ARIC Manuscript Proposal # 3604

PC Reviewed: 4/14/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of Interleukin-6 and Interleukin-18 levels in blood with Global Cardiovascular Disease in Older Adults: Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Proteomics of CV aging

2. Writing Group:(alphabetical):

Mahmoud Al Rifai
Christie M. Ballantyne
Leo Buckley
Joseph Coresh
Ivan Gorlov
Ron Hoogeveen
Xiaoming Jia
Vijay Nambi
Amil Shah
Olive Tang
Elizabeth Selvin
Carol Sun
Salim Virani
Bing Yu

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

First author:
Xiaoming Jia, MD
Baylor College of Medicine
Department of Medicine
One Baylor Plaza,
Houston TX 77030
Email: xiaoming.jia@bcm.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):
Christie M. Ballantyne, MD, FACP, FACC
Chief, Section of Cardiology
Chief, Section of Cardiovascular Research
Baylor College of Medicine
Department of Medicine
One Baylor Plaza, Room 524DA1, MS: BCM 285
Houston TX 77030
Email: cmb@bcm.edu

3. Timeline: Analysis is anticipated to begin as soon as approval is obtained. The manuscript is to be prepared as soon as analyses are available. The analysis and manuscript preparation is anticipated to take place within one year of approval of the proposal.

4. Rationale:

Inflammation is increasingly being recognized as a risk factor for cardiovascular disease (CVD). Signaling pathways of inflammation are multiple and complex[1]. We have seen in the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial that targeting interleukin-1β (IL-1β) resulted in cardiovascular risk reduction among secondary prevention patients with elevated high-sensitivity C-reactive protein (hs-CRP)[2]. Immediately downstream of IL-1β, the cytokine interleukin-6 (IL-6), which acts via both cell-bound and soluble IL-6 receptors (IL-6R), is an important mediator of both local and systemic inflammation[3,4]. Mendelian randomization studies have suggested that IL-6R mediated pathways have a causal relationship with coronary heart disease[5,6]. Meanwhile, serum IL-6 levels have also been associated with incident heart failure (HF) in patients without a history of HF as well as worse clinical outcomes in patients with established HF[7,8]. Though we now have a framework supporting the role of IL-6R mediated pathways and CVD, there are still many nuances that remain unclear. One such area is the intersection between inflammation and CVD in older age. Age related changes in plasma IL-6 concentrations have been observed, suggesting potential alterations in expression of inflammatory mediators with aging[9,10].

Another related cytokine of interest is interleukin-18 (IL-18). IL-18 is part of the IL-1β pathway but is instead a member of the same structural family as IL-1. Similar to IL-1, IL-18 is also activated inflammasomes, such as the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, and exerts pro-inflammatory effects downstream via binding to IL-18 receptor (IL-18R)[11]. In circulation, IL-18 exists in free form but also as a bound form with IL-18 binding protein (IL-18BP)[12]. In inflammatory conditions, both IL-18 and IL-18BP are often both elevated. Elevation in IL-18 has further been shown to be related to atherosclerotic plaque progression and vulnerability as well as increased risk for atherosclerotic CVD (ASCVD) events[13,14,15]. There is limited epidemiologic data of how IL-18 relates to HF.

To gain further biological insight into the relationship of IL-6 and IL-18 with CVD in older adults, we propose to assess for protein networks associated with IL-6/IL-6R and IL-18/IL-18BP/IL-18R and investigate how these proteins/networks may be related with CVD using the SomaLogic proteomics dataset from ARIC. This systems approach allows for the simultaneous evaluation of a vast number of serum proteins and has the potential to reveal important associations that may otherwise be overlooked using traditional methodology[16,17]. Ultimately, a better understanding of the proteins/networks associated with IL-6/IL-6R and IL-18/IL-18BP/IL-18R may help to identify additional therapeutic targets against inflammation and CVD as well as elucidate possible off-site effects of therapies targeting IL-6 and IL-18.

5. Main Hypothesis/Study Questions:

Hypothesis:

We hypothesize that there is a significant association between elevated IL-6/IL-6R and IL-18/IL-18BP/IL-18R with increased risk for global CVD (CHD, ischemic stroke and HF) in older adults. Furthermore, there exists significant protein networks associated with IL-6/IL-6R and IL-18/IL-18BP/IL-18R, and these networks are further associated with global CVD. However, protein networks of IL-6/IL-6R and IL-18/IL-18BP/IL-18R may have different associations with CHD, ischemic stroke and HF.

Study Aims:

The assessment of:

1. Association between IL-6/IL-6R and IL-18/IL-18BP/IL-18R in blood with global CVD and all-cause death in older adults.
2. Association between serum protein and protein networks with IL-6/IL-6R and IL-18/IL-18BP/IL-18R.
3. Association of protein networks of IL-6/IL-6R and IL-18/IL-18BP/IL-18R with global CVD and all-cause death.

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 methodological limitations or challenges if present).

