Title

Lipoprotein-associated phospholipase A2 and risk of incident cardiovascular disease in a multi-ethnic cohort: The multi ethnic study of atherosclerosis

Journal

Atherosclerosis, 241(1)

ISSN

0021-9150

Authors

Garg, Parveen K
McClelland, Robyn L
Jenny, Nancy S
et al.

Publication Date

2015-07-01

DOI

10.1016/j.atherosclerosis.2015.05.006

Peer reviewed

HHS Public Access

Author manuscript

Atherosclerosis. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Atherosclerosis. 2015 July ; 241(1): 176–182. doi:10.1016/j.atherosclerosis.2015.05.006.

Lipoprotein-Associated Phospholipase A_2 and Risk of Incident Cardiovascular Disease in a Multi-Ethnic Cohort: The Multi Ethnic Study of Atherosclerosis

Parveen K. Garg[a], **Robyn L. McClelland**[b], **Nancy S. Jenny**[c], **Michael H Criqui**[d], **Philip Greenland**[e,f], **Robert S. Rosenson**[g], **David S. Siscovick**[h], **Neal Jorgensen**[b], and **Mary Cushman**[c,i]

[a]Division of Cardiovascular Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California

[b]Department of Biostatistics, University of Washington, Seattle, Washington

[c]Department of Pathology and Laboratory Medicine, University of Vermont College of Medicine, Burlington, Vermont

[d]Department of Family & Preventive Medicine, University of California in San Diego, La Jolla, California

[e]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

[f]Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

[g]Mount Sinai Heart, Icahn School of Medicine at Mount Sinai, New York, New York

[h]New York Academy of Medicine, New York, New York

[i]Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont

Abstract

Objective—Prospective studies reporting a positive association of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) mass and activity with incident cardiovascular disease (CVD) have included primarily white individuals. We evaluated associations of Lp-PLA_2 and first-time cardiovascular events in a healthy multi-ethnic cohort characterized by presence or absence of baseline subclinical atherosclerosis.

Methods—Lp-PLA_2 mass and activity were measured at baseline in 5456 participants in the Multi-Ethnic Study of Atherosclerosis. Individuals were characterized for presence of baseline

Address for Correspondence: Mary Cushman, Department of Medicine, University of Vermont, 208 South Park Drive, Colchester, VT 05446, Phone: 802-656-8968, Fax: 802-656-8965, mary.cushman@uvm.edu.

Disclosures:
None

subclinical disease (coronary artery calcium score>0 or carotid intima-media thickness value>80th percentile) and followed prospectively for development of CVD events (coronary heart disease, ischemic stroke, and cardiovascular death).

Results—516 incident CVD events occurred over median follow-up of 10.2 years. In adjusted Cox proportional hazards models, each higher standard deviation of both Lp-PLA$_2$ activity and mass was associated with an increased risk of cardiovascular events; hazard ratios (HR; 95% confidence intervals (CI)) 1.12 (1.01–1.26) for Lp-PLA$_2$ activity and 1.10 (1.01–1.21) for mass. Associations did not differ by subclinical disease status (p-value for interaction 0.99 for Lp-PLA$_2$ activity and 0.32 for Lp-PLA$_2$ mass) and there was no confounding by subclinical atherosclerosis measures. Associations of Lp-PLA$_2$ activity but not mass were weaker in Chinese participants but there were relatively few events among Chinese in race-stratified analysis.

Conclusion—In this multi-ethnic cohort, Lp-PLA$_2$ was positively associated with CVD risk, regardless of the presence of coronary artery calcium or a thickened carotid-intimal media.

Keywords

Lipoprotein-associated Phospholipase A$_2$; Cardiovascular Disease; Inflammation; Ethnicity; Biomarker

Introduction

Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) is a 50-kd calcium-independent enzyme highly expressed by macrophages in atherosclerotic lesions.[1,2] Lp-PLA$_2$ is responsible for the hydrolysis of oxidized phospholipids on LDL particles.[3,4] The presence and activity of Lp-PLA$_2$ within a plaque appear to be associated with vulnerable, rupture-prone plaques.[5] Thus, Lp-PLA$_2$ may be a marker specific to vascular inflammation.[6]

Prior studies in individuals free of prevalent cardiovascular disease (CVD) have documented an association between higher Lp-PLA$_2$ mass and elevated Lp-PLA$_2$ activity with incident coronary heart disease and ischemic stroke.[7–12] These studies included primarily white individuals, with data in non-whites largely limited to Asian populations. Additionally, prior studies did not evaluate whether the risk of incident cardiovascular events associated with Lp-PLA$_2$ differed based on presence of subclinical atherosclerosis. Individuals with subclinical atherosclerosis are at higher risk for developing incident CVD compared to those without subclinical atherosclerosis.[13] If associations of Lp-PLA$_2$ with incident CVD are larger for those with subclinical disease compared to those without subclinical disease, this might identify a group more likely to experience a reduction in primary cardiovascular disease with Lp-PLA$_2$ inhibition. In patients with stable coronary heart disease (CHD), however, oral Lp-PLA$_2$ inhibition did not significantly reduce the composite outcome cardiovascular death, myocardial infarction (MI), or stroke although there was a reduced risk of coronary events.[14]

We evaluated associations of both Lp-PLA$_2$ mass and activity with incident cardiovascular events in a healthy multi-ethnic cohort characterized at baseline for subclinical atherosclerosis. We hypothesized larger associations of Lp-PLA$_2$ with cardiovascular events in those with subclinical atherosclerosis.

