

ARIC Manuscript Proposal # 3673

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1.a.Full Title: The Role of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Matrix Metalloproteinases (TIMPs) in Predicting Cardiovascular Outcomes and Cardiac Dysfunction: Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title: MMPs and Heart Failure

2. Writing Group:

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

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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. Both the analysis and manuscript preparation are anticipated to take place within one year of approval of the proposal.

4. Rationale:

Matrix metalloproteinases (MMPs) are responsible for the remodeling of the extracellular matrix (ECM) of the myocardium, which plays a significant role in the myocardial response to stress. Changes in the collagen network surrounding the myocardium can influence the geometry of the left ventricle (LV) [1]. Alterations in the

levels of MMPs and tissue inhibitors of metalloproteinase (TIMPs) have been observed in the setting of heart failure (HF) and cardiac remodeling after myocardial infarction (MI) [1, 2]. Furthermore, genetic mutations which affect MMP levels have been associated with the progression of atherosclerotic cardiovascular disease (ASCVD) [3, 4].

There are many types of MMPs, including collagenases, gelatinases, and membranous type MMPs [5]. Of note, The SOMALogic® SOMAscan assay is an aptamer-based technology that can quantify the levels of thousands of proteins in the serum and was used to quantify the levels of many MMPs and TIMPs of ARIC visit 5 participants. In particular, the assays for MMP-1 and TIMP-1 demonstrated a strong correlation with ELISA-based assays, and the assay for MMP-7 demonstrated a strong correlation with Olink® assay.

There is a great deal of evidence demonstrating that alterations in expression of the aforementioned MMPs and TIMPs is associated with cardiac dysfunction. For example, one study demonstrated that patients with deteriorating heart failure (i.e. heart failure requiring left ventricular assist device (LVAD) placement) have increased expression of both TIMP1 and MMP1 mRNA [6]. Another study revealed that in the days following an acute MI, both MMP-1 and TIMP-1 demonstrate a time-dependent alteration in expression, and fluctuations in levels of MMP-1 and TIMP-1 may lead to cardiac remodeling [7]. This suggests that the ratio of these two counterregulatory proteins at any specific point in time may influence the subsequent development/progression of left ventricular hypertrophy (LVH).

With respect to MMP-7, increased MMP-7 levels have been noted among patients who develop both left ventricular hypertrophy (LVH) and diastolic dysfunction, respectively [8]. Additionally, a recent study demonstrated an association between MMP-7 and all-cause death or heart failure-related hospital admission [9]. In mice, deletion of the gene responsible for MMP-7 expression has led to improved survival after MI [10]. This study, along with many similar studies, have encouraged the possibility of developing therapeutic targets which alter MMP and TIMP expression to improve outcomes in the setting of acute myocardial infarction and heart failure [11].

Other promising markers, such as MMP-2, are being studied (and may also be studied if SOMALogic® SOMAscan assay is validated against the ELISA by the time of analysis). Increased MMP-2 levels have been demonstrated to impair contraction and minimize inotropic response, even in the absence of myocardial injury [5]. In both heart failure and post-MI, increased levels of MMP-2 are responsible for cardiac remodeling and cardiac mechanical dysfunction [12, 13].

Patients may demonstrate structural abnormalities on echocardiogram, which may suggest they are at an increased risk of developing prospective adverse events. These structural abnormalities include measures of systolic function (left ventricular [LV] ejection fraction, global longitudinal strain [14]), cardiac structure (LV mass index [LVMI]), diastolic function (left atrial volume index [LAVi], tissue doppler imaging [TDI]

septal e' and septal E/e' ratio [15]), and pulmonary artery pressure (tricuspid regurgitation maximum jet velocity [TR Vmax]). ARIC participants had echocardiograms performed concomitantly with cardiac biomarker measurements (including MMPs/TIMPs) at visit 5, allowing for the assessment of the cross-sectional relationship between MMP-1, MMP-7, TIMP-1 (in addition to MMP-1/TIMP-1 ratio) and these echocardiographic parameters.

