Journal of the American Heart Association

ORIGINAL RESEARCH

Lipoprotein Subclasses Associated With High-Risk Coronary Atherosclerotic Plaque: Insights From the PROMISE Clinical Trial

Robert W. McGarrah, MD; Maros Ferencik, MD, PhD, MCR; Stephanie N. Giamberardino, ScM; Udo Hoffmann, MD, MPH; Borek Foldyna, MD; Julia Karady, MD; Geoffrey S. Ginsburg, MD, PhD; William E. Kraus, MD; Pamela S. Douglas, MD; Svati H. Shah, MD, MS, MHS

BACKGROUND: More than half of major adverse cardiovascular events (MACE) occur in the absence of obstructive coronary artery disease and are often attributed to the rupture of high-risk coronary atherosclerotic plaque (HRP). Blood-based biomarkers that associate with imaging-defined HRP and predict MACE are lacking.

METHODS AND RESULTS: Nuclear magnetic resonance–based lipoprotein particle profiling was performed in the biomarker substudy of the PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) trial (N=4019) in participants who had stable symptoms suspicious for coronary artery disease. Principal components analysis was used to reduce the number of correlated lipoproteins into uncorrelated lipoprotein factors. The association of lipoprotein factors and individual lipoproteins of significantly associated factors with core laboratory determined coronary computed tomographic angiography features of HRP was determined using logistic regression models. The association of HRP-associated lipoproteins with MACE was assessed in the PROMISE trial and validated in an independent coronary angiography biorepository (CATHGEN [Catheterization Genetics]) using Cox proportional hazards models. Lipoprotein factors composed of high-density lipoprotein (HDL) subclasses were associated with HRP. In these factors, large HDL (odds ratio [OR], 0.70 [95% CI, 0.56–0.85]; $P<0.001$) and medium HDL (OR, 0.84 [95% CI, 0.72–0.98]; $P=0.028$) and HDL size (OR, 0.82 [95% CI, 0.69–0.96]; $P=0.018$) were associated with HRP in multivariable models. Medium HDL was associated with MACE in PROMISE (hazard ratio [HR], 0.76 [95% CI, 0.63–0.92]; $P=0.004$), which was validated in the CATHGEN biorepository (HR, 0.91 [95% CI, 0.88–0.94]; $P<0.001$).

CONCLUSIONS: Large and medium HDL subclasses and HDL size inversely associate with HRP features, and medium HDL subclasses inversely associate with MACE in PROMISE trial participants. These findings may aid in the risk stratification of individuals with chest pain and provide insight into the pathobiology of HRP.

REGISTRATION: URL: https://clinicaltrials.gov; Unique identifier: NCT01174550

Key Words: atherosclerotic plaque ■ biomarkers ■ circulating lipoproteins ■ computed tomography angiography

Although most coronary artery disease (CAD) diagnosis and treatment guidelines focus on individuals with obstructive CAD (oCAD), more than half of major adverse cardiovascular events (MACE) occur in individuals with nonobstructive CAD. Many of these events associated with nonobstructive CAD are attributed to the rupture of "vulnerable" high-risk coronary atherosclerotic plaque (HRP). Studies using intravascular coronary imaging and tissue samples in individuals with sudden cardiac death and acute coronary syndromes have defined HRP as those with a large necrotic lipid pool, large plaque burden, and thin-cap fibroatheroma.[1–4]

Coronary computed tomographic angiography (coronary CTA) has emerged as an effective, noninvasive

Correspondence to: Svati H. Shah, MD, MS, MHS, Division of Cardiology, Department of Medicine, Duke University School of Medicine, 300 North Duke St, Durham, NC, 27701. Email: svati.shah@duke.edu

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.122.026662

For Sources of Funding and Disclosures, see page 8.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In this biomarker substudy of the PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) clinical trial of individuals presenting with stable symptoms suspicious for coronary artery disease, distinct high-density lipoprotein subclasses were inversely associated with features of high-risk coronary atherosclerotic plaque on coronary computed tomography angiography both in the presence and absence of obstructive coronary artery disease.
- Medium high-density lipoprotein subclasses were inversely associated with major adverse cardiovascular events in PROMISE and in the greater risk CATHGEN (Catheterization Genetics) biorepository of individuals undergoing coronary angiography for concern of ischemic heart disease.

What Are the Clinical Implications?

- Because more than half of major adverse cardiovascular events occur in the absence of obstructive coronary artery disease, high-density lipoprotein subclass measurement may aid in the risk stratification of individuals presenting with chest pain.

Nonstandard Abbreviations and Acronyms

CATHGEN	Catheterization Genetics
CTA	computed tomographic angiography
HRP	high-risk coronary atherosclerotic plaque
MACE	major adverse cardiovascular events
NMR	nuclear magnetic resonance
oCAD	obstructive coronary artery disease
PCA	principal components analysis
PROMISE	Prospective Multicenter Imaging Study for Evaluation of Chest Pain
TRL	triglyceride-rich lipoprotein

way to detect oCAD and provide risk stratification in individuals with chest pain.[5–7] Recent studies have also demonstrated that coronary CTA is able to effectively identify HRP features—including positive remodeling, low computed tomography (CT) attenuation, or napkin-ring sign—that correlate with HRP characteristics seen on intravascular imaging or pathologic specimens and are predictive of future MACE.[8,9]

In addition to coronary CTA, the development of blood-based biomarkers also offers a promising, noninvasive means to identify individuals with HRP and at increased risk of MACE. Consistent with what is known about HRP pathophysiology, studies to date have reported on lipids (eg, oxidized low-density lipoprotein [LDL], lipoprotein-associated phospholipase A_2, secretory type II phospholipase A_2), inflammation (eg, C-reactive protein, interleukin-6, interleukin-18, myeloperoxidase), and extracellular matrix dynamics (eg, matrix metalloproteinases) that associate with HRP features (reviewed in Koenig and Khuseyinova[10]).

Although traditional lipid parameters are well-established risk factors for CAD, studies have demonstrated the clinical utility of more granular lipoprotein parameter measurements. In fact, lipoprotein subclasses are easily measured via nuclear magnetic resonance (NMR) spectroscopy and are now used clinically for potential improved risk prediction. Greater LDL particle numbers and small, dense LDL particles are associated with an increased risk for incident cardiovascular disease (CVD), independent of other lipid parameters.[11] Similarly, greater high-density lipoprotein (HDL) particle numbers and medium HDL subclasses are associated with a decreased risk of CVD.[12,13]

We have previously shown that HRP detected by coronary CTA in the PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) trial is predictive of MACE independent of oCAD in this population of outpatients undergoing evaluation for new-onset stable chest pain.[14] In the current PROMISE trial biomarker substudy, we sought to determine whether distinct lipoprotein subclasses are associated with coronary CTA–defined HRP features and predict MACE. We hypothesized that triglyceride-rich lipoproteins (TRLs) and LDLs would be positively associated and that HDLs would be inversely associated with HRP and MACE.

