Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2009 November ; 29(11): 1975–1980. doi:10.1161/ATVBAHA.109.190405.

ION MOBILITY ANALYSIS OF LIPOPROTEIN SUBFRACTIONS IDENTIFIES THREE INDEPENDENT AXES OF CARDIOVASCULAR RISK

Kiran Musunuru, M.D., Ph.D.[*], **Marju Orho-Melander, Ph.D.**[*], **Michael P. Caulfield, Ph.D.**, **Shuguang Li, Ph.D.**, **Wael A. Salameh, M.D.**, **Richard E. Reitz, M.D.**, **Göran Berglund, M.D., Ph.D.**, **Bo Hedblad, M.D., Ph.D.**, **Gunnar Engström, M.D., Ph.D.**, **Paul T. Williams, Ph.D.**, **Sekar Kathiresan, M.D.**, **Olle Melander, M.D., Ph.D.**[†], and **Ronald M. Krauss, M.D.**[†]
: Center for Human Genetic Research and Cardiovascular Research Center, Cardiology Division, Massachusetts General Hospital, Boston, Massachusetts, and Broad Institute of MIT and Harvard, Cambridge, Massachusetts (K.M., S.K.); University Hospital Malmö, Lund University, Malmö, Sweden (M.O.-M., G.B., B.H., G.E., O.M.); Quest Diagnostics Nichols Institute, San Juan Capistrano, California (M.P.C., S.L., W.A.S., R.E.R.); Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, California (P.T.W.); Children's Hospital Oakland Research Institute, Oakland, California (R.M.K.)

Abstract

Objective—Whereas epidemiologic studies show that levels of low-density-lipoprotein cholesterol (LDL-C) and high-density-lipoprotein cholesterol (HDL-C) predict incident cardiovascular disease (CVD), there is limited evidence relating lipoprotein subfractions and composite measures of subfractions to risk for CVD in prospective cohort studies.

Methods and Results—We tested whether combinations of lipoprotein subfractions independently predict CVD in a prospective cohort of 4,594 initially healthy men and women (the Malmö Diet and Cancer Study, mean follow-up 12.2 years, 377 incident cardiovascular events). Plasma lipoproteins and lipoprotein subfractions were measured at baseline with a novel, high-resolution ion mobility technique. Principal component analysis (PCA) of subfraction concentrations identified three major independent (i.e., zero correlation) components of CVD risk, one representing LDL-associated risk, a second representing HDL-associated protection, and the third representing a pattern of decreased large HDL, increased small/medium LDL, and increased triglycerides. The last corresponds to the previously described "atherogenic lipoprotein phenotype." Several genes that may underlie this phenotype—*CETP, LIPC, GALNT2, MLXIPL, APOA1/A5, LPL*—are suggested by SNPs associated with the combination of small/medium LDL and large HDL.

Corresponding Authors: Ronald M. Krauss, M.D. Children's Hospital Oakland Research Institute 5700 Martin Luther King Jr Way Oakland, CA 94609 rkrauss@chori.org / Phone: 510-450-7908 / Fax: 510-450-7909. Olle Melander, M.D., Ph.D. Clinical Research Centre, Lund University Entrance 72, Building 91, Floor 12 Malmö University Hospital SE-205 02 Malmö, Sweden olle.melander@med.lu.se / Phone: +46 40 391209 / Fax: +46 40 391222.
[*]These authors contributed equally to this work
[†]These authors contributed equally to this work

Disclosures: Dr. Musunuru has served as a consultant for Alnylam Pharmaceuticals. Dr. Kathiresan has received research support from Alnylam Pharmaceuticals and serves on an Advisory Board for Merck & Co. Dr. Krauss has served as a consultant to Celera Genomics, has received research support from Quest Diagnostics, and is a co-inventor on patents for the technology underlying the ion mobility method. Drs. Caulfield, Li, Salameh and Reitz are employees of Quest Diagnostics. The other authors declare no conflicts of interest or relevant financial interests. None of the authors has been specifically compensated by any of the listed companies in the writing of this manuscript. Drs. Musunuru, Orho-Melander, and Melander served as independent analysts with no relationship with Quest Diagnostics.

Conclusion—PCA on lipoprotein subfractions yielded three independent components of CVD risk. Genetic analyses suggest these components represent independent mechanistic pathways for development of CVD.

Keywords

Lipids; lipoproteins; cardiovascular diseases; genetics

INTRODUCTION

Prospective epidemiologic studies have shown that plasma levels of LDL-C and HDL-C predict incident atherosclerotic cardiovascular disease (CVD).[1] However, there is considerable heterogeneity among lipoproteins, e.g., ranging from small, dense, lipid-depleted LDL particles to large, buoyant, cholesterol-enriched LDL particles.[2] Various studies have evaluated the associations of small LDL particles,[3] total LDL particle number,[3] specific HDL subfractions,[4,5] and combined measures such as the LDL-C/HDL-C and apoB/apoA-I ratios[6-8] with cardiovascular risk. While a number of these studies suggest clinically useful metrics, no study has systematically addressed the interrelationships among the lipoproteins and asked whether there are distinct combinations of lipoprotein subfractions—possibly reflecting specific underlying mechanistic pathways—that *independently* confer cardiovascular risk.

