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Inheritance of Low-Density Lipoprotein Subclass Patterns: Results of Complex Segregation Analysis

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Summary

Heterogeneity in the size of low-density lipoprotein (LDL) particles was used to identify two distinct patterns based on gradient gel electrophoresis analysis. These two phenotypes, LDL subclass pattern A and pattern B, were characterized by a predominance of large, buoyant LDL particles and small, dense LDL particles, respectively. The inheritance of these LDL subclass patterns was investigated in a sample of 61 healthy families including 301 individuals. LDL subclass pattern B was present in 31% of the subjects, with the prevalence varying by gender, age, and (in women) menopausal status. Complex segregation analysis suggested a major locus controlling LDL subclass patterns. The model providing the best fit to the data included a dominant mode of inheritance with a frequency of .25 for the allele determining LDL subclass pattern B and reduced penetrance for men under age 20 and for premenopausal women. Thus, the allele for the LDL subclass pattern characterized by a predominance of small, dense LDL particles appears to be very common in the population, although not usually expressed until adulthood in men and until after menopause in women. The presence of a major gene controlling LDL subclasses could explain much of the familial aggregation of lipid and apolipoprotein levels and may be involved in increased risk of coronary heart disease.

Introduction

Elevated levels of low-density lipoprotein (LDL) cholesterol in plasma influence the development of atherosclerosis and coronary heart disease (Kannel et al. 1971; Lipid Research Clinics Program 1984; National Heart, Lung and Blood Institute Consensus Development Panel 1985; Brown and Goldstein 1986; Ross 1986). In contrast, increased levels of high-density lipoproteins (HDL) cholesterol are associated with reduced heart disease risk (Miller and Miller 1975; Castelli et al. 1977*b*, Pearson et al. 1979). Elevation of other lipoprotein and apolipoprotein measures, including plasma triglyceride, intermediate-density lipoproteins (IDL), and apolipoprotein (apo) B, have also been associated with increased coronary heart disease risk (Brunzell et al. 1984; Criqui et al. 1987; Krauss et al. 1987). In a recent report, we presented evidence that heterogeneity within the LDL range of particle size influences risk of myocardial infarction (Austin et al. 1988); that is, a lipoprotein profile characterized by a predominance of small, dense LDL subspecies (LDL subclass pattern B) was associated with a significantly increased risk of myocardial infarction.

It is well established that lipid levels aggregate in families (Sing and Orr 1978; Sosenko et al. 1980; Namboodiri et al. 1984). With the exception of relatively uncommon diseases such as familial hypercholesterolemia (Goldstein et al. 1973), however, the degree to which familial aggregation of lipids contributes to the clustering of coronary heart disease in families is unknown (Robertson 1981; Neufeld and Goldbourt 1983; Perkins 1986). In addition, it is not fully understood whether the familial clustering of either lipids or coronary heart disease is due to underlying genetic traits,

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to common environmental or behavioral traits among relatives, or to a combination of both (Segal et al. 1982; King et al. 1984). Results from twin studies support the hypothesis that a substantial proportion of the familial aggregation of lipids is genetic (Feinleib et al. 1977; Austin et al. 1987). Polymorphisms in the apo AI and apo B genes have been associated with coronary heart disease (Hegele et al. 1986; Ordovas et al. 1986). We have presented preliminary data indicating that LDL subclass patterns are also genetically controlled (Austin and Krauss, 1986). The present report uses complex segregation analysis to investigate the inheritance of LDL subclass patterns.

Material and Methods

Families

Sixty-one nuclear families in 29 kindreds were recruited for this study between 1984 and 1987. The kindreds ranged in size from six to 44 individuals, and a total of 301 individuals were screened. With one exception, all kindreds were Mormons and had at least one relative living in the San Francisco Bay area. Mormon families were selected for this study because carefully maintained genealogical records were available and because Mormon subjects generally do not smoke tobacco and do not drink alcohol or caffeine-containing beverages. Since these factors can alter lipid and lipoprotein levels (Castelli et al. 1977*a*; Goldbourt and Medalie 1977; Criqui et al. 1980; Ernst et al. 1980; Williams et al. 1985), there were fewer confounders in the genetic analysis of the data. The one non-Mormon kindred was large ($n = 28$) and informative. Since the segregation of LDL subclass patterns in this kindred was not different from that in the Mormon kindreds, it was included in the analysis.

Families were not selected for history of cardiovascular disease or lipid disorders, although extended kindreds were sequentially sampled when matings informative for LDL subclass patterns A and B were found (Cannings and Thompson 1977). Family members who were not pregnant, had no serious diseases, and were at least six years of age were eligible for the study. Among the eligible relatives, the response rate was 93%. All participants gave signed, informed consent. Blood samples were obtained from each subject after an overnight fast. For nonlocal relatives, samples were delivered to Donner Laboratory by overnight mail. In addition, each subject completed a medical interview.