*Note: This study will focus on ARIC visit 5 because heart failure adjudication was not done until after visit 4 and there is also echo data available at visit 5. However power is limited after visit 5 in regards to the total number of events, and therefore we may repeat the same approach using the Visit 2 or 3 Somalogics data depending upon availability.

Aim 1: Association between IL-6/IL-6R and IL-18/IL-18BP/IL-18R in blood with global CVD in older adults.

Study Design:

We will perform prospective analyses using ARIC visit 5 as the index visit. We will exclude participants without proteomics assay measurements, those of race other than white or African American, nonwhite participants at the Minneapolis and Washington County centers because of small numbers, and participants with prevalent CHD, stroke, or HF at index visit.

Exposure Variables:

IL-6 and IL-6R (IL-18/IL-18BP/IL-18R) measured in blood using the aptamer-based SomaLogic (SOMAscan v.4) proteomics assay. Variables will be treated as continuous

variable (variable will be log-transformed if not normally distributed) as well as categorical variable by quartiles.

Outcome Variables:
Incident events beyond ARIC visit 5: CHD, ischemic stroke, HF hospitalization, global CVD (composite of CHD, ischemic stroke and HF hospitalization), all-cause death.

Statistical Analysis:
Cox proportional hazard models assessing association between IL-6 and IL-6R levels (IL-18/IL-18BP/IL-18R) with incident CVD events.

Model 1 = adjust for age, sex and race-center
Model 2 = model 1 + total cholesterol, HDL-C, SBP, use of anti-hypertensive medication, current smoking, diabetes status
Model 3 = model 2 + BMI + eGFR + statin use

Sensitivity analysis: The above Cox regression analysis will be repeated with death as a competing risk for incident CVD events.

For IL-18, we will also perform tertile analysis assessing association of IL-18 and IL-18BP together with CVD outcomes.

Aim 2: Association between serum protein and protein networks with IL-6 and IL-6R (IL-18/IL-18BP/IL-18R).

Study Design:
Cross sectional analyses to assess for proteins/protein networks associated with IL-6 and IL-6R (IL-18/IL-18BP/IL-18R).

Exposure Variables:
Serum proteome (4,931 serum proteins) from SOMAscan v.4 obtained from ARIC visit 5 excluding IL-6 and IL-6R (IL-18/IL-18BP/IL-18R).

Outcome Variables:
IL-6 and IL-6R (or IL-18/IL-18BP/IL-18R) measured using from SOMAscan v.4 at ARIC visit 5, treated as continuous and categorical variables (highest tertile vs lowest 3 tertile).

Statistical Analysis:
We will perform linear regression (when interleukins are treated as continuous variable) or logistic regression (when interleukins are treated as categorical variable) to evaluate the association of each protein measured by SomaScan with IL-6 or IL-6R. P-values were adjusted for multiple testing using Benjamini–Hochberg False Discovery Rate (FDR) procedure. FDR <0.05 will be used as a threshold for statistical significance. We will adjust for model 3 co-variables (see Aim 1).

We will then use Ingenuity Pathway Analysis (IPA) (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) based on significantly associated proteins while using all proteins/associated genes in the study as a background/reference.

Finally, we will use an elastic net regularized regression model with 10-fold cross validation to identify significant proteins (selected ≥7/10 times) within the serum proteome based on model contribution to IL-6 and IL-6R (or IL-18/IL-18BP/IL-18R) levels. Elastic net regularization allows for handling of multicollinearity which is important for inference of distinct proteins/pathways that may be associated with IL-6/IL-6R.

Exploratory Analysis:
For IL-6, the single nucleotide polymorphism (SNP) Asp358Ala have been shown in Mendelian randomized studies to be causally associated with CHD[5]. We propose to assess association of the presence vs absence of Asp358Ala with each serum protein using linear regression analyses adjusted for model 3 (see above) variables. We will then use IPA analysis to assess for protein networks/pathways associated with the Asp358Ala polymorphism.

Aim 3: Prospective evaluation of proteins/pathways, IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) and global CVD.

Study Designs:
Prospective analyses assessing how proteins from the serum proteome relate to the association between IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) with global CVD.

Exposure Variables:
IL-6 and IL-6R (IL-18/IL-18BP/IL-18R) treated as continuous variable as well as categorical variable by quartiles.

Major Co-variables:
1) Proteins from the serum proteome selected by elastic net to be significantly associated with IL-6/IL-6R (IL-18/IL-18BP/IL-18R) levels (see Aim 2).
2) All serum proteome (4,931 serum proteins) from SOMAscan v.4 obtained from ARIC visit 5 excluding IL-6 and IL-6R (or IL-18/IL-18BP/IL-18R).

Outcome Variables:
Incident events beyond ARIC visit 5: CHD, ischemic stroke, HF hospitalization, global CVD (composite of CHD, ischemic stroke and HF hospitalization), all-cause death.

Statistical Analysis:

Cox proportional hazard models assessing association between IL-6 and IL-6R (or IL-18/IL-18BP/IL-18R) levels with incident CVD events adjusting for model 3 covariable (see above).

Assuming there is significant association between IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) with CVD based on the above Cox models, we will perform further adjustments with backward elimination using the list of candidate proteins derived from our elastic net. We will select for proteins that significantly attenuate the association between IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) with CVD, which are likely proteins that are within the same "pathway" as IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) as related to CVD.

We will then assess each protein from the elastic net analysis as a moderator or mediator of the association between IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) and CVD.

To test these proteins as moderators, we will perform tests for interaction based on $\geq$ vs < the median concentration for that protein.

For mediation analysis, we propose to use the natural effect model (Lange et al) to decompose total effect into direct effect and indirect effect via mediators[18,19,20]. Each protein will be treated as a categorical variable grouped by quartiles.

Limitations:

- IL-6/IL-6R and IL-18/IL-18BP/IL-18R are measured by aptamer-based assay which is not as well-validated as immunoassays. However, as the main intent of this study is to provide a systems approach to assessing proteins/networks associated with IL-6/IL-6R (or IL-18/IL-18BP/IL-18R) and CVD, we believe the use of this assay is appropriate.
- Residual confounding, survival bias.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X_ 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 __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

_____ 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)?

This proposal shares some overlap with regards to IL-18 with the ARIC ancillary study - Ancillary 2019.03 ("Interleukin-18, Cardiac Structure and Function, and Heart Failure in Older Adults". The writing groups have discussed and are working together.

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 *

___ 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.

References

1. Williams JW, Huang LH, Randolph GJ. Cytokine Circuits in Cardiovascular Disease. *Immunity.* 04 2019;50(4):941-954.
2. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med.* 09 2017;377(12):1119-1131.

3. Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci.* 2012;8(9):1237-1247.

4. Simon TG, Trejo MEP, McClelland R, et al. Circulating Interleukin-6 is a biomarker for coronary atherosclerosis in nonalcoholic fatty liver disease: Results from the Multi-Ethnic Study of Atherosclerosis. *Int J Cardiol.* 05 2018;259:198-204.

5. Sarwar N, Butterworth AS, Freitag DF, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet.* Mar 2012;379(9822):1205-1213.

6. Swerdlow DI, Holmes MV, Kuchenbaecker KB, et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet.* Mar 2012;379(9822):1214-1224.

7. Vasan RS, Sullivan LM, Roubenoff R, et al. Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. *Circulation.* Mar 2003;107(11):1486-1491.

8. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, et al. The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. *Eur J Heart Fail.* Aug 2019;21(8):965-973.

9. Daynes RA, Araneo BA, Ershler WB, Maloney C, Li GZ, Ryu SY. Altered regulation of IL-6 production with normal aging. Possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. *J Immunol.* Jun 1993;150(12):5219-5230.

10. Ng A, Tam WW, Zhang MW, et al. IL-1β, IL-6, TNF- α and CRP in Elderly Patients with Depression or Alzheimer's disease: Systematic Review and Meta-Analysis. *Sci Rep.* 08 2018;8(1):12050.

11. Yasuda K, Nakanishi K, Tsutsui H. Interleukin-18 in Health and Disease. *Int J Mol Sci.* Feb 2019;20(3).

12. Novick D, Schwartsburd B, Pinkus R, et al. A novel IL-18BP ELISA shows elevated serum IL-18BP in sepsis and extensive decrease of free IL-18. *Cytokine.* Jun 2001;14(6):334-342.

13. Blankenberg S, Luc G, Ducimetière P, et al. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation.* Nov 2003;108(20):2453-2459.

14. O'Brien LC, Mezzaroma E, Van Tassell BW, et al. Interleukin-18 as a therapeutic target in acute myocardial infarction and heart failure. *Mol Med.* Jun 2014;20:221-229.

15. Trøseid M, Seljeflot I, Hjerkinn EM, Arnesen H. Interleukin-18 is a strong predictor of cardiovascular events in elderly men with the metabolic syndrome: synergistic effect of inflammation and hyperglycemia. *Diabetes Care.* Mar 2009;32(3):486-492.

16. Ngo D, Sinha S, Shen D, et al. Aptamer-Based Proteomic Profiling Reveals Novel Candidate Biomarkers and Pathways in Cardiovascular Disease. *Circulation.* Jul 2016;134(4):270-285.

17. Robinson SW, Fernandes M, Husi H. Current advances in systems and integrative biology. *Comput Struct Biotechnol J.* Aug 2014;11(18):35-46.

18. Lange T, Vansteelandt S, Bekaert M. A simple unified approach for estimating natural direct and indirect effects. *Am J Epidemiol.* Aug 2012;176(3):190-195.

19. Lange T, Rasmussen M, Thygesen LC. Assessing natural direct and indirect effects through multiple pathways. *Am J Epidemiol.* Feb 2014;179(4):513-518.

20. Fritz J, Shiffman D, Melander O, Tada H, Ulmer H. Metabolic Mediators of the Effects of Family History and Genetic Risk Score on Coronary Heart Disease-Findings From the Malmö Diet and Cancer Study. *J Am Heart Assoc.* Mar 2017;6(3).