We set out to identify any such combinations of lipoproteins in a systematic fashion in a prospective cohort study. To directly measure lipoprotein particles with high resolution, we used a novel ion mobility method.[9,10]

Our study had two objectives. First, we applied *principal component analysis* to identify interrelated combinations of subfractions and investigated their relationships to incident CVD in a large prospective cohort study of men and women. Second, to better understand the mechanistic pathways underlying these combinations, we tested their associations with common variants in 29 genetic loci that we have recently shown to be associated with LDL-C, HDL-C, or triglyceride levels.[11]

METHODS

The Malmö Diet and Cancer Study Cardiovascular Cohort is a prospective, community-based epidemiological cohort of 6,103 residents of Malmö, Sweden. The women in the cohort were born between 1923 and 1950, and the men between 1923 and 1945. At baseline examinations (conducted between 1991 and 1996), all participants underwent a complete history, physical examination, and laboratory assessment of cardiovascular risk factors, including cigarette smoking, family history of myocardial infarction, blood pressure, presence of diabetes mellitus, C-reactive protein, lipid-lowering therapy, and a fasting lipid profile measured according to standard procedures. LDL-C levels were calculated with Friedewald's formula, except when triglycerides exceeded 400 mg/dl. Apolipoprotein B (apoB) and A-I (apoA-I) levels were not available. Blood samples were obtained and placed in long-term storage at −80°C.

An ion mobility method was used on these blood samples to directly quantify the full spectrum of lipoprotein particles, from the smallest, most dense HDL particles to large, buoyant VLDL particles, in archived baseline plasma samples (Supplementary Table I). This method employs an ion separation/particle detector system that separates ions by size and which can count lipoprotein particles over a wide range of sizes. A full description of this technique is published elsewhere.[9,10] Since the original publication of the method, refinements to the technique have been made that address concerns about the method.[10,12] These refinements were fully

incorporated into the ion mobility measurements used for this study and are described in Supplementary Methods.

We pre-specified a primary composite cardiovascular endpoint of myocardial infarction, stroke, and death from coronary heart disease, as well as a secondary coronary endpoint of myocardial infarction and death from coronary heart disease. Events were ascertained from local or national records on the basis of ICD-9/-10 codes as previously described.[13] All subjects still being followed were censored after December 31, 2005. The mean follow-up time was 12.2 years.

We selected 29 single nucleotide polymorphisms (SNPs) in 29 loci that displayed associations with HDL-C, LDL-C, and/or triglycerides at genomewide significance ($P < 5 \times 10^{-8}$) in a recently completed meta-analysis of seven genome-wide association studies (Supplementary Table II).[12] Genotyping was performed as previously described.[13] All SNPs were in Hardy-Weinberg equilibrium ($P > 0.001$). The estimated genotyping error rate based on a subset of samples was < 1%.

Non-parametric Spearman rank correlations were computed for all combinations of each of the eleven lipoprotein subfractions and HDL-C, LDL-C, and triglycerides. Principal component analysis was performed on the eleven lipoprotein subfractions with or without lipids, using all individuals (4,594 total, 2,749 women, 1,845 men) with complete data for all subfractions. The principal component (PC) vectors were used to calculate PC values for each individual from the sum of weighted terms for each lipoprotein or lipid, with each term being the product of the vector-derived weighting and the particle concentration minus the subfraction mean, divided by the standard deviation for the subfraction (all values used for the first three principal components are available in Supplementary Table I and Table 2).

For analyses of associations between lipoprotein subfractions, PCs, or lipid measures and incident cardiovascular events or coronary events, we excluded subjects with prior myocardial infarction/stroke or on baseline lipid-lowering therapy. For the remaining 4,368 subjects, we constructed multivariable proportional-hazards models treating the PCs, lipid levels, particle concentrations/ratios, and peak LDL particle diameter as continuous variables and used the time to the first cardiovascular or coronary event as the dependent variable, adjusting for age, gender, systolic blood pressure, use of antihypertensive medications, diabetes, and current smoking status. Proportionality of hazards for each variable was assessed by Wald chi-square testing. The three PC measures were tested for association with the endpoint separately and together in a single model; they were also tested in models additionally adjusting for HDL-C, LDL-C, and triglyceride levels, as well as in analyses stratified by gender.

For the 29 SNPs and each of the subfractions, PCs, or lipid measures, multivariable linear regression analyses were used to test whether each measure differed according to an increasing copy number of the SNP minor allele, adjusted for age, gender, and diabetes status. $P < 0.001$ was considered statistically significant, accounting for multiple testing.

SPSS (version 16.0) and STATA (version 10) were used for the analyses.