METHODS

Study Design and Population

The data that support the findings of this study are available from the corresponding author upon reasonable request. The PROMISE trial was a pragmatic comparative effectiveness trial that enrolled 10 003 outpatients without known CAD who presented with stable chest pain requiring noninvasive cardiovascular testing (NCT01174550). Participants were randomly assigned to either coronary CTA or clinician-determined standard of care, which was most commonly noninvasive stress testing with imaging. Results of the primary trial have been described elsewhere.[15] The current study included 4019 individuals who also consented to participate in the PROMISE biomarker substudy (Figure 1).

Coronary CTA Phenotypes/Outcomes

ECG-gated or triggered coronary CT scans were performed on CT scanners with at least 64 detector rows (64 rows, 128 rows, 256 rows, 320 rows, and dual source) from 4 vendors (Siemens, General Electric, Toshiba, and Philips). All CTA data set images were transferred to a core laboratory for the analysis at a cardiac image–viewing workstation (TeraRecon, Foster City, CA). Coronary CTA data sets were randomly assigned to 1 of 6 level 3–trained core laboratory readers with 3 to 10 years of experience interpreting coronary CTA images. Furthermore, 50 randomly selected coronary CTA images were analyzed by all 6 readers to determine interobserver agreement as assessed by κ (oCAD defined as ≥70% stenosis in any coronary arteries or left main ≥50% stenosis, κ =0.69; high-risk plaque, κ =0.56). Coronary CTA analysis was performed per coronary segment, and qualitative stenosis severity was reported according to the current Society of Cardiovascular Computed Tomography Guidelines (normal, absence of plaque and no luminal stenosis; minimal, 1%–29% stenosis; mild, 30%–49% stenosis; moderate, 50%–69% stenosis; severe, 70%–99% stenosis; occluded, 100% stenosis).[16] In this current analysis, we defined oCAD as lesions with ≥70% stenosis in any coronary artery or ≥50% in the left main coronary artery. Furthermore, coronary artery segments with plaque were evaluated for HRP, defined as the presence of at least 1 of the following features: low CT attenuation (voxels in the plaque <30 Hounsfield units), positive remodeling (remodeling index >1.1), and napkin-ring sign (plaque with a ring-like higher attenuation peripheral rim with a low CT attenuation central core).[14]

For the primary analyses for this study, HRP cases were defined as the presence of any HRP feature (with or without oCAD); HRP controls were defined as no HRP features and no oCAD. In sensitivity analyses, to determine whether identified associations were unique to the presence of HRP independent of oCAD, HRP cases were restricted to those with the presence of any HRP feature in the absence of oCAD.

Lipoprotein Subclass Measurements

Lipoprotein subclass measurements were performed by LabCorp, Inc. NMR spectra were acquired on a Vantera Clinical Analyzer for the NMR LipoProfile test. The NMR MetaboProfile analysis, which reports lipoprotein particle concentrations and sizes, was performed using the recently developed LP4 lipoprotein profile deconvolution algorithm.[17] Mean TRL, LDL, and HDL particle sizes are weighted averages derived from the sum of the diameter of each subclass multiplied by its relative mass percentage (Table S1). In the LP4

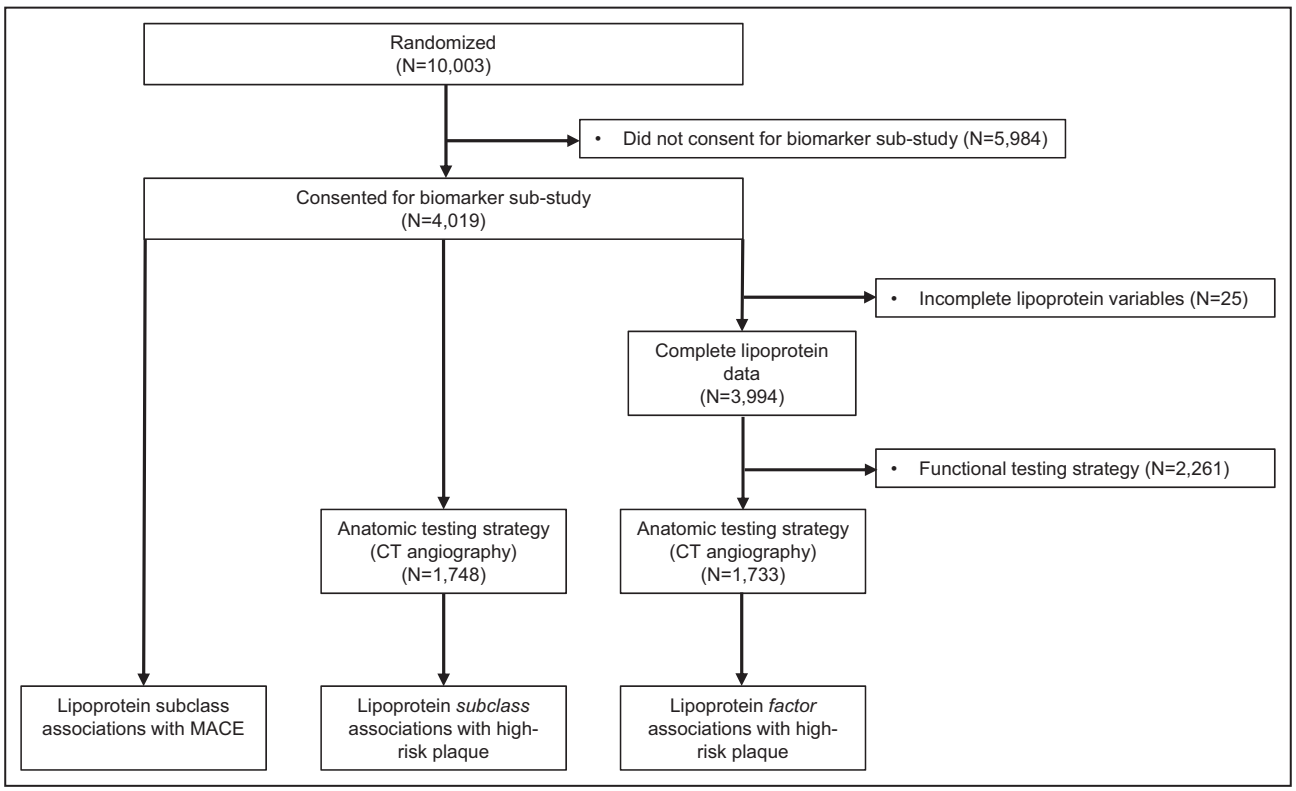

Figure 1. Consort flow diagram: patient inclusion and exclusion criteria.
CT indicates computed tomography; and MACE, major adverse cardiovascular events.

profile, medium HDL encompasses H3P and H4P subclasses, and large HDL encompasses H5P, H6P, and H7P subclasses. Linear regression of the lipoprotein subclass signal areas against serum lipid and apolipoprotein levels measured chemically in a large reference range study population provided the conversion factors to generate NMR-derived concentrations of total cholesterol, triglyceride, LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) fractions. NMR-derived concentrations of these parameters are highly correlated ($r \geq 0.95$) with those measured by standard methods.