Analysis of LDL Subclass Patterns

Heterogeneity within the major lipoprotein classes—very-low-density lipoproteins (VLDL), LDL, and HDL—is well recognized. In our laboratory, multiple, discrete subclasses of LDL particles have been identified and characterized using analytic ultracentrifugation, density gradient ultracentrifugation, and gradient gel electrophoresis (Lindgren et al. 1972; Shen et al. 1981; Krauss and Burke 1982). For this analysis, nondenaturing polyacrylamide gradient gel electrophoresis of whole plasma and the $d < 1.063$ plasma fraction was performed on 2%–16% gels according to a method described elsewhere (Krauss and Burke 1982; Nichols et al. 1986). On the basis of this technique, two distinct LDL subclass patterns have been identified, denoted pattern A and pattern B. Pattern A is characterized by a major peak of large, buoyant LDL particles and a minor peak of smaller, denser LDL. In contrast, pattern B has a major peak of small, dense LDL with a skewing of the curve toward the larger particle diameters. Thus, it is the distribution of LDL particles by size *within* the LDL density range that distinguishes these patterns. In general, the peak particle diameter determined from gradient gel electrophoresis is greater than 255 Å for pattern A and 255 Å or less for pattern B. In the present study, 87% of subjects could be classified into one of these two patterns. The remaining 13% of subjects had an intermediate pattern, with some of the characteristics of both patterns A and B. The mean peak particle diameters for A, intermediate, and B patterns were 266 Å, 259 Å, and 248 Å, respectively.

A family including members with each of these LDL subclass patterns is shown in figure 1. The mother has LDL subclass pattern A (peak particle diameter 266 Å), as do three of the offspring (peak particle diameters 268, 270, and 268 Å). The father has LDL subclass pattern B (peak particle diameter 248 Å). Two of the offspring have intermediate patterns (peak particle diameters 257 and 259 Å. These two peak particle diameters are slightly greater than 255 Å, but the distribution of LDL particle sizes resembles that of pattern B. Segregation analysis was carried out by classifying subjects with the intermediate pattern as either pattern B or pattern A. These classifications will be termed "broad" and "narrow" definitions of pattern B, respectively.

Segregation Analysis

The segregation of LDL subclass patterns in the families was investigated using the mixed model with

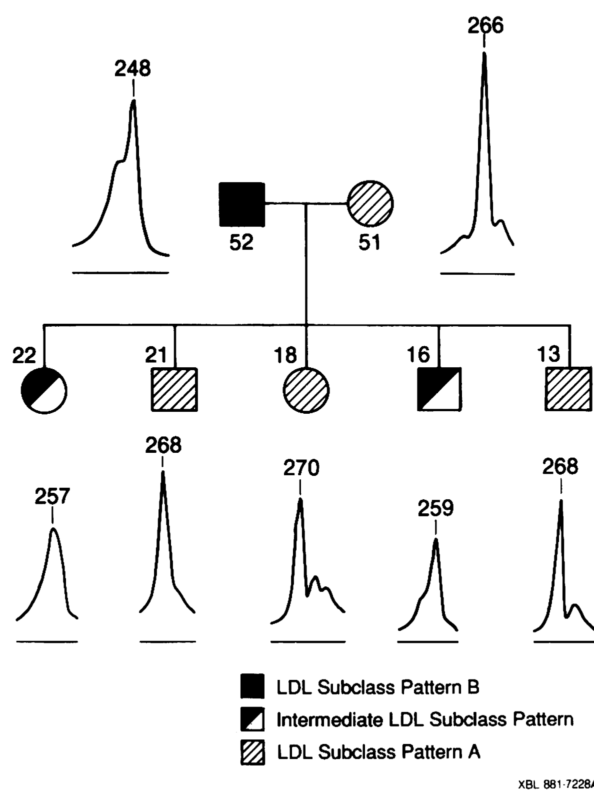

Figure 1 Pedigree of family 01, showing LDL subclass pattern B (solid symbols), LDL subclass pattern A (striped symbols), and the intermediate LDL subclass pattern (half-filled symbol). For each individual, the age is given and the LDL subclass pattern from gradient gel electrophoresis is shown with the peak particle diameter expressed in angstrom units.

pointers (Lalouel et al. 1983). The unit of analysis is the nuclear family, and "pointers" are used to include individuals outside the nuclear family that lead to its ascertainment (Lalouel and Morton 1981). The mixed model assumes an underlying continuous liability for the trait under study. It incorporates a major locus with two alleles, multifactorial (polygenic or cultural) inheritance, and environmental effects. These effects are assumed to be additive on the liability scale, so that individuals above a threshold value express the trait (Morton and MacLean 1974). In addition, discrete liability classes can be defined with the probability of expressing the trait depending on age, gender, or other determinants (Morton et al. 1983). The parameters of the mixed model are multifactorial (polygenic or cultural) inheritance (H), dominance of the major locus (D), difference in means of the liability distributions for the homozygous genotypes, expressed in SD units (T), and frequency of the susceptibility allele for the trait (Q). Transmission probabilities—$T1$, $T2$, and $T3$—are also incorporated into the model, and $T2$ is used to test for departure from Mendelian inheritance (Elston and Stewart 1971). Maximum likelihood estimation is used, and likelihood ratio tests compare nested models by using χ^2 statistics. This analysis was based on likelihood values conditional on the phenotypes of parents and pointers (Morton 1982). All calculations were performed using the computer program POINTER (Morton et al. 1983).