Materials and Methods

Multi-Ethnic Study of Atherosclerosis (MESA) Cohort

MESA recruited 6814 adults aged 45 to 84 years from 6 field centers (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York; and St Paul, Minnesota) to a baseline examination between July 2000 and September 2002.[15] The study participants were white (38%), African American (28%), Hispanic (22%), and Chinese American (12%) and without known clinical CVD. MESA conducted 3 subsequent examinations of the cohort between 2002 and 2007. Institutional review boards at each site approved the study, and all participants gave written informed consent.

Risk Factor Assessments

At baseline, standardized questionnaires were used to obtain demographic information, level of education, annual household income, smoking history, and medication usage for high blood pressure, high cholesterol, or diabetes. Cigarette smoking was calculated in pack-years and also defined as current, former, or never. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic resting blood pressures were measured in seated participants.[16]

Serum measurements

Total and high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels were measured from blood samples obtained after a 12-h fast. Low-density lipoprotein cholesterol was calculated by the Friedewald equation. Diabetes was defined as fasting glucose >125 mg/dl or use of hypoglycemic medication. C-reactive protein (CRP) was quantified by a high-sensitivity assay (N-High-Sensitivity CRP; Dade Behring, Deerfield, IL; inter-assay coefficient of variation: 2.1–5.7%).

Plasma Lp-PLA$_2$ measurement

Both Lp-PLA$_2$ mass and activity were measured in plasma samples from the baseline examination. Measurements were performed by diaDexus Inc. (South San Francisco, CA).[17] Lp-PLA$_2$ mass was measured with a sandwich enzyme immunoassay (PLAC™ Test; diaDexus). Lp-PLA$_2$ activity was measured by an enzymatic assay using a tritium-labeled platelet activating factor (PAF) analog as the substrate. The interassay coefficients of variation were 6.0% for Lp-PLA$_2$ mass and 5.0% for Lp-PLA$_2$ activity. LpPLA$_2$ values were not available in 1328 participants, mostly due to lack of consent for research involving a commercial entity.

Subclinical atherosclerosis measurement

Scanning centers assessed coronary artery calcium (CAC) by CT using either a cardiac-gated electron-beam CT scanner or a multidetector CT system.[18] Participants were scanned twice consecutively over phantoms of known physical calcium concentration. The phantom contained 4 bars of known calcium density and was used to calibrate the x-ray attenuation level between measurements conducted on different machines. A radiologist or cardiologist read all CT scans at a central reading center (Harbor-UCLA Medical Center/Los Angeles

Biomedical Research Institute, Torrance, California). An abnormal CAC was defined as a value greater than zero. The mean phantom-adjusted Agatston score was used in all analyses.[19]

Carotid intimal medial thickness (CIMT) ultrasound measurements were interpreted at Tufts-New England Medical Center, Boston, Massachusetts and have been previously described.[20] Maximal IMT was defined as the mean maximum IMT value taken from the near and far walls of the right and left sides for both the internal carotid artery (ICA) and common carotid artery (CCA). An abnormal CIMT was defined as either an ICA or CCA value in the highest overall quintile.

Follow-up

At 9–12 month intervals, participants or family members were contacted regarding interim hospital admissions, outpatient diagnoses of CVD, and deaths. Follow-up for this analysis extended through 2011. To verify self-reported diagnoses, trained personnel abstracted data from hospital records. Next of kin and physicians were contacted for participants with out-of-hospital cardiovascular deaths. Two physician members of the MESA mortality and morbidity review committee independently classified events. The full committee made final classifications if there were disagreements.

Events were classified as due to CVD or CHD. CVD events included nonfatal MI, resuscitated cardiac arrest, CVD death, definite angina and probable angina associated with revascularization, and ischemic stroke. CHD events included nonfatal MI, resuscitated cardiac arrest, CHD death, definite angina and probable angina associated with revascularization. A subset of CHD events was defined as ‘hard’ CHD events and included CHD death or nonfatal MI. Revascularizations not preceded by a diagnosis of angina were not included in the CVD endpoint. The diagnosis of MI was based on symptoms, electrocardiographic findings, and levels of circulating cardiac biomarkers. A death was considered related to CHD if it occurred within 28 days after a myocardial infarction, if the participant had had chest pain within 72 hours before death, or if the participant had a history of CHD and there was no known nonatherosclerotic, non-cardiac cause of death. Reviewers classified resuscitated cardiac arrest when a patient successfully recovered from full cardiac arrest through cardiopulmonary resuscitation (including cardioversion). Adjudicators graded angina using their clinical judgment. A classification of definite or probable angina required clear and definite documentation of symptoms distinct from the diagnosis of MI. Classification of definite angina also required objective evidence of reversible myocardial ischemia or obstructive coronary artery disease. A more detailed description of the MESA follow-up methods is available at http://www.mesa-nhlbi.org/followup.aspx.

Statistical analysis

Baseline characteristics were compared between CVD event cases and non-cases using t-tests for continuous variables and chi-square tests for categorical variables.

