LBL-33745
UC-000

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

Presented at the Genetic Analysis Workshop 8,
Watsonville, CA, November 8–10, 1992,
and to be published in the Proceedings

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October 1992

Donner Laboratory

Biology & Medicine Division

Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

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LBL 33745

Lipoprotein Subclasses in Genetic Studies:

The Berkeley Data Set

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KEYWORDS: LDL, HDL, cholesterol, genetics, apolipoproteins, electrophoresis

Running title: Lipoprotein subclasses in genetic analysis

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This work was supported by the National Institutes of Health Program Project Grant HL 18574 from the National Heart, Lung, and Blood Institute, a grant from the National Dairy Promotion and Research Board administered in cooperation with the National Dairy Council, and was conducted at the Lawrence Berkeley Laboratory through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Lipoprotein subclasses and coronary disease risk. There is considerable evidence that high levels of total cholesterol and low-density lipoprotein (LDL)-cholesterol and low levels of high-density lipoprotein (HDL)-cholesterol are associated with increased coronary heart disease (CHD) risk [Miller et al., 1975]. More recently attention has focused on subclasses of HDL, LDL and very-low-density lipoproteins (VLDL) as determined by analytic ultracentrifugation, gradient get electrophoresis, and other techniques [Lindgren et al., 1972]. Lindgren, Elliott and Gofman identified two subfractions of HDL with approximate hydrated densities of 1.075 (HDL_2) and 1.12 g/ml (HDL_3) [Lindgren, et al., 1951]. LDL may also be divided into three major groups by their flotation rates in the analytic ultracentrifuge: small LDL (S_f 0-7), large LDL (S_f 7-12), and intermediate-density lipoproteins (IDL; S_f 12-20) [Lindgren et al., 1972].

Although exceptions exist, most studies show that high HDL_2 levels are associated with reduced CHD risk [Miller et al., 1981; Ballantyne et al., 1982; Gofman, et al., 1966]. Increased concentrations of small LDL and IDL were associated with coronary disease progression in the National Heart, Lung and Blood Institute Type II Coronary Intervention Study [Krauss et al., 1987]. Less certain is the relationship between HDL_3 and CHD [Miller et al., 1981; Ballantyne et al., 1982; Gofman et al., 1966].

Analysis of lipoprotein subclasses by gradient gel electrophoresis: HDL may be further divided into two HDL_2 and three HDL_3 subclasses by nondenaturing polyacrylamide gradient gel electrophoresis (GGE) (Figure 1). These subclasses are defined by their estimated particle diameters: HDL_{3c} (approximately 7.2-7.8 nm), HDL_{3b} (7.8-8.2 nm), HDL_{3a} (8.2-8.8 nm), HDL_{2a} (8.8-9.7) and HDL_{2b} (9.7-12.9 nm) [Blanche et al., 1981]. HDL particles may contain both apoA-I and apoA-II or apoA-I without apo-AII [Cheung et al., 1984]. In normal men, the HDL(A-I *with* A-II) includes major components within HDL_{3b}, HDL_{3a} and HDL_{2a} subclasses [Nichols et al.,

1989]. The HDL(A-I *without* A-II) particle distribution includes major components within the HDL_{3c}, HDL_{3a} and HDL_{2b} subclasses. The total HDL profile is the sum of these two overlapping distributions. The HDL_{3c}, HDL_{3a} and HDL_{2b} subclasses appear to contain two, three and four molecules of apolipoprotein A-I, respectively. Figure 1 shows that as compared to women, men have significantly higher values for HDL_{3b} and HDL_{3c} and significantly lower HDL_{2b} and HDL_{2a}. Case control and angiographic studies suggest that CHD risk may be increased when HDL_{2b} is reduced relative to HDL_{3c} and HDL_{3b} [Wilson et al., 1990; Johansson et al., 1991; Cheung et al. 1991].

Low-density lipoproteins also contain subpopulations of particles that are distinguished by GGE [Krauss and Burke, 1982; McNamara et al., 1987]. The LDL-profiles may be characterized by peak diameter and by LDL subclass pattern. The LDL-peak diameter refers to the size of the predominant LDL-peak (Figure 2). These variables have been used to study the relationships to other lipoproteins [Austin et al., 1990a, 1990b, Hokanson et al., in press], acute and long-term effects of exercise [Lamon-Fava et al. 1989a, 1989b], diet-induced and exercise-induced weight loss [Wood et al., 1976], menopause in women [Campos et al., 1988], and estrogen therapy in postmenopausal women [Campos et al., 1990]. A high concentration of small LDL particles and a gradient gel electrophoretic LDL profile with a major peak usually less than 25.5 nm characterizes LDL subclass pattern B, whereas a predominance of larger LDL particles and a major peak usually greater than 260 nm with skewing of the curve towards larger particle diameters characterizes pattern A (Figure 2) [Austin et al., 1988b]. Approximately 25-30% of healthy subjects have patttern B, and an additional 15-20% have a pattern with multiple or symmetrical peaks with diameters in an intermediate size range. The prevalence of pattern B is associated with age and gender, and is relatively low in males under age 20 and in premenopausal women [Austin et al., 1988b, 1990b]. In comparison with pattern A,