Results

Liability classes were defined for the segregation analysis on the basis of the observed distribution of LDL subclass patterns by gender, age, and (in women) hormonal status. The distribution of patterns A and B in the sample of relatives, when the broad definition is used, is shown in table 1. The overall prevalence of LDL subclass pattern B was 31%. The prevalence differed by gender: it was 37% among males and 25% among females. For males in the sample, the prevalence also differed considerably by age: under age 20 years it was 17%, and over age 20 years it was 44%. Even larger differences were seen among females when considered in terms of menopausal status. The prevalence of LDL subclass pattern B was 13% among premenopausal women and 49% among postmenopausal women (including women who reported having had a hysterectomy). Among the postmenopausal women, the prevalence of pattern B did not differ by hormone use. For the segregation analysis, the liability classes consisted of two age groups in males—ages 6–19 year and ages 20 and over—and of two hormonal status groups in women—premenopausal and postmenopausal. Two additional classes were defined for deceased or nonsampled family members on the basis of the overall rates for males and females. The frequency of the intermediate LDL subclass pattern in these groups ranged from 8% in premenopausal women to 19% in males age 20 years and over, but no trends by age or gender were observed. The prevalence of LDL subclass pattern B in each liability class was entered into the POINTER program along with family data.

An example of an informative kindred for the segregation of LDL subclass patterns A and B is shown in figure 2. In this family, LDL subclass pattern B appeared in 3 generations. Of the four siblings sampled in generation II, two had pattern B, one had the intermediate pattern, and one had pattern A. Despite the relatively

Table 1

Distributions of LDL Subclass Patterns[a] by Gender, Age, and Hormone Status

Group	LDL Subclass Pattern A (%)	LDL Subclass Pattern B (%)	Total
Males:			
Age 6–19 years	29 (82.9)	6 (17.1)	35
Age ≥20	63 (56.3)	49 (43.7)	112
Total	92 (62.6)	55 (37.4)	147
Females:			
Premenopausal	90 (87.4)	13 (12.6)	103
Postmenopausal or hysterectomy	26 (51.0)	25 (49.0)	51
Total	116 (75.3)	38 (24.7)	154
All subjects	208 (69.1)	93 (30.9)	301

[a] The broad definition of LDL subclass pattern B was used; that is, subjects with an intermediate LDL subclass pattern are grouped with subjects having pattern B.

low prevalence of pattern B among young males (table 1), an 8-year-old in generation III had the trait.

The observed segregation ratios for patterns A and B among the 49 nuclear families with both parents sampled, when the broad definition of pattern B is used, are summarized in table 2*A*. Of the 41 offspring of 14 A×A matings, all had LDL subclass pattern A. Among the 94 offspring of the A×B matings, 70% had pattern A and 30% had pattern B. Similarly, among the 20 offspring of B×B matings, 65% had pattern A and 35% had pattern B. In table 2*B* are given the observed segregation ratios from the 22 nuclear families in which at least one child had LDL subclass pattern B.

The results of the complex segregation analysis, when the broad definition of pattern B was used, are given in table 3. For each model, the maximum likelihood estimates of parameters *H*, *T2*, *D*, *T*, and *Q* and the value of $-2 \ln L + C$ (where *L* is the likelihood) are given and χ^2 tests comparing appropriate models are shown. Parameter values in parentheses were not iterated. The lower the value of $-2 \ln L + C$, the greater the likelihood and the better the fit of the model. Probability values are given uncorrected for multiple comparisons.

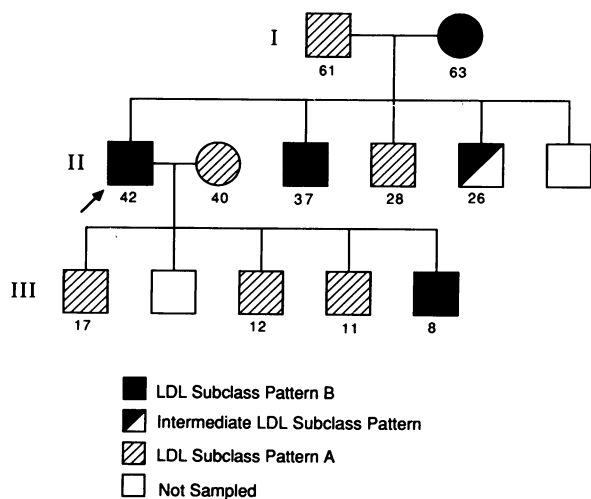

Figure 2 Pedigree of family 17, showing presence of LDL subclass pattern B in each of 3 generations (solid symbols). The age of each sampled relative is also shown.