The associations of standard deviation increments of baseline Lp-PLA_2 mass and activity with incident cardiovascular events were evaluated using Cox proportional-hazard models,

adjusting for age, gender, race/ethnicity, body-mass index (BMI), diabetes mellitus, smoking status, systolic blood pressure, total and HDL cholesterol, statin use, anti-hypertensive use, education, CRP, and continuous measures of subclinical atherosclerosis (maximal CCA IMT, maximal ICA IMT, and CAC). Models were run with and without adjustment for subclinical disease measures because they could be in the pathway between Lp-PLA_2 and CVD. We used the transformation ln (CAC+1) for analysis. These analyses were performed for each of the following cardiovascular endpoints: (1) CVD, (2) CHD, and (3) CHD (hard). We used generalized additive models to evaluate whether the assumption of linearity was significantly violated. All relationships seemed well represented by the linear model. We tested for multiplicative interactions between the Lp-PLA_2 variables and age, gender, race/ethnicity and subclinical CVD. A p-value for interaction of less than 0.05 was considered statistically significant and stratified results shown by race/ethnicity and presence of absence of subclinical atherosclerosis by design.

Results

5,486 (80.5%) MESA participants had Lp-PLA_2 mass and activity measured at baseline, of which 30 were excluded because they did not have follow-up data. Chinese individuals were less likely to have missing Lp-PLA_2 measurements while Black individuals were more likely to have missing Lp-PLA_2 measurements. Otherwise, there were no meaningful differences in baseline characteristics between participants with versus without Lp-PLA_2 measurements.

Of 516 validated cardiovascular events occurring during follow-up, 358 were due to CHD and 223 of these were ‘hard’ events. Baseline characteristics of the study population are shown in Table 1. Compared with noncases, individuals who developed CVD were older and more likely male. They had significantly higher systolic and diastolic blood pressures, and lower HDL-C levels. Diabetes, statin use, and anti-hypertensive use were more prevalent in CVD cases. Individuals who developed CVD had a higher proportion of current and former smokers compared with individuals who did not. Mean levels of both Lp-PLA_2 mass and Lp-PLA_2 activity were higher in CVD cases, CHD cases, and hard CHD cases when compared to noncases (Table 2).

In a Cox proportional hazards model adjusted for age, sex, race/ethnicity, diabetes, smoking, total and HDL cholesterol, systolic blood pressure, lipid and anti-hypertensive medication use, BMI, education, and CRP, higher Lp-PLA_2 mass and activity were both associated with an increased risk of incident CVD, CHD, and ‘hard’ CHD with the largest associations for hard CHD (Table 3). After additional adjustment for continuous measures of subclinical atherosclerosis (maximal CCA IMT, maximal ICA IMT, and CAC), the hazard ratios for incident CVD, CHD and hard CHD were nearly identical for both Lp-PLA_2 mass and activity.

In the subset of patients on baseline statin therapy (n=879), higher Lp-PLA_2 mass was not associated with an increased risk of incident CVD (HR 1.06, 95% CI 0.87– 1.29), CHD (HR 1.14, 95% CI 0.91–1.41), or ‘hard’ CHD (HR 1.24, 95% CI 0.93–1.64) after adjustment for age, gender, and race/ethnicity. Similarly, higher Lp-PLA_2 activity was also not associated

with an increased risk of incident CVD (HR 1.12, 95% CI 0.89–1.41), CHD (HR 1.24, 95% CI 0.96–1.60), or 'hard' CHD (HR 1.39, 95% CI 0.99–1.97).

When performing tests for interaction between Lp-PLA_2 and sex a significant interaction was seen for Lp-PLA_2 mass and the endpoints of CHD (p interaction=0.01) and 'hard' CHD (p interaction=0.02). Lp-PLA_2 mass was more strongly associated with incident CHD in women than men. The interaction terms for Lp-PLA_2 activity and sex for CHD and 'hard' CHD were 0.19 and 0.12 respectively. No significant interactions were seen between Lp-PLA_2 mass and activity and either CRP or sex for the CVD endpoint.

There was some evidence of effect modification by race/ethnicity for Lp-PLA_2 activity and the endpoints of CVD (p interaction=0.16), CHD (p interaction=0.06), and 'hard' CHD (p interaction=0.07), but it did not meet our stringent significance level. In age and gender adjusted analysis, higher Lp-PLA_2 activity was not associated with an increased risk of incident CVD (HR 0.95, 95% CI 0.71–1.28), CHD (HR 0.90, 95% CI 0.64–1.26), or 'hard' CHD (HR 0.79, 95% CI 0.50–1.26) in Chinese participants. The number of events for incident CVD, CHD, and 'hard' CHD among Chinese participants were 41, 32, and 17 respectively. The association of higher Lp-PLA_2 activity and incident CVD in Blacks reached borderline significance (p=0.10) but all other associations of higher Lp-PLA_2 activity and all three endpoints were significant and similar in Blacks, Hispanics, and Whites (Table 4).

