ARIC Manuscript Proposal #2566

PC Reviewed: 6/9/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1. a. Full Title:
Lipoprotein-associated Phospholipase A_2 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-PLA_2 & AF in ARIC, CHS, & MESA

2. Writing Group:
Parveen K Garg, Robyn McClelland, Nancy S Jenny, PhD, Neal Jorgensen, Philip Greenland, Robert Rosenson, Mary Cushman, Alvaro Alonso, Traci Bartz, John Gottdiener, Susan Heckbert, Jorge Kizer, Christie Ballantyne, Lin Y Chen, Ron Hoogeveen, Faye Norby, Elsayed Soliman

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]**

First author: **Parveen Garg**
Address: 1510 San Pablo St, #322
Los Angeles, CA
Phone: (323)-442-6131
Fax: (323)-442-6133
E-mail: parveeng@med.usc.edu

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).
Name: Alvaro Alonso
E-mail: alonso@umn.edu

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 9th 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.[21] 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.[22]

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 (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.

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.[23] 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 A_2 (Lp-PLA_2)

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-PLA_2 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-PLA_2 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-PLA_2 as determined by their distributions across the cohorts to help identify potential confounders.
2) Associations of Lp-PLA_2 with incident AF
   Cox proportional hazards models to investigate the association of baseline Lp-PLA_2 (mass and activity separately if both are available) with incident AF. In this analysis, we will model Lp-PLA_2 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 anti-hypertensive 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-PLA_2. 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-PLA_2 mass and Lp-PLA_2 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.cscc.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-PLA_2 and incident of stroke and CHD (Hoogeveen) and MS2063: Lp-PLA_2 and incident VTE (Folsom); however, the author identifies no overlap with these manuscript proposals. In addition, representatives from the lab where Lp-PLA_2 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

1. Go AS, Hylek EM, Phillips KA, et al. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA* 2001;285:2370-2375.
2. Benjamin EJ, Levy D, Vaziri SM, et al. Independent risk factors for atrial fibrillation in a population-based cohort: the Framingham Heart Study. *JAMA* 1994;271:840–844.
3. Kannel WB, Abbott RD, Savage DD, et al. Epidemiologic features of chronic atrial fibrillation: the Framingham study. *N Engl J Med* 1982;306:1018–1022.
4. Kannel WB, Benjamin EJ. Status of the epidemiology of atrial fibrillation. *Med Clin North Am* 2008; 92:17–40, ix.
5. Nakamura Y, Nakamura K, Fukushima-Kusano K, et al. Tissue factor expression in atrial endothelia associated with nonvalvular atrial fibrillation: possible involvement in intracardiac thrombogenesis. *Thromb Res* 2003;111:137-142.
6. Prage P, Hisham D, Peter T, Nasser L. Update on the association of inflammation and atrial fibrillation. *J Cardiovasc Electrophysiol* 2010;21:1064-1070.
7. Aviles RJ, Martin DO, Apperson-Hansen C, et al. Inflammation as a risk factor for atrial fibrillation. *Circulation* 2003;108:3006-3010.
8. Marott SC, Nordestgaard BG, Zacho J, et al. Does elevated C-reactive protein increase atrial fibrillation risk? A Mendelian randomization of 47,000 individuals from the general population. *JACC* 2010;56:789-795.
9. Conen D, Ridker PM, Everett BM, et al. A multimarker approach to assess the influence of inflammation on the incidence of atrial fibrillation in women. *Eur Heart J* 2010;31: 1730-1736.
10. Stafforini DM, Elstad MR, McIntyre TM, Zimmerman GA, Prescott SM. Human macrophages secret platelet-activating factor acetylhydrolase. *J Biol Chem*. 1990;265:9682-9687
11. Asano K, Okamoto S, Fukunaga K, Shiomi T, Mori T, Iwata M, Ikeda Y, Yamaguchi K. Cellular source(s) of platelet-activating-factor acetylhydrolase activity in plasma. *Biochem Biophys Res Commun*. 1999;261:511-514
12. Tarbet EB, Stafforini DM, Elstad MR, Zimmerman GA, McIntyre TM, Prescott SM. Liver cells secrete the plasma form of platelet-activating factor acetylhydrolase. *J Biol Chem*. 1991;266:16667-16673
13. 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
14. Hakkinen T, Luoma JS, Hiltunen MO, Macphee CH, Milliner KJ, Patel L, Rice SQ, Tew DG, Karkola K, Yla-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
15. Macphee C, Benson GM, Shi Y, Zalewski A. Lipoprotein-associated phospholipase A_2: A novel marker of cardiovascular risk and potential therapeutic target. *Expert Opin Investig Drugs*. 2005;14:671-679
16. 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(pt 2):479-487
17. Winkler K, Winkelmann BR, Scharnagl H, et al. 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.
18. Schnabel RB, Larson MG, Yamamoto JF, et al. Relation of multiple inflammatory biomarkers to incident atrial fibrillation. *Am J Cardiol* 2009;104:92-96.
19. Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. *Arterioscler Thromb Vasc Biol*. Jun 2007;27(6):1411-1416.
20. Iribarren C, Gross MD, Darbinian JA, Jacobs DR, 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;25:216-21.
21. Psaty BM, Manolio TA, Kuller LH, et al. Incidence of and risk factors for atrial fibrillation in older adults. *Circulation* 1997;96:2455-61.
22. Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol* 2002;156:871-81.
23. The ARIC investigators. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. *Am J Epidemiol* 1989;129:687-702.
24. Alonso A, Krijthe BP, Aspelund T, et al. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium