ARIC Manuscript Proposal #3988

PC Reviewed: 1/11/22 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1. **a. Full Title:**
Proteomic Markers of Aortic Stenosis Progression: the Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length: 26 characters): Aortic Stenosis Proteomics

2. Writing Group:
Writing group members: Khaled Shelbaya, Amil Shah, Bing Yu, Brian Claggett, Hicham Skali, Kunihiro Matsushita, Josef Coresh, Christie Ballantyne, Suma Konety, Kenneth Butler, Dalane Kitzman, OTHERS WELCOME.

I, the first author, confirm that all the co-authors have given their approval for this manuscript proposal. KS

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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:
We will begin analysis once the proposal is approved and anticipate manuscript completion in approximately 6 months following proposal approval.

4. **Rationale and Motivation:**

Aortic stenosis (AS) is the most common moderate to severe valvular heart disease in the U.S, and its prevalence increases with aging.(1) The prevalence of severe AS among persons > 75 years of age is approximately 3.4 %. The number of persons eligible for aortic valve intervention is therefore expected to increase as average life expectancy increases in Western countries.(2) Calcific aortic valve disease is the leading cause of AS in this context and is characterized by progression from leaflet thickening and calcification (aortic sclerosis) to significant hemodynamic stenosis.(3)

Common risk factors of atherosclerosis, including hypertension, diabetes, and dyslipidemia, are independently associated with severe AS in older populations.(4) However, no therapy currently exists to prevent the progression of aortic calcification and stenosis.(5) Several mechanistic pathways have been related to aortic calcification, including inflammation, neurohormonal activation, lipid oxidization, and biomineralization.(6) While biomarkers of LV wall stress (NT-proBNP) and injury (high sensitivity Troponin) are elevated in higher grades of AS (7) where they may be used to guide the intervention decisions,(8,9) little is known regarding other circulating biomarkers predictive of the development of hemodynamic valve stenosis. Such data may provide novel insights into mechanisms underlying AS and facilitate early prediction of persons at risk for the development of AS in late life.

We propose to leverage proteomic data available in mid- (Visit 3) and late-life (Visit 5) and serial echocardiographic assessments of aortic valve hemodynamics available in ARIC to identify circulating biomarkers predictive of hemodynamic valve stenosis and stenosis progression. We will also perform Mendelian randomization analyses on candidate proteins to identify novel proteins with potential causal associations with AS.

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

We hypothesize that individual proteins and protein networks will be associated with AS severity and its progression.

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):**

Study design:

This is a longitudinal analysis using data from ARIC Visits 3, 5, and 7. We will use aptamer-based proteomics measurements from Visits 3 and 5, and echocardiographic data on aortic valve hemodynamics available at Visits 5 and 7, and clinical covariates from all three study visits. First, we will identify proteins associated cross-sectionally with aortic valve hemodynamics at Visit 5. We will then identify the sub-set proteins associated with progressive changes in AV hemodynamics between Visits 5 and 7.

Inclusion/Exclusion criteria:
We will include ARIC participants who underwent echocardiographic assessment at Visit 5 and were free of mitral or aortic valve replacement. Participants with prior aortic valve replacement at Visit 5 will be excluded.

Key variables of interest:

1. ***Exposure:***

   Proteomic data from Visits 3 and 5: SOMAscan assay (Somalogic Inc., Boulder, CO).

2. ***Outcome:***

   Aortic valve parameters:
   Primary measures of interest: Aortic valve peak velocity, calculated aortic valve area (AVA), aortic valve mean gradient, and aortic valve dimensionless index.
   Additional measures: Aortic valve velocity time integral (VTI), LV outflow tract (LVOT) VTI, LVOT diameter, calculated stroke volume (SV) and stroke volume indexed to BSA (SVi), aortic regurgitation severity.

3. Co-variates:

- Clinical covariates Visit 5 and 7:
  Age, gender, race/ethnicity, body mass index (BMI), history of hypertension, diabetes, dyslipidemia, prior or current smoking, blood pressure, heart rate, eGFR, serum creatinine, hemoglobin, hematocrit, hemoglobin HbA1c, prevalent chronic kidney disease, coronary heart disease (prior myocardial infarction (MI) or revascularization procedure), atrial fibrillation, prior stroke, prevalent heart failure (HFpEF or HFrEF) and cardiac symptoms including dyspnea, angina, and exhaustion

- Echocardiographic covariates (Visits 5 and 7):

  Measures of cardiac structure and function: (1) left ventricular end-diastolic volume (LVEDV), end-systolic volume (LVESV), and mass (LVM); mean wall thickness (MWT), relative wall thickness (RWT); (2) LV diastolic function (E wave, A wave, E', E/e' ratio, left atrial volume index (LAVi), and pulmonary artery systolic pressure (PASP); (3) LV systolic function (Left ventricular ejection fraction (LVEF), global longitudinal strain, global circumferential strain)

- Cardiac biomarkers covariates (Visit 5): NT-proBNP, hs-cTnT, CRP.

Data Analysis:

Cross-sectional association with AV hemodynamic measures at Visit 5

Aim: To estimate the association of proteomics at Visit 5 with measures of AV hemodynamics (Vmax and AVA) at Visit 5.

Population: All participants who were free from previous valve replacement through Visit 5 and had available exposure measurements at Visit 5.

Exposures: Proteomic data from Visit 5: SOMAscan assay

Outcomes: AV Vmax and AVA at Visit 5, modeled as continuous variables. Secondary outcomes will include AV dimensionless index and mean gradient at Visit 5.

Analyses

- The association of proteins with Vmax and AVA will be estimated using multivariable linear regression with statistical significance defined using Bonferroni correction for multiple testing. We will sequentially adjust regression models for Visit 5 demographic characteristics (model 1: age, sex, race/ethnicity, heart rate and blood pressure of Visits 5), and clinical characteristics (model 2: coronary heart disease, HF, atrial fibrillation, diabetes mellitus, hypertension, and BMI). Additional sensitivity analyses will be performed excluding participants with abnormal LVEF and Visit 5.
- We will utilize LASSO regularized regression with an initial Bonferroni filter as additional analyses.
- For protein candidates associated with AV stenosis measures in cross-sectional analysis at Visit 5, association of their V3 levels with AV stenosis measures at V5 will also be assessed.

Association with progression in measures of AS severity from Visit 5 to Visit 7

Aim: To estimate the association of AS-related circulating proteins with changes in measures of AS severity between Visit 5 & 7 (ΔVmax and ΔAVA)

Population: All participants without previous valve replacement through Visit 7, proteomic data at Visit 5, and echocardiographic data at Visits 5 and 7.

Exposures: Candidate proteins associated with AS severity measures from the cross-sectional analysis above.

Outcomes: Changes in AV Vmax and AVA between Visit 5 and 7. Secondary outcomes will include changes in AV dimensionless index and mean gradient.

Analyses

- We will use linear regression models to estimate the association of AS-related proteins (derived as above) with changes in measures of AS severity modeled as a continuous variable. In addition, we will sequentially adjust regression models for Visit 5 demographic characteristics (model 1: age, sex, race/ethnicity, and heart rate and blood pressure at Visits 5 and 7), and clinical characteristics (model 2: coronary artery disease, HF, atrial fibrillation, diabetes mellitus, hypertension,

and, BMI). Additional sensitivity analyses will be performed excluding participants with abnormal LVEF and Visit 5 and/or Visit 7.
- Additional analyses will employ LASSO regularized regression with an initial Bonferroni filter.
- We will repeat analyses after excluding patients who experienced a cardiovascular event (MI, HF, stroke) between Visit 5 & 7 as sensitivity analysis.

For key candidate proteins, we will evaluate for non-linear associations using polynomial terms and restricted cubic splines. Given established differences in aortic stenosis prevalence by gender and race/ethnicity, we will also conduct separate analyses according to gender and race/ethnicity. For candidate proteins demonstrating consistent associations with AV measures cross-sectionally at Visit 5, and their change from Visit 5 to Visit 7, we will perform Mendelian randomization analyses to assess for possible causal associations with valve stenosis. (10)

Anticipated methodologic limitations.

- Survivor and attendance bias between Visit 5 and Visit 7 may lead to underestimation of changes in AV measures between visits, which may limit our power to detect associations with protein measures. We will perform sensitivity analyses using the inverse probability of attrition weights to avoid the non-random attendance bias, which may direct our result toward the null. We will use clinical characteristics at visit 5 to estimate the probability weights.
- Peak transvalvular velocity and pressure gradient are flow (stroke volume) dependent. We will there assess associations with peak velocity, but also with with AVA and dimensionless index which account for transvalvular flow.
- The relation between AV Vmax and proposed proteomics may be non-linear. We will evaluate the non-linear associations in our analysis.
- Absolute quantification of protein biomarkers is not available.

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

8. a. Will the DNA data be used in this manuscript? ___ Yes __ X __ No

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 inactive status.** ARIC Investigators have access to the publications lists under the Study Members Area of the website 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)?**

    ARIC Manuscript Proposal # 3632 The Association Between Lipid Profiles and the Incidence of Aortic Stenosis in Older Adults: Atherosclerosis Risk in Communities Study

    ARIC Manuscript Proposal #3644 Prevalence and predictors of changes in aortic valve hemodynamics in late life: Atherosclerosis Risk in Communities (ARIC) Study

    ARIC Manuscript Proposal #3784 The association of aortic and mitral valve calcification with echocardiographic valvular parameters: The Atherosclerotic Risk in Community (ARIC) Study

    ARIC Manuscript Proposal #2739 Valvular heart disease and cardiac remodeling, damage, and overload in older adults

    ARIC Manuscript Proposal #3389 Proteomic Profiling and Heart Failure Risk in the Atherosclerosis Risk in Communities (ARIC) Study

    ARIC Manuscript Proposal #3795 Proteomic Measures Linking Kidney Disease to Heart Failure and Adverse Cardiac Remodeling in Late-Life: the Atherosclerosis Risk in Communities Study

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

    b. If yes, is the proposal _X__ A. primarily the result of an ancillary study (list number* _2015.34; 2017.27, 2018.19_) ___ 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/

12. **a. 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.**

    b. 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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