RESULTS

Of the 6,103 individuals in the MDC-CC cohort, 4,822 individuals had available plasma samples that were subjected to ion mobility analysis for quantification of lipoprotein subfractions. Of these, 4,758 samples yielded complete lipoprotein subfraction profiles, of which 4,594 (2,749 women, 1,845 men) had standard lipid data (LDL-C, HDL-C, and triglycerides) available and were used for analyses. Percentiles, means, and standard deviations for these measurements are listed in Supplementary Table I. All subjects were of self-reported

European ancestry. Other baseline characteristics of the MDC-CC cohort have been reported previously.[13]

In the correlation analyses, we found small and medium-sized LDL particle concentrations to be inversely correlated with large HDL particle concentrations ($\rho = -0.46, -0.55$; Table 1) and directly correlated with triglycerides ($\rho = 0.45, 0.54$). Notably, these correlations were far stronger than that between small/medium LDL and small HDL ($\rho = 0.04, 0.04$), as well as stronger than that between small/medium LDL and LDL-C ($\rho = 0.22, 0.29$). Large HDL but not small HDL was highly correlated with HDL-C ($\rho = 0.82, 0.17$).

Principal component analysis is a statistical technique that analyzes the interrelationships between numerous variables and yields a fewer number of components that explain most of the correlation information of the original variables. Each of these components is an independent, linear combination of the original variables. We reasoned that the correlations observed among the lipoprotein subfractions may reflect hitherto unmeasured biological processes that may be more relevant to cardiovascular risk than the standard lipoprotein measures. Thus, we applied principal component analysis to the ion mobility measurements of the MDC-CC cohort.

Three major principal components (PC1, PC2, PC3) were found to account for 76% of the overall variance among the subfractions (Table 2). Similar results were obtained when HDL-C, LDL-C, and triglycerides were included in the model (Table 2). The strongest contributors to PC1 were the LDL and VLDL subfractions, whereas HDL subfractions were the strongest contributors to PC3, with lesser contributions from LDL subfractions. Notably, PC2 had the strongest contributions from small LDL/medium LDL/triglycerides in the positive direction and large HDL in the negative direction, indicating that as the first group of subfractions increase, large HDL decreases.

Of 4,368 individuals, 377 (8.6%) experienced an incident cardiovascular event during the follow-up period, with 206 (4.7%) being coronary events. With all three major PCs incorporated into a single model, PC2 and PC3 were strongly associated with cardiovascular events ($P = 0.001$ for both), and PC1 was associated at borderline significance ($P = 0.07$; Table 3). The same levels of association were seen when each of the major PCs was analyzed in a model separately (data not shown). The fourth through eleventh (minor) PCs were not significantly associated with events (data not shown).

Associations of individual subfractions, composite subfraction measures, and traditional lipid traits with cardiovascular events are shown in Table 3. Among individual subfractions, the strongest effects on risk (either increasing or decreasing risk) for cardiovascular events were seen for small LDL (HR 1.14; 95% CI, 1.04-1.25; $P = 0.004$), medium-sized LDL (HR 1.17; 95% CI, 1.07-1.28; $P = 0.001$), and large HDL (HR 0.76; 95% CI, 0.66-0.88; $P < 0.001$) particles. The other subfractions were either nominally or not associated with cardiovascular events. Analyses performed with coronary events yielded similar results (Table 3).

The weak association of PC1 with cardiovascular events was eliminated by adjustment for LDL-C and HDL-C but not for triglycerides (Table 4). For both PC2 and PC3, the association with cardiovascular events was weakened by adjustment for HDL-C but not for either LDL-C or triglycerides. Of the three PCs, only PC2 was associated with cardiovascular events in both men and women in subgroup analyses (Table 5).

We assessed the strength of association between each of 29 SNPs and the three PCs (Supplementary Table II). For those SNPs showing association with HDL-C, LDL-C, or triglycerides, we also tested for association with large HDL, small LDL, and medium LDL (Table 6). In total, eleven loci were highly associated ($P < 0.001$) with HDL-C, LDL-C, or

triglycerides (Table 6). Of these, six loci—*CETP, LIPC, GALNT2, MLXIPL, APOA1/A5, LPL*—were highly associated with PC2 and were associated to some degree with both small/medium LDL and large HDL (Table 6). None of the six loci was associated with large LDL or small HDL (data not shown). *CETP* alone was associated with PC3 and was also weakly associated with PC1 ($P = 0.02$). Of the other five loci, three were strongly associated with both LDL-C and PC1—*APOB, APOE, CELSR2/PSRC1/SORT1*.

DISCUSSION

In this prospective cohort of healthy individuals, we used principal component analysis to identify three independent combinations of correlated lipoprotein subfractions and assess the contribution of these combinations to cardiovascular risk. One of the components (PC2) represents a relationship among the lipoprotein particle classes—small/medium LDL and large HDL—that were most highly associated with risk for CVD in our study, consistent with a recently published NMR lipoprotein analysis of the Women's Health Study.[5] PC2 was associated with cardiovascular events in both men and women, and it was strongly associated with six genetic loci.