Table 2

Observed Segregation Ratios of LDL Subclass Patterns A and B

Mating Type	No. of Matings	No. of Offspring: Pattern A	Pattern B	Total
A. In 49 Nuclear Families				
A×A	14	41 (1.00)	0 (.00)	41 (1.00)
A×B	27	66 (.70)	28 (.30)	94 (1.00)
B×B	8	13 (.65)	7 (.35)	20 (1.00)
B. Among Families With at Least One Pattern B Offspring				
A×B	16	36 (.56)	28 (.44)	64 (1.00)
B×B	6	6 (.46)	7 (.54)	13 (1.00)

Table 3

Results of Complex Segregation Analysis Based on Broad Definition of LDL Subclass Pattern B

Model	H	$T2$	D	T	Q	$-2 \ln L + C$	Comparison of Models	χ^2 (df)
1. Unrestricted	.01	.63	.62	2.68	.22	141.88		
2. No inheritance of susceptibility	(.0)	...	...	...	(.0)	184.51	2 vs. 1	42.63 (2)***
3. Multifactorial inheritance only	.29	(.0)	(.0)	(.0)	(.0)	163.81	3 vs. 1	21.93 (4)***
4. No multifactorial component	(.0)	.58	1.00	1.95	.25	143.05	4 vs. 1	1.17 (1)
5. General single locus	(.0)	(.5)	.93	2.20	.29	144.22	5 vs. 4	1.17 (1)
6. Dominant single locus	(.0)	(.5)	(1.0)	2.12	.25	144.45	6 vs. 5	0.23 (1)
7. Additive single locus	(.0)	(.5)	(.5)	3.30	.23	147.35	7 vs. 5	3.13 (1)†
8. Recessive single locus	(.0)	(.5)	(.0)	1.97	.71	147.78	8 vs. 5	3.56 (1)†

† $.05 < P < .10$.
*** $P < .001$.

Hypotheses of no inheritance of pattern B (model 2) and multifactorial inheritance only (model 3) were rejected in comparison with the unrestricted model (model 1). A major gene model (model 4) fit the data very well, and $T2$ did not vary significantly from 0.5 (model 5 vs. model 4). The single-locus dominant model (model 6) was consistent with the general single-locus model (model 5). Neither the additive nor the recessive single-locus models (models 7 and 8, respectively) fit the data as well as did the dominant model (model 6). The differences in likelihoods of the additive and recessive models compared with the general single-locus model were of borderline significance ($.05 < P < .10$). Of the single-locus models in table 3, the best likelihood value was provided by the dominant model (model 6) with $T = 2.12$ and $Q = .25$.

On the basis of model 6, the penetrance of pattern B for genotypes BB and AB was .39 for males younger than age 20 years and increased to 1.00 for males age 20 years and over. Pattern B was not expected among genotype AA males, regardless of age. For females of genotypes BB and AB, penetrance values of pattern B were .30 before menopause and 1.00 after menopause. Among postmenopausal women with genotype AA, the prevalence of pattern B was .11, indicating that pattern B may occasionally be present in the absence of the pattern B allele in this group of women. Full penetrance of pattern B was thus only observed in males ages 20 years and over and in postmenopausal females. Among subjects in these groups with pattern B, the estimated frequencies for genotypes BB, AB, and AA were .14, .86, and .00, respectively, for adult males and .13, .75, and .12 for postmenopausal females. Most subjects with LDL subclass pattern B are, then, expected to have a heterozygous AB genotype.

The complex segregation analysis was repeated using the narrow definition of pattern B (table 4). The maximum likelihood model was obtained by iterating major gene parameters with $H = .00$ (model 1). Models with H values varying from .01 to .5 were considered, but none provided a better fit to the data than model 1. Hypotheses of no inheritance (model 2) and multifactorial inheritance only (model 3) were again rejected. A general single-locus model (model 4) fit the data well, and $T2$ did not differ significantly from .5 (model 4 vs. model 1). Dominant major gene inheritance (model 5) had a likelihood identical to that of the general single-locus model (model 4). However, the additive and recessive models (models 6 and 7, respectively) could not be rejected using this definition. The penetrance values for model 5 were generally lower than penetrances based on the broad definition of pattern B. Specifically, the penetrance values for genotypes BB and AB, when the narrow definition was used, were .11 for premenopausal women, .91 for postmenopausal women, .02 for males under age 20 years, and .57 for males age 20 years and over. Prevalence of pattern B in individuals with genotype AA was zero for all liability classes.

