

Atherogenic Lipoprotein Phenotype

A Proposed Genetic Marker for Coronary Heart Disease Risk

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In a community-based study of 301 subjects from 61 nuclear families, two distinct phenotypes (denoted A and B) were identified by nondenaturing gradient gel electrophoretic analysis of low density lipoprotein (LDL) subclasses. Phenotype A was characterized by predominance of large, buoyant LDL particles, and phenotype B consisted of a major peak of small, dense LDL particles. Previous analysis of the family data by complex segregation analysis demonstrated that these phenotypes appear to be inherited as a single-gene trait. In the present study, the phenotypes were found to be closely associated with variations in plasma levels of other lipid, lipoprotein, and apolipoprotein measurements. Specifically, phenotype B was associated with increases in plasma levels of triglyceride and apolipoprotein B, with mass of very low and intermediate density lipoproteins, and with decreases in high density lipoprotein (HDL) cholesterol, HDL₂ mass, and plasma levels of apolipoprotein A-I. Thus, the proposed genetic locus responsible for LDL subclass phenotypes also results in an atherogenic lipoprotein phenotype. (*Circulation* 1990;82:495–506)

Epidemiological studies have established several lipoprotein-related risk factors for coronary heart disease (CHD). Elevated plasma levels of low density lipoprotein (LDL) cholesterol are believed to increase risk,^{1–3} whereas high density lipoprotein (HDL) cholesterol levels are inversely related to risk.^{4–6} Plasma concentrations of apolipoprotein (apo) B and apo A-I, the major protein components of LDL and HDL, respectively, have also been associated with atherosclerosis.^{7,8} The relation of plasma triglyceride levels and triglyceride-rich lipoproteins such as very low density lipoproteins (VLDL) to heart disease risk is less well understood.^{9,10} In addition, there is evidence that other classes of lipoproteins, such as intermediate density lipoproteins (IDL) and Lp(a), are involved in the development of atherosclerosis.^{11,12}

Genetic influences on lipoproteins have also been demonstrated. Based on population studies, lipid levels have been shown to cluster in families^{13–15}; studies of twins indicate significant genetic influences as well.^{16,17}

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Familial forms of hypercholesterolemia may result from deficient or defective LDL receptors¹⁸ or from mutations in apo B leading to defective receptor binding.¹⁹ More common polymorphisms of apo A-I, apo B, and apo E have been associated with variations in lipid and lipoprotein levels.^{20–22} Recently, Lp(a) levels and isoforms have also been shown to be under genetic control.²³

In our laboratory, we have identified distinct lipoprotein phenotypes based on analysis of LDL subclasses.²⁴ Specifically, two phenotypes, A and B, are characterized by a predominance of large, buoyant LDL particles

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and small, dense LDL particles, respectively. We have recently demonstrated that phenotype B is associated with increased risk of myocardial infarction,²⁴ consistent with two previous studies.^{25,26} Based on complex segregation analysis of 61 nuclear families, we have also shown that phenotype B appears to be inherited as a single-gene trait with a dominant mode of inheritance.²⁷ In the present report, we show that in these families, LDL subclass phenotypes are closely associated with other lipoprotein and apolipoprotein profiles that are known to influence risk of atherosclerosis. Thus, we have designated the proposed genetic locus responsible for LDL subclass phenotypes as an atherogenic lipoprotein (ALP) phenotype locus.

Methods

Subjects

The recruitment of families took place primarily among the Mormon community in the San Francisco

Bay area between 1984 and 1987, although nonlocal relatives were also screened.²⁷ Families were not selected for lipid disorders or family history of cardiovascular disease, but sequential sampling of informative kindreds was used.²⁸ With the exception of one kindred of Portuguese descent ($n=28$) and one small Asian nuclear family ($n=4$), all families were non-Hispanic Caucasian. Three hundred one family members of 29 kindreds participated in the study; included were 61 nuclear families. Of the 301 participants, 100 had married into the kindreds and were not related to each other. Subjects ranged in age from 6 to 95 years, with approximately equal proportions of men and women (49% men and 51% women). Only 22 eligible subjects declined participation, a response rate of 93%. Each subject gave written informed consent.

All participants provided blood samples after an overnight fast and completed a medical history questionnaire. For local family members, heights and weights were measured in the clinic, whereas among nonlocal subjects, reported heights and weights were used. Mormon families were selected for this study because they usually do not smoke tobacco and do not drink beverages containing alcohol or caffeine. Abstinence from these factors among the majority of family members was confirmed by questionnaire and reduced possible confounding in the genetic analysis because these factors have been associated with variations in lipid and lipoprotein levels.²⁹⁻³³ In addition, because Mormon families are generally large and genealogical records are carefully maintained, they are especially informative for genetic analysis. The segregation results in the non-Caucasian kindreds were not different from the remaining kindreds; therefore, all families were analyzed as a single group.