We believe our data are of mechanistic relevance for several reasons. First, the PCs represent three *independent* axes of CVD risk that are not weakened by combining them into a single regression model, unlike traditional lipid measures. PC1 mainly comprises the LDL and VLDL subfractions and appears to best capture the component of CVD risk related to LDL-C; hazard ratios and confidence intervals of PC1 and LDL-C for cardiovascular events were very similar (Table 3), and PC1 was the only PC whose association with events was eliminated by adjustment for LDL-C (Table 4).

PC2 is unique because it represents an inverse correlation between small LDL/medium LDL/triglycerides and large HDL particles, i.e., the magnitude of PC2 is largest for individuals with high levels of lipoproteins in the first group and low levels of large HDL particles, or vice versa. This pattern matches the "atherogenic lipoprotein phenotype" first proposed two decades ago.[14] This phenotype comprises a triad of decreased HDL-C, increased smaller LDL particles, and increased triglycerides that confers substantially higher risk for CVD[15,16] and is characteristic of type 2 diabetes, insulin resistance, and the metabolic syndrome.[17,18] Thus, principal component analysis—which by design yields *independent* components with zero correlation—provides unbiased evidence that the atherogenic lipoprotein phenotype represents an *independent* mechanism of cardiovascular risk.

PC3, which has the greatest contributions from both large and small HDL particles, was inversely associated with events and appears to reflect a contribution of HDL-C to CVD risk that, in contrast to PC2, has smaller contributions from non-HDL lipoproteins including an inverse correlation with large LDL particles (Table 3).

Second, we found that most of the SNPs that were strongly associated with HDL-C or triglycerides were associated with PC2, with opposite effects on LDL and HDL subfraction particle concentrations. Four of the implicated genes—*CETP, LIPC, APOA1/A5, LPL*—have been reported to be associated with LDL or HDL particle size/distribution,[19-27] while the roles of two genes—*GALNT2, MLXIPL*—in lipoprotein metabolism are less clear. *MLXIPL* encodes a transcriptional regulator whose targets include genes involved in triglyceride metabolism.[28] The genes implicated by SNPs in these loci appear to define a distinct biological pathway that (when dysregulated) gives rise to the atherogenic lipoprotein phenotype.

Third, we note that the PCA technique used in this study can be viewed as a "redistribution" of the CVD risk associated with the traditional lipid profile—HDL-C, LDL-C, and triglycerides—into three *independent* risk factors. As observed above, PC1 appears to be a proxy for plasma

LDL-C levels, with only PC1's association with cardiovascular events eliminated by adjustment for LDL-C. In contrast, all three PCs' associations with events were weakened by adjustment for HDL-C, indicating that the HDL-C-associated CVD risk overlaps with and is "redistributed" among the three PCs.

Thus, we hypothesize that the strong epidemiological association of plasma HDL-C levels with CVD seen in numerous studies emerges as a result of HDL lipoprotein particles contributing to cardiovascular risk through at least three independent mechanisms, embodied by the three PCs. As suggested by the SNP analyses, several genes—*LIPC, APOA1/A5, LPL, GALNT2, MLXIPL*—appear to act primarily through one of these mechanisms (PC2), whereas *CETP* is unique in acting through multiple risk factors (PC2 and PC3 and, perhaps more weakly, PC1).

Finally, the present findings help clarify the conflicting conclusions of previously published reports on lipoprotein particles and cardiovascular risk.[3] While a number of studies have found that small LDL particle concentrations are associated with cardiovascular disease,[4,29-35] a few studies have not found this relationship.[36-38] Our study, demonstrating that (1) levels of small/medium LDL particles are associated with and (2) levels of large LDL particles are not significantly related to cardiovascular disease, is consistent with other large prospective cohort studies whose data were obtained by two different lipoprotein measurement techniques: the Québec Cardiovascular Study,[4,34] which used gradient gel electrophoresis, and the Multi-Ethnic Study of Atherosclerosis[35] and Women's Health Study,[5] which both used NMR.

The disproportionate influence of large LDL on the measured plasma LDL-C level accounts for the latter's weaker association with cardiovascular risk than small LDL particle concentration or total LDL particle number, which may be more appropriate than LDL-C as targets of therapeutic intervention. Measurement of the PCs may provide even greater specificity in evaluating the effectiveness of lipid-altering treatment. Accordingly, it would be of interest to know how lipid-modifying medications affect each of the three PCs. The application of principal component analysis in trials of lipid-modifying therapies, as well as specific analyses on the utility of the PCs for clinical risk prediction and as therapeutic targets with which to select and titrate medications, will be the focus of future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: K.M. is supported by a T32 grant in Cell and Molecular Training for Cardiovascular Biology from the United States National Institutes of Health (NIH). M.O.-M. and O.M. are supported by the Swedish Medical Research Council, the Swedish Heart and Lung Foundation, the Medical Faculty of Lund University, Malmö University Hospital, the Albert Påhlsson Research Foundation, the Crafoord foundation, the Ernhold Lundströms Research Foundation, the Region Skane, the Hulda and Conrad Mossfelt Foundation, the King Gustaf V and Queen Victoria Foundation and the Lennart Hanssons Memorial Fund. S.K. is supported by a Doris Duke Charitable Foundation Clinical Scientist Development Award, a charitable gift from the Fannie E. Rippel Foundation, the Donovan Family Foundation, and a K23 career development award from the NIH. R.M.K. is supported by U01HL069757 from the NIH and has received research support from Quest Diagnostics, Inc. For this study, ion mobility measurements of study participants were provided free of charge by Quest Diagnostics, which had no role in the analysis and interpretation of the data or in the preparation, review, or approval of the manuscript.