3,228 participants had evidence of baseline subclinical disease and 450 of these individuals experienced a cardiovascular event. After adjusting for age, sex, and total cholesterol, Lp-PLA_2 mass and activity had no significant correlation with either carotid IMT or CAC. Both Lp-PLA_2 mass and Lp-PLA_2 activity were associated with increased risk of incident CVD in the subgroup of individuals with baseline subclinical disease (Table 5). Among the 2,228 participants without evidence of baseline subclinical disease, only 66 experienced a cardiovascular event. Lp-PLA_2 activity but not mass was associated with increased risk for a CVD event. Despite the apparent difference in the association of Lp-PLA_2 mass with CVD by subclinical disease status, the interaction terms for Lp-PLA_2 mass with subclinical disease was not statistically significant (p= 0.32 in a model adjusted for age, sex and race/ethnicity).

Discussion

Higher Lp-PLA_2 mass and activity were both associated with increased incidence of CVD and CHD in a multiethnic cohort without clinical CVD at baseline. Higher Lp-PLA_2 activity was associated with a similar increased CVD risk in individuals with or without baseline subclinical disease, defined by the presence of calcified coronary artery disease or a thickened carotid intima-media. Although higher Lp-PLA_2 mass was associated with increased CVD risk in individuals with subclinical disease but not in those without subclinical disease, the difference by subclinical disease status failed to achieve statistical significance.

The association between higher Lp-PLA_2 and CVD risk has been previously reported in both white and Asian populations and our hazard ratios are consistent with prior studies.[8–10,12,21–24] In a recent meta-analysis of 32 prospective studies, associations with incident coronary heart disease and ischemic stroke were 1.11 and 1.14 respectively per standard deviation increment of Lp-PLA_2 mass and 1.10 and 1.08 respectively per standard deviation increment of Lp-PLA_2 activity.[7] In this meta-analysis, however, Lp-PLA_2 activity was not associated with incident coronary heart disease in the subset of patients without a history of vascular disease, which is in contrast to what we found.[7]

Our results extend these previous findings to a multi-ethnic population, which has been underrepresented in research to date. The increased risk was similar across different ethnicities with the exception of no association of Lp-PLA_2 activity in Chinese individuals. A higher prevalence of certain gene polymorphisms in Chinese individuals may be responsible for these findings. Mutations in the V279F allele of the PLA2G7 gene are associated with lower Lp-PLA_2 activity.[25, 26] The carrier frequency of these polymorphisms is high in Asian populations, 25% in Japanese individuals and greater than 10% in Korean and Chinese individuals.[27–29] In a cross-sectional study of Korean males, presence of this polymorphism was associated with a 20% less CAD prevalence. [30] The polymorphism, however, was not associated with risk of ischemic stroke in a study of Chinese individuals.[31] In two large meta-analyses that included individuals of Europoid ancestery, a reduction in incident CVD risk associated with these polymorphisms was not demonstrated.[29,32] Our null findings in Chinese subjects may also simply be due to small event numbers or chance.

Lp-PLA_2 can be measured by quantification of either its mass or activity. Previous studies that measured both mass and activity reported only moderate correlations between the two measurement methods.[22] Activity may be more reflective of the inflammatory state induced by Lp-PLA_2.[22] With respect to cardiovascular outcomes the two measurements perform similarly, with a recent meta-analysis reporting that the risk ratios for Lp-PLA_2 were similar whether mass or activity of the Lp-PLA_2 enzyme was measured, in agreement with our results.[7] Although our results suggest there is effect modification by sex for Lp-PLA_2 mass and CHD, these findings are likely due to chance since the same meta-analyses showed no difference by sex in the association between Lp-PLA_2 mass and CHD.[7]

There were a significantly higher proportion of patients on baseline statin therapy who experienced a cardiovascular event compared to those not on statin therapy. Hazard ratios of Lp-PLA_2 mass and activity with incident cardiovascular disease in the subset of participants on baseline statin therapy, although not statistically significant, were similar to the associations in the entire cohort. The lack of significance is likely attributed to limited power and Lp-PLA_2 levels are likely a risk factor regardless of statin use at baseline.

Since Lp-PLA_2 is considered a marker of vascular inflammation, we hypothesized that levels of Lp-PLA_2 in individuals with known subclinical atherosclerosis may be more predictive of incident CVD compared to levels in those without subclinical atherosclerosis; however, this was not observed in our study. By contrast, in a report from the Cardiovascular Health Study, higher CRP was associated with CVD risk only in those participants with detectable atherosclerosis on carotid ultrasound.[33]

Both Lp-PLA_2 mass and activity were weakly correlated with carotid IMT and CAC in our study. Studies on associations between Lp-PLA_2 and measures of subclinical atherosclerosis in individuals have reported mixed results. The Cardiovascular Health Study and the Malmo Diet and Cancer Study have both reported associations with higher Lp-PLA_2 activity and higher carotid IMT.[34,35] Conversely, other studies reported no associations between Lp-PLA_2 and carotid artery atherosclerosis.[36,37] Regarding CAC, in the Coronary Artery RIsk Development in Young Adults study higher Lp-PLA_2 mass, but not activity, was associated with presence and severity of calcified coronary plaque after adjustment for cardiovascular risk factors including both LDL and HDL cholesterol.[38] In other studies, there were no associations between Lp-PLA_2 and either coronary artery calcification or carotid artery atherosclerosis when adjusted for total and HDL cholesterol.[39,40]

Our findings suggest that the association of Lp-PLA_2 with incident CVD appears to be through mechanisms independent of those associated with the presence of measurable subclinical disease as assessed here. In a study of patients with a zero coronary artery calcium score, 11% had evidence of noncalcified plaque on CT coronary angiogram.[41] A prior study demonstrated that reduction in Lp-PLA_2 reduces progression of necrotic core volume but not total atheroma volume in human coronary atherosclerotic plaque.[42] Our findings suggest that Lp-PLA_2 identifies characteristics of vulnerable plaque not associated with traditional measures of subclinical disease. Lp-PLA_2 may, therefore, potentially help to identify individuals at higher risk for cardiovascular events without regard to presence of measurable subclinical atherosclerosis.