Lipid, Apolipoprotein, and LDL Subclass Analyses

Plasma lipid and apolipoprotein determinations and LDL subclass analyses were performed on fresh plasma samples after immediate centrifugation of whole blood. Total cholesterol and triglyceride levels were measured by enzymatic techniques with the Gilford 3500 autoanalyzer. HDL cholesterol was measured after precipitation with heparin-MnCl₂,^{34,35} and LDL cholesterol was calculated from the formula of Friedewald et al.³⁶ Plasma apo A-I levels and apo B levels were measured by maximal radial immunodiffusion using reagents from Tago, Inc.^{37,38} Lipoprotein mass measurements were determined as a function of Svedberg flotation rate using analytic ultracentrifugation in an unselected subset of 211 subjects.³⁹ The remaining 90 subjects did not have these measurements made due to funding and staff limitations. Measurements of VLDL mass of flotation rate (S_f^0) 20-400, IDL of S_f^0 12-20, large LDL of S_f^0 7-12, small LDL of S_f^0 0-7, HDL₂ of flotation rate ($F_{1.20}^0$) 3.5-9, and HDL₃ of $F_{1.20}^0$ 0-3.5 are reported here.

LDL subclass patterns were determined based on nondenaturing polyacrylamide gradient gel electro-

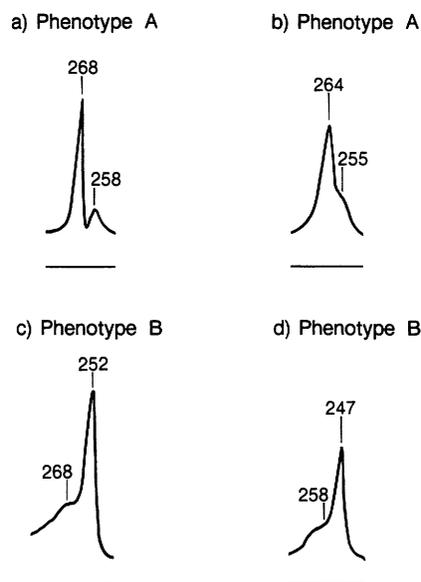


FIGURE 1. Atherogenic lipoprotein phenotypes for four sibs: 35-year-old man (a), 44-year-old woman (b), 38-year-old man (c), and 32-year-old man (d). Examples of atherogenic lipoprotein phenotype A are seen in panels a and b with peak particle diameters of 268 and 264 Å, respectively. Scans in panels c and d represent atherogenic lipoprotein phenotype B with peak particle diameters of 252 and 247 Å, respectively.

phoresis of whole plasma and of the density < 1.063 g/ml plasma fraction, using Pharmacia PPA 2-16% gradient gels as described previously.^{40,41} Stained gels were scanned with a Transidyne RFT Scanning Densitometer, and particle diameters were calculated from calibration curves using standards of known size.⁴⁰ The coefficient of variation of the calculated particle diameters has been estimated to be 3% by this procedure.⁴⁰ Based on the resulting scans, two distinct LDL subclass patterns were identified and are denoted here as ALP phenotype A and phenotype B.²⁴ Examples of these phenotypes among four sibs from a large kindred are shown in Figure 1. Phenotype A is characterized by a major peak of large, buoyant LDL particles and a minor peak of smaller, denser LDL subspecies, as shown in Figures 1a and 1b (peak particle diameters of 268 and 264 Å, respectively). The peak particle diameter for ALP phenotype A scans is generally more than 255 Å. In contrast, the major peak for ALP phenotype B is usually 255 Å or less, as shown in Figures 1c and 1d (peak particle diameters of 252 and 247 Å, respectively). The major peak in this phenotype consists of small, dense LDL particles, with a skewing of the curve toward the larger particle diameters.

Among the 301 family members, 87% could be classified into one of these two phenotypes. The remaining 13% of subjects had patterns of an intermediate phenotype. That is, either the peak particle diameter value was close to the 255 Å cutoff point and no skewing of curve was seen, or two distinct major peaks were seen. For the present analysis,

TABLE 1. Observed and Expected Segregation Ratios of Atherogenic Lipoprotein Phenotypes in 49 Nuclear Families

Parental mating type	Matings (n)		ALP phenotype A (n) (%)	ALP phenotype B (n) (%)	Total (n)
A×A	14	Observed	41 (100)	0 (0)	41
		Expected	41.0	0.0	
A×B	27	Observed	66 (70)	28 (30)	94
		Expected	64.3	29.7	
B×B	8	Observed	13 (65)	7 (35)	20
		Expected	9.7	10.3	

ALP, atherogenic lipoprotein phenotype.

Based on single-locus dominant model, with allele frequency of 0.25 for phenotype B and reduced penetrance among males less than 20 years old and premenopausal females, as determined by complex segregation analysis.²⁷ Only families with both parents sampled are included in table.

these subjects have been classified as ALP phenotype B.²⁷ By this definition, 31% of the study subjects had ALP phenotype B, although the prevalence varied by age, gender, and menopausal status in women. Specifically, among the males, phenotype B had a frequency of 17% in those less than 20 years old and 44% in those 20 years old or older; among females, phenotype B had a frequency of 13% before menopause and 49% after menopause.²⁷ Mean peak particle diameters were 266.2 ± 5.8 (\pm SD) and 252.7 ± 7.1 Å for subjects with phenotypes A and B, respectively.