The ARIC study provides a unique opportunity to determine the association between MMPs/TIMPs measured using the SOMALogic® SOMAScan assay (an aptamer-based technology that can quantify the levels of thousands of proteins in the serum) and other cardiac biomarkers measured at visit 5 (including high-sensitivity Troponin-I [hs-cTnI], high-sensitivity Troponin-T [hs-cTnT], and N-terminal pro B-type Natriuretic Peptide [NT-pro-BNP]). This will allow us to better understand the role of MMPs/TIMPs in the pathophysiology in heart disease. Additionally, determining the association between MMPs/TIMPs and echocardiographic abnormalities at ARIC visit 5 will allow us to better understand their role in cardiac remodeling. Finally, determining the association between MMPs/TIMPs and future adverse events (including fatal or non-fatal myocardial infarction [MI], ischemic stroke, or revascularization (i.e. Percutaneous coronary intervention [PCI] or coronary artery bypass graft [CABG]), heart failure hospitalizations, atrial fibrillation events, or mortality), in addition to other biomarker and echocardiographic abnormalities, will provide us with a more holistic understanding of their role in predicting cardiovascular outcomes. Of note, the occurrence of these adverse has been recorded for over half a decade since ARIC visit 5.

5. Main Hypothesis/Study Questions:

Hypothesis:

1. MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, will have a strong correlation and association with hs-cTnI, hs-cTnT, and NT-pro-BNP obtained at ARIC visit 5.
2. MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, will be cross-sectionally associated with echocardiographic measures of systolic function (left ventricular [LV] ejection fraction, global longitudinal strain), cardiac structure (LV mass index [LVMI]), diastolic function (left atrial volume index [LAVi], tissue doppler imaging [TDI] septal e' and septal E/e' ratio), and pulmonary artery pressure (tricuspid regurgitation maximum jet velocity [TR Vmax]) based on echocardiograms performed at ARIC visit 5.
3. MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio at ARIC visit 5 will be associated with prospective clinical events, including ASCVD events (fatal or non-fatal myocardial infarction [MI], ischemic stroke, or

revascularization (i.e. Percutaneous coronary intervention [PCI] or coronary artery bypass graft [CABG]), heart failure hospitalizations, atrial fibrillation events, and mortality.

Study Aims:

1. Assess whether MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio correlate and are associated with other cardiac biomarkers, including hs-cTnl, hs-cTnT, and NT-pro-BNP, obtained at ARIC visit 5.
2. Assess whether MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio are associated with echocardiographic measures of systolic function (left ventricular [LV] ejection fraction, global longitudinal strain), cardiac structure (LV mass index [LVMI]), diastolic function (left atrial volume index [LAVi], septal tissue doppler imaging [TDI], septal e' and septal E/e' ratio), and pulmonary artery pressure (tricuspid regurgitation maximum jet velocity [TR Vmax]) based on echocardiograms performed at ARIC visit 5.
3. Assess whether MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio at ARIC visit 5 are associated with prospective clinical events, including ASCVD events, heart failure hospitalizations, atrial fibrillation events, or mortality.

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).

Study design:

We will include subjects who have MMP-1, MMP-7, and/or TIMP-1 levels from visit 5 available for analysis.

We will assess the cross-sectional correlation and association between MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, with hs-cTnl, hs-cTnT, and NT-pro-BNP levels obtained at ARIC visit 5. We will then assess the cross-sectional association between visit 5 MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, with echocardiographic parameters at visit 5. In additional analyses, we will also study the association between visit 5 MMP-1, MMP-7, and TIMP-1 levels (in addition to MMP-1/TIMP-1 ratio) and future ASCVD events, heart failure hospitalizations, atrial fibrillation events, and mortality.

In secondary analysis performed to determine the role MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, in predicting initial adverse event. We will exclude those with aforementioned adverse events prior to visit 5.

Other promising markers, such as MMP-2, are being studied (and may be added if SOMALogic® SOMAscan assay is validated against the ELISA).

Inclusion/ exclusion criteria:

All eligible ARIC participants from visit 5 who had MMP-1, MMP-7, and/or TIMP-1 measured at ARIC visit 5 will be included in the study. The major exclusion criteria is missing information on MMP-1, MMP-7 and TIMP-1, or missing clinical/demographic information regarding ASCVD events (fatal or non-fatal myocardial infarction [MI], ischemic stroke, or revascularization (i.e. Percutaneous coronary intervention [PCI] or coronary artery bypass graft [CABG]), heart failure hospitalizations, atrial fibrillation events, and/or mortality.

Exposures:

1. MMP-1, MMP-7, TIMP-1 level, obtained during visit 5 using SOMALogic® SOMAscan assay, in addition to MMP-1/TIMP-1 ratio, will be modeled as a continuous variables. If distribution is non-normal, appropriate transformation will be performed.