Statistical Analysis

Given collinearity between lipoprotein subclasses and as an initial filtering process to reduce the burden of multiple comparisons, principal components analysis (PCA) was used for dimensionality reduction on the lipoproteins. All particle size subclasses of TRL, LDL, and calibrated HDL particle concentrations as well as mean TRL, LDL, and HDL lipoprotein size variables were included in the PCA (Table S1). Analytes with >25% of values below lower limits of quantification of the assay were not included in the PCA and were instead analyzed as binary variables (present/absent). These included the following 2 analytes: largest TRL particles (very large TRL particles, 90–240 nm) and largest calibrated HDL particles (H7P, 12 nm). Lipoprotein inputs for PCA were centered and scaled, and principal components were created using the prcomp function in R. A total of 5 resulting eigenvectors with eigenvalues >1 were carried forward to create varimax-rotated factors and tested in downstream association analyses.

PCA factors, very large TRL particles, and H7P were tested for associations with HRP case/control status in univariable and multivariable (adjusted for sex, age, hypertension, diabetes, body mass index, smoking status, statin use, and LDL-C) logistic regression models. To further assess for independent association, additional sensitivity analyses were performed using multivariable models for PCA factors heavily loaded with HDL subclasses and HDL-C. Individual lipoproteins from significant factors contributing a heavy load to a factor (ie, an absolute factor loading >0.4) were then analyzed individually for associations with HRP in sensitivity analyses; specifically, individual lipoproteins were converted to *z* scores and independently tested for association with our primary and sensitivity phenotype. Therefore, all odds ratios (ORs) and hazard ratios (HRs) presented for individual lipoproteins (excluding very large TRL particles and H7P) represent per 1 SD change in the lipoprotein concentration. Multiple-comparison testing was adjusted for using the false discovery rate Benjamini and Hochberg method[18] at the level of lipoprotein PCA factors analyzed in univariate models.

MACE in PROMISE were defined as based on the parent clinical trial composite outcome of all-cause death, myocardial infarction, or unstable angina hospitalization and were adjudicated by an independent clinical events committee.[15] Individual lipoproteins with a nominal univariate *P* value <0.05 in the primary HRP analysis were tested for association with time to event using univariate and multivariable (adjusted for age, sex, body mass index, LDL-C, statin use, hypertension, diabetes, and smoking) Cox proportional hazards models. As noted previously, for HDL subclasses, sensitivity analyses were performed including HDL-C as a covariate in the multivariable model. All models were assessed for violation of the proportional hazards assumption for the lipoprotein term. For lipoproteins with a proportional hazards assumption violation, we ran an additional Cox proportional hazards model that included a lipoprotein×log(time) interaction term. This model was then compared with a model excluding the lipoprotein and interaction term to determine whether the lipoprotein was significantly associated with events.

To validate these findings in a higher risk cohort of individuals, we also used the CATHGEN (Catheterization Genetics) cohort,[19] which consisted of sequential individuals undergoing cardiac catheterization for concern of ischemic heart disease at Duke University Medical Center. A total of 8707 individuals in the CATHGEN study had available lipoprotein data. Events in CATHGEN were defined as a composite of myocardial infarction or all-cause death. Univariable (adjusted only for NMR assay batch) and multivariable (adjusted for age, sex, body mass index, NMR assay batch, LDL-C, hypertension, diabetes, and smoking) Cox proportional hazards time-to-event models were constructed for individual lipoproteins that were significantly associated with time to event in the PROMISE trial. In CATHGEN multivariable analyses, statin use was not included as a covariate because of the incomplete collection of drug information for this cohort. Raw lipoprotein concentrations were converted to *z* scores before use in analyses, and all models were assessed for violation of the proportional hazards assumption for the lipoproteins of interest in each analysis.

This study was approved by the Duke University Institutional Review Board, and all subjects provided informed consent.

RESULTS

Baseline Participant Characteristics

Of the 4019 individuals who consented to participate in the PROMISE trial biomarker substudy, 1748 of these individuals were in the coronary CTA arm, and a total of 3994 individuals had lipoprotein profiling performed and had complete data for all lipoproteins (Figure 1).

Table 1. Baseline Characteristics of Individuals in the PROMISE Coronary CTA Arm With Lipoprotein Data

Variables	All patients (N=1748)	No HRP (N=1471)	HRP (N=277)
Age, y, mean±SD	60.1±8.0	60.1±8.0	60.4±8.0
Female sex, n (%)	935 (53.5)	833 (56.6)	102 (36.8)
Hypertension, n (%)	1127 (64.5)	950 (64.6)	177 (63.9)
Diabetes, n (%)	353 (20.2)	288 (19.6)	65 (23.5)
BMI, kg/m^2, mean±SD	30.6±5.9	30.7±6.0	29.7±5.1
Current or past tobacco use, n (%)	918 (52.5)	742 (50.4)	176 (63.5)
Statin, n/total n (%)	753/1689 (44.6)	634/1426 (44.5)	119/263 (45.2)
LDL cholesterol, mg/dL, mean±SD	120.7±34.6	119.9±34.7	124.6±34.1
HDL cholesterol, mg/dL, mean±SD	53.6±13.6	54.4±13.8	49.6±11.6

BMI indicates body mass index; CTA, computed tomographic angiography; HDL, high-density lipoprotein; HRP, high-risk coronary atherosclerotic plaque; LDL, low-density lipoprotein; and PROMISE, Prospective Multicenter Imaging Study for Evaluation of Chest Pain.

Baseline characteristics are presented in Table 1. The demographics for individuals with measured lipoproteins were similar to those in the entire PROMISE cohort (Table S2). Patients with HRP were more likely to be men, have a lower body mass index, have current or past tobacco use, have higher LDL-C, and have lower HDL cholesterol than HRP controls. There were no differences in age, prevalence of hypertension, prevalence of diabetes, or statin use.

Association of Lipoprotein Subclass Factors With HRP With or Without oCAD

A total of 16 lipoprotein subclass variables were input into PCA; PCA identified 5 orthogonal factors composed of biologically related lipoproteins (Table S3). PCA factors were analyzed for association with HRP in the subset of PROMISE individuals with HRP phenotyping (ie, from the CTA arm, N=1748). In univariable models, factor 2 (composed of small HDL subclasses, H1P and H3P; OR, 1.20 [95% CI, 1.06–1.37]; P=0.006; q=0.02) and factor 3 (composed of large LDL particles, HDL subclasses [H2P, H4P, H6P] and HDL size; OR, 1.32 [95% CI, 1.15–1.53]; P<0.001; q=0.001) were associated with HRP (Table 2). In multivariable models, factor 3 remained significant (adjusted OR, 1.23 [95% CI, 1.05–1.46]; P=0.013). After further adjustment of the multivariable model with HDL-C, neither factor was significant (Table S4). PCA factors that were primarily loaded with LDL and TRLs were not associated with HRP.

Individual lipoprotein subclasses highly loaded on factors 2 and 3 were then tested for association with HRP. In univariable models, large LDL particles, large HDL particles, medium HDL particles, and HDL size were inversely associated with HRP, but only large HDL (OR, 0.70 [95% CI, 0.56–0.85]; P<0.001), medium HDL (OR, 0.84 [95% CI, 0.72–0.98]; P=0.028), and HDL size (OR, 0.82 [95% CI, 0.69–0.96]; P=0.018) remained significant in multivariable models (Table 3, Figure 2); none were significant after adjustment for traditional HDL-C (Table S4).

