusi et al. 1987). In a recent study of unrelated individuals, HDL levels were significantly positively correlated with postheparin plasma LPL activity levels and significantly negatively correlated with HL activity levels (Kuusi et al. 1987). Kuusi et al. (1987) suggest that part of the genetic regulation of HDL and its subfraction distribution might be mediated through the activity of HL. Therefore, quantitative levels of apo AI might also be mediated through HL. Another locus, the structural locus for cholesterol ester transfer protein (CETP) on chromosome 16, may also be indirectly involved in determining quantitative levels of apo AI. CETP functions in the transfer of cholesterol esters among lipoproteins and is thought to play a role in regulation of plasma cholesterol homeostasis (Lusis 1988). The locus for CETP is close to the locus for lecithin:cholesterol acyltransferase, which also plays a role in esterification of plasma cholesterol. Recently, a family has been described with a deficiency of cholesterol ester transfer activity and high levels of both HDL and apo AI (Koizumi et al. 1985).

Our demonstration here of a single unmeasured polymorphic locus that explains 27% of the adjusted variation in apo AI levels in a subset of pedigrees selected from the general population suggests that a protein polymorphism may exist with effects on apo AI that are similar in magnitude to the effects of the variation, at the structural locus for apo E, on plasma levels of apo E. Recently, Boerwinkle and Utermann (1988) reported that the three common alleles, identified by amino acid substitutions, for the apo E gene on chromosome 19 account for 20% of the variability in plasma apo E. Even the common polymorphisms leave much of the variability for plasma levels of apo E unexplained. Variation at the apo E locus is known to influence the levels of low-density lipoprotein cholesterol and explains a large proportion of both the variation for serum cholesterol (approximately 7%) and the genetic variability for serum cholesterol (approximately 14%) (Sing and Davignon 1985; Boerwinkle and Sing 1987; Boerwinkle et al. 1987). Recently, it has been suggested that variation in the apo E gene locus may explain 2.8% of the variation in risk for CAD in the general population, through the effect of the locus on cholesterol levels (Davignon et al. 1988). With apo E as a paradigm, the next step with regard to apo AI is to establish the molecular basis for the single polymorphic locus suggested by our study to have a major effect on plasma levels of apo AI and then to relate that genetic variation to variation in HDL levels, total cholesterol levels, and the risk for CAD. The search will be made more challenging by the evidence, reported here, that in the general population there are multiple etiologies for high levels of plasma apo AI.

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