REFERENCES

1. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation; Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High

Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–3421. [PubMed: 12485966]

2. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23:97–104. [PubMed: 7057116]
3. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. Ann Intern Med 2009;150:474–484. [PubMed: 19349632]
4. Asztalos BF, Collins D, Cupples LA, Demissie S, Horvath KV, Bloomfield HE, Robins SJ, Schaefer EJ. Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent cardiovascular events in the Veterans Affairs HDL Intervention Trial. Arterioscler Thromb Vasc Biol 2005;25:2185–2191. [PubMed: 16123324]
5. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation 2009;119:931–939. [PubMed: 19204302]
6. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. JAMA 2005;294:326–333. [PubMed: 16030277]
7. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, Pencina MJ, Schoonmaker C, Wilson PW, D'Agostino RB, Vasan RS. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007;298:776–785. [PubMed: 17699011]
8. McQueen MJ, Hawken S, Wang X, Ounpuu S, Sniderman A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, Kazmi K, Yusuf S, INTERHEART study investigators. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. Lancet 2008;372:224–233. [PubMed: 18640459]
9. Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, Reitz RE, Krauss RM. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. Clin Chem 2008;54:1307–1316. [PubMed: 18515257]
10. Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, Reitz RE, Krauss RM. Concerns regarding lipoprotein particle measurement by ion mobility analysis. In reply. Clin Chem 2008;54:2088–2089.
11. Kathiresan S, Willer CJ, Peloso G, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, Voight BF, Bonnycastle LL, Jackson AU, Crawford G, Surti A, Guiducci C, Burtt NP, Parish S, Clarke R, Zelenika D, Kubalanza KA, Morken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kuusisto J, Bergman RN, Sundvall J, Laakso M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, Debakker PI, O'Donnell CJ, Chambers JC, Kooner JS, Hercberg S, Meneton P, Lakatta EG, Scuteri A, Schlessinger D, Tuomilehto J, Collins FS, Groop L, Altshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, Mohlke KL, Cupples LA. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 2009;41:56–65. [PubMed: 19060906]
12. Otvos JD, Rudel LL, McConenell JP. Concerns regarding lipoprotein particle measurements by ion mobility analysis. Clin Chem 2008;54:2086–2087. [PubMed: 19042998]
13. Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, Roos C, Hirschhorn JN, Berglund G, Hedblad B, Groop L, Altshuler DM, Newton-Cheh C, Orho-Melander M. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;358:1240–1249. [PubMed: 18354102]
14. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990;82:495–506. [PubMed: 2372896]
15. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917–1921. [PubMed: 3418853]
16. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276:882–888. [PubMed: 8782637]

17. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest 1993;92:141–146. [PubMed: 8325978]

18. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW, D'Agostino RB, Vasan RS, Robins SJ. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation 2006;113:20–29. [PubMed: 16380547]

19. Rotter JI, Bu X, Cantor RM, Warden CH, Brown J, Gray RJ, Blanche PJ, Krauss RM, Lusis AJ. Multilocus genetic determinants of LDL particle size in coronary artery disease families. Am J Hum Genet 1996;58:585–594. [PubMed: 8644718]

20. Allayee H, Aouizerat BE, Cantor RM, Dallinga-Thie GM, Krauss RM, Lanning CD, Rotter JI, Lusis AJ, de Bruin TW. Families with familial combined hyperlipidemia and families enriched for coronary artery disease share genetic determinants for the atherogenic lipoprotein phenotype. Am J Hum Genet 1998;63:577–585. [PubMed: 9683614]

21. Hokanson JE, Brunzell JD, Jarvik GP, Wijsman EM, Austin MA. Linkage of low-density lipoprotein size to the lipoprotein lipase gene in heterozygous lipoprotein lipase deficiency. Am J Hum Genet 1999;64:608–618. [PubMed: 9973300]

22. Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, Lahoz C, Coltell O, Wilson PW, Schaefer EJ. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler Thromb Vasc Biol 2000;20:1323–1329. [PubMed: 10807749]

23. Talmud PJ, Edwards KL, Turner CM, Newman B, Palmen JM, Humphries SE, Austin MA. Linkage of the cholesteryl ester transfer protein (CETP) gene to LDL particle size: use of a novel tetranucleotide repeat within the CETP promoter. Circulation 2000;101:2461–2466. [PubMed: 10831518]

24. Humphries SE, Berglund L, Isasi CR, Otvos JD, Kaluski D, Deckelbaum RJ, Shea S, Talmud PJ. Loci for CETP, LPL, LIPC, and APOC3 affect plasma lipoprotein size and sub-population distribution in Hispanic and non-Hispanic white subjects: the Columbia University BioMarkers Study. Nutr Metab Cardiovasc Dis 2002;12:163–172. [PubMed: 12514935]