Segregation Analysis

As we have recently reported, the inheritance of ALP phenotypes was investigated using complex segregation analysis based on the mixed model with pointers.^{27,42–45} The model that best explained the data was a single-locus model with a dominant mode of inheritance. Based on this model, the frequency of the allele leading to phenotype B was 0.25.²⁷ Full penetrance (the probability of expressing phenotype B given genotype AB or BB) was observed among men 20 years old or older and among postmenopausal women.²⁷ Penetrance was 0.4 for younger males and 0.3 for premenopausal females. Thus, complex segregation analysis suggested that ALP phenotype B is a common genetic trait with a dominant mode of inheritance and is fully expressed in adult men and postmenopausal women.

Statistical Procedures

Individual lipid, apolipoprotein levels, and lipoprotein mass measurements were adjusted by analysis of covariance^{46,47} for age, gender, and Quetelet index [measured as $(\text{wt} [\text{kg}])/(\text{ht} [\text{m}])^2$]. Main effects for these covariates were included in each model. For HDL cholesterol, apo A-I, HDL₂ mass, and HDL₃ mass, a significant gender by age interaction term was included in the model. For apo B, a significant Quetelet index by gender interaction term was included. Triglyceride, VLDL cholesterol, and VLDL mass were transformed logarithmically due to skewing of the distributions. Means and SDs of these variables are reported in antilog units, however, for ease of interpretation. All mean values were adjusted to

expected values for 50-year-old men. Significance levels for comparison of subjects with ALP phenotypes A and B were also based on analysis of covariance models. Skewness of frequency histogram distributions was calculated as the third central moment.⁴⁸ Thus, skewness values of more than 0 indicate a long tail to the right, and values less than 0 indicate a long tail to the left.

Interrelations among lipid, lipoprotein, and apolipoprotein variables were assessed by Pearson's product moment correlations.⁴⁷ To evaluate the simultaneous associations of lipid and apolipoprotein levels with the ALP phenotypes, multivariate analyses were performed with unconditional logistic regression.^{49,50} That is, ALP phenotype was the dependent variable and a series of lipid and apolipoprotein variables were the independent variables in the models. A prior probability of 0.30, based on the overall prevalence in the study sample, was used for phenotype B, and χ^2 goodness-of-fit statistics were determined for each model. All computations were performed with the Statistical Analysis System.^{51,52}

Results

Genetic Analysis

Based on the single-locus dominant model and incorporating both the estimated allele frequency and penetrance values,²⁷ expected segregation ratios were calculated. Table 1 compares these expected frequencies with the observed values based on 49 nuclear families in which both parents were sampled. Among the 14 families in which both parents had phenotype A, all of the 41 offspring had phenotype A, identical to the expected frequency. Among the 27 A×B families, 28 of the 94 total offspring (30%) had phenotype B, corresponding very closely to the expected frequency of 29.7 based on the model. Of the 20 offspring from B×B matings, seven had phenotype B, compared an expected 10.3. Although this comparison is not as close as the other mating types, the sample size in this category of families was small, and sampling variation was likely to be higher. A statistical comparison of the observed and expected was not significant ($\chi^2=1.021$, $df=5$, $p=0.961$). Thus, the results of the segregation

TABLE 2. Mean Values of Lipids for Study Subjects Compared With Lipid Research Clinics Program Prevalence Study Results

	Present study subjects (n=301)*	Lipid Research Clinics subjects (n=340)†
Total cholesterol	183±39	213±35
Triglyceride	100±63	153±101
VLDL cholesterol	20±13	27±20
LDL cholesterol	119±35	142±31
HDL cholesterol	43±10	44±11

VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

Values are given as mean±SD mg/dl.

*Mean values adjusted to 50-year-old men with analysis of covariance and adjusting for age, gender, and Quetelet index.

†Based on Lipid Research Clinics Program Prevalence Study data, visit 2 random sample, men 50–54 years old.⁵³

analysis appear to explain the observed familial clustering extremely well.

Lipid Levels

Mean lipid values of the 301 study subjects adjusted to values for 50-year-old men are given in Table 2 and are compared with results for men of a similar age from the Lipid Research Clinics Program Prevalence Study random sample.⁵³ Total cholesterol and triglyceride mean values were 30 and 50 mg/dl less, respectively, in the study subjects than in the Lipid Research Clinics populations. Differences were also seen for VLDL cholesterol and LDL cholesterol. However, the mean values for HDL cholesterol are similar in the two samples.

Lipid, Apolipoprotein, and Lipoprotein Associations

In the present study, ALP phenotypes were found to be closely associated with variation in other lipid, apolipoprotein, and lipoprotein mass measurements. As shown in Table 3, mean values of both total cholesterol and triglyceride were significantly higher among subjects with phenotype B ($p<0.001$). These mean values are within normal ranges, however, because these data are based on a sample of primarily healthy families. The difference in total cholesterol is due to relative increases of LDL cholesterol and VLDL cholesterol among phenotype B subjects, although the difference in mean values for LDL cholesterol was only 10 mg/dl. HDL cholesterol was significantly lower among subjects with phenotype B ($p<0.001$). Differences were also seen for plasma apolipoprotein levels; apo B levels were significantly higher among subjects with phenotype B ($p<0.001$), and apo A-I levels were lower ($p<0.05$).