Outcomes:

Endpoints to be assessed:

1. Biomarkers, including hs-cTnI, hs-cTnT, and NT-pro-BNP.
2. Echocardiographic measures of systolic function (left ventricular [LV] ejection fraction, global longitudinal strain), cardiac structure (LV mass index [LVMI]), diastolic function (left atrial volume index [LAVi], tissue doppler imaging [TDI] septal e' and septal E/e' ratio), and pulmonary artery pressure (tricuspid regurgitation maximum jet velocity [TR Vmax]).
3. Future ASCVD events (fatal or non-fatal myocardial infarction [MI], ischemic stroke, or revascularization (i.e. Percutaneous coronary intervention [PCI] or coronary artery bypass graft [CABG]), heart failure hospitalizations, atrial fibrillation events, and mortality.

Covariates will include age, sex, race, current history of smoking, diabetes, hypertension, hyperlipidemia, CKD (eGFR of less than 60 ml/min/1.73m²), body mass index (BMI), and ARIC field center.

Statistical Analysis:

We will use the Spearman rank correlation to assess the correlation of MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, with hs-cTnI, hs-cTnT, and NT-pro-BNP levels obtained at ARIC visit 5. We will also assess the cross-sectional association of MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, with hs-cTnI, hs-cTnT, and NT-pro-BNP using linear regression. Model 1 will be adjusted for age, sex, and race. Model 2 will be adjusted for all variables in model 1 plus traditional risk factors including total cholesterol, high-density lipoprotein cholesterol, smoking status, systolic blood pressure, antihypertensive medication use, and diabetes status. Model 3 will be adjusted for all of the variables in model 2 plus CKD status, BMI, lipid-lowering medication use, and hs-CRP.

We will use linear regression to assess the cross-sectional association of MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, with echocardiographic measures of systolic function (left ventricular [LV] ejection fraction, global longitudinal strain), cardiac structure (LV mass index [LVMI]), diastolic function (left atrial volume index [LAVI], tissue doppler imaging [TDI] septal e' and septal E/e' ratio), and pulmonary artery pressure (tricuspid regurgitation maximum jet velocity [TR Vmax]) obtained at ARIC visit 5. Adjustments will be made as in models 1-3 as above. Model 4 will include all variables in model 3 plus hs-cTnI, hs-cTnT, and NT-proBNP.

Additionally, we will use Cox proportional hazards models to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the prospective associations of MMP-1, MMP-7, and TIMP-1 levels (in addition to MMP-1/TIMP-1 ratio) measured at visit 5 with time to ASCVD events (fatal or non-fatal myocardial infarction [MI], ischemic stroke, or revascularization (i.e. Percutaneous coronary intervention [PCI] or coronary artery bypass graft [CABG]), heart failure hospitalization, atrial fibrillation events, or mortality (these will be analyzed as both composite and individual outcomes). Adjustments will be made as in models 1-4 above. Analyses of clinical events will account for competing risk of non-cardiovascular death using the method of Fine and Gray. Bonferroni correction will be performed (when appropriate) to account for multiple testing.

Furthermore, we will perform internal validation of our results by determining the association between visit 2 MMP-1, MMP-7, and TIMP-1 levels (in addition to MMP-1/TIMP-1 ratio) and prospective events through ARIC visit 5, and comparing it to the association between MMP-1, MMP-7, and TIMP-1 levels (in addition to MMP-1/TIMP-1 ratio) obtained at ARIC visit 5 and prospective events to date (if visit 2 data is not available at the time of analysis, we will implement visit 3 data instead).

For each study aim, we will perform secondary analysis excluding those with ASCVD events prior to visit 5 in order to determine the role MMP-1, MMP-7, and TIMP-1 levels, in addition to MMP-1/TIMP-1 ratio, in predicting future risk of cardiovascular dysfunction and aforementioned adverse events.

Other promising markers, such as MMP-2, are being studied (and may be added if SOMALogic® SOMAscan assay is validated against the ELISA).

Limitations / Major Challenges:

- SomaLogic assays provide only relative concentrations of biomarkers such as MMP-1, MMP-7, and TIMP-1. However, in one pilot study, we found a strong correlation between SomaLogic TIMP-1 ($r = 0.54$) and MMP-1 ($r=0.89$) levels compared to ELISA. Additionally, there is a strong correlation with MMP-7 levels and Olink® assay ($r = 0.69$).

7a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes
 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 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 in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:

<http://www.csc.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)?

N/A

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.csc.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.csc.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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