Thus, when both the broad and narrow definitions of pattern B were used, a dominant, single-locus model accurately represented the data from this sample of families. On the basis of these models, the parameter values for the distance between homozygous means (T) and the allele frequency (Q) were nearly identical when both the broad and narrow definitions of pattern B were used ($T = 2$; $Q = .25$). The primary difference between

Table 4

Results of Complex Segregation Analysis Based on Narrow Definition of LDL Subclass Pattern B

Model	H	$T2$	D	T	Q	$-2 \ln L + C$	Comparison of Models	χ^2 (df)
1. Maximum likelihood model	.00	.60	1.00	2.01	.25	93.08		
2. No inheritance of susceptibility	(.0)	...	...	...	(.0)	107.40	2 vs. 1	14.32 (2)***
3. Multifactorial inheritance only	.28	(.0)	(.0)	(.0)	(.0)	102.66	3 vs. 1	9.58 (4)*
4. General single locus	(.0)	(.5)	1.00	1.94	.25	93.82	4 vs. 1	.74 (1)
5. Dominant single locus	(.0)	(.5)	(1.0)	1.94	.25	93.82	5 vs. 4	.00 (1)
6. Additive single locus	(.0)	(.5)	(.5)	3.05	.69	94.80	6 vs. 4	.98 (1)
7. Recessive single locus	(.0)	(.5)	(.0)	1.96	.69	94.80	7 vs. 4	.98 (1)

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

the models based on different definitions was the lower penetrance values observed when the narrow definition was used.

Discussion

In this sample of healthy kindreds, complex segregation analysis suggests that the LDL subclass pattern characterized by a predominance of small, dense LDL particles (pattern B) is controlled by a single major locus. The model providing the best fit to the data included a dominant mode of inheritance, and the allele for pattern B was quite common ($Q = .25$). A recessive mode of inheritance could not be rejected at the $\alpha = .05$ level, however, with an estimated allele frequency of $Q = .70$. On the basis of the dominant model and with the broad definition of pattern B, full penetrance was observed in adult males and postmenopausal women, while penetrance was low in young males and premenopausal females. When the allele frequency estimate of .25 from the dominant model is used and Hardy-Weinberg equilibrium is assumed, the proportion of individuals homozygous for the pattern B allele is estimated to be 6% and the proportion of heterozygotes is 38%. Thus, among males age 20 years and over and among postmenopausal women, 44% of the population can be expected to express the pattern B trait. When the narrow definition is used, penetrance in these two groups is reduced to .57 and .91, respectively. This suggests that the broad definition, classifying intermediate LDL subclass pattern as pattern B, may be the more appropriate definition.

Genetic influence on LDL size has been reported elsewhere (Hammond and Fisher 1971; Fisher et al. 1975). In five families, the molecular weight of "monodisperse" LDL appeared to be inherited, possibly reflecting the same trait reported here. A high frequency of small, dense LDL has also been observed among patients with familial combined hyperlipidemia (Krauss et al. 1983). This disorder is characterized by the presence of elevated cholesterol and/or triglyceride among family members and by increased risk of coronary heart disease (Goldstein et al. 1973; Brunzell et al. 1983). A dominant model of inheritance for familial combined hyperlipidemia was initially proposed (Goldstein et al. 1973), but this has not yet been established. It is possible that the gene controlling LDL subclass patterns may also be involved in the development of familial combined hyperlipidemia.

Many other studies, using a variety of approaches and analytic techniques, have considered potential genetic influences on total and LDL cholesterol levels (Segal et al. 1982). Family studies have generally confirmed that total cholesterol and LDL cholesterol levels are significantly correlated among first-degree relatives but are not strongly correlated between spouses (Sing and Orr 1978; Garrison et al. 1979; Sosenko et al. 1980; Namboodiri et al. 1984). Path analyses have revealed high heritability for LDL cholesterol ($h^2 = .62$) (Rao et al. 1979). Twin studies have reported a heritability of .57 for LDL cholesterol in men (Feinleib et al. 1977) and higher heritability ($h^2 = .91$) in women, even after adjusting for shared environmental influences between cotwins (Austin et al. 1987). The best-understood genetic abnormality responsible for hypercholesterolemia is the LDL receptor defect in familial hypercholesterolemia (Brown and Goldstein 1986). However, this disease is relatively uncommon and can-

not account for most of the variation in LDL cholesterol levels found in the general population.

Genetic influences on plasma levels of apo B (the primary protein on LDL particles) are less well understood. A recent investigation in Utah reported evidence for a single major gene controlling elevated plasma apo B levels (Hasstedt et al. 1987), and twin studies also suggest significant heritability (Berg 1983). Variation at the apo B locus, as reflected by DNA polymorphisms, has been associated with increased risk of myocardial infarction (Hegele et al. 1986) and with increases in plasma triglyceride levels (Law et al. 1986). The Ag(x) allele of the immunologically determined Ag marker system of apo B has also been related to increases in both total cholesterol and triglyceride levels (Berg et al. 1976). Recently, a disorder designated familial defective apo B-100 has been identified, in which hypercholesterolemia is due to LDL with abnormal receptor binding (Innerarity et al. 1987). This disorder is most likely due to a structural defect in the apo B-100 on LDL particles.