Based on analytic ultracentrifugal analyses in a subsample of all subjects, VLDL mass was significantly higher among subjects with phenotype B ($p<0.001$), consistent with results for triglyceride and estimated VLDL cholesterol. The differences in large and small LDL reflect primarily the definitions of ALP phenotypes based on LDL subspecies. Mean IDL mass was also higher among phenotype B subjects. HDL₂ mass was significantly lower among subjects with phenotype B, but no difference was seen in HDL₃ mass.

Triglyceride and HDL Cholesterol Distributions

Both triglyceride and HDL cholesterol have been related to risk of CHD in numerous studies.^{4–6,9,10,54}

TABLE 3. Adjusted Plasma Lipid, Apolipoprotein, and Lipoprotein Mass Levels by Atherogenic Lipoprotein Phenotype

	ALP phenotype A		ALP phenotype B	
	n	Mean±SD	n	Mean±SD
Total cholesterol*	208	177±37	93	197±40
Triglyceride†*	208	69±26	93	141±79
VLDL cholesterol**	208	14±5	93	28±16
LDL cholesterol‡	208	116±35	92	126±36
HDL cholesterol*	208	46±15	92	37±14
Apo A-I‡	206	131±29	92	122±31
Apo B*	206	76±34	93	98±36
VLDL mass**	151	18±31	60	111±68
LDL mass				
Large*	151	119±38	60	87±34
Small*	151	164±55	60	221±64
IDL mass*	151	20±14	60	38±17
HDL ₂ mass*	151	55±44	60	13±26
HDL ₃ mass	151	189±47	60	180±59

ALP, atherogenic lipoprotein phenotype; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein.

Values are given as mean±SD mg/dl.

Mean values are adjusted to 50-year-old men with analysis of covariance and adjusting for age, gender, and relative weight.

* $p<0.001$, † $p<0.05$, for difference in means between phenotype A and phenotype B subjects based on analysis of covariance.

‡Log₁₀ transformation used in calculations; reported values based on antilogs.

Because of their close association with ALP phenotypes as well, the distributions of these variables, adjusted for age, gender, and relative weight, were examined further.

The percent frequency distribution of triglyceride levels is shown in Figure 2A. The overall distribution has a long tail to the right, with skewness estimated to be 2.35. This has been observed in many other investigations, and log transformations are often used in statistical analyses.^{9,54,55} The distribution of triglyceride by ALP phenotype is also shown. As expected from the mean values given in Table 3, the triglyceride distribution for phenotype B subjects is shifted upward in comparison to that for phenotype A subjects. Of particular interest is that the phenotype B subjects appear to be responsible for the skewing of the overall distribution; that is, skewing of the triglyceride distribution is markedly reduced when considered by phenotype; the skewness value was 0.65 for phenotype A subjects, and 1.49 for phenotype B subjects.

In Figure 2B, the cumulative triglyceride distributions for all subjects and for phenotype A and B subjects are shown. The differences in the distributions for phenotypes A and B are clearly demonstrated. The 50th percentile values differ by more than 60 mg/dl (71 versus 133 mg/dl, respectively), and the 90th percentile values are even more divergent (104 versus 278 mg/dl, respectively).

In Figure 2C, the cumulative distribution for phenotype A subjects is reversed to compare the degree of overlap of the distributions for the two phenotypes. The distributions for subjects with phenotypes A and B cross at a triglyceride level of approximately 95 mg/dl. Of subjects with phenotype A, only 17% have triglyceride values of more than 95 mg/dl, whereas 17% of subjects with phenotype B have triglyceride values of less than 95 mg/dl. Because there is very little overlap in these distributions, triglyceride levels of more than and less than 95 mg/dl discriminate ALP phenotype in approximately 83% of the study subjects.

A similar analysis for HDL cholesterol is shown in Figure 3. In Figure 3A, the percent frequency distribution of HDL cholesterol values is shown. In contrast to the triglyceride distribution, little skewness is seen in the overall distribution. As was seen in Table 2, the mean value of this overall distribution is very similar to the Lipid Research Clinics results (43 versus 44 mg/dl, respectively). However, the distribution for phenotype B subjects is shifted downward in comparison to that for phenotype A subjects. This difference is seen even more clearly in the cumulative distributions shown in Figure 3B. For pattern A subjects, the 50th and 10th percentiles were approximately 44 and 34 mg/dl, respectively. The comparable values for phenotype B subjects were considerably lower at 36 and 27 mg/dl, respectively. In Figure 3C, the cumulative distribution for phenotype B subjects is reversed. The distribution curves cross at approximately 39 mg/dl, with 28% of phenotype A

subjects having HDL cholesterol values of less than 39 mg/dl and an equal percent of phenotype B subjects having values of more than 39 mg/dl. The HDL cholesterol distributions overlap more than the triglyceride distributions, but the 39 mg/dl threshold discriminates phenotype A and phenotype B subjects relatively well.

A similar cumulative distribution analysis for apo A-I and apo B is shown in Figures 4A and 4B. The apo B distributions cross at 81 mg/dl and 37% of phenotype B subjects have values below this level, whereas an equal percent of phenotype A subjects have values above this level. The apo A-I distribution curves cross at 124 mg/dl, with a similar percent overlap. Thus, apo A-I and apo B levels also discriminate subjects with the two phenotypes but not as well as triglyceride and HDL cholesterol.

Correlations

The interrelations of selected lipid, lipoprotein, and apolipoprotein variables associated with ALP phenotypes, based on correlation coefficients, are shown in Table 4. As expected from the structure of lipoprotein particles, triglyceride, LDL cholesterol, apo B, VLDL mass, and IDL were highly intercorrelated, as were HDL cholesterol, HDL₂ mass, and apo A-I. In addition, triglyceride-related variables were generally inversely correlated with HDL-related variables. For example, the correlation for plasma triglyceride and HDL cholesterol and for VLDL mass and HDL₂ mass was -0.24 ($p < 0.001$) and -0.47 ($p < 0.001$), respectively.

Multivariate Analysis

Because of these interrelations, the simultaneous associations of lipids and apolipoproteins with ALP phenotypes were investigated by performing logistic regression analysis. That is, the associations of lipid and apolipoprotein variables with ALP phenotypes were investigated by using phenotype as the dependent variable and including various combinations of lipid and apolipoprotein measures as independent variables. The results in Table 5 summarize three models that include age, gender, and Quetelet index as covariates and show χ^2 goodness-of-fit statistics for each model.

In model 1, both HDL cholesterol and plasma triglyceride were strongly and independently associated with ALP phenotypes. In addition to these variables, LDL cholesterol, apo A-I, and apo B were also considered independent variables; however, they did not make a significant contribution to the fit of the model and are not reported in the table. Model 2 shows that apo A-I and apo B, without other lipid variables, were both significantly associated with ALP phenotypes. The model χ^2 values show that model 1 provides a better fit to the data than model 2, however. In model 3, VLDL mass and HDL₂ mass were considered and found to be independently associated with ALP phenotypes in the subset of 211 study subjects with analytic ultracentrifuge data.

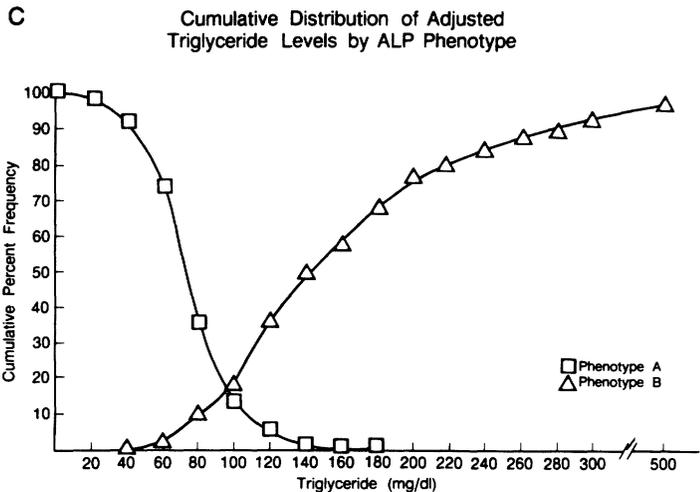
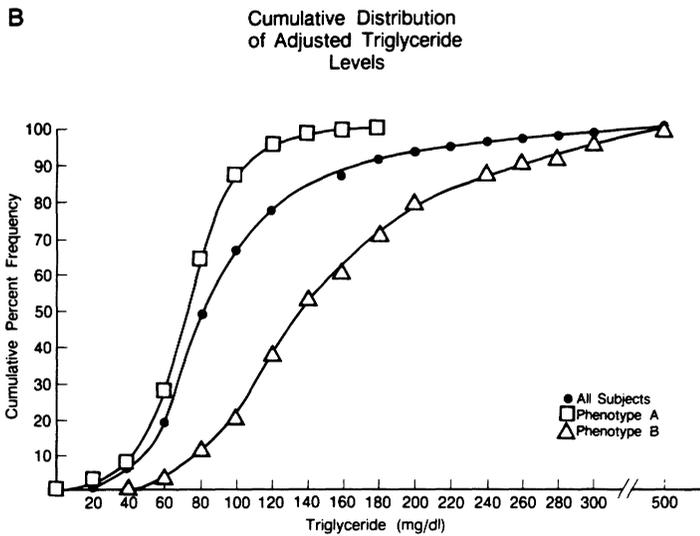
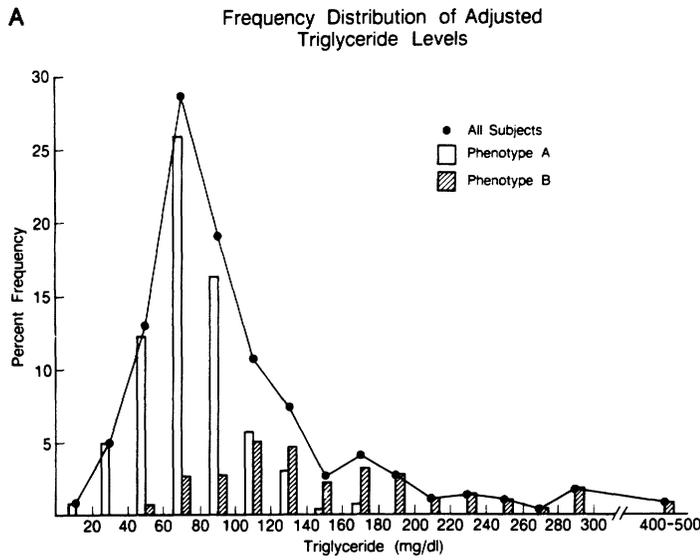


