ARIC Manuscript Proposal #4214

PC Reviewed: 3/18/23	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of Plasma Coagulation Factor XI Levels with Cardiovascular Outcomes and Cardiac Structure and Function

b. Abbreviated Title (Length 26 characters): **Factor XI and cardiovascular events**

2. Writing Group:

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*,† contributed equally to the paper

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

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3. Timeline: Data analysis: 2 months
Manuscript preparation: 6 month(s)
Anticipated first draft: Summer 2023

4. Rationale:

Coagulation factor XI (FXI) inhibition is a promising and novel class of therapeutics for thromboembolism prevention and anticoagulation.[1] High plasma FXI levels are associated with a higher risk of ischemic stroke and venous thromboembolism,[2-5] but people with genetic FXI deficiency rarely experience spontaneous bleeding.[6] Therefore targeting this coagulation factor may decrease the risk of stroke and venous thromboembolism without increasing the risk of hemorrhage.[7]

Apart from its role in coagulation, a recent mechanistic animal study has identified a novel role for FXI as a protective anti-fibrotic factor in heart failure with preserved ejection fraction (HFpEF).[8] The investigators found that overexpression of FXI ameliorated heart failure, reduced diastolic dysfunction and decreased fibrosis in a murine model of HFpEF. They also found that higher plasma FXI levels were correlated with a lower E/e' ratio (better diastolic function) in patients with HFpEF. However, the investigators were unable to find a significant difference in plasma FXI levels between patients with and without HFpEF in a small clinical study (n=40). Given the positive findings from animal experiment, the non-significant clinical data was most likely the result of a limited sample size.

In ARIC, plasma FXI levels were measured at Visit 5 as part of the Somalogic proteomics platform. Participants also concurrently underwent a comprehensive echocardiographic examination. Therefore, the aim of this proposed study is to examine the association of plasma FXI levels with incident HF and AF in ARIC. In addition, we will examine the association of plasma FXI levels with measures of cardiac structure and function cross-sectionally at Visit 5 and change in these measures from Visit 5 to Visit 7. Finally, we propose validating the causal relationship between plasma FXI levels and cardiovascular events and cardiac structure and function using Mendelian randomization analysis.

5. Main Hypothesis/Study Questions:

Aim 1: Examine the association of plasma FXI levels with incident cardiovascular events.

Hypothesis 1: Lower plasma FXI levels are associated with a higher risk of atrial fibrillation (AF) and heart failure (HF).

Aim 2A: Examine the cross-sectional association of plasma FXI levels with measures of cardiac structure and function at Visit 5

Hypothesis 2A: Lower plasma FXI levels are associated with lower LA reservoir strain, lower LA contractile strain, lower LA conduit strain, larger LA volume index, higher E/e' ratio, higher E/A ratio, and lower LV global longitudinal strain.

Aim 2B: Examine the association of plasma FXI levels with change in measures of cardiac structure and function from Visit 5 to Visit 7.

Hypothesis 2B: Lower plasma FXI levels are associated with a greater increase in LA volume index, E/e' ratio, E/A ratio, and greater decrease in LV global longitudinal strain.

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:
Aim 1: Prospective observational analysis for the association between plasma FXI levels (from visit 5) and incident AF and HF until 2019.

Aim 2: Cross-sectional analysis for the association between FXI plasma levels and diastolic function at ARIC Visit 5. Prospective observational analysis for the association between plasma FXI levels and diastolic function change from ARIC Visit 5 to Visit 7.

Study population:

1) Aim 1:
    a. Inclusion criteria: ARIC participants attending Visit 5 with available plasma FXI data
    b. Exclusion criteria: participants with prevalent HF or AF, missing covariates, anticoagulant use, race other than Black or White, Black participants in Minneapolis or Washington County field centers.
2) Aim 2A:
    a. Inclusion criteria: ARIC participants attending Visit 5 with plasma FXI and echocardiographic data.
    b. Exclusion criteria: missing covariates, anticoagulant use, race other than Black or White, Black participants in Minneapolis or Washington County field centers.
3) Aim 2B:
    a. Inclusion criteria: ARIC participants attending Visit 5 and Visit 7, with available plasma FXI data at visit 5 and echocardiographic data at Visit 5 and Visit 7.
    b. Exclusion criteria: missing covariates, anticoagulant use, race other than Black or White, Black participants in Minneapolis or Washington County field centers.

Variables:

Exposure: Plasma FXI levels at Visit 5 as measured by SOMAscan at Visit 5 (in relative fluorescence unit). Log_2 transformed values of plasma FXI levels will be analyzed as a continuous exposure, we will consider categorical FXI levels if significant non-linear associations are found.

Outcomes:

1) Cardiovascular events
    a. Incident AF
    b. Incident HF
        i. Incident heart failure with reduced ejection fraction (HFrEF)
        ii. Incident heart failure with preserved ejection fraction (HFpEF)

2) Cardiac function measures
   A: Diastolic and systolic function at visit 5 (and systolic function for comparison)

a. E/e' ratio
b. E/A ratio
c. LA maximal volume index (aka LA volume index)
d. LA function (LA reservoir strain, conduit strain, contractile strain)
e. LV mass index
f. LV ejection function
g. LV global longitudinal strain
h. Pulmonary artery systolic pressure

B: Diastolic and systolic function from visit 5 to visit 7(and systolic function for comparison)

a. E/e' ratio
b. E/A ratio
c. LA maximal volume index (aka LA volume index)
d. LV mass index
e. LV ejection function
f. LV global longitudinal strain
g. Pulmonary artery systolic pressure

Covariates: age, sex, race/center, body mass index (BMI), height, weight, cigarette smoking status, alcohol intake, systolic and diastolic blood pressure, estimated glomerular filtration rate ($eGFR_{cr\text{-}cys}$, creatine-cystatin using 2021 CKD epi equation), total and high density lipoprotein (HDL) cholesterol, prevalent diabetes, prevalent coronary heart disease, anti-hypertensive medication use, antiplatelet use, anticoagulant use.