On the basis of the single-locus dominant model, the reduced penetrance in premenopausal females and young males could indicate that environmental, behavioral, and/or genetic background may modify the expression of LDL subclass pattern B. In a small study, we found that patterns A and B were stable for a year in a free-living sample of eight adults without dietary intervention or drug treatment (R. M. Krauss, M. A. Austin, and W. L. Fitch, unpublished data). However, data from the present study and one other (McNamara et al. 1987) suggest that age, gender, and hormonal status are important. It has also been reported that the use of antihypertensive medication, particularly beta-blockers, may influence the size of LDL particles (Schaefer et al. 1987) and that the ratio of abdominal to hip girth is associated with small LDL mass (Terry et al. 1985).

The gene apparently controlling LDL subclass patterns may have pleiotropic effects, and from the present study alone it is not possible to determine the primary effect of this locus. We have demonstrated that LDL subclass pattern B is associated with relative increases in plasma triglyceride, IDL mass, and apo B levels and with decreases in HDL cholesterol, HDL_2 mass, and apo AI (Austin and Krauss 1986; Austin et al. 1988). These results are comparable with previous reports of interrelationships among lipoprotein subclasses (Krauss et al. 1980, 1988). Thus it is possible that the production of small, dense LDL subclass pattern B is one step in a complex pathway that also involves the metabolism of VLDL and HDL (Deckelbaum et al. 1984; Eisenberg et al. 1984; Krauss 1987). In addition, we have shown that LDL subclass pattern B is associated with increased risk of myocardial infarction (Austin et al. 1988). It will be important to determine whether this increased risk is due directly to the presence of small LDL particles or is a consequence of one or more of the other lipoprotein variations found in association with this trait. Regardless of the mechanisms involved, the present results suggest that a single genetic locus is responsible for this common trait that may predispose to coronary heart disease.

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References

Austin, M. A., J. L. Breslow, C. H. Hennekens, J. E. Buring, W. C. Willett, and R. M. Krauss. 1988. Low density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 260:1917–1921.

Austin, M. A., M.-C. King, R. D. Bawol, S. B. Hulley, and G. D. Friedman. 1987. Risk factors for coronary heart disease in adult female twins: genetic heritability and shared environmental effects. Am. J. Epidemiol. 125:308–318.

Austin, M. A., and R. M. Krauss. 1986. Genetic control of low density lipoprotein subclasses. Lancet 2:592–595.

Berg, K. 1983. Genetics of coronary heart disease. Pp. 35–90 *in* A. G. Steinberg, A. G. Bearn, A. R. Motulsky, and B. Childs, eds. Progress in medical genetics. W. B. Saunders, Philadelphia.

Berg, K., C. Hames, G. Dahlen, M. H. Frick, and I. Krishan. 1976. Genetic variation in serum low density lipoproteins and lipid levels in man. Proc. Natl. Acad. Sci. USA 73:937–940.

Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. Science 232:34–47.

Brunzell, J. D., J. J. Albers, A. Chait, S. M. Grundy, E. Groszek, and G. B. McDonald. 1983. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. J. Lipid Res. 4:614–624.

Brunzell, J. D., A. D. Sniderman, J. J. Albers, and P. O. Kwiterovich. 1984. Apoprotein B and A-I and coronary artery disease in humans. Arteriosclerosis 4:79–83.

Cannings, C., and E. A. Thompson. 1977. Ascertainment in the sequential sampling of pedigrees. Clin. Genet. 12:208–212.

Castelli, W. P., J. T. Doyle, T. Gordon, C. G. Hames, M. C. Hjortland, S. B. Hulley, A. Kagan, and W. J. Zukel. 1977*a*. Alcohol and blood lipids: the Cooperative Lipoprotein Phenotyping Study. Lancet 2:153–155.

———. 1977*b*. HDL cholesterol and other lipids in coronary heart disease: the Cooperative Lipoprotein Phenotyping Study. Circulation 55:767–772.

Criqui, M. H., G. Heiss, R. Cohn, C. M. Suchindran, and S. Bangeliwala. 1987. Triglycerides and coronary heart disease mortality: the Lipid Research Clinics Follow-up Study. Paper presented at the American Heart Association 27th Conference on Cardiovascular Disease Epidemiology, Charleston, March 19–20.

Criqui, M. H., R. B. Wallace, G. Heiss, M. Mishkel, G. Schonfeld, and G. T. L. Jones. 1980. Cigarette smoking and plasma high-density lipoprotein cholesterol: the Lipid Research Clinics Program Prevalence Study. Circulation 62:70–76.

Deckelbaum, R. J., E. Granot, Y. Oschry, L. Rose, and S. Eisenberg. 1984. Plasma triglyceride determines structure-composition in low and high density lipoproteins. Arteriosclerosis 4:225–231.

Eisenberg, S., D. Gavish, Y. Oschry, M. Fainaru, and R. J. Deckelbaum. 1984. Abnormalities in very low, low, and high density lipoproteins in hypertriglyceridemia: reversal toward normal with bezafibrate treatment. J. Clin. Invest. 74:470–482.

Elston, R. C., and J. Stewart. 1971. A general method for the genetic analysis of pedigree data. Hum. Hered. 21: 523–542.