25. Skoglund-Andersson C, Ehrenborg E, Fisher RM, Olivecrona G, Hamsten A, Karpe F. Influence of common variants in the CETP, LPL, HL and APO E genes on LDL heterogeneity in healthy, middle-aged men. Atherosclerosis 2003;167:311–317. [PubMed: 12818414]

26. Austin MA, Talmud PJ, Farin FM, Nickerson DA, Edwards KL, Leonetti D, McNeely MJ, Viernes HM, Humphries SE, Fujimoto WY. Association of apolipoprotein A5 variants with LDL particle size and triglyceride in Japanese Americans. Biochim Biophys Acta 2004;1688:1–9. [PubMed: 14732475]

27. Mar R, Pajukanta P, Allayee H, Groenendijk M, Dallinga-Thie G, Krauss RM, Sinsheimer JS, Cantor RM, de Bruin TW, Lusis AJ. Association of the APOLIPOPROTEIN A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. Circ Res 2004;94:993–999. [PubMed: 15001527]

28. Kooner JS, Chambers JC, Aguilar-Salinas CA, Hinds DA, Hyde CL, Warnes GR, Pérez FJ Gómez, Frazer KA, Elliott P, Scott J, Milos PM, Cox DR, Thompson JF. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. Nat Genet 2008;40:149–151. [PubMed: 18193046]

29. Kuller L, Arnold A, Tracy R, Otvos J, Burke G, Psaty B, Siscovick D, Freedman DS, Kronmal R. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 2002;22:1175–1180. [PubMed: 12117734]

30. Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002;106:1930–1937. [PubMed: 12370215]

31. Soedamah-Muthu SS, Chang YF, Otvos J, Evans RW, Orchard TJ, Pittsburgh Epidemiology of Diabetes Complications Study. Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease in type 1 diabetes. A prospective

report from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia 2003;46:674–682. [PubMed: 12743701]

32. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002;90:89–94. [PubMed: 12106834]
33. Arsenault BJ, Lemieux I, Després JP, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, Boekholdt SM. Cholesterol levels in small LDL particles predict the risk of coronary heart disease in the EPIC-Norfolk prospective population study. Eur Heart J 2007;28:2770–2777. [PubMed: 17947216]
34. St-Pierre AC, Cantin B, Dagenais GR, Mauriège P, Bernard PM, Després JP, Lamarche B. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Québec Cardiovascular Study. Arterioscler Thromb Vasc Biol 2005;25:553–559. [PubMed: 15618542]
35. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC Jr, O'Leary DH, Saad MF, Tsai MY, Sharrett AR. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2007;192:211–217. [PubMed: 16765964]
36. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006;113:1556–1563. [PubMed: 16534013]
37. Kuller LH, Grandits G, Cohen JD, Neaton JD, Prineas R, Multiple Risk Factor Intervention Trial Research Group. Lipoprotein particles, insulin, adiponectin, C-reactive protein and risk of coronary heart disease among men with metabolic syndrome. Atherosclerosis 2007;195:122–128. [PubMed: 17011566]
38. Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. JAMA 2001;286:1468–1474. [PubMed: 11572739]

Table 1

Spearman correlation coefficients (ρ) for lipid and lipoprotein measures

Measure	HDL-C	LDL-C	TG	HDL-S	HDL-L	LDL-VS	LDL-S	LDL-M	LDL-L	IDL-S	IDL-L	VLDL-S	VLDL-M	VLDL-L
HDL-C	1.0	−0.08	−0.54	0.17	0.82	−0.22	−0.44	−0.54	−0.19	0.50	−0.13	0.09	−0.22	−0.34
LDL-C	—	1.0	0.27	−0.002	−0.18	0.26	0.22	0.29	0.50	0.27	0.62	0.61	0.52	0.38
TG	—	—	1.0	0.08	−0.50	0.34	0.45	0.54	0.22	−0.35	0.34	0.16	0.48	0.64
HDL-S	—	—	—	1.0	0.24	0.15	0.04	0.04	0.09	0.11	0.19	0.19	0.15	0.14
HDL-L	—	—	—	—	1.0	−0.23	−0.46	−0.55	−0.18	0.55	−0.11	0.11	−0.20	−0.34
LDL-VS	—	—	—	—	—	1.0	0.77	0.64	0.23	−0.18	0.41	0.28	0.46	0.48
LDL-S	—	—	—	—	—	—	1.0	0.93	0.34	−0.37	0.37	0.17	0.42	0.47
LDL-M	—	—	—	—	—	—	—	1.0	0.51	−0.38	0.46	0.22	0.48	0.53
LDL-L	—	—	—	—	—	—	—	—	1.0	0.31	0.65	0.54	0.54	0.43
IDL-S	—	—	—	—	—	—	—	—	—	1.0	0.35	0.51	0.13	−0.08
IDL-L	—	—	—	—	—	—	—	—	—	—	1.0	0.88	0.82	0.64
VLDL-S	—	—	—	—	—	—	—	—	—	—	—	1.0	0.82	0.56
VLDL-M	—	—	—	—	—	—	—	—	—	—	—	—	1.0	0.87
VLDL-L	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0

