ARIC Manuscript Proposal #4013

PC Reviewed: 3/8/22 **Status: _____** **Priority: 2**

SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Iron metabolism, incident heart failure and adverse cardiac remodeling in community-dwelling adults: the Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Iron and heart failure

2. Writing Group:

Writing group members: Iman Aboelsaad, Leo Buckley, Amil Shah, Youssef Farag, Bing Yu, Joseph Coresh, Kunihiro Matsushita, Thomas Mosley, Pamela Lutsey, Lynne Wagenknecht, Brian Claggett, Pranav Dorbala, Victoria Arthur

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

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3. Timeline:

We will begin statistical analysis after manuscript proposal approval. We expect to complete analysis within 3-4 months of the manuscript approval date. We anticipate submitting an abstract for presentation at the American Heart Association Scientific Sessions in June 2022, and the manuscript for publication in August 2022.

4. Rationale

An estimated 4.5% to 18% of Americans of all ages and sexes have iron deficiency (without anemia), with the highest prevalence among children and women of childbearing age.[1,2] The prevalence of iron deficiency, however, begins to rise in mid-life, affecting 5% of women between the ages of 50-69 years, 7% of women 70 years or older, 2% of men ages 50-69 years and 4% of men aged 70 years or older.[2]

Iron plays an essential role in oxygen transport, mitochondrial respiration and free radical protection in cells with high energy requirements, such as cardiomyocytes.[3] Pre-clinical research indicates that disordered iron metabolism impairs cardiac function independent of anemia.[4-7] In patients who have established heart failure, iron deficiency is associated with a higher risk of heart failure hospitalization and impaired functional capacity.[8] Intravenous iron repletion can reduce the risk of heart failure hospitalization and improve functional capacity in patients with heart failure with reduced or mid-range ejection fraction.[9-12]

Disordered iron metabolism also may contribute to an increased risk of incident heart failure. In a nested cohort of 1063 ARIC Visit 1 participants who were free from prevalent heart failure, both low (<30 ng/mL) and high (>200 ng/mL in women and >300 ng/mL in men) ferritin concentrations associated with an increased risk of incident heart failure compared to normal levels.[13] In the community-based PREVEND cohort, higher ferritin and hepcidin levels associated with an increased risk of incident heart failure in women, but not men.[14]

Important questions about the contributions of disordered iron metabolism to the development of incident heart failure remain unanswered. The only available data on the association of iron metabolism biomarkers with incident heart failure with reduced and preserved ejection fraction comes from the aforementioned PREVEND study, which analyzed only 79 incident heart failure cases with available ejection fraction within the female subgroup and none within the male subgroup.[14] Limited data exist regarding the associations of iron metabolism biomarkers with cardiac structure and function from large, observational cohorts. Although serum ferritin and transferrin saturation are widely used for the diagnosis of iron deficiency or iron overload, alternative biomarkers have stronger correlations with bone marrow iron stores, the gold-standard measurement.[15-17] Among such biomarkers, the SomaScan Platform captures hepcidin, which regulates expression of ferroportin, a transmembrane protein responsible for intestinal iron absorption and extracellular iron release, and the transferrin receptors-1 and -2 (TfR), which facilitate intracellular iron uptake. Evaluating the associations between these biomarkers of iron metabolism and heart failure and cardiac structure and function may provide insight into the pathophysiology of iron-related cardiac

dysfunction, potential therapeutic targets and candidate biomarkers for diagnostic and therapeutic monitoring.

We propose to estimate the association between circulating iron biomarkers and incident heart failure and cardiac structure and function in community-dwelling adults. Based upon the differential prevalence and mechanisms of iron deficiency in mid-life and late-life, we will assess associations with incident heart failure at ARIC Visit 2 and Visit 5. We will also assess effect modification by birth sex and anemia status.

5. Specific Aims:

Specific Aim 1: Determine the relationship between plasma ferritin, hepcidin and soluble transferrin receptors levels and incident heart failure in mid- and late-life. We hypothesize that lower ferritin levels, higher hepcidin levels and higher soluble transferrin receptor levels associate with an increased risk of incident heart failure.

Specific Aim 2: Determine the relationship between plasma ferritin, hepcidin and soluble transferrin receptors levels and cardiac structure and function. We hypothesize that lower ferritin levels, higher hepcidin levels and higher soluble transferrin receptor levels associate with impaired systolic and diastolic function in late-life.

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).

Aim 1

- Population:
    - For analysis of post-Visit 2 HF, we will include all participants who were free from HF through Visit 2 and had the required iron biomarkers measurements at Visit 2
    - For analysis of post-Visit 5 HF, we will include all participants who were free from HF through Visit 5 and had the iron biomarker measurements at Visit 5.
- Exposures:
    - Plasma measurements of ferritin (FTH1|FTL, UniProt P02794|P02792), hepcidin (HAMP, UniProt P81172), transferrin receptor 1 (TFRC, UniProt P02786), transferrin receptor 2 (TFR2, UniProt Q9UP52) and transferrin (TF, UniProt P02787) measured using the SomaLogic platform
    - The ratio of ferritin to transferrin, as a surrogate of transferrin saturation
- Outcomes:
    - Incident heart failure post-Visit 2 (truncating follow-up at 15-years to avoid overlap with Visit 5)
    - Incident heart failure post-Visit 5