FIGURE 2. Plots of percent frequency distribution of adjusted triglyceride values for all study subjects and for atherogenic lipoprotein (ALP) phenotype A and phenotype B subjects (A), cumulative distributions of adjusted triglyceride values for all study subjects and for phenotype A and phenotype B subjects (B), and cumulative triglyceride distributions by ALP phenotype (C). Distribution for phenotype A subjects is reversed to compare overlap of two distributions. Triglyceride values are adjusted to mean level for 50-year-old men based on analysis of covariance.

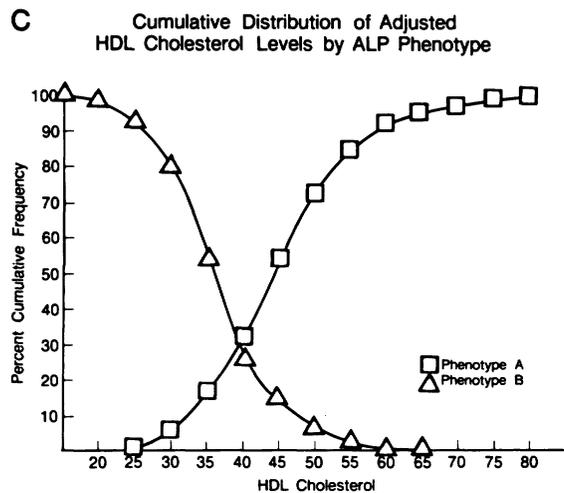
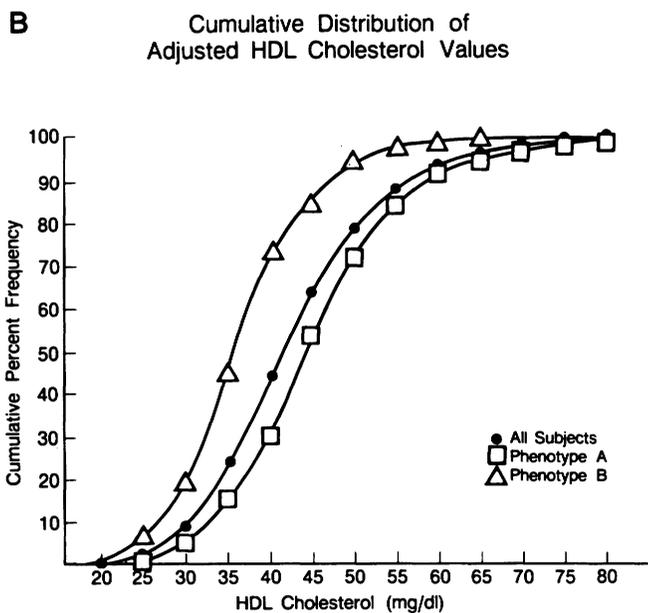
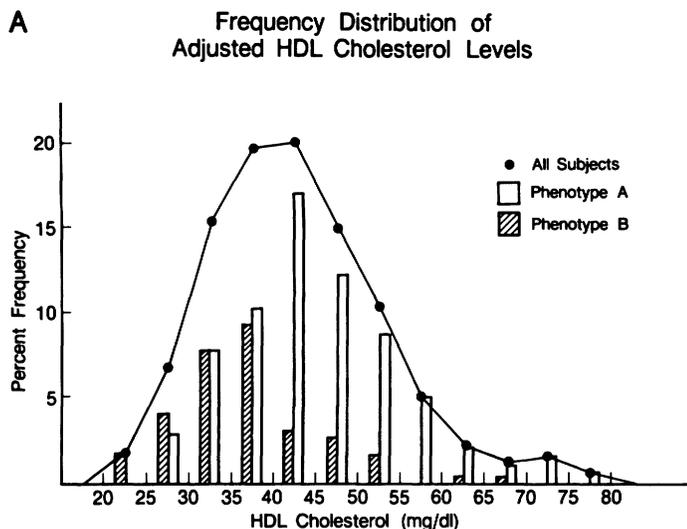


FIGURE 3. Plots of percent frequency of adjusted high density lipoprotein (HDL) cholesterol values for all subjects and for subjects with atherogenic lipoprotein (ALP) phenotype A and phenotype B (A), cumulative distributions of adjusted HDL cholesterol values for all study subjects and for phenotype A and phenotype B subjects (B), and cumulative distributions of HDL cholesterol by ALP phenotype (C). Distribution for phenotype B is reversed to compare overlap of distributions. HDL cholesterol values are adjusted to mean level for 50-year-old men by analysis of covariance.