HRP in the Absence of oCAD

To determine whether associations between lipoprotein factors and HRP were unique to the presence of HRP independent of oCAD, sensitivity analyses were performed analyzing patients with HRP who did not have oCAD (N=206). In these analyses in univariable models, both factor 2 (OR, 1.20 [95% CI, 1.04–1.39]; P=0.014) and factor 3 (OR, 1.18 [95% CI, 1.02–1.38]; P=0.034) remained associated with HRP with similar ORs; however, these factors were no longer significant in multivariable analyses, which may have been attributed to the smaller sample size (Table S5). In similar

Table 2. Association of Lipoprotein Factors With HRP

	Basic OR (95% CI)	*P* value	Q value	Adjusted OR (95% CI)	*P* value
Factor 1	0.95 (0.83–1.09)	0.486	0.589		
Factor 2	1.20 (1.06–1.37)	0.006	0.020	1.05 (0.91–1.21)	0.495
Factor 3	1.32 (1.15–1.53)	<0.001	0.001	1.23 (1.05–1.46)	0.013
Factor 4	0.97 (0.85–1.10)	0.641	0.641		
Factor 5	1.12 (0.99–1.27)	0.063	0.147		
H7P	0.87 (0.67–1.13)	0.291	0.509		
VL-TRLP	0.91 (0.70–1.19)	0.505	0.589		

Adjusted models included covariates for sex, age, hypertension, diabetes, body mass index, smoking status, statin use, and low-density lipoprotein cholesterol. HRP indicates high-risk coronary atherosclerotic plaque; OR, odds ratio; and VL-TRLP, very large triglyceride-rich lipoprotein particle.

Table 3. Association of Individual Lipoprotein Subclasses and HRP

Lipoprotein	Basic OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
L-LDLP	0.85 (0.73–0.97)	0.018	0.86 (0.73–1.01)	0.070
H6P	0.68 (0.57–0.81)	<0.001	0.70 (0.56–0.85)	<0.001
H4P	0.80 (0.69–0.92)	0.003	0.84 (0.72–0.98)	0.028
H3P	0.86 (0.75–0.98)	0.020	0.97 (0.84–1.12)	0.672
H2P	0.94 (0.83–1.07)	0.378		
H1P	1.07 (0.94–1.21)	0.315		
HDL size	0.75 (0.65–0.86)	<0.001	0.82 (0.69–0.96)	0.018

Adjusted models included covariates for sex, age, hypertension, diabetes, body mass index, smoking status, statin use, and low-density lipoprotein cholesterol. HDL indicates high-density lipoprotein; HRP, high-risk coronary atherosclerotic plaque; L-LDLP, large low-density lipoprotein particle; and OR, odds ratio.

analyses of individual lipoprotein subclasses loaded on each factor, large HDL and HDL size were inversely associated with HRP in univariable models, and large HDL remained significantly associated with HRP without oCAD in multivariable models (OR, 0.77 [95% CI, 0.62–0.94]; *P*=0.013) (Table S5). However, after additionally adjusting for HDL-C in sensitivity analyses, large HDL was no longer significantly associated (OR, 0.85 [95% CI, 0.65–1.10]; *P*=0.228).

Association of Lipoprotein Subclasses With Cardiovascular Events

The presence of HRP in the PROMISE cohort has previously been shown to be associated with an increased risk of MACE.[14] Thus, we next sought to determine if individual lipoprotein subclasses from PCA factors associated with HRP were also associated with MACE using the full PROMISE biomarker substudy samples (N=4019). In these analyses, medium HDL was the only lipoprotein subclass associated with time to MACE (unadjusted HR, 0.76 [95% CI, 0.63–0.92]; *P*=0.004), although it was no longer significant in multivariable models with and without HDL-C (Table 4 and Table S6). In the CATHGEN study, which contained >2-fold the number of individuals than the PROMISE substudy, in both unadjusted (HR, 0.91 [95% CI, 0.88–0.94]; *P*<0.001) and adjusted (HR, 0.93 [95% CI, 0.90–0.96]; *P*<0.001) models, medium HDL was inversely associated with time to death/myocardial infarction, with a similar magnitude as in the PROMISE study (Table 4). Large HDL and HDL size were positively associated with MACE in CATHGEN, whereas these lipoproteins were not significantly associated with MACE

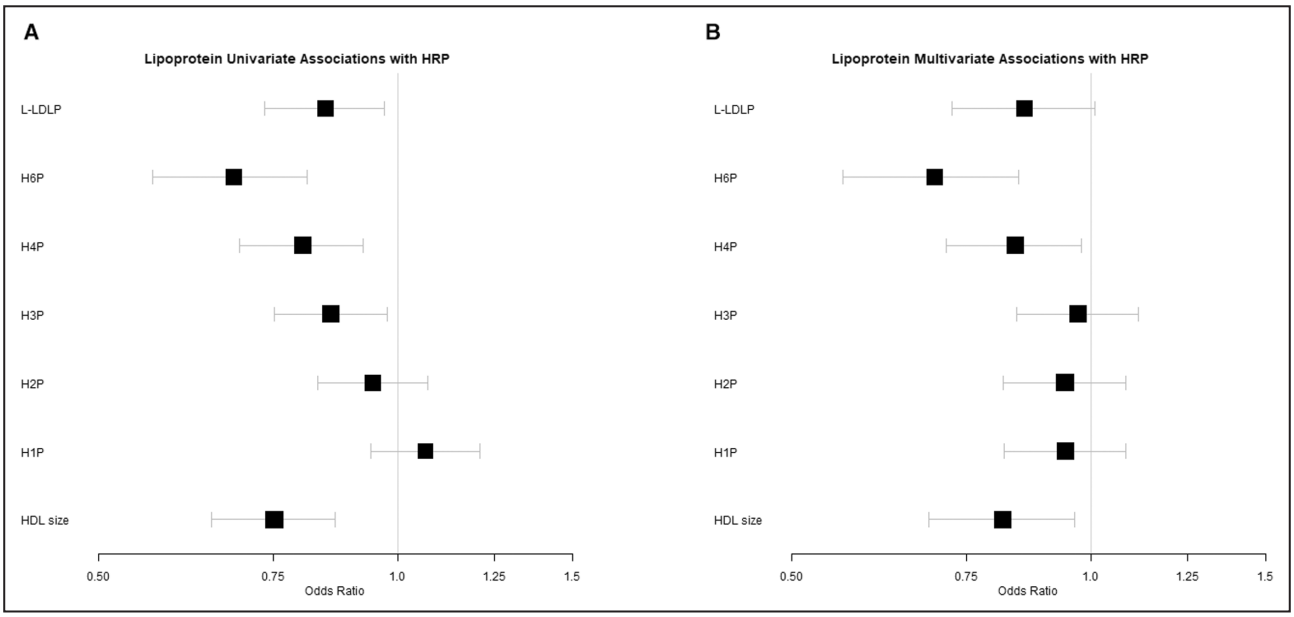

Figure 2. Forest plots of the association of individual lipoprotein subclasses with HRP in univariable (A) and multivariable (B) models.
HDL indicates high-density lipoprotein; HRP, high-risk coronary atherosclerotic plaque; and L-LDLP, large low-density lipoprotein particle.

