1.a. Full Title: Comparative proteomic signatures of high sensitivity cardiac troponin T and high sensitivity cardiac troponin I

b. Abbreviated Title (Length 26 characters): Proteomics of cardiac troponins

2. Writing Group:
   Writing group members: Olive Tang; Josef Coresh; Jingsha Chen; Adrienne Tin; Kuni Matsushita; Eric Boerwinkle; Bing Yu; Xiaoming Jia; Ron Hoogeveen; James Pankow; Christie Ballantyne; Elizabeth Selvin; others welcome

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]

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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:
The SOMAScan proteomics data from Visit 5 are available. We aim to complete the manuscript <1 year from the time of approval.

4. Rationale:
   Cardiac troponin T and I are functional components of the cardiomyocyte myosin-actin complex released into circulation with cellular injury. In the setting of cardiac ischemia, both
troponins undergo a biphasic release with the initial peak corresponding to release of free cytosolic troponin and the second corresponding to degradation of the troponin complex. Given the biologic similarity between the two, they are used interchangeably in the diagnosis of acute myocardial infarctions.

In the general population, subclinical levels of hs-cTnT and hs-cTnI have been associated with subsequent risk of cardiovascular and mortality outcomes. The growing availability of high sensitivity assays for cardiac troponin T (hs-cTnT) and cardiac troponin I (hs-cTnI) has allowed for the evaluation of the implications of hs-cTnT and hs-cTnI concentrations below those diagnostic of myocardial infarctions. While the diagnostic thresholds for myocardial infarctions have focused on levels above the 99th percentile in the healthy population, values even below the lower limit of detection appear prognostic of future cardiovascular risk.

Few studies have simultaneous measurements of both biomarkers; thus, work directly comparing hs-cTnT and hs-cTnI are very limited. In the Generation Scotland Scottish Family Health Study and in ARIC, there was only moderate correlation between the two troponins. Elevations in both troponins appear indicative of greater risk of cardiovascular outcomes as compared to isolated elevations in either marker. This suggests that, in addition to their shared etiology, there may be independent drivers of either circulating cardiac troponin T or cardiac troponin I.

There are currently no therapeutics targeting circulating cardiac troponins; in fact, biologically meaningful reduction in hs-cTnT or hs-cTnI may require addressing underlying causes of their release or accumulation. For example, analysis of certain statin trials have shown statin-associated reductions in troponin, which independently predict risk reductions even beyond LDL-cholesterol lowering. This suggests there may be meaningful biology beyond known traditional cardiovascular risk factors driving elevations in cardiac troponins. Therefore, greater understanding of this underlying biology will be instrumental in developing interventions for high risk populations with elevated cardiac troponins.

Proteomic libraries allow for a snapshot of an individual’s biologic state and has been used to identify pathways and markers associated with cardiovascular outcomes. Using nucleotide-based aptamers, the newest SOMAScan platform is able to detect ~5,000 circulating proteins, which are normalized and reported in relative units. This newly available SOMAScan proteomics data in ARIC opens the possibility of furthering the biological understanding of the joint and independent drivers of circulating hs-cTnT and hs-cTnI and identifying potential therapeutic targets for intervention beyond traditional risk factors.

5. Main Hypothesis/Study Questions:
1. What are the proteomic signatures of hs-cTnT and hs-cTnI?
2. What is the proteomic signature of joint elevations in hs-cTnT and hs-cTnI?
3. What are the proteomic signatures of elevations in hs-cTnT independent of hs-cTnI and vice versa?

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: Cross-sectional analysis at visit 5, among participants with simultaneous measurements of hs-cTnT, hs-cTnI, and SOMAScan proteomics.
**Exposures:** SOMAScan proteins (~5,000 proteins measured using SOMAScan’s newest aptamer-based proteomics platform)

**Outcomes**
1) Roche hs-cTnT
2) Abbott hs-cTnI
3) Joint elevations in Roche hs-cTnT and Abbott hs-cTnI
   a. Elevated: Top tertile of Roche hs-cTnT and Abbott hs-cTnI
   b. Reference: Lowest tertile of Roche hs-cTnT and Abbott hs-cTnI
4) Elevated Roche hs-cTnT beyond Abbott hs-cTnI
   a. Residuals greater than the 80th percentile from linear regression of log-transformed hs-cTnT (dependent variable) and log-transformed hs-cTnI (independent variable)
5) Elevated Abbott hs-cTnI beyond Roche hs-cTnT
   a. Residuals less than the 20th percentile from linear regression of log-transformed hs-cTnT (dependent variable) and log-transformed hs-cTnI (independent variable)

**Covariates:** age, sex, race-center, prevalent cardiovascular disease, total cholesterol, LDL-C, HDL-C, SBP, DBP, hypertension medication use, current smoking status, estimated GFR, diabetes status, BMI

**Exclusions:**
- standard ARIC race-center exclusions
- missing either hs-cTnT or hs-cTnI at visit 5
- Participants missing covariate data
- Sex-mismatched SOMALogic samples
- Non-human proteins detected on the SOMAScan due to cross-reactivity of aptamers

**Statistical Analysis:**

**Regression analyses**
1) Linear regression for continuous outcomes (#1, #2)
2) Logistic regression for binary outcomes (#3, #4, #5)
3) Regularized regression using Elastic Net

**Pathway analysis**
1) Assess the association of known cardiovascular biomarkers such as NT-proBNP, GDF15, CRP, etc. with cardiac troponin outcomes
2) Qiagen Ingenuity Pathway Analysis Program with Causal Pathway Analysis

**Significance:** Proteome-wide significance will be defined based on a Bonferroni-corrected p-value of 9.46e-6.

**Sensitivity analyses:**
- Analytic population: We will conduct a sensitivity analysis among the population
- without prevalent atherosclerotic cardiovascular disease (prevalent coronary heart disease or stroke)
- without prevalent atherosclerotic cardiovascular disease (prevalent coronary heart disease or stroke) or stage 3+ chronic kidney disease (eGFR-cr-cys<60mL/min per 1.73m²)
- SOMAScan flagged proteins: Some proteins have been flagged by SOMALogic based on its analytic performance on a given assay plate. We will conduct sensitivity analyses including proteins with differing levels of “allowable” flagging by the SOMAScan system.

Limitations:
1) Cross-sectional analysis – inability to establish temporality between troponin measurements and proteomic signatures
2) These findings would benefit from external validation for confirmation.

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)?

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
A. primarily the result of an ancillary study (list number* __AS2017.27 ____)
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.

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:


