ARIC Manuscript Proposal #3411

PC Reviewed: 6/18/19  Status: _____  Priority: 2
SC Reviewed: _________  Status: _____  Priority: _____

1.a. Full Title: The Association of Growth Differentiation Factor-15 and Risk of Incident Atherosclerotic Cardiovascular Disease, Heart Failure Hospitalization, and All-Cause Mortality: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): The Association of GDF-15 and ASCVD, HF Hospitalization, and Death

2. Writing Group:

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

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Name: Christie Ballantyne
3. Timeline:

The data needed for this analysis are currently available; we plan to submit for publication within 1 year.

4. Rationale:

Cardiovascular disease (CVD) remains the leading cause of death in the US accounting for nearly a third of overall deaths. Older adults carry a high burden of CVD with an estimated incidence rate of 1,122 per 100,000 person-years in 2011. Importantly, older adults experience significantly higher rates of incident heart failure (HF) and mortality. As the predictive value of traditional risk factors is lessened with age, novel biomarkers may be used to not only identify older adults at high risk of these adverse outcomes but also evaluate disease mechanisms linking such biomarkers with events.

Growth-differentiation factor-15 (GDF-15) is a distant member of the transforming growth factor-β superfamily. The expression of GDF-15 in rat cardiac myocytes is induced by stressors including inflammation and tissue injury. Increased cardiac expression of GDF-15 has been observed in mouse models with myocardial infarction, pressure overload, and cardiomyopathy. GDF-15 is also produced by endothelial cells in response to angiogenic stress, and is expressed in human atherosclerotic plaque macrophages.

GDF-15 may have important prognostic implications with respect to atherosclerotic CVD (ASCVD), HF, and mortality outcomes. For example, a nested case-control analysis of the Women’s Health Study showed that GDF-15 was associated with incident CVD among elderly women without prior history of CVD. GDF-15 levels were also associated with risk of death or HF rehospitalization in HF patients with reduced or preserved ejection fraction (HFrEF and HFpEF respectively). Importantly the prognostic value of GDF-15 in these studies was independent of traditional cardiovascular risk factors as well as cardiac markers, suggesting a potentially distinct pathophysiological mechanism linking GDF-15 with adverse outcomes.

Although GDF-15 appears to be a powerful indicator of the adverse outcomes of HF and death in older adults and has a very strong association with age, the biological pathways which lead to increased levels of GDF-15 in elderly individuals is not well understood. Furthermore, there are no therapies that have been examined in clinical trials to see if treatment of older individuals with high levels could reduce incident CVD events or reduce mortality. Identification of biological pathways associated with high levels of GDF-15 in older participants in ARIC may be informative in designing future trials to test if targeted therapies could prevent incident CVD events or reduce mortality. Genome wide association studies (GWAS) suggest that genetic factors may also regulate levels of GDF-15.
In this study we plan to evaluate the prognostic value of GDF-15 for CVD events and mortality in the ARIC cohort. The ARIC study is ideal for this analysis given the comprehensive assessment of cardiovascular risk factors, adjudicated follow-up of incident events, as well as extensive proteomic data consisting of SomaLogic and immunoassays allowing for a pathway analysis to establish disease mechanisms linking GDF-15 with adverse outcomes. Importantly, a large number of ARIC participants are older adults and therefore we are able to examine the prognostic significance of GDF-15 in this important group.

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

**Aim 1:** Study the cross-sectional and prospective association between GDF-15 and adverse outcomes.

1. **Hypothesis 1:** There is a graded relationship between baseline levels of GDF-15 with prevalence of ASCVD and adjudicated HF hospitalization independent of cardiovascular risk factors
2. **Hypothesis 2:** There is a graded relationship between baseline levels of GDF-15 with incident ASCVD, HF hospitalization, and all-cause mortality independent of cardiovascular risk factors.

**Aim 2:** Examine the biological pathways associated with elevated plasma GDF-15 protein levels in the participants in ARIC V5 using proteomic data available in ARIC.