Table 4. Association of Individual Lipoprotein Subclasses With Time to Event

	Full PROMISE cohort (N=4019)				CATHGEN (N=8707)			
Lipoprotein	Basic HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value	Basic HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
L-LDLP	1.03 (0.86–1.22)	0.757			1.02 (0.99–1.05)	0.134		
H6P	1.00 (0.84–1.20)	0.976			1.10 (1.07–1.13)	<0.001	1.09 (1.06–1.12)	<0.001
H4P	0.84 (0.69–1.03)	0.089			1.05 (1.02–1.08)	<0.001	1.08 (1.05–1.11)	<0.001
H3P	0.76 (0.63–0.92)	0.004	0.86 (0.71–1.05)	0.132	0.91 (0.88–0.94)	<0.001	0.93 (0.90–0.96)	<0.001
HDL size	0.97 (0.81–1.16)	0.730			1.27 (1.23–1.30)	<0.001	1.26 (1.22–1.29)	<0.001

PROMISE-adjusted models included covariates for sex, age, hypertension, diabetes, body mass index, smoking status, statin use, and low-density lipoprotein cholesterol.

CATHGEN basic models included a covariate for nuclear magnetic resonance assay batch, and adjusted models included covariates for age, sex, body mass index, nuclear magnetic resonance assay batch, low-density lipoprotein cholesterol, hypertension, diabetes, and smoking. CATHGEN indicates Catheterization Genetics; HDL, high-density lipoprotein; HR, hazard ratio; L-LDLP, large low-density lipoprotein particle; and PROMISE, Prospective Multicenter Imaging Study for Evaluation of Chest Pain.

in PROMISE. These differences may be related to sample size or may reflect the differences in patient characteristics. Because many of the lipoprotein subclasses in CATHGEN violated the proportional hazards assumption, we reran the analyses while incorporating a lipoprotein×log(time) interaction term in the models with these violations and compared them with models without these additional terms. These analyses demonstrated that the lipoproteins remained statistically significantly associated with events (Table S7).

DISCUSSION

Leveraging a large cardiovascular diagnostic strategy trial, the PROMISE trial, here we perform, to our knowledge, the largest study of lipoprotein subclass associations with high-risk coronary plaque. In outpatients free of known CAD presenting with stable chest pain, HDL subclasses were associated with HRP, even in individuals without oCAD, and were also associated with MACE. These results simultaneously identify a circulating lipoprotein signature that identifies individuals with HRP in the absence of oCAD and adds to our knowledge about HRP pathobiology.

Specifically, we found that greater concentrations of large (H6P) and medium (H4P) HDL particles and HDL size were associated with less risk of HRP, and greater concentrations of medium HDL particles (H3P) were associated with a reduced risk of incident MACE. These HDL findings may represent impaired reverse cholesterol transport in association with HRP, indicated by the accumulation of smaller, lipid-poor immature HDL subclasses. Of note, although associations with HRP remained significant in multivariable models, the collinearity of these HDL subclasses resulted in attenuation of the association after adjustment for traditional HDL-C, a composite of these HDL subclasses. Additional studies will need to be performed to determine if distinct HDL subclasses better predict HRP and MACE than HDL-C; however, prior investigations in different cohorts have shown this to be the case.[12,13]

Lipoprotein subclasses are also associated with CVD risk[11,13] and, more recently, have been shown to be related to coronary plaque composition as measured by multiple different imaging modalities.[20–22] In the ATLANTA (Assessment of Tissue Characteristics, Lesion Morphology, and Hemodynamics by Angiography With Fractional Flow Reserve, Intravascular Ultrasound and Virtual Histology, and Noninvasive Computed Tomography in Atherosclerotic Plaques) study of 60 individuals without oCAD presenting with chest pain, small, dense LDL and small HDL are associated with HRP features (namely, larger plaque volume, more noncalcified plaque, and higher volume of necrotic core) on coronary CTA and intravascular ultrasound, whereas larger HDL particles are associated with lower plaque volume and lower volume of necrotic core.[21] Supportive of the role of impaired reverse cholesterol transport, in a separate study using carotid magnetic resonance imaging assessment to detect HRP, the Chicago Healthy Aging Study found that HDL efflux capacity correlates with large and medium HDL subclasses; however, after multivariable adjustment, neither efflux capacity nor subclasses associate with HRP.[23] In another study of statin-treated patients with diabetes, the triglyceride/HDL-C ratio was significantly associated with HRP features detected by frequency-domain optical coherence tomography.[24] Although that study did not measure HDL subclasses, an elevated triglyceride/HDL-C ratio has been associated with a shift toward smaller HDL size, suggesting impaired reverse cholesterol transport.[25–27] Taken together, our findings in the PROMISE trial are consistent with and extend these prior, smaller studies. We interpret the inverse association of medium and large HDL particles and HDL size with HRP as indicating possible

impaired reverse cholesterol transport, which would be expected to contribute to HRP features such as a large noncalcified plaque burden and necrotic lipid core. Measuring HDL subclasses and size in individuals with chest pain or at risk for CAD may therefore help in risk stratification to identify those who need additional evaluation or more intensive preventive therapy. One interesting area of future investigation is to determine whether therapies that shift HDL particle size distributions toward medium HDL subclasses are associated with reduced HRP.

We found that medium HDL particles also inversely associate with MACE in addition to an association with HRP; however, this association was not significant after adjustment for established cardiovascular risk factors. This finding was replicated in CATHGEN, which is composed of individuals who have, or are at high risk for, CVD. In CATHGEN, medium HDL particles were associated with incident events even after an adjustment for established risk factors, suggesting that these results are independent of traditional risk factors. As noted previously, medium HDL particles may report on HDL efflux capacity, which is associated with a decreased risk of MACE across a variety of populations.[28–30] We note, however, that large HDL (H6P) and HDL size, which were negatively associated with HRP in PROMISE, were positively associated with MACE in CATHGEN. Therefore, although medium and large HDL particles and HDL size may help in identifying individuals with HRP, medium HDL particles may be unique in predicting MACE in individuals with HRP.

The strengths of this study include the relatively large sample size, leveraging samples collected as part of a clinical trial with adjudicated clinical outcomes and CTA plaque characteristics analyzed by a core laboratory. Our major findings center around NMR-derived HDL particle subclasses. It is possible that other methods to measure HDL subclasses might determine different associations with HRP; however, given that our study is consistent with one that demonstrated similar associations of large HDL particles measured by gel electrophoresis with HRP,[21] we believe this is unlikely. We also note that the associations of HDL subclasses with HRP were attenuated when including HDL-C in our multivariable regression models. This finding is likely the result of a correlation of HDL-C and HDL subclasses. The PROMISE trial was composed of low-risk individuals with no history of CVD, so the results cannot necessarily be generalized to high-risk individuals with known CAD and HRP; however, the finding that medium HDL subclasses are also inversely associated with MACE in the higher risk CATHGEN biorepository suggests that this HDL subclass may be a reliable biomarker for adverse events in broad populations of individuals presenting with chest pain or at risk for CAD.

In conclusion, we find that medium and large HDL subclasses and HDL size were inversely associated with HRP features in PROMISE trial participants and that medium HDL subclasses were inversely associated with MACE in PROMISE and the greater risk CATHGEN cohort. These findings may aid the risk stratification of individuals with chest pain and suspected CAD and provide insight into the pathobiology of HRP.