Table 2

Principal component analysis of lipoprotein subfractions without and with conventional lipid measures*

Measure	Principal components with lipoproteins only			Principal components with lipoproteins and conventional lipid measures		
	PC1	PC2	PC3	PC1+	PC2+	PC3+
HDL-C	—	—	—	−0.181	−0.356	+0.319
LDL-C	—	—	—	+0.265	−0.158	−0.243
TG	—	—	—	+0.247	+0.277	+0.113
HDL-S (7.7-10.5 nm)	+0.098	−0.164	+0.637	+0.061	−0.151	+0.457
HDL-L (10.5-14.5 nm)	−0.091	−0.384	+0.529	−0.149	−0.360	+0.473
LDL-VS (18.0-20.8 nm)	+0.259	+0.287	+0.366	+0.258	+0.139	+0.411
LDL-S (20.8-21.4 nm)	+0.258	+0.424	+0.245	+0.272	+0.286	+0.325
LDL-M (21.4-22.0 nm)	+0.314	+0.344	+0.030	+0.320	+0.234	+0.125
LDL-L (22.0-23.3 nm)	+0.302	−0.195	−0.327	+0.270	−0.196	−0.311
IDL-S (23.3-25.0 nm)	+0.100	−0.514	+0.016	+0.037	−0.471	−0.040
IDL-L (25.0-29.6 nm)	+0.421	−0.187	−0.050	+0.361	−0.242	−0.023
VLDL-S (29.6-33.5 nm)	+0.370	−0.311	−0.060	+0.302	−0.349	−0.045
VLDL-M (33.5-42.4	+0.430	−0.079	−0.079	+0.379	−0.151	−0.022
VLDL-L (42.4-52.0 nm)	+0.388	+0.071	−0.018	+0.356	−0.009	+0.051
% variance explained	41%	24%	11%	41%	21%	10%

The first three out of eleven/fourteen principal components (PCs) are included here, with individual weightings of each lipoprotein/lipid for each PC, as well as the proportion of variance explained by each PC. The particle size ranges for each lipoprotein subfraction is indicated.

* HDL-C, LDL-C, TG

Table 3

Multivariable-adjusted associations of PC, lipid, and lipoprotein measures with incident cardiovascular or coronary events in 4,368 individuals

Measure	Cardiovascular events (N=377) HR	95% CI	*P* value	Coronary events (N=206) HR	95% CI	*P* value
PC1*	1.10	0.99–1.22	0.07	1.11	0.97–1.28	0.12
PC2*	1.22	1.08–1.36	0.001	1.24	1.06–1.45	0.008
PC3*	0.81	0.71–0.92	0.001	0.76	0.64–0.91	0.003
HDL-C	0.72	0.63–0.82	< 0.001	0.72	0.61–0.86	< 0.001
LDL-C	1.10	0.99–1.21	0.08	1.20	1.04–1.37	0.01
TG	1.12	1.00–1.25	0.04	1.07	0.92–1.25	0.36
Non-HDL-C	1.10	1.00–1.21	0.06	1.13	0.99–1.28	0.08
HDL-S	0.87	0.77–0.99	0.04	0.78	0.64–0.95	0.01
HDL-L	0.76	0.66–0.88	< 0.001	0.75	0.61–0.91	0.003
LDL-VS	1.03	0.92–1.14	0.66	1.02	0.88–1.18	0.82
LDL-S	1.14	1.04–1.25	0.004	1.16	1.04–1.31	0.01
LDL-M	1.17	1.07–1.28	0.001	1.19	1.06–1.34	0.005
LDL-L	1.09	0.98–1.20	0.11	1.09	0.95–1.24	0.23
IDL-S	0.91	0.81–1.02	0.10	0.93	0.79–1.09	0.37
IDL-L	1.05	0.95–1.17	0.31	1.09	0.95–1.24	0.23
VLDL-S	1.02	0.91–1.13	0.79	1.05	0.91–1.21	0.52
VLDL-M	1.05	0.95–1.16	0.38	1.06	0.93–1.22	0.38
VLDL-L	1.06	0.96–1.17	0.24	1.06	0.93–1.21	0.40
LDL total	1.16	1.05–1.28	0.004	1.17	1.03–1.34	0.02
Non-HDL	1.10	1.00–1.21	0.06	1.13	0.99–1.28	0.08
HDL total	0.78	0.68–0.90	0.001	0.71	0.58–0.87	0.001
LDL/HDL	1.22	1.12–1.32	< 0.001	1.21	1.09–1.35	< 0.001
LDL/HDL-L	1.25	1.14–1.36	< 0.001	1.22	1.09–1.37	0.001
Non-HDL/HDL	1.20	1.11–1.30	< 0.001	1.20	1.08–1.33	0.001
Non-HDL/HDL-L	1.25	1.15–1.37	< 0.001	1.23	1.09–1.38	0.001
LDL-S+M/HDL-L	1.20	1.10–1.30	< 0.001	1.20	1.08–1.33	0.001
Peak LDL diameter	0.87	0.78–0.97	0.009	0.84	0.73–0.97	0.02

Hazard ratios (HR) and 95% confidence intervals (CI) are per one standard deviation of the measure. Values shown are adjusted for age, gender, systolic blood pressure, use of antihypertensive medications, diabetes status, and current smoking status.