1. **Hypothesis 2:** There are unique biological pathways that are associated with high levels of GDF-15 in older adults and that some of these pathways may be amenable to targeted therapies in future clinical trials to examine if specific treatments could improve outcomes.

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:**

**Study Design:** prospective at ARIC visit 5 (n= 6,538) and visit 6 (n= 4,214)

**Exclusions:**
- Missing measurements of covariates
- Participants with prior ASCVD or HF hospitalization will be excluded in analyses of incident ASCVD and HF hospitalization respectively.

**Exposure:**
GDF-15 at visit 5 was measured using SOMAScan proteins, excluding non-human proteins detected due to cross-reactivity (n ~ 5,000).

GDF-15 at visit 6 was measured in EDTA plasma of individuals who participated in the ARIC visit 6 examination (n=3,799) using an automated electrochemiluminescence immunoassay (Elecsys GDF-15, Roche Diagnostics).

GDF-15 levels will be categorized using tertiles or expressed continuously per 1 standard deviation (SD)

Outcomes:
1. Incident ASCVD: defined as myocardial infarction, coronary revascularization, coronary death, or stroke.
2. Incident HF hospitalization (adjudicated): defined using International Statistical Classification of Diseases and Related Health Problems (ICD) codes of 428.x (9th Revision) or I50 (10th Revision) in any position on the hospital discharge list or on a death certificate with death from heart failure in any position.
3. All-cause mortality: determined as identification of death by telephone contact with participant proxy, obituaries, hospital records, death certificates, or vital statistics from the National Death Index.

Covariates:
Age, sex, race/ethnicity, hypertension, diabetes mellitus, dyslipidemia, obesity, cigarette smoking, alcohol use, diet, physical activity, body mass index (BMI), systolic blood pressure (SBP), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), hemoglobin A1c (HbA1c), lipid-lowering medication use, antihypertensive medication use, glucose-lowering medication use, estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hsCRP), high-sensitivity cardiac troponin T (hs-cTnT), and N-terminal pro-brain natriuretic peptide (NT-proBNP).

*Non-normally-distributed covariates will be log-transformed.

Statistical Analyses:
Baseline characteristics will be tabulated by tertiles of GDF-15. Continuous variables will be reported using mean (SD) or median (IQR) depending on normality of the data, while categorical variables will be expressed as count (percentage). Differences will be tested using ANOVA, non-parametric testing, or chi-square testing as appropriate.

Multivariable linear regression models will be used to study the association between GDF-15 levels with clinical and biochemical variables: age (per 5 years), sex, race/ethnicity, BMI (per 5 kg/m²), hypertension, diabetes mellitus, cigarette smoking, LDL-C (per 1 SD), HDL-C (per 1 SD), eGFR (per 1 SD), hsCRP (per 1 SD), hs-cTnT (5ng/L >0.01), and NT-proBNP (per 1 SD).

Models will be adjusted for lipid-lowering medication use, antihypertensive medication use, and glucose-lowering medication use.
GDF-15 and Prevalent Outcomes

Logistic regression models will be used to calculate prevalence odds ratios for the cross-sectional association between tertiles of GDF-15 and prevalent ASCVD and HF hospitalization. We will also examine prevalence ratios. Analyses will be performed separately for visits 5 and 6.

Models will be sequentially adjusted for age, sex, race/ethnicity (Model 1) and hypertension, diabetes mellitus, dyslipidemia, obesity, cigarette smoking, alcohol use, diet, physical activity, BMI, SBP, LDL-C, HDL-C, HbA1c, lipid-lowering medication use, antihypertensive medication use, glucose-lowering medication use, eGFR, hsCRP, hs-cTnT, and NT-proBNP (Model 2).

GDF-15 and Incident Outcomes

Kaplan Meier curves will be constructed to graphically depict the association between tertiles of GDF-15 (at visit 5 and visit 6) and cumulative incidence of ASCVD, HF hospitalization, and death.

