

ARIC Manuscript Proposal #2730

PC Reviewed: 4/12/16
SC Reviewed: _____

Status: A
Status: _____

Priority: 2
Priority: _____

1. a. Full Title:

Lipoprotein-associated Phospholipase A₂ and risk of incident peripheral arterial disease: Findings from The Atherosclerosis Risk in Communities Study (ARIC)

b. Abbreviated Title (Length 26 characters):

Lp-PLA₂ & incident PAD in ARIC

2. Writing Group:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. PKG [**please confirm with your initials electronically or in writing**]

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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

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3. Timeline:

March-April 2016 – Submission and review of proposal

May-June-July 2016 – Complete primary data analysis

August-September 2016 – Submit as abstract to AHA EPI/Lifestyle

October-November-December-January 2017 – Additional data analysis/Manuscript preparation

February-March 2017 – anticipate submitting manuscript for P&P review

4. Rationale:

Lipoprotein-associated Phospholipase A₂ (Lp-PLA₂) is a 50-kd calcium-independent enzyme highly expressed by macrophages in atherosclerotic lesions.¹ Lp-PLA₂ is responsible for the hydrolysis of oxidized phospholipids on LDL particles.² The presence and activity of Lp-PLA₂ is thought to be associated with vulnerable, rupture-

prone plaques. This close association with plaque activity and low correlation with circulating inflammatory markers suggests specificity of Lp-PLA₂ for vascular inflammation.³

The association between elevated Lp-PLA₂ levels and both incident coronary heart disease and ischemic stroke is well established.⁴ The association of Lp-PLA₂ with incident peripheral arterial disease (PAD) however has not been similarly assessed. Current evidence suggests a relationship between higher Lp-PLA₂ concentrations and increased risk of incident PAD exists but is limited to a single prospective study of elderly, predominantly white individuals.⁵ In this study a significant p-value for interaction was noted, however, between Lp-PLA₂ mass and black race for the outcome of incident PAD in this study and the association of Lp-PLA₂ mass with incident PAD was not significant for this subgroup.

8 million people in the United States alone and over 200 million people worldwide are estimated to have PAD.^{6,7} Prevalence of PAD varies substantially according to age and ethnicity with significantly higher rates reported in older populations and African American individuals.⁸⁻¹⁰ Established risk factors alone do not explain ethnic-specific variations in PAD prevalence.^{10,11} It is important to determine if high Lp-PLA₂ levels are associated with the development of PAD as well. If an association is observed, future studies could investigate if these individuals may benefit from more intensive measures to modify cardiovascular risk and, potentially, pharmacologic inhibition of Lp-PLA₂.

The Atherosclerosis Risk in Communities (ARIC) offers an opportunity to prospectively examine the relationship between Lp-PLA₂ levels and the development of PAD in a large, well-defined population with long-term follow-up and better explore whether associations may differ by race.

5. Main Hypothesis/Study Questions:

- To investigate the association of Lp-PLA₂ activity with risk of incident PAD, defined by either an incident low ABI or development of clinical PAD.
- To investigate whether associations of Lp-PLA₂ activity with risk of incident PAD differ when stratified by race
- To determine whether Lp-PLA₂ contributes to the prediction of PAD beyond traditional risk factors and other inflammatory markers

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Data:

Study participants

In 1987-89, the ARIC Study recruited to an initial examination a cohort of 15,792 men and women aged 45-64 years from four U.S. communities. Participants were re-examined in 1990-92 (93% response), 1993-95 (86%), 1996-98 (80%), and 2011-13 (65%) and followed long-term for cardiovascular events. Due to the availability of Lp-PLA₂ activity, participants in ARIC Visit 4 (1996-98) will serve as the eligible cohort and baseline visit for the present analysis.

Lipoprotein-associated Phospholipase A₂ (Lp-PLA₂)

ARIC assessed Lp-PLA₂ activity in Visit 4 plasma by an automated Colorimetric Activity Method (CAM) assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer.

Ankle-brachial index

The ABI was measured on nearly all participants at visit 1 (96%), on a select random number of participants at visits 3 (n=4197), 4 (n=5882), and most participants at visit 5 (86%). At visits 1 and 5, ABI was computed by dividing the average of ankle SBP measurements by the average of brachial SBP measurements. Two ankle BP measurements were taken 5 to 8 min apart at the posterior tibial artery in a randomly selected leg while the participant was prone. Two brachial artery BP were measured, usually in the right arm, with the participant supine. At visits 3 and 4, the ABI was defined as the ratio of a single ankle SBP to a single brachial BP, both measured with the participant supine.

An abnormal ABI at baseline was defined as a recorded ABI of <0.9 or >1.4 at study visits 1, 3, or 4.

Clinical PAD

A positive Rose Questionnaire or a hospitalization ICD-9 code consistent with the diagnosis of PAD were used to determine clinical PAD.