Our study has limitations. The population included individuals with no known baseline clinical CVD and findings cannot be generalized to dissimilar populations. The number of CVD events was low for some strata in our stratified analyses. The lack of an association between Lp-PLA_2 and incident cardiovascular disease when stratified by subclinical disease or across certain ethnicities may be due to limited power. Other studies or longer term follow-up in MESA is required to further investigate these questions. Non-ischemic cardiac causes cannot be excluded as an etiology for resuscitated cardiac arrest; however, only 7 of 516 CVD events were exclusively due to resuscitated cardiac arrest and it is unlikely to affect the results. Lastly, our detection of atherosclerosis is based on surrogate measures and does not capture all participants with evidence of subclinical disease.

In conclusion, Lp-PLA_2 mass and activity were both associated with CVD and CHD risk in a multiethnic cohort characterized for the presence of subclinical disease at baseline using presence of calcified coronary disease or a thickened carotid intima-media. The increased CVD risk was similar in individuals with or without baseline subclinical disease. Our findings suggest that the association of Lp-PLA_2 with incident CVD appears to be through mechanisms independent of those associated with the measures of subclinical disease as assessed in this cohort.

Acknowledgements

We thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org

Sources of funding:

This research was supported by contracts N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169 and grant K12-HL083790 from the National Heart, Lung, and Blood Institute and by grants UL1-TR-000040 and UL1-TR-001079 from the National Center for Research Resources. Additional support was from an investigator initiated grant from GlaxoSmithKline.

References

1. Häkkinen T, Luoma JS, Hiltunen MO, Macphee CH, Milliner KJ, Patel L, Rice SQ, Tew DG, Karkola K, Ylä-Herttuala S. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 1999; 19:2909–2917. [PubMed: 10591668]
2. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, Virmani R. Lipoprotein-associated phospholipase A_2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2006; 26:2523–2529. [PubMed: 16960105]
3. MacPhee CH, Moores KE, Boyd HF, Dhanak D, Ife RJ, Leach CA, Leake DS, Milliner KJ, Patterson RA, Suckling KE, Tew DG, Hickey DM. Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. Biochem J. 1999; 338:479–487. [PubMed: 10024526]
4. Rosenson RS, Stafforini DM. Modulation of oxidative stress, inflammation, and atherosclerosis by lipoprotein-associated phospholipase A_2. J Lipid Res. 2012 epub ahead of print.
5. Weintraub HS. Identifying the vulnerable patient with rupture-prone plaque. Am J Cardiol. 2008; 101:3F–10F. [PubMed: 18243856]
6. Winkler K, Winkelmann BR, Scharnagl H, Hoffmann MM, Grawitz AB, Nauck M, Böhm BO, März W. Platelet-activating factor acetylhydrolase activity indicates angiographic coronary artery disease independently of systemic inflammation and other risk factors: the Ludwigshafen Risk and Cardiovascular Health Study. Circulation. 2005; 111:980–987. [PubMed: 15710755]
7. Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hofman A, Packard C, Thompson SG, Collins R, Danesh J. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet. 2011; 375:1536–1544. [PubMed: 20435228]
8. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A_2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation. 2005; 111:570–575. [PubMed: 15699277]
9. Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A_2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med. 2000; 343:1148–1155. [PubMed: 11036120]
10. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A_2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 2004; 109:837–842. [PubMed: 14757686]
11. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A_2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med. 2005; 165:2479–2484. [PubMed: 16314544]
12. Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A_2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. Circulation. 2004; 110:1903–1908. [PubMed: 15451783]

13. Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, Budoff MJ, Liu K, Shea S, Szklo M, Tracy RP, Watson KE, Burke GL. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). Arch Intern Med. 2008; 168:1333–1339. [PubMed: 18574091]

14. White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, Budaj A, Harrington RA, Steg PG, Ardissino D, Armstrong PW, Avezum A, Aylward PE, Bryce A, Chen H, Chen MF, Corbalan R, Dalby AJ, Danchin N, De Winter RJ, Denchev S, Diaz R, Elisaf M, Flather MD, Goudev AR, Granger CB, Grinfeld L, Hochman JS, Husted S, Kim HS, Koenig W, Linhart A, Lonn E, López-Sendón J, Manolis AJ, Mohler ER 3rd, Nicolau JC, Pais P, Parkhomenko A, Pedersen TR, Pella D, Ramos-Corrales MA, Ruda M, Sereg M, Siddique S, Sinnaeve P, Smith P, Sritara P, Swart HP, Sy RG, Teramoto T, Tse HF, Watson D, Weaver WD, Weiss R, Viigimaa M, Vinereanu D, Zhu J, Cannon CP, Wallentin L. Darapladib for preventing ischemic events in stable coronary heart disease. N Engl J Med. 2014; 370:1702–1711. [PubMed: 24678955]

15. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, Liu K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156:871–881. [PubMed: 12397006]

16. Ramsey M 3rd. Blood pressure monitoring: automated oscillometric devices. J Clin Monit. 1991; 7:56–67. [PubMed: 1999699]

17. Lenzini L, Antezza K, Caroccia B, Wolfert RL, Szczech R, Cesari M, Narkiewicz K, Williams CJ, Rossi GP. A twin study of heritability of plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2) mass and activity. Atherosclerosis. 2009; 205:181–185. [PubMed: 19110247]

18. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, Sidney S, Bild DE, Williams OD, Detrano RC. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. Radiology. 2005; 234:35–43. [PubMed: 15618373]

19. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. Journal of the American College of Cardiology. 1990; 15:827–832. [PubMed: 2407762]

20. O'Leary DH, Polak JF, Wolfson SK Jr, Bond MG, Bommer W, Sheth S, Psaty BM, Sharrett AR, Manolio TA. Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. CHS Collaborative Research Group. Stroke; a journal of cerebral circulation. 1991; 22:1155–1163.

21. Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. A prospective evaluation of lipoprotein-associated phospholipase A(2) levels and the risk of future cardiovascular events in women. J Am Coll Cardiol. 2001; 38:1302–1306. [PubMed: 11691499]

22. Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA_2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. Arterioscler Thromb Vasc Biol. 2007; 27:1411–1416. [PubMed: 17431184]

23. Daniels LB, Laughlin GA, Sarno MJ, Bettencourt R, Wolfert RL, Barrett-Connor E. Lipoprotein-associated phospholipase A_2 is an independent predictor of incident coronary heart disease in an apparently healthy older population: the Rancho Bernardo Study. J Am Coll Cardiol. 2008; 51:913–919. [PubMed: 18308160]

24. Hou L, Chen S, Yu H, Lu X, Chen J, Wang L, Huang J, Fan Z, Gu D. Associations of PLA2G7 gene polymorphismis with plasa lipoprotien-associated phospholipase A2 activity and coronary heart disease in a Chinese Han population: the Beijing Atherosclerosis Study. Hum Genet. 2009; 125:111–120.

25. Stafforini DM, Satoh K, Atkinson DL, Tjoelker LW, Eberhardt C, Yoshida H, Imaizumi T, Takamatsu S, Zimmerman GA, McIntyre TM, Gray PW, Prescott SM. Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. The Journal of clinical investigation. 1996; 97:2784–2791. [PubMed: 8675689]

26. Miwa M, Miyake T, Yamanaka T, Sugatani J, Suzuki Y, Sakata S, Araki Y, Matsumoto M. Characterization of serum platelet-activating factor (PAF) acetylhydrolaseCorrelation between

deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. The Journal of clinical investigation. 1988; 82:1983–1991. [PubMed: 3198761]