pattern B is associated with relatively higher plasma levels of triglyceride-rich lipoproteins and apoB, and lower levels of HDL-cholesterol and apoA-I [Austin et al., 1990b]. Men with LDL subclass pattern B have significantly higher levels of HDL_{3b} and significantly lower HDL_{2b} and HDL_{2a} than men with pattern A.

Analyses carried out in a case-control study of men and women with myocardial infarction survivors indicated a three-fold increased risk of acute myocardial infarction associated with subclass pattern B [Austin et al., 1988a]. This relationship was recently confirmed in a study of subjects with coronary disease documented by angiography [Campos et al., 1992]. This relation of the pattern B lipoprotein profile to CHD risk has led to its designation as the atherogenic lipoprotein phenotype [Austin et al., 1990b].

The Berkeley Data Set: The inheritance of LDL subclass patterns was first investigated in a sample of primarily healthy families using complex segregation analysis [Austin et al., 1988b]. Subsequent studies have confirmed these initial results using both family studies and twins (deGraaf et al., 1992, Lamon-Fava et al., 1991, Austin et al., in press]. Following is a description of the methods used to obtain the data for this study, designated here as the Berkeley Data Set.

Lipoprotein measurements were obtained in 301 individuals in 27 kindreds ascertained between 1984 and 1987. With one exception, all kindreds were Mormons and had at least one relative living in the San Francisco Bay area. The kindreds ranged in size from 6 to 44, with lipoprotein subclass data available for 301 individuals. The families were not selected on the basis of history of CHD or lipid disorders. The study was begun with an "open-ended" screening of families who volunteered for the study. The first families were chosen strictly on the basis of large family size. Beginning with kindred 33, the focus of attention shifted to LDL subclass pattern. The goal in this second phase of the study was to

assemble large kindreds in which this phenotype was segregating. Nuclear families who volunteered were screened for the pattern B, and were enrolled if two or more members shared this phenotype. These families were expanded by recruiting every available relative. All family members were retained for segregation analysis.

Participants completed questionnaires during their clinic visits, which included questions on date of birth, drug and medication use, current and recent pregnancy, lactation, medication and hormone use, cigarette use and usual intake of alcoholic beverages. Ounces of alcohol consumed per week were calculated on the basis of 0.48 oz per 12 oz bottle of beer, 0.48 oz per 4-oz glass of wine, 0.60 oz per drink of hard liquor (including cocktails and mixed drinks), and 0.4 oz per after dinner drink. Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared.

All participants provided blood samples after an overnight fast. Plasma total cholesterol and triglyceride concentrations were measured by enzymatic techniques using the Gilford 3500 autoanalyzer. HDL-cholesterol was measured after precipitation with heparin-$MnCl_2$ [Warnick et al., 1982; Lipid Research Clinics Program 1975]. Plasma was ultracentrifuged at $d<1.006$ g/ml, cholesterol determined in the infranant, and LDL-cholesterol was calculated from the formula by Friedewald et al [1972]. Plasma apolipoprotein A-I concentrations were determined by radialimmunodiffusion [Cheung et al., 1977].

Electrophoresis of HDL in the ultracentrifuged $d \leq 1.20$ g/ml fraction was performed on Pharmacia Electrophoresis Apparatus (GE 4-II) using slab gradient gels (PAA 4/30, Pharmacia, Piscataway, NJ) as described by Blanche et al. [1981]. Protein-stained gels were obtained by agitating the gels in a 50-75 ml solution of 0.04% Coomassie G-250 and 3.5% perchloric acid after fixing the protein in 10% sulfosalicylic acid for 1 hour. The protein-stained gradient gels were scanned with a model RFT densitometer (Transidyne Corp., Ann Arbor, MI) at a wave length of 603

nm. A mixture of four globular proteins (High Molecular Weight Calibration Kit, Pharmicia) was run on the central lane to calibrate for particle size. Electrophoresis of LDL on the d < 1.063 fraction were done using Pharmacia 2-16% polyacrylamide gradient gels [Krauss et al., 1982; Nichols et al., 1986] and scanned at a wave length 596 nm [Nichols et al., 1986]. Latex beads were added to the high molecular weight standard to determine particle diameter.