Rose Questionnaire

Interviewers contacted participants annually by telephone to identify intermittent claudication symptoms and all hospitalizations. The Rose Questionnaire was used to evaluate whether participants had developed intermittent claudication, which was defined as exertional leg pain relieved within 10 min by resting.¹²

Hospitalized PAD

When a hospitalization had occurred, a trained abstractor obtained and recorded all International Classification of Disease, Ninth Revision hospital discharge diagnoses. All records with an International Classification of Disease, Ninth Revision code of 443.9 (peripheral vascular disease, unspecified), 84.11 (toe amputation), 84.12 (foot amputation), 84.15 (below-knee amputation), 84.17 (above-knee amputation), 38.18 (leg endarterectomy), 39.25 (aorto-iliac-femoral bypass), and 39.29 (leg bypass surgery) qualified as hospitalized PAD.

Clinical PAD at baseline was defined as a positive Rose Questionnaire or a qualifying hospitalized PAD diagnosis that occurred prior to visit 4.

Incident PAD

In individuals without prevalent PAD (defined as above by either the presence of an abnormal ABI or clinical PAD at baseline), PAD incidence was characterized by one of the following criteria: (1) A new ABI<0.9 at visit 5; (2) new intermittent claudication based on Rose Questionnaire; or (3) a hospital discharge diagnosis consistent with PAD

Other Variables of Interest (all cohorts)

Demographic - Age, Race, Sex, height, weight, clinic site

Comorbidities - Cigarette smoking, SBP, DBP, anti-HTN med use, fasting glucose, anti-DM med use, and cardiovascular disease.

Laboratory data – CRP, Fibrinogen, eGFR

Lipid profile – TC, LDL, HDL, TG

Medication use – Statin & Aspirin use

Others – Alcohol consumption, Physical activity levels

Exclusion criteria

Individuals without a baseline Lp-PLA₂ activity measurement, with baseline PAD (ABI<0.9, ABI>1.4, or evidence of clinical PAD), or without follow-up data will be excluded from the analysis.

Analysis plan:

1) Comparison of baseline characteristics

The comparison groups will be those who did not develop incident PAD and those who did develop incident PAD. Adjusting for age and sex, we will use a *t*-test for continuous variable and a χ^2 test for dichotomous variables to test differences between these two groups.

2) Associations of Lp-PLA₂ activity with incident PAD

Cox proportional hazards models will be used to investigate the association of baseline Lp-PLA₂ activity with incident PAD. In this analysis, we will model Lp-PLA₂ activity continuously (per SD increment) and also categorized into quartiles (lowest quartile as referent category).

The analysis will be adjusted for age, sex, race, clinic site, smoking, alcohol consumption, diabetes, systolic and diastolic blood pressure, CHD, total and HDL cholesterol, BMI, physical activity, eGFR, anti-hypertensive medication, aspirin use, and statin use. The analyses will be additionally adjusted for CRP and fibrinogen.

3) Subgroup analyses

Step 2 will be repeated stratified by race (Whites and Blacks) for the incident PAD outcome. P-value for interaction will also be determined.

4) Sensitivity analysis

This will be performed to determine whether associations of Lp-PLA₂ activity differ based on how incident PAD is assessed (Clinical PAD and Low ABI analyzed separately) and to assess for possible bias introduced from the ABI not being measured in all participants at study visits.

A) Clinical PAD: Step 2 will be repeated for all eligible study participants as mentioned above to investigate the association of baseline Lp-PLA₂ activity with incident PAD defined only by the clinical PAD outcome

B) Low ABI: Step 2 will be repeated only in eligible individuals with ABI measured at both visit 4 and visit 5 to investigate the association of baseline Lp-PLA₂ activity with incident PAD defined only by the low ABI outcome.

5) Inflammatory markers

AUC comparison will be calculated for predictive ability of Lp-PLA₂ activity beyond traditional RF's and markers of inflammation

**7.a. Will the data be used for non-CVD analysis in this manuscript? Yes
 No**

**b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? Yes
 No**

(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?

Yes No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"?

Yes No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: <http://www.csc.unc.edu/ARIC/search.php>

Yes No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

Potentially related proposals include: MS1642: Lp-PLA₂ and incidence of stroke and CHD (Hoogeveen) and MS2063: Lp-PLA₂ and incident VTE (Folsom); however, the author identifies no overlap with these manuscript proposals. In addition, representatives from the lab where Lp-PLA₂ was measured are included as co-authors on this proposal.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes No

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (list number* _____)

B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____)

*ancillary studies are listed by number at <http://www.csc.unc.edu/aric/forms/>

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does

not and be in compliance with this policy. Four files about the public access policy from <http://publicaccess.nih.gov/> are posted in <http://www.csc.unc.edu/aric/index.php>, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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