27. Jang Y, Kim OY, Koh SJ, Chae JS, Ko YG, Kim JY, Cho H, Jeong TS, Lee WS, Ordovas JM, Lee JH. The Val279Phe variant of the lipoprotein-associated phospholipase A_2 gene is associated with catalytic activities and cardiovascular disease in Korean men. The Journal of clinical endocrinology and metabolism. 2006; 91:3521–3527. [PubMed: 16787988]
28. Yamada Y, Yoshida H, Ichihara S, Imaizumi T, Satoh K, Yokota M. Correlations between plasma platelet-activating factor acetylhydrolase (PAF-AH) activity and PAF-AH genotype, age, and atherosclerosis in a Japanese population. Atherosclerosis. 2000; 150:209–216. [PubMed: 10781653]
29. Casas JP, Ninio E, Panayiotou A, Palmen J, Cooper JA, Ricketts SL, Sofat R, Nicolaides AN, Corsetti JP, Fowkes FG, Tzoulaki I, Kumari M, Brunner EJ, Kivimaki M, Marmot MG, Hoffmann MM, Winkler K, März W, Ye S, Stirnadel HA, Boekholdt SM, Khaw KT, Humphries SE, Sandhu MS, Hingorani AD, Talmud PJ. PLA2G7 genotype, lipoprotein-associated phospholipase A_2 activity, and coronary heart disease risk in 10 494 cases and 15 624 controls of European Ancestry. Circulation. 2010; 121:2284–2293. [PubMed: 20479152]
30. Jang Y, Waterworth D, Lee JE, et al. Carriage of the V279F null allele within the gene encoding Lp-PLA(2) is protective from coronary artery disease in South Korean males. PLoS One. 2011; 6:e18208. [PubMed: 21490708]
31. Liu X, Zhu R, Tian Y, Li Q, Li L, Deng S, He Z. Association of PLA2G7 gene polymorphisms with ischemic stroke in northern Chinese Han population. Clin Biochem. 2014; 47:404–408. [PubMed: 24463064]
32. Grallert H, Dupuis J, Bis JC, et al. Eight genetic loci associated with variation in lipoprotein-associated phospholipase A_2 mass and activity and coronary heart disease: meta-analysis of genome-wide association studies from five community-based studies. European heart journal. 2012; 33:238–251. [PubMed: 22003152]
33. Cao JJ, Arnold AM, Manolio TA, Polak JF, Psaty BM, Hirsch CH, Kuller LH, Cushman M. Association of carotid artery intima-media thickness, plaques, and C-reactive protein with future cardiovascular disease and all-cause mortality: the Cardiovascular Health Study. Circulation. 2007; 116:32–38. [PubMed: 17576871]
34. Furberg CD, Nelson JJ, Solomon C, Cushman M, Jenny NS, Psaty BM. Distribution and correlates of lipoprotein-associated phospholipase A_2 in an elderly cohort: the Cardiovascular Health Study. J Am Geriatr Soc. 2008; 56:792–799. [PubMed: 18363676]
35. Persson M, Nilsson J, Nelson JJ, Hedblad B, Berglund G. The epidemiology of Lp-PLA_2: Distribution and correlation with cardiovascular risk factors in a population-based cohort. Ather osclerosis. 2007; 190:388–396.
36. Campo S, Sardo MA, Bitto A, Bonaiuto A, Trimarchi G, Bonaiuto M, Castaldo M, Saitta C, Cristadoro S, Saitta A. Platelet-Activating Factor Acetylhydrolase is not associated with carotid intima-media thickness in hypercholesteremic sicilian individuals. Clin Chem. 2004; 50:2077–2082. [PubMed: 15364890]
37. Kiortsis DN, Tsouli S, Lourida ES, Xydis V, Argyropoulou MI, Elisaf M, Tselepis AD. Lack of association between carotid intima-media thickness and PAF-acetylhydrolase mass and activity in patients with primary hyperlipidemia. Angiology. 2005; 56:451–457. [PubMed: 16079929]
38. Iribarren C, Gross MD, Darbinian JA, Jacobs DR Jr, Sidney S, Loria CM. Association of lipoprotein-associated phospholipase A_2 mass and activity with calcified coronary plaque in young adults: the CARDIA study. Arterioscler Thromb Vasc Biol. 2005; 25:216–221. [PubMed: 15499045]
39. Kardys I, Oei HH, van der Meer IM, Hofman A, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A_2 and measures of extracoronary atherosclerosis: the Rotterdam Study. Arterioscler Thromb Vasc Biol. 2006; 26:631–636. [PubMed: 16373603]
40. Kardys I, Oei HH, Hofman A, Oudkerk M, Witteman JC. Lipoprotein-associated phospholipase A_2 and coronary calcification. The Rotterdam Coronary Calcification Study. Atherosclerosis. 2007; 191:377–383. [PubMed: 16678183]

41. Iwasaki K, Matsumoto T, Aono H, Furukawa H, Samukawa M. Prevalence of noncalcified coronary plaque on 64-slice computed tomography in asymptomatic patients with zero and low coronary artery calcium. Can J Cardiol. 2010; 7:377–380. [PubMed: 20847965]

42. Serruys PW, Garcia-Garcia HM, Buszman P, et al. Effects of the direct lipoprotein-associated phospholipase A_2 inhibitor Darapladib on human coronary atherosclerotic plaque. Circulation. 2008; 118:1172–1182. [PubMed: 18765397]

Highlights

- We evaluated associations of Lp-PLA_2 and first-time cardiovascular events
- Participants comprised a multi-ethnic cohort characterized at baseline for subclinical disease
- Both Lp-PLA_2 activity and mass were associated with incident cardiovascular events
- Associations of Lp-PLA_2 activity were weaker in Chinese participants
- There was no confounding by subclinical atherosclerosis measures

Table 1

Baseline characteristics of study participants*

Baseline Characteristics	Noncases (n=4940)	Cardiovascular Disease Cases (n=516)	*p*-value†
Age, y	62 ± 10	68 ± 10	<0.001
Male	2280 (46%)	315 (61%)	<0.001
Race/ethnicity			
Black	1264 (26%)	135 (26%)	0.001
Chinese	677 (14%)	41 (8%)	
Hispanic	1113 (22%)	110 (21%)	
White	1886 (38%)	230 (45%)	
Body mass index, kg/m^2	28 ± 5	29 ± 5	0.054
Systolic blood pressure, mm Hg	125 ± 21	136 ± 23	<0.001
Diastolic blood pressure, mm Hg	72 ± 10	74 ± 11	<0.001
Cholesterol, mg/dl			
Total	194 ± 36	196 ± 37	0.461
LDL	117 ± 31	120 ± 33	0.063
HDL	52 ± 15	48 ± 14	<0.001
Cigarette smoking			
Current	601 (12%)	73 (14%)	0.005
Former	1784 (36%)	215 (42%)	
Never	2539 (52%)	227 (44%)	
Diabetes	565 (11%)	116 (22%)	<0.001
Statin use	697 (14%)	102 (20%)	<0.001
Anti-hypertensive use	1726 (35%)	279 (54%)	<0.001
C-reactive protein, mg/L	3.7 ± 6	4.2 ± 5.9	0.093
Lp-PLA$_2$ activity, nmol/min/ml	148 ± 37	158 ± 36	<0.001
Lp-PLA$_2$ mass, ng/ml	177 ± 42	186 ± 43	<0.001

* Values are means ± SD or numbers (percentages of total).

