ARIC Manuscript Proposal #4256

PC Reviewed: 5/09/23	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title:
Mid-Life Plasmalogens and Other Metabolites with AntiInflammatory Properties are Inversely Associated with Long term Cardiovascular Disease Events.

b. Abbreviated Title (Length 26 characters): Anti inflammatory Metabolites and Cardiovascular Disease

2. Writing Group:

Anum Saeed
Jiaxuan Duan
Christopher McKennan
Kevin E. Kip
David Finegold
Michael Vu
Justin Swanson
Oscar Lopez
Ann Cohen
Mark Mapstone
Steven E. Reis
Christie Ballantyne
Bing Yu

(More authors to be added as needed)

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

First author
Anum Saeed, MD

Address:
200 Lothrop St, South Tower 3rd Floor, Pittsburgh, PA 15213
Phone: 2036061032 **E-mail:** saeeda2@upmc.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: Christie Ballantyne
Address: One Baylor Plaza, Houston, Texas Room 524DA, MS BCM 285,
Tel: 713-798-5034 E: cmb@bcm.edu

3. Timeline: 1-3 months

4. Rationale:

Atherosclerotic cardiovascular diseases (ASCVD) are a leading cause of mortality and morbidity worldwide (1). The process of atherosclerosis begins decades before clinical manifestations of ASCVD events (2,3). Therefore, identification of pathophysiologic and protective markers of atherosclerosis in mid-life is essential in primordial and primary prevention of ASCVD events in late-life (4,5). It is well known that inflammation-induced oxidative stress, dysregulated lipid metabolism and elevated remnant cholesterol contribute to the residual risk of cardiovascular disease (CVD). This residual risk is beyond what is captured by traditional lipid profiles (6) and clinically available risk assessment tools. The use of metabolomic analyses for comprehensive profiling of molecular markers in the lipid, amino acid and other pathways in systemic disease processes (7-9) provides an opportunity to identify the role of these metabolites in the development of ASCVD.

Preclinical data indicate that low levels of plasmalogens, a class of membrane glycerophospholipids, modulate cardiometabolic changes (10) which could lead to atherosclerosis. However, population level associations of mid-life plasmalogens and other metabolites with anti-inflammatory properties with incident ASCVD events are lacking.
In the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study, a community-based participatory research study of race-related disparities in ASCVD, we showed that mid-life levels of three plasmalogens, two amino acid metabolites, and a bilirubin degradation product, all of which have anti-inflammatory properties, are associated with lower risk of late-life ASCVD events (11). Secondary cohort validation of these findings are warranted.

The ARIC study, with its longitudinal data and complimentary metabolomic, biomarker and serologic data will be used as a validation cohort for these findings.

5. Main Hypothesis/Study Questions:

Primary: We hypothesize that a subset of plasmalogen metabolites (with anti inflammatory properties including *1-(1-enyl-palmitoyl)-2-arachidonoyl-glycerophosphatidylcholine [GPC]* (P-16:0/20:4). *1-(1-enyl-palmitoyl)-2-arachidonoyl-glycerophosphatidylethanolamine [GPE]* (P-16:0/20:4); and *1-methylnicotinamide1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)* in mid-life will be inversely associated with ASCVD events including coronary heart disease, stroke and global cardiovascular events (CHD, stroke and HF) over a longitudinal follow up time period of >10 years.

Secondary: We will assess amino acids pathway metabolites, specifically arginine, methionine, and cysteine sub pathways (*2-oxoarginine, alpha-ketobutyrate*) as well as bilirubin derived

metabolites (*C16H18N2O5*) with anti-inflammatory properties and their association with ASCVD events including coronary heart disease, stroke, and global cardiovascular events (CHD, stroke and HF) over a longitudinal follow up time period of >10 years.

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).

Metabolomic and serologic data from ARIC visit 1 will be used. These values will serve as the exposure variable and incident CHD, ischemic stroke and HF will be the outcomes. Follow up will be upto Demcember 31st 2019

Metabolites to be assessed:
1-(1-enyl-palmitoyl)-2-arachidonoyl-glycerophosphatidylcholine [GPC] (P-16:0/20:4).
1-(1-enyl-palmitoyl)-2-arachidonoyl-glycerophosphatidylethanolamine [GPE] (P-16:0/20:4);
1-methylnicotinamide1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4) and other plasmalogen and lipid pathway derivatives
2-oxoarginine,
alpha-ketobutyrate
C16H18N2O5 and other bilirubin derivatives

Endpoints to be assessed:

1. *Total/All CHD (fatal CHD, definite/probable MI, cardiovascular revascularization)*
2. *Hard CHD ((fatal CHD, definite/probable MI)*
3. *Stroke (ischemic/ thrombotic stroke)*
4. *HF hospitalization consists of definite and probable acute decompensated HF (as adjudicated in the ARIC study)*
5. *Global CVD (CHD + stroke+ heart failure)*
6. *CV mortality and total mortality*

Covariates will include age, gender, race, heart rate, body mass index (BMI), lipids, current smoking, diabetes, hypertension, systolic blood pressure, eGFR, prediabetes (HbA1c 5.7-≤6.6%), impaired fasting glucose (fasting glucose>100mg/dl), hs-CRP, IL-6.

Inclusion/ exclusion criteria:
All eligible ARIC participants will be included in the study.
Standard ARIC exclusions (race exclusions for different communities) will apply. The major exclusion criteria include participants without data on exposure, outcome, or covariates.

Analysis:

Participant characteristics will be reported as means and standard deviations, as medians and inter-quartile ranges (IQR), or as frequencies and percent, where appropriate. If lack of normality is not a concern and transformation is not required, then conventional statistics will be used. For non-normal data, transformations and/ or non-parametric testing will be used.

Identification of Cardiovascular Disease Metabolite Associations
Latent factors that might confound the relationship between metabolite levels and ASCVD events will be estimated using the method described by McKennan, et al. (12). For each metabolite, we will use logistic regression to regress ASCVD (yes/no) onto that metabolite's log-abundance while controlling for the baseline covariates in two models. First, we will use the "*Basic model*," including the variables of the Pooled Cohorts Equations (PCE)(age, sex, race, hypertension medication use, systolic BP, smoking status, diabetes mellitus, high density lipoprotein (HDL) cholesterol, total cholesterol) (13,14). We will then control for inflammatory markers (hs-CRP and IL-6) by using a second "*Complete model*," which included the covariates in the Basic model and log-concentrations of hs-CRP and IL-6. Both models were adjusted for latent factors. The Benjamini-Hochberg procedure (15) will be used to control the false discovery rate (FDR) at 10%.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes xxx____ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The 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 _xxx___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 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/mantrack/maintain/search/dtSearch.html

__xxxx____ 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)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __xx__ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.

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