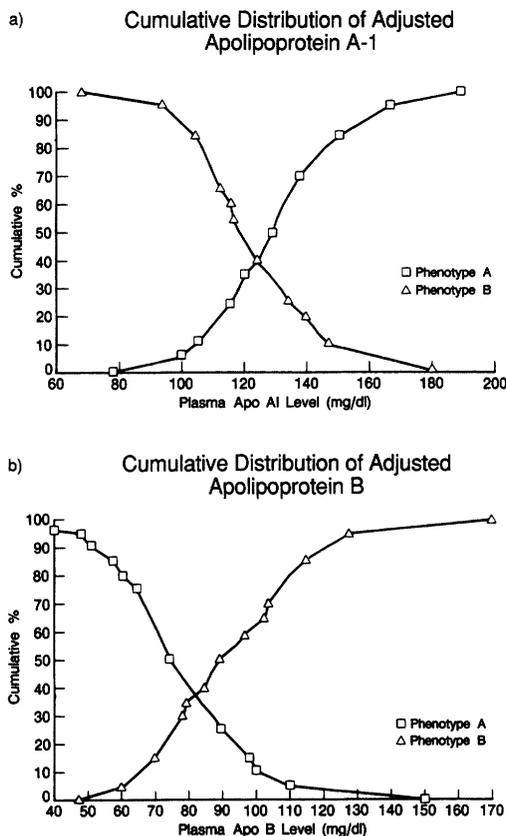


FIGURE 4. Cumulative distributions of apo A-I levels by ALP phenotype (a). Distribution for phenotype B subjects is reversed. Plots of cumulative distributions of adjusted apo B values by atherogenic lipoprotein (ALP) phenotype (b). Distribution for phenotype A subjects is reversed. All apolipoprotein values are adjusted for 50-year-old men by analysis of covariance.

When IDL mass was added to this model, it did not significantly increase the fit of the model to the data.

Taken together, these results demonstrate that a predominance of small, dense LDL particles is strongly associated with an apparently high-risk lipoprotein profile characterized by relative increases in plasma triglyceride, VLDL, and apo B levels and by decreases in HDL cholesterol, HDL₂ mass, and apo A-I levels.

Discussion

We have previously demonstrated that the phenotype characterized by a predominance of small, dense LDL particles (ALP phenotype B) segregates in families consistent with the presence of a single major genetic locus.²⁷ Based on estimates from the complex segregation analysis, approximately 44% of study subjects would be expected to carry at least one copy of the proposed phenotype B allele. In the same sample of primarily healthy families, we also demonstrate that phenotype B is associated with increased levels of plasma triglyceride, VLDL, IDL, and apo B and with decreased levels of HDL cholesterol, HDL₂ mass, and apo A-I. These results are consistent based on comparisons of mean values, cumulative distributions, and multivariate analysis. Thus, ALP phenotype B may be a common genetic marker for increased susceptibility to CHD.

Although the genetic results reported above are based on a community-based sample of families, we have recently identified a similar mode of inheritance for phenotype B in members of families with familial combined hyperlipidemia.⁵⁶ A similar allele frequency was found, and penetrance estimates by age and gender showed comparable trends. However, both studies used complex segregation analysis, a technique that has many advantages as well as limitations. It allows comparisons of a variety of genetic and environmental models, including multifactorial inheritance (polygenic or cultural), single major gene models, and horizontal (environmental) transmission only, using likelihood statistics.⁴⁵ However, this is a statistical modeling technique, and the use of this "mixed model" can give spurious results if ascertainment bias is present. Thus, although the two family studies conducted so far provide strong evidence for the presence of a major gene, this can be proven only by using linkage studies to identify the chromosomal location.

In a recent case-control study, we demonstrated that phenotype B was associated with both increased risk of myocardial infarction (odds ratio, 3.0) and a high-risk lipoprotein profile.²⁴ Specifically, subjects in that study with phenotype B had significantly increased levels of triglyceride, VLDL mass, IDL

TABLE 4. Correlations of Lipid, Apolipoprotein, and Lipoprotein Mass Levels

	LDL cholesterol	HDL cholesterol	Apo B	Apo A-I	VLDL mass*	IDL mass*	HDL ₂ mass*
Triglyceride	0.31†	-0.24†	0.54†	-0.004	0.93†	0.61†	-0.43†
LDL cholesterol		-0.06	0.87†	0.10	0.29†	0.67†	-0.24†
HDL cholesterol			-0.09	0.73†	-0.38†	-0.25†	0.81†
Apo B				0.11	0.52†	0.74†	-0.34†
Apo A-I					0.09	0.07	0.60†
VLDL mass						0.63†	-0.47†
IDL mass							-0.35†

LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein.

*Sample size was 211 study subjects for correlations including these variables.

† $p < 0.001$.