Previous Genetic findings using the Berkeley Data Set

LDL subclass pattern B. Complex segregation analysis using the program POINTER gave results consistent with a single major gene influencing LDL subclass patterns [Austin et al., 1988b]. The best-fitting genetic model was an autosomal dominant mode of inheritance for LDL subclass pattern B, with an allele frequency of 0.25, and maximal penetrance in men over age 20 and in postmenopausal women. The basic features of this model were confirmed in a second study in 243 members of seven large kindreds with familial combined hyperlipidemia [Austin et al., 1990a]. However, the estimated frequency of the pattern B allele(s) was somewhat higher (0.3) and the possibility of an additive mode of inheritance with a multifactorial component could not be ruled out. Recently, linkage of pattern B to the vicinity of the LDL receptor gene locus on chromosome 19p has been reported [Nishina et al., 1992]. Preliminary analyses based on sib pairs in another study population have also indicated linkage to two other genetic loci: the apoA-I/apoC-III, apoA-IV gene cluster on chromosome 11, and the Mn-SOD gene on chromosome 8 [Rotter et al., 1992].

HDL subclasses. Data from the Berkeley Data Set have also been used recently to investigate familial correlations of HDL-subclasses [Williams et al., 1992]. Figure 3 displays the sibling intraclass correlation coefficient by HDL particle diameter. The figure shows that sibling HDL levels were significantly correlated

for HDL_{2b}, HDL_{3a} and HDL_{3b} subclasses. Figure 4 displays the percentage of the offsprings' variance explained by their two parents. Our finding that parents and offspring have the highest correlation for HDL_{2b} is consistent with published reports that show higher heritability estimates for HDL_2 compared with HDL_3-cholesterol [Kuusi et al., 1987].

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health Program Project Grant HL 18574 from the National Heart, Lung, and Blood Institute, a grant from the National Dairy Promotion and Research Board administered in cooperation with the National Dairy Council, and was conducted at the Lawrence Berkeley Laboratory through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. We appreciate the assistance of Linda Abe, Darlene Dreon, and Beverly Paigen in subject recruitment, and gratefully acknowledge Linda Harrison, Elina Golder, Rita Harvey-Turner, Janet Selmek-Halsey, Dennis Duncan, and Gerald Adamson for their technical expertise.

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FIGURE CAPTIONS

Figure 1. Mean absorbance of protein-stained HDL by particle size in men and women (top) and their difference (bottom). The solid portions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance ($p < 0.01$) for 2 sample t-test.

Figure 2. Low-density lipoprotein (LDL) subclass patterns A and B as determined by GGE in two selected individuals. LDL-peak diameters are given in angstroms.

Figure 3. Intraclass correlation coefficients for HDL levels by particle diameter for 83 sibling in 29 families. The solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly at $p \leq 0.05$ [Williams et al. 1992].

Figure 4. Multiple correlation coefficient showing the percentage of the offspring variance explained by the parents' HDL levels.

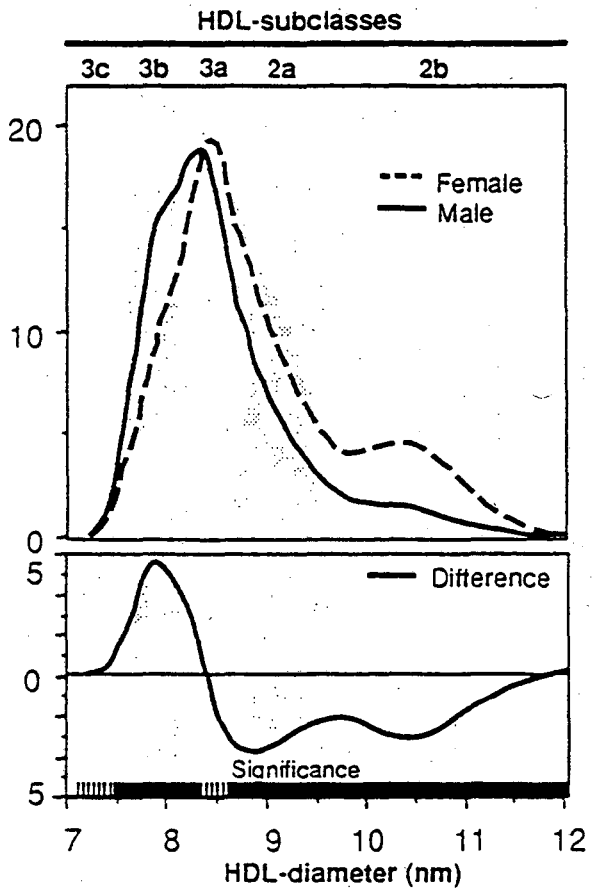
HDL-subclasses
3c
3b
3a
2a
2b
20
10
0
Female
Male
5
0
5
Difference
Significance
7
8
9
10
11
12
HDL-diameter (nm)