After testing and confirming the proportionality assumption using log-log plots, multivariable-adjusted Cox regression models will be used to study the association between baseline levels of GDF-15 (tertile 1 as reference or per 1 SD increase) and incident ASCVD, HF hospitalization, and all-cause mortality. Analyses of incident ASCVD and HF hospitalization will exclude individuals with prior ASCVD and HF respectively. Analyses will be performed separately for visits 5 and 6.

Models will be sequentially adjusted for age, sex, race/ethnicity (Model 1) and hypertension, diabetes mellitus, dyslipidemia, obesity, cigarette smoking, alcohol use, diet, physical activity, BMI, SBP, LDL-C, HDL-C, HbA1c, lipid-lowering medication use, antihypertensive medication use, glucose-lowering medication use, eGFR, hsCRP, hs-cTnT, and NT-proBNP (Model 2).

Sensitivity Analyses:

Results will be stratified by sex and race/ethnicity and multiplicative interaction testing will be performed between GDF-15 and each of the aforementioned groups and tested for significance in multivariable models (Models 1 and 2 as described above). Analyses of incident ASCVD will account for competing risk of non-cardiovascular death using the method of Fine and Gray.23

Aim 2:

Study Design: cross-sectional analysis at ARIC visit 5 (n= 6,538)

Exclusions:
  - Standard race-center exclusions
• Sex-mismatched SOMAScan samples
• Missing measurements of covariates

Exposure: SOMAScan proteins, excluding non-human proteins detected due to cross-reactivity (n ~ 5,000)

Outcomes:
• Log2-transformed SOMAScan GDF15
• Elevated SOMAScan GDF15 vs reference
  o As there are no clinically established references ranges for GDF15 and the SOMAScan does not report absolute quantification of proteins, we will define an “Elevated” GDF15 as values greater than the 80th percentile compared to the “Reference” of less than the 20th percentile in order to achieve a differential between the two populations.

Regression Analyses:
• Linear regression of log-transformed GDF-15
• Logistic regression of Elevated GDF-15
• Regularized regression using Elastic Net

GDF-15 Proteomic Signature and Pathway Analyses:
  1) Targeted analysis assessing associations of GDF15 with other known cardiovascular biomarkers such as NT-proBNP, hsCRP, cTnT, etc.
  2) Exploratory pathway analysis using Qiagen Ingenuity Pathway Analysis with Causal Pathway Analysis to elucidate potential upstream regulators associated with circulating GDF15 levels

Given the prognostic implications of GDF15 in HF, we will further compare the pathways associated with GDF15 levels among those with and without prevalent HF at visit 5.

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

Sensitivity Analyses:
• Study population
  o Exclusion of prevalent ASCVD (CHD or stroke)
  o Exclusion of prevalent heart failure
• 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.
• Correlation between GDF-15 measured by the Roche immunoassay in visit 6 with 100 case controls from visit 5 measured using SOMAscan. In addition, 20 unblinded plasma samples have already been measured with both SOMAscan and the Roche assay. Plasma GDF15 measured by ELISA assay in another study was
correlated with the measure from the SOMAscan at with a correlation of 0.821.\textsuperscript{18}
To further analyze the agreement between the two assays we will also perform Bland Altman analyses of GDF-15 measurements obtained using SOMAscan and ELISA assay.

Limitations:

\begin{itemize}
\item Small sample size: analyses of events may be underpowered.
\item There is the potential for residual confounding.
\item Ideally we would have GDF-15 measured by Roche at visit 5.
\end{itemize}

7.a. Will the data be used for non-CVD analysis in this manuscript? 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? NA
(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? 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”? NA

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.cscu.unc.edu/ARIC/search.php

Yes

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 relevant proposals have been identified.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes

11.b. If yes, is the proposal
\begin{itemize}
\item[X] A. primarily the result of an ancillary study (list number* AS#2015.26

\end{itemize}
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/

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