Ernst, N., M. Fisher, W. Smith, T. Gordon, B. M. Rifkind, J. A. Little, M. A. Mishkel, and D. O. Williams. 1980. The association of plasma high-density lipoprotein cholesterol with dietary intake and alcohol consumption: the Lipid Research Clinics Program Prevalence Study. Circulation 62:41–52.

Feinleib, M., R. J. Garrison, R. Fabsitz, J. C. Christian, Z. Hrube, N. O. Borhani, W. B. Kannel, R. Rosenman, J. T. Schwartz, and J. O. Wagner. 1977. The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. Am. J. Epidemiol. 106:284–295.

Fisher, W. R., M. G. Hammond, M. C. Mengel, and G. L. Warmke. 1975. A genetic determinant of the phenotypic variance of the molecular weight of low density lipoprotein. Proc. Natl. Acad. Sci. USA 72:2347–2351.

Garrison, R. J., W. P. Castelli, M. Feinleib, W. B. Kannel, R. F. Havlick, S. J. Padgett, and P. M. McNamara. 1979. The association of total cholesterol, triglycerides and plasma lipoprotein cholesterol levels in first degree relatives and spouse pairs. Am. J. Epidemiol. 110:313–321.

Goldbourt, V., and J. H. Medalie. 1977. Characteristics of smokers, non-smokers, and ex-smokers among 10,000 adult males in Israel. II. Physiologic, biochemical, and genetic characteristics. Am. J. Epidemiol. 105:75–86.

Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J. Clin. Invest. 52:1544–1568.

Hammond, M. G., and W. R. Fisher. 1971. The characterization of a discrete series of low density lipoproteins in the disease, hyper-pre-β-lipoproteinemia. J. Biol. Chem. 246: 5454–5465.

Hasstedt, S. J., L. Wu, and R. R. Williams. 1987. Major locus inheritance of apolipoprotein B in Utah Pedigrees. Genet. Epidemiol. 4:67–76.

Hegele, R. A., L.-S. Huang, P. N. Herbert, C. B. Blum, J. E. Buring, C. H. Hennekens, and J. L. Breslow. 1986. Apolipoprotein B-gene DNA polymorphisms associated with myocardial infarction. N. Engl. J. Med. 315: 1509–1515.

Innerarity, T. L., K. H. Weisgraber, K. S. Arnold, R. W. Mahley, R. M. Krauss, G. L. Vega, and S. M. Grundy. 1987. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. Proc. Natl. Acad. Sci. USA 84:6919–6923.

Kannel, W. B., W. P. Castelli, and T. Gordon. 1971. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. Ann. Intern. Med. 74:1–12.

King, M.-C., G. M. Lee, N. B. Spinner, G. Thomson, and M. R. Wrensch. 1984. Genetic epidemiology. Annu. Rev. Public Health 5:1–52.

Krauss, R. M. 1987. Relationship of intermediate and low-density lipoprotein subspecies to risk of coronary artery disease. Am. Heart J. 113:578–582.

Krauss, R. M., J. J. Albers, and J. D. Brunzell. 1983. An apolipoprotein B-enriched low density lipoprotein subspecies in familial combined hyperlipidemia. Clin. Res. 31:503a.

Krauss, R. M., and D. J. Burke. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J. Lipid Res. 23:97–104.

Krauss, R. M., F. T. Lindgren, and R. M. Ray. 1980. Interrelationships among subgroups of serum lipoproteins in normal human subjects. Clin. Chim. Acta 104:275–290.

Krauss, R. M., F. T. Lindgren, P. T. Williams, S. F. Kelsey, J. Brensike, K. Vranizan, K. M. Detre, and R. I. Levy. 1987. Intermediate density lipoproteins and progression of coronary artery disease in hypercholesterolemic men. Lancet 2:62–66.

Krauss, R. M., P. T. Williams, F. T. Lindgren, and P. D. Wood. 1988. Coordinate changes in levels of human serum low and high density lipoprotein subclasses in healthy men. Arteriosclerosis 8:155–162.

Lalouel, J. M., and N. E. Morton. 1981. Complex segregation analysis with pointers. Hum. Hered. 31:312–321.

Lalouel, J. M., D. C. Rao, N. E. Morton, and R. C. Elston. 1983. A unified model for complex segregation analysis. Am. J. Hum. Genet. **35**:816–826.

Law, A., S. C. Wallis, L. M. Powell, R. J. Pease, H. Brunt, L. M. Priestley, T. J. Knott, J. Scott, D. G. Altman, G. J. Miller, J. Rajput, and N. E. Miller. 1986. Common DNA polymorphism within coding sequence of apolipoprotein B gene associated with altered lipid levels. Lancet **1**: 1301–1303.

Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. Pp. 181–274 *in* G. J. Nelson, ed. Blood lipids and lipoproteins. Wiley Interscience, New York.

Lipid Research Clinics Program. 1984. The Lipid Research Clinics Coronary Primary Prevention Trial. I. Reduction in incidence of coronary heart disease. JAMA **251**:351–364.