Statistical analysis:

1) Aim 1: Prospective observational analysis of plasma FXI levels and cardiac events
Unadjusted disease event trends will be presented by Kaplan-Meier curve. We will use Cox regression to estimate the hazard ratio of plasma FXI levels to AF, and HF (further stratified into HFpEF and HFrEF). Follow up time will be set from Visit 5 (plasma sample measuring time) until 2019.

For the AF outcome, we will apply the following models:

- Model 1: Adjusted for demographic variables as age, sex, race/center.
- Model 2: Model 1 further adjusted for height, weight, systolic and diastolic blood pressure, smoking and alcohol status, prevalent diabetes, prevalent heart failure, prevalent coronary heart disease, anti-hypertensive medication use
- Model 3: Model 2 further adjusted for $eGFR_{cr\text{-}cys}$,
- Model 4: Model 3 further adjusted for antithrombotic medication (antiplatelet + anticoagulation)

For the HF outcome, we will apply the following models:

- Model 1: Adjusted for demographic variables as age, sex, race/center.
- Model 2: Model 1 further adjusted for BMI, systolic blood pressure, smoking and alcohol status, total and HDL cholesterol, prevalent diabetes, prevalent coronary heart disease, anti-hypertensive medication use

- Model 3: Model 2 further adjusted for $eGFR_{cr\text{-}cys}$,
- Model 4: Model 3 further adjusted for antithrombotic medication (antiplatelet + anticoagulation)

2) Aim 2A: Cross-sectional analysis of plasma FXI levels and diastolic function.
   We will use linear regression to relate plasma FXI levels continuously with echocardiographic measures. All covariates in the statistical model will be obtained from ARIC Visit 5. For all outcomes, we will apply the following models:
   - Model 1: Adjusted for demographic variables as age, sex, race/center
   - Model 2: Model 1 further adjusted for BMI, systolic blood pressure, smoking and alcohol status, $eGFR_{cr\text{-}cys}$, prevalent diabetes, anti-hypertensive medication use
   - Model 3: Model 2 further adjusted for antithrombotic medication (antiplatelet + anticoagulation)

3) Aim 2B: Prospective observational analysis of plasma FXI levels and changes in diastolic function.
   We will use linear regression or generalized estimating equations to relate plasma FXI levels continuously with changes in echocardiographic measures from visit 5 to visit 7. All covariates in the statistical model will be obtained from ARIC Visit 5. For all outcomes, we will apply the following models:
   - Model 1: Adjusted for demographic variables as age, sex, race/center.
   - Model 2: Model 1 further adjusted for BMI, systolic blood pressure, smoking and alcohol status, $eGFR_{cr\text{-}cys}$, prevalent diabetes, anti-hypertensive medication use
   - Model 3: Model 2 further adjusted for antithrombotic medication (antiplatelet + anticoagulation)

4) Sensitivity analyses
   a) For Aim 1, we will exclude participants with incident AF in the analysis of incident HF and vice versa (exclude incident HF in analysis of incident AF).
   b) For Aim 1, we will use a Fine-Gray model to adjust for the competing risk of incident AF in the analysis of incident HF and vice versa (competing risk of incident HF in analysis of incident AF).
   c) For Aim 2A and B, we will perform inverse probability of attrition weighting and multiple imputation to account for missing data and attrition of participants between Visit 5 and Visit 7.[9,10] For multiple imputation, pooled estimates of 10 iterations of imputation will be reported.
   d) For Aim 2B, we will further adjust for baseline (visit 5) diastolic function parameters. As the direction and size of changes in diastolic function may be different in different levels of baseline diastolic function.
   e) We will explore adjusting for FVII levels as a Model 4, because FVII interacts with FXI during coagulation,[11] it is also associated with adverse cardiovascular outcomes.[12]
   f) We will also explore age, sex, and race interactions.

5) Validation analysis

We will seek to replicate both aims in the Cardiovascular Health Study (CHS). Plasma FXI levels by sandwich ELISA (orthogonal platform) were obtained in 1992-1993 and measured in 2014.[13] Echocardiograms were obtained in 1994-1995.[14]

6) Mendelian Randomization analysis
Although the Cao *et al* study mechanistically demonstrated the causal relationship between plasma FXI levels and cardiac function and heart failure, the study was largely confined to a murine platform. We will leverage the availability of large human datasets for plasma FXI protein quantitative trait loci (pQTL) and AF and HF genome-wide association studies to examine causal relationships between plasma FXI and AF and HF in humans. We will obtain genetic variants for MR from the deCODE study plasma FXI pQTL,[15] AFGen consortium for AF,[16] and HERMES consortium for HF.[17]

7) Limitations: Our major limitation is the measurement of plasma FXI by Somalogic. The FXI levels from this platform has not been fully validated against established ELISA methods. However, the measurement of plasma FXI levels at ARIC Visit 5 has good CoV and Pearson correlation (7.11 and 0.85, respectively). We will also attempt to validate our findings in CHS.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes __X__ 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 __X__ 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

_____ Yes ___X____ 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)?

No other related manuscript proposals

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* _2012.04, 2014.18, 2015.13, 2015.29, 2015.34, 2017.14, 2017.27, 2022.03_____)

___ 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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