† *p* values were obtained using t-tests for continuous variables and chi-square test for categorical variables

Table 2

Average Lp-PLA$_2$ mass and activity by subsequent case status (n=5456)*

LpPLA$_2$	**CVD (n=498)**	**CHD (n=346)**	**CHD (Hard) (n=215)**
Mass (ng/mL)			
Cases	186 ± 43	189 ± 44	193 ± 45
Noncases	177 ± 42	177 ± 42	177 ± 42
p-value†	0.003	0.001	<0.001
	CVD (n=506)	CHD (n=349)	CHD (Hard) (n=217)
Activity (nmol/min/mL)			
Cases	158 ± 35	161 ± 35	161 ± 33
Noncases	148 ± 36	148 ± 36	149 ± 37
p-value	<0.001	<0.001	0.001

*Values are means ± SD

†P-values compare the means between cases and noncases adjusting for age, gender and race/ethnicity.

Table 3

Association of Lp-PLA$_2$ levels and risk of incident cardiovascular disease (n=5456)

	Model 1*		Model 2*	
	Hazard Ratio† (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Cardiovascular disease (516 events)				
Lp-PLA$_2$ activity	1.12 (1.01, 1.26)	0.04	1.11 (0.99, 1.25)	0.06
Lp-PLA$_2$ mass	1.10 (1.01, 1.21)	0.03	1.10 (1.01, 1.21)	0.04
Coronary heart disease (358 events)				
Lp-PLA$_2$ activity	1.18 (1.03, 1.34)	0.01	1.17 (1.02, 1.34)	0.02
Lp-PLA$_2$ mass	1.15 (1.04, 1.28)	0.01	1.14 (1.03, 1.27)	0.01
Coronary heart disease – Hard (223 events)				
Lp-PLA$_2$ activity	1.22 (1.04, 1.44)	0.02	1.22 (1.02, 1.44)	0.03
Lp-PLA$_2$ mass	1.29 (1.14, 1.46)	<0.001	1.31 (1.15, 1.49)	<0.001

*Model 1: Adjusted for age, gender and race/ethnicity, BMI, diabetes, smoking status, high school education, systolic blood pressure, use of anti-hypertensive medication, total and HDL cholesterol, use of lipid lowering medications, and CRP.

Model 2: Model 1 plus maximal common carotid intimal-medial thickness, maximal internal carotid intimal-medial thickness, and coronary artery calcification score

†Cox Proportional Hazard ratios expressed per 1 standard deviation increment: 36 nmol/min/mL for activity, 42 ng/mL for mass.

Table 4

Association of Lp-PLA$_2$ activity and risk of incident cardiovascular disease stratified by race/ethnicity*

	Cardiovascular Disease		Coronary Heart Disease		Coronary Heart Disease - Hard	
	Hazard Ratio† (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Race/Ethnicity‡						
Black	1.17 (0.97, 1.40)	0.10	1.27 (1.02, 1.59)	0.03	1.33 (1.01, 1.75)	0.04
Chinese	0.95 (0.71, 1.28)	0.74	0.90 (0.64, 1.26)	0.53	0.79 (0.50, 1.26)	0.32
Hispanic	1.41 (1.16, 1.71)	0.001	1.52 (1.21, 1.92)	<0.001	1.49 (1.13, 1.95)	0.004
White	1.25 (1.09, 1.44)	0.001	1.26 (1.07, 1.48)	0.007	1.31 (1.05, 1.62)	0.015

*Mean Lp-PLA$_2$ activity values (mmol/min/mL) according to race/ethnicity: Black 137, Chinese 152, Hispanic 152, White 156

†Cox Proportional Hazard ratios adjusted for age and gender and expressed per 1 standard deviation increment.

‡p-value for interaction by race/ethnicity for Lp-PLA$_2$ activity and the endpoints of CVD, CHD, and 'hard' CHD are 0.16, 0.06, and 0.07 respectively.

Table 5

Association of Lp-PLA$_2$ levels and risk of incident cardiovascular disease stratified by subclinical disease (n=5456)

	Cardiovascular disease			
	Model 1*		Model 2*	
	Hazard Ratio† (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Subclinical disease‡, n=3228 (450 events)				
Lp-PLA$_2$ activity	1.17 (1.06, 1.30)	0.002	1.10 (0.97, 1.23)	0.14
Lp-PLA$_2$ mass	1.17 (1.06, 1.28)	0.001	1.13 (1.03, 1.24)	0.01
No subclinical disease, n=2228 (66 events)				
Lp-PLA$_2$ activity	1.30 (1.01, 1.68)	0.04	1.26 (0.92, 1.72)	0.15
Lp-PLA$_2$ mass	1.01 (0.78, 1.30)	0.95	0.96 (0.74, 1.24)	0.73

*Model 1: Adjusted for age, gender and race/ethnicity

Model 2: Model 1 plus BMI, diabetes, smoking status, high school education, systolic blood pressure, use of anti-hypertensive medication, total and HDL cholesterol, use of lipid lowering medications, and CRP.

†Cox Proportional Hazard ratios expressed per 1 standard deviation increment: 36 nmol/min/mL for activity, 42 ng/mL for mass.

‡p-value for interaction by subclinical disease status for both Lp-PLA$_2$ activity and Lp-PLA$_2$ mass with the endpoint of CVD are 0.99 and 0.32 respectively.