1. a. Full Title:
Lipoprotein-associated Phospholipase A2 and risk of incident atrial fibrillation: Findings from The Atherosclerosis Risk in Communities Study (ARIC), The Cardiovascular Health Study (CHS), and The Multi-Ethnic Study of Atherosclerosis (MESA)
b. Abbreviated Title (Length 26 characters):
LP-PLA2 & AF in ARIC, CHS, & MESA

2. Writing Group:

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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3. Timeline:
June 2015 – Submit proposal
July-August-September 2015 – Complete primary data analysis
October 2015 – Submit as abstract to AHA EPI/Lifestyle
November-December-January-February 2016 – Additional data analysis/Manuscript preparation
March-April 2016 – anticipate submitting manuscript for P&P review
4. **Rationale:**

Atrial Fibrillation (AF) is the most commonly presenting cardiac arrhythmia in clinical practice, affecting over 2 million people in the United States alone.\(^1\) AF is a major source of cardiovascular morbidity and mortality.\(^2-4\) Although the pathophysiology of AF is complex and incompletely understood, inflammation is thought to play a role in this process. At the atrial tissue level inflammatory activity has been shown to be elevated in manifest AF, suggesting the contribution of inflammation to structural remodeling of the atria.\(^5\)

Multiple prospective epidemiological studies have confirmed an association between various markers of inflammation and increased risk of incident AF, including C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), and fibrinogen.\(^6-9\) Although the association between many elevated serum markers of inflammation and incident AF is well established, the association between Lipoprotein-associated phospholipase A\(_2\) (Lp-PLA\(_2\)) levels and incident AF has not been studied in a large, comprehensive, long-term follow-up study population. Lp-PLA\(_2\) is an enzyme synthesized primarily by inflammatory cells and is highly expressed by macrophages in atherosclerotic lesions.\(^10-14\) Lp-PLA\(_2\) hydrolyzes oxidized phospholipids found on the LDL particle in plaques, resulting in proinflammatory and proatherogenic products.\(^15,16\)

Considering the specificity of Lp-PLA\(_2\) for vascular inflammation, it would be important to assess whether Lp-PLA\(_2\) levels were associated with incident AF independent of traditional biomarkers of inflammation.\(^17\) Existing literature on this association comes from a single prospective study that was limited by a small number of events and included 12 biomarkers.\(^18\) Despite these limitations, Lp-PLA\(_2\) mass showed a borderline association with incident AF (p=0.02, significance defined as p<0.01).

The Cardiovascular Health Study (CHS), the Multi-Ethnic Study of Atherosclerosis (MESA), and the Atherosclerosis Risk in Communities (ARIC) offer an opportunity to prospectively examine the relationship between Lp-PLA\(_2\) levels and the development of AF in multiple a large, well-defined populations with long-term follow-up. Examination of multiple prospective cohorts with very different baseline demographic characteristics will allow for increased generalizability of findings. Additionally, inclusion of multiple cohorts will provide better power to evaluate this relationship in gender- and race-specific analyses. Lp-PLA\(_2\) levels can be measured by quantification of either its mass or activity. Previous studies that have measured both mass and activity have reported only moderate correlations between the two.\(^19,20\)

Considering the potential for differences are possible, we will study the associations of both Lp-PLA\(_2\) mass and Lp-PLA\(_2\) activity with risk of incident AF.

5. **Main Hypothesis/Study Questions:**

- To investigate the associations of both Lp-PLA\(_2\) mass and activity with risk of incident AF in a multi-cohort analysis including ARIC, CHS, & MESA
- To investigate whether associations of both Lp-PLA\(_2\) mass and activity with risk of incident AF differ when stratified by gender or race
- To determine whether Lp-PLA\(_2\) contributes to the prediction of incident AF beyond current risk prediction models
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:

**CHS**

**Study participants**

Eligible participants will be from the original cohort (n=5201) and the primarily African-American cohort (n=687) at baseline.

**Lipoprotein-associated Phospholipase A$_2$ (Lp-PLA$_2$)**

Plasma Lp-PLA$_2$ mass was measured at baseline from stored specimens using a commercially available ELISA kit (second generation PLAC Test; diaDexus Inc, South San Francisco, CA). Plasma Lp-PLA$_2$ activity was measured at GlaxoSmithKline (Research Triangle Park, NC) by high-throughput radiometric assay using a tritium-labeled form of platelet activating factor as substrate in a 96-well microplate.

**Atrial Fibrillation**

An annual resting ECG was obtained yearly through the 9$^{th}$ year of follow-up and discharge diagnoses for all hospitalizations were collected. AF was identified in 3 ways. Annual outpatient study ECGs were interpreted by the EPICARE ECG reading center, here the diagnoses of AF or atrial flutter were verified. In addition, hospital discharge diagnoses with ICD-9 codes for AF and flutter were also included (427.4, 427.31, 427.32) except for those made during the same hospitalization as coronary artery bypass surgery or heart valve surgery. Finally, outpatient diagnoses of AF and flutter were identified from Medicare claims.