ARTICLE INFORMATION

Received May 2, 2022; accepted November 23, 2022.

Affiliations

Division of Cardiology, Department of Medicine (R.W.M., W.E.K., P.S.D., S.H.S.); and Duke Molecular Physiology Institute (R.W.M., S.N.G., W.E.K., S.H.S.), Duke University School of Medicine, Durham, NC; Knight Cardiovascular Institute, Oregon Health and Science University, Portland, OR (M.F.); Cardiovascular Imaging Research Center, Harvard Medical School–Massachusetts General Hospital, Boston, MA (U.H., B.F., J.K.); MTA-SE Cardiovascular Imaging Research Group, Heart and Vascular Center, Semmelweis University, Budapest, Hungary (J.K.); Duke Center for Applied Genomics & Precision Medicine (G.S.G.) and Duke Clinical Research Institute, Duke University School of Medicine, Durham, NC (P.S.D., S.H.S.).

Sources of Funding

This study was supported by the National Heart, Lung, and Blood Institute (R01HL146145) and LabCorp, Inc., who performed the lipoprotein particle profiling.

Disclosures

Dr Ferencik reports consulting for Biograph, Inc, and Dr Foldyna reports unrelated research funding from AstraZeneca and Medtrace. The remaining authors have no conflicts to disclose.

Supplemental Material

Table S1–S7

REFERENCES

1. Stefanadis C, Antoniou C-K, Tsiachris D, Pietri P. Coronary atherosclerotic vulnerable plaque: current perspectives. *J Am Heart Assoc*. 2017;6:6. doi: 10.1161/JAHA.117.005543
2. Stone GW, Maehara A, Lansky AJ, de Bruyne B, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N, Marso SP, et al. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med*. 2011;364:226–235. doi: 10.1056/NEJMoa1002358
3. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. *J Am Coll Cardiol*. 2006;47:47–C18. doi: 10.1016/j.jacc.2005.10.065
4. Bom MJ, Van Der Heijden DJ, Kedhi E, Van Der Heyden J, Meuwissen M, Knaapen P, Timmer SAJ, Van Royen N. Early detection and treatment of the vulnerable coronary plaque: can we prevent acute coronary syndromes? *Circ Cardiovasc Imaging*. 2017;10(5):e005973. doi: 10.1161/CIRCIMAGING.116.005973
5. Bittencourt MS, Hulten E, Ghoshhajra B, O'Leary D, Christman MP, Montana P, Truong QA, Steigner M, Murthy VL, Rybicki FJ, et al. Prognostic value of nonobstructive and obstructive coronary artery disease detected by coronary computed tomography angiography to identify cardiovascular events. *Circ Cardiovasc Imaging*. 2014;7:282–291. doi: 10.1161/CIRCIMAGING.113.001047
6. Hadamitzky M, Täubert S, Deseive S, Byrne RA, Martinoff S, Schömig A, Hausleiter J. Prognostic value of coronary computed tomography angiography during 5 years of follow-up in patients with suspected coronary artery disease. *Eur Heart J*. 2013;34:3277–3285. doi: 10.1093/eurheartj/eht293
7. Hoffmann U, Ferencik M, Udelson JE, Picard MH, Truong QA, Patel MR, Huang M, Pencina M, Mark DB, Heitner JF, et al. Prognostic value of noninvasive cardiovascular testing in patients with stable chest pain:

Insights from the PROMISE trial (prospective multicenter imaging study for evaluation of chest pain). *Circulation*. 2017;135:2320–2332. doi: 10.1161/CIRCULATIONAHA.116.024360
8. Motoyama S, Ito H, Sarai M, Kondo T, Kawai H, Nagahara Y, Harigaya H, Kan S, Anno H, Takahashi H, et al. Plaque characterization by coronary computed tomography angiography and the likelihood of acute coronary events in mid-term follow-up. *J Am Coll Cardiol*. 2015;66:337–346. doi: 10.1016/j.jacc.2015.05.069
9. Puchner SB, Liu T, Mayrhofer T, Truong QA, Lee H, Fleg JL, Nagurney JT, Udelson JE, Hoffmann U, Ferencik M. High-risk plaque detected on coronary CT angiography predicts acute coronary syndromes independent of significant stenosis in acute chest pain: results from the ROMICAT-II trial. *J Am Coll Cardiol*. 2014;64:684–692. doi: 10.1016/j.jacc.2014.05.039
10. Koenig W, Khuseyinova N. Biomarkers of atherosclerotic plaque instability and rupture. *Arterioscler Thromb Vasc Biol*. 2007;27:15–26. doi: 10.1161/01.ATV.0000251503.35795.4f
11. Ip S, Lichtenstein AH, Chung M, Lau J, Balk EM. Systematic review: association of low-density lipoprotein subtractions with cardiovascular outcomes. *Ann Intern Med*. 2009;150:474–484. doi: 10.7326/0003-4819-150-7-200904070-00007
12. Mackey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *J Am Coll Cardiol*. 2012;60:508–516. doi: 10.1016/j.jacc.2012.03.060
13. McGarrah RW, Craig DM, Haynes C, Dowdy ZE, Shah SH, Kraus WE. High-density lipoprotein subclass measurements improve mortality risk prediction, discrimination and reclassification in a cardiac catheterization cohort. *Atherosclerosis*. 2016;246:229–235. doi: 10.1016/j.atherosclerosis.2016.01.012
14. Ferencik M, Mayrhofer T, Bittner DO, Emami H, Puchner SB, Lu MT, Meyersohn NM, Ivanov AV, Adami EC, Patel MR, et al. Use of high-risk coronary atherosclerotic plaque detection for risk stratification of patients with stable chest pain: a secondary analysis of the promise randomized clinical trial. *JAMA Cardiol*. 2018;3:144–152. doi: 10.1001/jamacardio.2017.4973
15. Douglas PS, Hoffmann U, Patel MR, Mark DB, Al-Khalidi HR, Cavanaugh B, Cole J, Dolor RJ, Fordyce CB, Huang M, et al. Outcomes of anatomical versus functional testing for coronary artery disease. *N Engl J Med*. 2015;372:1291–1300. doi: 10.1056/NEJMoa1415516
16. Abbara S, Blanke P, Maroules CD, Cheezum M, Choi AD, Han BK, Marwan M, Naoum C, Norgaard BL, Rubinshtein R, et al. SCCT guidelines for the performance and acquisition of coronary computed tomographic angiography: a report of the society of cardiovascular computed tomography guidelines committee: Endorsed by the north American Society for Cardiovascular Imaging (NASCI). *J Cardiovasc Comput Tomogr*. 2016;10:435–449. doi: 10.1016/j.jcct.2016.10.002
17. Chung ST, Cravalho CKL, Meyers AG, Courville AB, Yang S, Matthan NR, Mabundo L, Sampson M, Ouwerkerk R, Gharib AM, et al. Triglyceride paradox is related to lipoprotein size, visceral adiposity and stearoyl-CoA desaturase activity in black versus White women. *Circ Res*. 2020;126:94–108. doi: 10.1161/CIRCRESAHA.119.315701
18. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B*. 1995;57:289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x
19. Kraus WE, Granger CB, Sketch MH, Donahue MP, Ginsburg GS, Hauser ER, Haynes C, Newby LK, Hurdle M, Dowdy ZE, et al. A guide for a cardiovascular genomics biorepository: the CATHGEN experience. *J Cardiovasc Transl Res*. 2015;8:449–457. doi: 10.1007/s12265-015-9648-y
20. Zambon A, Puato M, Faggin E, Grego F, Rattazzi M, Pauletto P. Lipoprotein remnants and dense LDL are associated with features of unstable carotid plaque: a flag for non-HDL-C. *Atherosclerosis*. 2013;230:106–109. doi: 10.1016/j.atherosclerosis.2013.06.024
21. Voros S, Joshi P, Qian Z, Rinehart S, Vazquez-Figueroa JG, Anderson H, Elashoff M, Murrieta L, Karmpaliotis D, Kalynych A, et al. Apoprotein B, small-dense LDL and impaired HDL remodeling is associated with larger plaque burden and more noncalcified plaque as assessed by coronary CT angiography and intravascular ultrasound with radiofrequency backscatter: results from the ATLANTA I study. *J Am Heart Assoc*. 2013;2:e000344.
22. Lin A, Nerlekar N, Rajagopalan A, Yuvaraj J, Modi R, Mirzaee S, Munnur RK, Seckington M, Doery JC, Seneviratne S, et al. Remnant cholesterol and coronary atherosclerotic plaque burden assessed by computed tomography coronary angiography. *Atherosclerosis*. 2019;284:24–30. doi: 10.1016/j.atherosclerosis.2019.02.019
23. Mutharasan RK, Thaxton CS, Berry J, Daviglus ML, Yuan C, Sun J, Ayers C, Lloyd-Jones DM, Wilkins JT. HDL efflux capacity, HDL particle size, & high-risk carotid atherosclerosis in a cohort of asymptomatic older adults: the Chicago healthy aging study. *J Lipid Res*. 2017;58:600–606. doi: 10.1194/jlr.P069039
24. Takata K, Kataoka Y, Andrews J, Puri R, Hammadah M, Duggal B, Kapadia SR, Tuzcu EM, Nissen SE, Nicholls SJ. Triglyceride-to-high-density lipoprotein cholesterol ratio and vulnerable plaque features with statin therapy in diabetic patients with coronary artery disease: frequency-domain optical coherence tomography analysis. *JACC Cardiovasc Imaging*. 2018;11:1721–1723. doi: 10.1016/j.jcmg.2018.02.017
25. Tian L, Fu M. The relationship between high density lipoprotein subclass profile and plasma lipids concentrations. *Lipids Health Dis*. 2010;9:118. doi: 10.1186/1476-511X-9-118
26. Yang Y, Yan B, Fu M, Xu Y, Tian Y. Relationship between plasma lipid concentrations and HDL subclasses. *Clin Chim Acta*. 2005;354:49–58. doi: 10.1016/j.cccn.2004.11.015
27. Jia L, Long S, Fu M, Yan B, Tian Y, Xu Y, Gou L. Relationship between total cholesterol/high-density lipoprotein cholesterol ratio, triglyceride/high-density lipoprotein cholesterol ratio, and high-density lipoprotein subclasses. *Metabolism*. 2006;55:1141–1148. doi: 10.1016/j.metabol.2006.04.004
28. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med*. 2014;371(25):2383–2393. doi: 10.1056/NEJMoa1409065
29. Mody P, Joshi PH, Khera A, Ayers CR, Rohatgi A. Beyond coronary calcification, family history, and C-reactive protein: cholesterol efflux capacity and cardiovascular risk prediction. *J Am Coll Cardiol*. 2016;67:2480–2487. doi: 10.1016/j.jacc.2016.03.538
30. Ebtehaj S, Gruppen EG, Bakker SJL, Dullaart RPF, Tietge UJF. HDL (high-density lipoprotein) cholesterol efflux capacity is associated with incident cardiovascular disease in the general population. *Arterioscler Thromb Vasc Biol*. 2019;39:1874–1883. doi: 10.1161/ATVBAHA.119.312645

SUPPLEMENTAL MATERIAL

Table S1. Description of measured lipoprotein particles

Parameter	Particle Subclass	Description	Diameter Estimate (nm)
TG-Rich Lipoprotein Particle (TRLP) Concentrations (nmol/L)			
TRLP		Total TRLP	24- 240
	VL-TRLP	Very Large TRLP	90-240
	L-TRLP	Large TRLP	50-89
	M-TRLP	Medium TRLP	37-49
	S-TRLP	Small TRLP	30-36
	VS-TRLP	Very Small TRLP	24-29
LDL Particle (LDLP) Concentrations (nmol/L)			
cLDLP		Total cLDLP	19-23
	L-cLDLP	Large LDLP	21.5-23
	M-cLDLP	Medium LDLP	20.5-21.4
	S-cLDLP	Small LDLP	19-20.4
Calibrated HDL Particle (cHDLP) Concentrations (µmol/L)			
cHDLP		Total cHDLP	7.5-13
	L-cHDLP	Large HDLP (H5+H6+H7)	
	M-cHDLP	Medium HDLP (H3+H4)	
	S-cHDLP	Small HDLP (H1+H2)	
	H7P	HDLP subspecies (12 nm)	12.0
	H6P	HDLP subspecies (10.8 nm)	10.8
	H5P	HDLP subspecies (10.3 nm)	10.3
	H4P	HDLP subspecies (9.5 nm)	9.5
	H3P	HDLP subspecies (8.7 nm)	8.7
	H2P	HDLP subspecies (7.8 nm)	7.8
	H1P	HDLP subspecies (7.4 nm)	7.4
Mean Particle Sizes (nm)			
TRLZ	---	TRL Size	30-100
LDLZ	---	LDL Size	19-22.5
HDLZ	---	HDL Size	7.4-13
Derived Lipid and Apolipoprotein Concentrations (mg/dL)			
TG		Total Triglycerides	
TC		Total Cholesterol	
LDLC		LDL Cholesterol	
HDLC		HDL Cholesterol	

TG: triglyceride; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Table S2. Baseline characteristics in full PROMISE cohort and within the subset with measured lipoproteins

Variables	PROMISE Cohort (N=10003)	Sub-Cohort with Measured Lipoproteins (N=4019)
Mean age - years ± std. dev.	60.8 ± 8.3	60.5 ± 8.1
Female sex - no. (%)	5270 (52.7)	2144 (53.3)
Hypertension - no. (%)	6501 (65.0)	2628 (65.4)
Diabetes - no. (%)	2144 (21.4)	881 (21.9)
Mean BMI - kg/m2 ± std. dev.	30.5 ± 6.1	30.8 ± 6.2
Current or past tobacco use - no. (%)	5104 (51.0)	2055 (51.1)
Statin - no./total no. (%)	4389/9569 (45.9)	1775/3885 (45.7)
LDL cholesterol - mg/dL ± std. dev.	120.9 ± 33.9	120.9 ± 33.9
HDL cholesterol - mg/dL ± std. dev.	53.5 ± 13.8	53.5 ± 13.8

BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Table S3. Lipoprotein loading on principal components analysis factors

	Varimax Rotated Loadings				
Lipoprotein	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
L-TRLP	0.60				
M-TRLP	0.65				0.45
S-TRLP					0.90
VS-TRLP				0.51	
L-LDLP			-0.60		0.40
M-LDLP	-0.75				
S-LDLP	0.88				
H6P			-0.75		
H5P				-0.73	
H4P			-0.55	0.51	
H3P		-0.75			
H2P			0.61		
H1P		0.80			
TRLZ				0.44	
LDLZ	-0.79				
HDLZ			-0.86		

L-TRLP: large triglyceride-rich lipoprotein particle; M-TRLP: medium TRLP; S-TRLP: small TRLP; L-LDLP: large low-density lipoprotein particle; M-LDLP: medium LDLP; S-LDLP: small LDLP; TRLZ: triglyceride-rich lipoprotein size; LDLZ: low-density lipoprotein size; HDLZ: high-density lipoprotein size

Table S4. Sensitivity analyses for association between lipoprotein classes with HRP, including HDL-C as a covariate in multivariable regression models

	Basic OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value	Adjusted OR + HDL-C (95% CI)	P-value
Factor 2	1.20 (1.06-1.37)	0.006	1.05 (0.91-1.21)	0.495		
Factor 3	1.32 (1.15-1.53)	<0.001	1.23 (1.05-1.46)	0.013	1.06 (0.88-1.29)	0.524
L-LDLP	0.85 (0.73-0.97)	0.018	0.86 (0.73-1.01)	0.070		
H6P	0.68 (0.57-0.81)	<0.001	0.70 (0.56-0.85)	<0.001	0.80 (0.62-1.02)	0.081
H4P	0.80 (0.69-0.92)	0.003	0.84 (0.72-0.98)	0.028	0.93 (0.78-1.10)	0.400
H3P	0.86 (0.75-0.98)	0.020	0.97 (0.84-1.12)	0.672		
H2P	0.94 (0.83-1.07)	0.378				
H1P	1.07 (0.94-1.21)	0.315				
HDL size	0.75 (0.65-0.86)	<0.001	0.82 (0.69-0.96)	0.018	1.08 (0.84-1.37)	0.538

OR: Odds Ratio; 95% CI: 95% Confidence Interval; L-LDLP: large low-density lipoprotein particle; HDL: high-density lipoprotein
Adjusted models included covariates for sex, age, hypertension, diabetes, BMI (body mass index), smoking status, statin use, and LDL-C (low-density lipoprotein cholesterol)

Table S5. Sensitivity analyses of lipoprotein factor associations with HRP (restricted to participants free of oCAD; N=1665)

	Basic OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Factor 2	1.20 (1.04-1.39)	0.014	1.08 (0.93-1.27)	0.316
Factor 3	1.18 (1.02-1.38)	0.034	1.13 (0.95-1.35)	0.174
H7P	0.94 (0.70-1.26)	0.662		
VL-TRLP	0.88 (0.65-1.18)	0.389		
L-LDLP	0.92 (0.79-1.06)	0.251		
H6P	0.78 (0.64-0.92)	0.006	0.77 (0.62-0.94)	0.013
H4P	0.87 (0.74-1.01)	0.069		
H3P	0.89 (0.77-1.03)	0.112		
H2P	0.89 (0.77-1.03)	0.116		
H1P	1.06 (0.92-1.23)	0.391		
HDL size	0.85 (0.72-0.98)	0.033	0.90 (0.75-1.07)	0.246

OR: Odds Ratio; 95% CI: 95% Confidence Interval; VL-TRLP: very large triglyceride-rich lipoprotein particle; L-LDLP: large low-density lipoprotein particle; HDL: high-density lipoprotein
Adjusted models included covariates for sex, age, hypertension, diabetes, BMI (body mass index), smoking status, statin use, and LDL-C (low-density lipoprotein cholesterol).

Table S6. Sensitivity analyses of lipoprotein factor associations with cardiovascular events (including HDL-C in multivariable models)

	Full PROMISE cohort (N=4019)				CATHGEN (N=8707)			
Lipoprotein	**Basic HR (95% CI)**	**P-value**	**Adjusted HR (95% CI)**	**P-value**	**Basic HR (95% CI)**	**P-value**	**Adjusted HR (95% CI)**	**P-value**
L-LDLP	1.03 (0.86-1.22)	0.757			1.02 (0.99-1.05)	0.134		
H6P	1.00 (0.84-1.20)	0.976			1.10 (1.07-1.13)	<0.001	1.28 (1.23-1.32)	<0.001
H4P	0.84 (0.69-1.03)	0.089			1.05 (1.02-1.08)	<0.001	1.13 (1.10-1.17)	<0.001
H3P	0.76 (0.63-0.92)	0.004	0.95 (0.76-1.20)	0.683	0.91 (0.88-0.94)	<0.001	0.96 (0.93-0.99)	0.016
HDL size	0.97 (0.81-1.16)	0.730			1.27 (1.23-1.30)	<0.001	1.46 (1.41-1.50)	<0.001

HR: Hazards Ratio; 95% CI: 95% Confidence Interval; L-LDLP: large low-density lipoprotein particle; HDL: high-density lipoprotein

PROMISE adjusted models included covariates for sex, age, hypertension, diabetes, BMI (body mass index(, smoking status, statin use, LDL-C (low-density lipoprotein cholesterol), and HDL-C (high-density lipoprotein cholesterol)

CATHGEN basic models included a covariate for NMR (nuclear magnetic resonance) assay batch and adjusted models included covariates for age, sex, BMI, NMR assay batch, LDL-C, hypertension, diabetes, smoking, and HDL-C

Table S7. Hazard ratios at different time intervals and P-values for sensitivity models incorporating a lipoprotein*log(time) coefficient for lipoproteins where the proportional hazards assumption was violated

		Estimated Hazard Ratios					
Cohort	**Lipoprotein**	**6 months**	**1 year**	**2 years**	**4 years**	**10 years**	**P-value**
PROMISE	HDL size	1.06	1.16	1.26	1.37	*N/A*	0.120
CATHGEN	L-LDLP	1.13	1.11	1.09	1.07	1.04	<0.001
	H6P	1.11	1.10	1.10	1.09	1.08	<0.001
	H4P	1.14	1.12	1.10	1.08	1.05	<0.001
	H3P	0.91	0.92	0.92	0.93	0.93	<0.001
	HDL size	1.39	1.33	1.28	1.23	1.17	<0.001

HDL: high-density lipoprotein; L-LDLP: large low-density lipoprotein particle
Estimated hazards ratios calculated at certain time intervals based on adjusted models with a lipoprotein*log(time) interaction covariate (PROMISE: sex, age, hypertension, diabetes, BMI (body mass index), smoking status, statin use, LDL-C (low-density lipoprotein cholesterol), and lipoprotein*log(time); CATHGEN: age, sex, BMI, NMR (nuclear magnetic resonance) assay batch, LDL-C, hypertension, diabetes, smoking, and lipoprotein*log(time)). Natural log (default in R) was used for the lipoprotein*log(time) interaction terms in both cohorts.