TABLE 5. Logistic Regression Equations Associating Atherogenic Lipoprotein Phenotypes With Lipid, Lipoprotein, and Apolipoproteins

	Regression coefficients		
	Model 1 (n=300)	Model 2 (n=298)	Model 3 (n=211)
Age (yr)	0.018	0.016	0.028*
Gender (0, female; 1, male)	0.133	0.459	0.212
Quetelet index (wt [kg]/(ht [m]) ²)	-0.118*	0.030	-0.127*
Triglyceride (mg/dl)	0.049†	...‡	...
HDL cholesterol (mg/dl)	-0.070§
VLDL mass (mg/dl)	0.041†
HDL ₂ mass (mg/dl)	-0.036§
Apo B (mg/dl)	...	0.032†	...
Apo A-I (mg/dl)	...	-0.014*	...
Model χ^2	141.54	60.06	112.28
df	5	5	5
p	<0.001	<0.001	<0.001

* $p < 0.05$, † $p < 0.001$, § $p < 0.01$.

‡This variable was not included in the model.

mass, and apo B and decreased levels of HDL cholesterol, HDL₂ mass, and apo A-I (Reference 24 and personal observations), each of which has been associated with increased risk of CHD.^{4-11,57-59} The variations in lipid and apolipoprotein levels seen in the present study of primarily healthy relatives confirm these associations, although mean levels are within normal ranges. In addition, mean total cholesterol and LDL cholesterol levels are slightly, but significantly, higher among phenotype B subjects in this study.

Another recent study has shown similar associations among healthy blood donors.⁶⁰ Although the underlying mechanism for phenotype B has not been identified, it is tempting to speculate that this constellation of lipid and apolipoprotein variations is the result of pleiotropic effects of a single gene. That is, the proposed ALP gene may simultaneously influence both LDL particle size and these other lipoprotein-related variables through a common metabolic mechanism. In addition, a borderline association between increased Quetelet index and phenotype B was observed ($p = 0.054$) after adjustment for age and gender.

The particularly strong association between ALP phenotype B and plasma triglyceride level reported in the present study could indicate that phenotype B is a marker for a defect having a primary action on triglyceride metabolism. As shown in Figure 2C, there is very little overlap in the cumulative triglyceride distributions of phenotype A and phenotype B subjects. In addition, the skewing of the triglyceride distribution appears to be largely explained by ALP phenotype B (Figure 2A). However, the triglyceride cutoff point that best distinguishes the two phenotypes (95 mg/dl) may be low because of the healthy study sample (Table 2). Many studies have demonstrated relations between LDL particle size and triglyceride metabolism.⁶¹⁻⁶⁴ In particular, a recent kinetic study of relatives of probands with primary hypertriglyceridemia provided evidence for genetic

control of triglyceride removal.⁶² Changes in both the core and surface of LDL particles have been shown to occur when plasma triglyceride levels are increased, possibly due to exchange of core lipids between lipoproteins.^{64,65} Studies of postprandial lipemia in normal individuals have also suggested that HDL cholesterol is influenced by triglyceride metabolism through the action of lipolytic enzymes.⁶⁶

The association of phenotype B with variations in lipid and apolipoprotein levels among these family members also suggests that phenotype B may be involved in other reported familial lipid disorders. A predominance of small, dense LDL has been shown to be common in families with familial combined hyperlipidemia.⁶⁷ This disorder is characterized by elevations of plasma total cholesterol and/or triglyceride levels in family members, and affected relatives have variable lipid phenotypes and increased risk of myocardial infarction.^{68,69} As mentioned above, we have recently demonstrated that ALP phenotype B appears to be inherited as a single-gene trait in a sample of families with this disorder, and phenotype B was closely associated with the hypertriglyceridemia found in family members.⁵⁶ In addition, both ALP phenotype B and familial combined hyperlipidemia are characterized by relative increases in plasma apo B levels. Two recent studies, also using complex segregation analysis, have provided data to indicate that apo B levels are controlled by a single, major locus.^{70,71} Finally, a condition termed hyperapobetalipoproteinemia, in which a subset of coronary artery disease patients were found to have elevated apo B levels but normal LDL cholesterol levels,⁷² could also involve or interact with ALP phenotype B. To date, the interrelations of these proposed genes and lipid disorders have not been investigated.

It should be noted, however, that the study of genetic control of ALP phenotypes is complicated by many factors. Phenotype B, as determined by gradi-

ent gel electrophoresis analysis, is often not expressed in young males and premenopausal women. This finding suggests that hormonal factors might be involved in the apparent full penetrance of phenotype B in adult men and postmenopausal women. Severe elevations of triglyceride due to mechanisms other than the genetic model proposed here may give rise to phenocopies. Behavioral and environmental influences such as diet, exercise, and use of lipid-altering medications may also affect the expression of the trait.⁷³ Other genes could potentially influence the expression of ALP phenotypes through epistatic effects, and segregation analysis cannot rule out the possibility of genetic heterogeneity of the ALP phenotypes. For example, a syndrome named "familial dyslipidemic hypertension" has recently been described and could involve the proposed ALP locus.⁷⁴

If genetic control of lipoprotein and apolipoprotein levels by the proposed ALP locus is confirmed, there are important clinical implications for reduction of risk of CHD. For example, intervention strategies might be designed specifically for individuals who carry an ALP B allele. These strategies may need to differ from general recommendations to be effective in reducing risk in these individuals.

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