*Values for PC1, PC2, PC3 were calculated in a single regression model including all three PCs.

Table 4

Multivariable-adjusted associations of PCs with CVD, with adjustment for lipids

Subfraction	HR	95% CI	*P* value	Adjusted for LDL-C	Adjusted for HDL-C	Adjusted for TG
PC1*	1.10	0.99–1.22	0.07	0.23	0.41	0.09
PC2*	1.22	1.08–1.36	0.001	0.001	0.51	0.003
PC3*	0.81	0.71–0.92	0.001	0.003	0.08	0.001

Hazard ratios (HR) and 95% confidence intervals (CI) are per one standard deviation of the measure. Values shown are adjusted for age, gender, systolic blood pressure, use of antihypertensive medications, diabetes status, and current smoking status.

* Values for PC1, PC2, PC3 were calculated in a single regression model including all three PCs.

Table 5

Multivariable-adjusted associations of PCs with CVD by gender

Subfraction	Women (N=2,653, events=195) HR	95% CI	*P* value	Men (N=1,715, events=182) HR	95% CI	*P* value
HDL-C	0.77	0.65–0.90	0.002	0.67	0.55–0.82	< 0.001
LDL-C	1.12	0.97–1.29	0.13	1.08	0.93–1.26	0.34
TG	1.07	0.91–1.26	0.42	1.15	0.99–1.34	0.07
PC1*	1.13	1.00–1.28	0.12	1.07	0.92–1.24	0.40
PC2*	1.19	1.07–1.40	0.03	1.28	1.07–1.52	0.006
PC3*	0.93	0.79–1.10	0.42	0.69	0.57–0.85	< 0.001

Hazard ratios (HR) and 95% confidence intervals (CI) are per one standard deviation of the measure. Values shown are adjusted for age, gender, systolic blood pressure, use of antihypertensive medications, diabetes status, and current smoking status.

* Values for PC1, PC2, PC3 were calculated in a single regression model including all three PCs.

Table 6

Multivariable-adjusted associations of SNPs to selected lipoprotein measures

SNP	Locus	Alleles maj/min/ MAF	HDL-C *P* value	LDL-C *P* value	TG *P* value	HDL-L *P* value	LDL-S *P* value	LDL-M *P* value	PC1 *P* value	PC2 *P* value	PC3 *P* value
rs693	*APOB*	G/A/0.48	0.002	3×10^{-11}	0.0002	0.03	0.04	0.01	2.7×10^{-8}	0.74	0.17
rs4420638	*APOE*	A/G/0.20	0.02	3×10^{-21}	0.006	0.16	2×10^{-10}	3×10^{-12}	8.7×10^{-7}	0.029	0.94
rs6511720	*LDLR*	G/T/0.10	0.36	1×10^{-15}	0.49	0.39	0.71	0.37	0.003	0.028	0.10
rs646776	*CELSR2/ PSRC1/ SORT1*	A/G/0.24	0.17	2×10^{-11}	0.21	0.75	0.03	0.02	5.7×10^{-5}	0.65	0.28
rs1800775	*CETP*	C/A/0.49	3×10^{-29}	0.002	0.23	8×10^{-16}	0.003	0.03	0.02	4×10^{-5}	0.0005
rs1800588	*LIPC*	C/T/0.21	2×10^{-10}	0.87	0.16	5×10^{-10}	8×10^{-5}	0.01	0.93	1×10^{-9}	0.69
rs4846914	*GALNT2*	A/G/0.40	1×10^{-6}	0.34	0.003	0.0006	0.009	0.002	0.37	2×10^{-5}	0.42
rs1260326	*GCKR*	C/T/0.36	0.28	0.78	5×10^{-9}	0.22	0.02	0.003	0.16	0.005	0.24
rs17145738	*MLXIPL*	C/T/0.13	0.003	0.34	3×10^{-8}	0.0007	0.0001	1×10^{-4}	0.04	9×10^{-7}	0.85
rs3133506	*APOA1/A5*	T/G/0.07	0.003	4×10^{-5}	2×10^{-15}	0.004	6×10^{-5}	4×10^{-7}	0.004	7×10^{-5}	0.40
rs328	*LPL*	C/G/0.09	3×10^{-12}	0.50	2×10^{-9}	4×10^{-6}	4×10^{-5}	0.0001	0.002	7×10^{-6}	0.04

P values shown are adjusted for age, gender, and diabetes status. The major allele, minor allele, and minor allele frequency (MAF) for each SNP is indicated; all modeling was performed with the major allele as the reference allele.