McNamara, J. R., H. Campos, J. M. Ordovas, J. Peterson, P. D. F. Wilson, and E. J. Schaefer. 1987. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution: results from the Framingham Offspring Study. Arteriosclerosis **7**:483–490.

Miller, C. J., and N. E. Miller. 1975. Plasma high density lipoprotein concentrations and development of ischemic heart disease. Lancet **1**:16–19.

Morton, N. E. 1982. Segregation analysis of familial data. Pp. 69–87 *in* M. A. Klingberg, ed. Outline of genetic epidemiology. Karger, Basel.

Morton, N. E., and C. S. MacLean. 1974. Analysis of family resemblance. III. Complex segregation of quantitative traits. Am. J. Hum. Genet. **26**:489–503.

Morton, N. E., D. C. Rao, and J.-M. Lalouel. 1983. Methods in genetic epidemiology. Pp. 62–102 *in* M. A. Klingberg, ed. Contributions to epidemiology and biostatistics. Vol. **4**. Karger, Basel.

Namboodiri, K. K., P. P. Green, E. B. Kaplan, J. A. Morrison, G. A. Chase, R. C. Elston, A. R. G. Owen, B. M. Rifkind, C. J. Glueck, and H. A. Tyroler. 1984. The Collaborative Lipid Research Clinics Program Family Study. IV. Familial associations of plasma lipid and lipoproteins. Am. J. Epidemiol. **119**:975–996.

National Heart, Lung and Blood Institute Consensus Development Panel. 1985. Lowering blood cholesterol to prevent heart disease. JAMA **253**:2080–2086.

Neufeld, H. N., and U. Goldbourt. 1983. Coronary heart disease: genetic aspects. Circulation **67**:943–954.

Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. Pp. 417–431 *in* J. P. Segrest and J. J. Albers, eds. Methods in enzymology. Vol. **128**: Plasma lipoproteins. A. Preparation, structure, and molecular biology. Academic Press, Orlando.

Ordovas, J. M., E. J. Schaefer, D. Salen, R. H. Ward, C. J. Glueck, C. Vergani, P. W. F. Wilson, and S. K. Karathanasis. 1986. Apolipoprotein A–I gene polymorphism associated with premature coronary artery disease and familial hypoalphalipoproteinemia. N. Engl. J. Med. **314**:671–677.

Pearson, T. A., B. H. Bulkely, S. C. Ashuff, P. O. Kwiterovich, and L. Gordis. 1979. The association of low levels of HDL cholesterol and arteriographically defined coronary heart disease. Am. J. Epidemiol. **109**:285–295.

Perkins, K. A. 1986. Family history of coronary heart disease: is it an independent risk factor? Am. J. Epidemiol. **124**:182–194.

Rao, D. C., N. E. Morton, C. L. Gulbrandsen, G. G. Rhoads, A. Kagen, and S. Yee. 1979. Cultural and biological determinants of lipoprotein concentrations. Ann. Hum. Genet. **42**:467–477.

Robertson, F. W. 1981. The genetic component in coronary heart disease—a review. Genet. Res. **37**:1–16.

Ross, R. 1986. The pathogenesis of atherosclerosis: an update. N. Engl. J. Med. **314**:488–500.

Schaefer, E. J., J. R. McNamara, J. Genest, M. M. Schaefer, P. W. F. Wilson, J. J. Albers, and J. M. Ordovas. 1987. LDL particle size, lipoproteins, and apolipoproteins in premature coronary artery disease (CAD): confounding effects of beta blockers. Circulation **76**(Suppl. 4): 4–531.

Segal, P., B. M. Rifkind, and W. J. Schull. 1982. Genetic factors in lipoprotein variation. Epidemiol. Rev. **4**:137–160.

Shen, M. M. S., R. M. Krauss, F. T. Lindgren, and T. M. Forte. 1981. Heterogeneity of serum low density lipoproteins in normal human subjects. J. Lipid Res. **22**:236–244.

Sing, C. F., and J. D. Orr. 1978. Analysis of genetic and environmental sources of variation in serum cholesterol in Tecumseh, Michigan. IV. Separation of polygene from common environmental effects. Am. J. Hum. Genet. **30**:491–504.

Sosenko, J. M., J. L. Breslow, R. C. Ellison, and O. S. Miettinen. 1980. Familial aggregation of total cholesterol, high density lipoprotein cholesterol and total triglyceride levels in plasma. Am. J. Epidemiol. **112**:656–660.

Terry, R. B., M. L. Stefanick, R. M. Krauss, W. L. Haskell, and P. D. Wood. 1985. Relationships between abdomen to hip ratio, plasma lipoproteins, and sex hormones. Circulation **72**(Suppl. 3): 452.

Williams, P. T., P. D. Wood, K. M. Vranizan, J. J. Albers, S. C. Garay, and C. B. Taylor. 1985. Coffee intake and elevated cholesterol and apolipoprotein B levels in men. JAMA **253**:1407–1411.