Figure 1

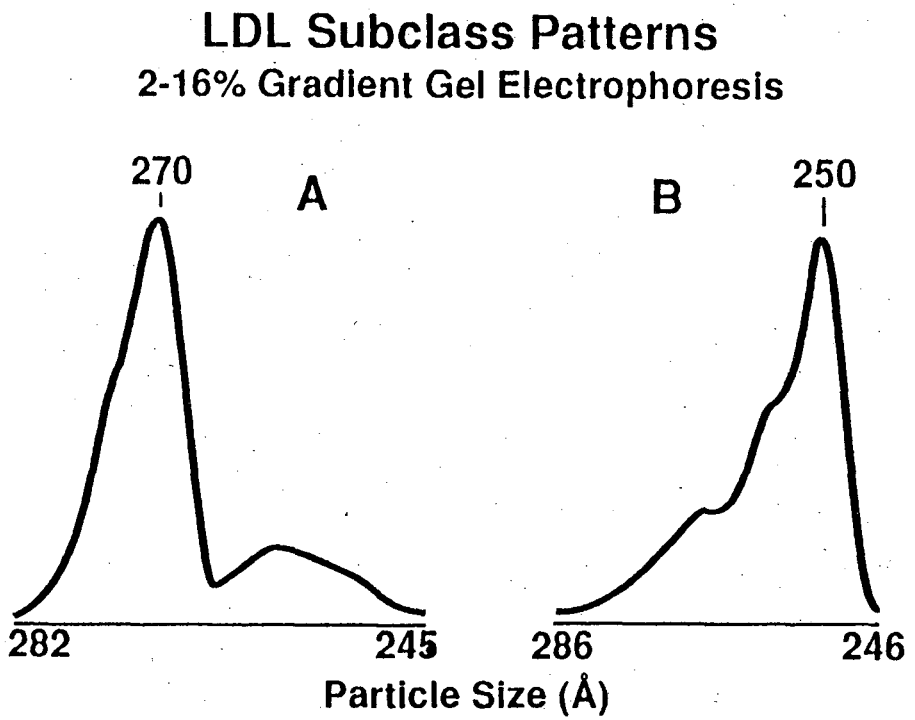
LDL Subclass Patterns
2-16% Gradient Gel Electrophoresis
270
A
B
250
282
245
286
246
Particle Size (Å)

Figure 2

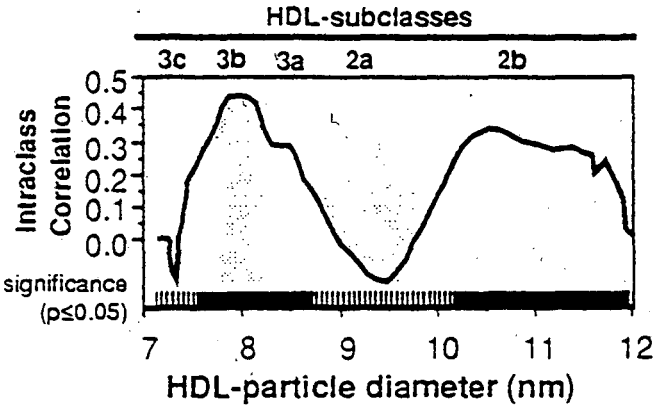
HDL-subclasses
3c
3b
3a
2a
2b
Intraclass Correlation
0.5
0.4
0.3
0.2
0.1
0.0
significance (p≤0.05)
7
8
9
10
11
12
HDL-particle diameter (nm)

Figure 3

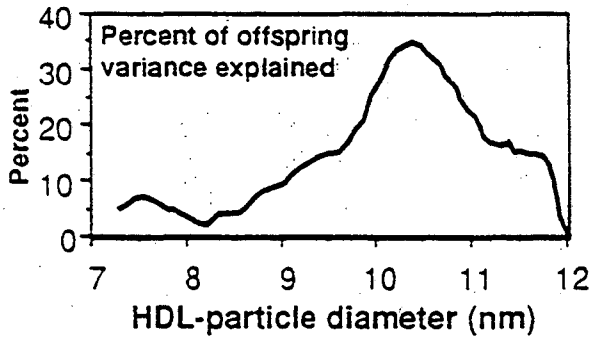
Percent of offspring
variance explained
Percent
40
30
20
10
0
7
8
9
10
11
12
HDL-particle diameter (nm)

Figure 4

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