    - Incident HFrEF (<50%) and HFpEF (≥50%) post-Visit 5
    - Incident heart failure or all-cause death after Visit 2 and Visit 5
- Covariates:
    - Model 1 will adjust for age, sex, and the interaction between race and field center
    - Model 2 will adjust for Model 1 covariates plus diabetes mellitus, hypertension, smoking, BMI, eGFR, coronary artery disease and atrial fibrillation
- Analysis:
    - Exposures will be log2-transformed and centered with a mean of 0 and standard deviation of 1 prior to analysis. We will use Cox proportional hazards regression models to estimate the association of each exposure to the specified incident heart failure outcomes. We will use restricted cubic splines to assess for non-linear associations. For analysis of incident HFpEF, we will censor patients at the time of incident HFrEF or HF with unknown LVEF. For analysis of incident HFrEF, we will censor patients at the time of incident HFpEF or HF with unknown LVEF
    - We will assess effect modification by birth sex and anemia status (defined as Hgb < 13 g/dL for men and 12 g/dL for women)

Aim 2:

- Population:
    - For cross-sectional associations with echocardiographic measures at Visit 5, we will include participants free of HF through Visit 5 with available echocardiography measures at Visit 5 and iron biomarkers measurements at Visit 5
    - For association with change in echocardiographic measures from Visit 5 to Visit 7, we will include participants also undergoing echocardiography at Visit 7.
- Exposures:
    - Same as for Aim 1 above.
- Outcomes:
    - Echocardiographic measures of cardiac structure and function (1) at Visit 5
    - Changes in those same echocardiographic measures from Visits 5 to 7.
    - Primary measures of interest will be those reflecting LV structure (left ventricular wall thickness, dimensions, mass, RWT), LV diastolic function (E wave, e', E/e', LA diameter, LA volume index), left ventricular systolic function (LVEF, longitudinal strain, circumferential strain), PASP, RV measures (area, fractional area change, tricuspid annular s')
- Covariates:
    - Model 1 and Model 2 covariates will be similar to those previously specified for Aim 1 above. In addition, we will adjust all models for systolic blood pressure and heart rate at Visit 5. For Models with change in echocardiographic measures of cardiac structure and function as an outcome, we will also adjust for systolic blood pressure and heart rate at Visit 7.
- Analysis:
    - Multivariable linear regression models will be used to assess for linear associations between the exposures and outcomes of interest. Restricted cubic splines will be used to consider non-linear associations.

- We will assess effect modification by birth sex and anemia status (defined as Hgb < 13 g/dL for men and 12 g/dL for women)

Detailed Statistical Analysis Plan:

Continuous variables will be summarized using means and standard deviations, medians and (25^{th} and 75^{th} percentiles), and categorical data as numbers and percentages.

We will estimate the association of iron biomarker levels to incident heart failure using Cox proportional hazards regression models as described above in Aim 1. We will estimate associations between iron biomarker levels and echocardiographic measures using linear regression as described above in Aim 2.

We will perform sensitivity analyses using inverse probability of attrition weights to account for non-random Visit 7 attendance. For Visit 2 and 5, we assess effect modification by birth sex and anemia status

There are certain limitations to this analysis:

1. The number of classifiable HFpEF and HFrEF events will be reduced due to missing LVEF data from time of incident heart failure hospitalization. We will conduct sensitivity analysis by imputing all incident HF cases with missing LVEF as either HFpEF or HFrEF and re-evaluating our models.
2. Under-reporting of HFpEF cases or misclassification of other conditions as HFpEF is possible since diagnosing this condition is challenging. It is unlikely that the number of misclassified cases will affect our estimates since independent committee has adjudicated all events according to standardized definitions. However, underreporting of HFpEF cases may still bias estimates towards the null.
3. Survivor bias and attendance bias resulting from non-random non-attendance at Visit 7 may bias the findings of our echocardiographic analyses towards the null and underestimate any true associations. We will adjust for non-random non-attendance at Visit 7 using inverse probability of attrition weights as described above.
4. Serum iron and total iron binding capacity were not measured in ARIC
5. Absolute quantification of protein biomarkers is not available. The Appendix provides comparisons of Somalogic with Olink data and availability of cis-pQTL.

Appendix: cis-pQTL and Olink comparisons with Somalogic

Protein	cis-pQTL	Olink Comparison
Ferritin	Yes (Ferkingstad, 2021 Nature Genetics)	Not measured
Hepcidin	Yes (Ferkingstad, 2021 Nature Genetics)	Not measured
Transferrin receptor 1	Yes (Ferkingstad, 2021 Nature Genetics)	Not measured
Transferrin receptor 2	No (trans only)	Not measured

Transferrin	Yes (Ferkingstad, 2021 Nature Genetics)	SeqId_8795_48: r = 0.72 (0.68-0.77); P<.001; SeqId_6895_1: r = 0.43 (0.35-0.50); P<.001

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/mantrack/maintain/search/dtSearch.html

___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)?

MPF #2481: Silvestre OM, Goncalvez A, Nadruz W, Claggett B, Couper D, Eckfeldt JH, Pankow JS, Anker SD, Solomon SD. Ferritin levels and risk of heart failure-the Atherosclerosis Risk in Communities study. Eur J Heart Fail. 2017;19:340-347.

MPF #2772: Selvaraj S, Seidelmann S, Silvestre OM, Claggett B, Ndumele CE, Cheng S, Yu B, Fernandes-Silva MM, Grove ML, Boerwinkle E, Shah AM, Solomon SD. HFE H63D Polymorphism and the

Risk for Systemic Hypertension, Myocardial Remodeling, and Adverse Cardiovascular Events in the ARIC Study. Hypertension 2019;73:68-74.

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

11.b. If yes, is the proposal

_X__ A. primarily the result of an ancillary study (list number* 2015.34; 2017.27)

___ 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 https://www2.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.
The writing group agrees to complete the manuscript within this timeframe.

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.

The writing group agrees to upload the manuscript to PubMed Central.

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