**MESA**

**Study participants**

Eligible participants will be from the original cohort (n=6814) at baseline. Details of MESA have been reported previously.

**Lipoprotein-associated Phospholipase A$_2$ (Lp-PLA$_2$)**

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). Lp-PLA$_2$ mass was measured with a sandwich enzyme immunoassay (PLACTM Test; diaDexus). Lp-PLA$_2$ activity was measured by an enzymatic assay using a tritium-labeled platelet activating factor (PAF) analog as the substrate.

**Atrial Fibrillation**

Incident AF was identified from hospital discharge diagnosis (International Classification of Diseases, Ninth Revision [ICD-9]) codes for AF or atrial flutter, (classified as ICD-9 diagnosis codes 427.31 and 427.32) ascertained by the MESA follow-up protocol or from Medicare claims for inpatient or outpatient care for AF or atrial flutter. Follow-up consisted of phone calls or field center visits every 9 to 12 months to identify hospitalizations and medical records, including discharge diagnoses. Medicare inpatient and outpatient claims data from the CMS were collected for MESA participants aged 65 years or older and enrolled in fee-for-service Medicare.

**ARIC**

**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. 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.

**Atrial Fibrillation**

Study participants underwent ECG recordings at baseline and at each follow-up exam. All ECG recordings automatically coded as AF were visually re-checked by a trained cardiologist to confirm the diagnosis. A trained abstractor obtained and recorded all ICD-9 hospital discharge diagnoses from each participant's hospitalizations reported in the annual follow-up interview. AF was defined as the presence of ICD-9 code 427.31 or 427.32 in the discharge codes. AF hospitalization diagnoses occurring simultaneously with heart revascularization surgery or other cardiac surgery involving heart valves or septa, without evidence of AF in subsequent hospitalizations or study exams will also be excluded. ARIC participants were also labeled as AF cases if the underlying cause of death was AF. The incidence date of AF was defined as the date for the first ECG showing AF or the first hospital discharge with an AF diagnosis.

**Other Variables of Interest (all cohorts)**

Demographic - Age, Race/Ethnicity, Sex, height, weight, clinic site
Comorbidities - Cigarette smoking, SBP, DBP, anti-HTN med use, fasting glucose, anti-DM med use, Coronary heart disease (CHS/ARIC), Congestive heart failure (CHS/ARIC)
Laboratory data – CRP, NT-proBNP
Lipid profile – TC, LDL, HDL
Medication use – Statin use
Others – Alcohol consumption, Physical activity levels

**Exclusion criteria (all cohorts)**

Individuals without a baseline Lp-PLA2 measurement, with baseline AF, or without follow-up data will be excluded.

**Analysis plan:**

In ARIC and CHS a p-test for interaction for Lp-PLA2 and incident AF between those with and without prevalent CHD/CHF will be performed. If a significant interaction exists, then patients with prevalent CHD/CHF will be excluded to reduce heterogeneity. The following analysis will be performed separately in CHS, MESA and ARIC.

1) **Comparison of baseline characteristics**

Participants will be stratified according to set levels of Lp-PLA2 as determined by their distributions across the cohorts to help identify potential confounders.

2) **Associations of Lp-PLA2 with incident AF**

Cox proportional hazards models to investigate the association of baseline Lp-PLA2 (mass and activity separately if both are available) with incident AF. In this analysis, we will model Lp-PLA2 continuously (per a prespecified increment if high inter-cohort variability exists) and also categorized into groups defined by the same cutpoints in each cohort.
The above analyses will need to be adjusted for age, race, sex, clinic site, coronary heart disease, congestive heart failure, cigarette smoking, DM, SBP, DBP, use of antihypertensive medication, LDL, BMI, physical activity, and alcohol consumption. The analyses will then be additionally adjusted for CRP and NT-proBNP.

3) Meta-analysis
   Assuming significant heterogeneity does not exist across the three cohorts a meta-analysis will be performed combining results from all included studies according to prespecified increments of Lp-PLA₂. Results will be displayed in forest plot format.

4) Subgroup analyses
   Steps 2 & 3 will be repeated stratified by gender and race (Whites and Blacks)

5) Risk prediction
   If a positive association is found, then an AUC comparison will be calculated for predictive ability of Lp-PLA₂ mass and Lp-PLA₂ activity beyond CHARGE-AF risk model for the entire study population or, if significant heterogeneity exists, for each cohort separately.²⁴

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ 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? __X__ 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  __X__ 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.cscce.unc.edu/ARIC/search.php

   __X__ 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 incident 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  ____X__ 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.cscc.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.cscc.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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes  ____X__ No.
References


