ARIC Manuscript Proposal Form

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Publication Committee Review Date: **_04/08/25**
ARIC Manuscript Proposal Number: **# 4649**

1.a. Full Title: GDF3 promotes adipose tissue macrophage-mediated inflammation via altered chromatin accessibility during aging

b. Abbreviated Title (Length 26 characters): GDF3 promotes inflammation

2. Writing Group [please provide a middle initial if available; EX: Adam L Williams]:

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I, the ~~first~~ corresponding author, confirm that all the coauthors have given their approval for this manuscript proposal. __CC___ **[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 (The ARIC author should be involved enough in ARIC to be able to point the lead author to appropriate ancillary study PIs and to be able to search ARIC manuscript proposals if the lead author doesn't have the access needed to do such a search).

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3. Timeline: Analyses will be completed within two months, and the results will be incorporated into a basic science manuscript on GDF3 that is already under review.

4. Rationale: GDF3, a TGFβ superfamily cytokine, regulates cell fates by modulating embryonic stem cell pluripotency[1]. GDF3 has contrasting functions, such as promoting metabolic dysfunction, improving muscle regeneration during injury, and reducing inflammation during sepsis[2-10], which likely stem from its divergent signaling capabilities[11, 12]. Recent studies have linked GDF3 to disease risk in myocardial infarction, metabolic dysfunction-associated steatohepatitis, obesity, sepsis, and Alzheimer's disease[2, 3, 9, 13-15].

Our manuscript under review (Nature Aging and as a pre-print here: PMID 39386655 {Jang, 2024 #11}) has investigated the role of GDF3 in inflammation in old organisms. We used two transgenic mouse models and two pharmacological methods to inhibit the GDF3 signaling pathway. Our results reveal that GDF3 increases inflammation in macrophages from old mice. We also identified that inhibition of the GDF3 signaling pathway reduces the severity of endotoxemia in old mice. Upon initial review, reviewers requested translation of findings to human populations. Around the same time, we learned of the existence of GDF3 in the ARIC SomaLogic SomaScan data. We therefore wish to evaluate the pathway between GDF3 as measured by SomaScan and inflammation in ARIC study participants. GDF3 expression may vary by age. We aim to assess the associations of GDF3 with inflammation at 2 timepoints to provide insight into the associations of GDF3 with inflammation in both middle-age and older adulthood.

5. Main Hypothesis/Study Aims:

Aim 1. Evaluate the cross-sectional association of GDF3 with biomarkers of inflammation at both middle age and older adulthood.

Aim 2. GDF3 and risk of incident hospitalized infection. at both middle age and older adulthood.

Hypotheses:

1. GDF3 expression is positively associated with biomarkers of inflammation in older individuals, but not younger individuals.
2. GDF3 expression is associated with increased risk for hospitalized infection in older adulthood, but not in midlife. Secondary analyses may consider other inflammatory-related conditions.

6. Design and analysis - please address the following aspects:

a) inclusion/exclusion

Inclusion: information on GD3 and inflammatory biomarkers present
Exclusion: ARIC usual race*center exclusions

b) Study design

Aim 1: Cross-sectional (or quasi-cross-sectional) at visits 2 and 5.
Aim 2: Prospective cohort, with baseline at visits 2 and 5.

c) Outcome and other variables of interest with specific reference to the time of their collection

Exposure: GDF3 via SomaScan at visit 2 and 5

Outcomes

- Cross-sectional: Markers of systemic inflammation at visits 1/2 and 5. The primary outcome will be CRP levels, which have been previously measured at Visits 2, 4, and 5 for all ARIC participants. Secondarily, we will also conduct an exploratory analysis using a Visit 1 inflammation composite score created using inflammatory biomarkers available at Visit 1 (i.e., WBC, fibrinogen, albumin, von Willebrand factor, factor VIII), as used in prior ARIC manuscripts. The inflammatory composite score will be created by summing the biomarker levels after each is rescaled to a z-scores based on the sample mean.
- Prospective: Incident hospitalized infection based on ICD-9 and ICD-10 codes, as in previous ARIC papers (e.g. PMID: 36442663). Both visit 2 and visit 5 will be used as baseline.

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Covariates

Age, sex, race, educational level, BMI. It is possible that we may explore additional covariates as the paper evolves. Example covariates that may be considered include smoking, physical activity, asthma, COPD, diabetes, hypertension, chronic kidney disease.

d) Summary of data analysis

Descriptive statistics (means and proportions) will be provided, stratified by quintiles of GDF3. Pearson's correlations between GDF3 and markers of inflammation will be assessed.

Aim 1: Linear regression will be used to explore the cross-sectional association between GDF3 and markers of inflammation. Linearity will be assessed and the dependent variables transformed as needed. Model 1 will adjust for age, sex, race/center, and educational attainment. Model 2 will further adjust for BMI.

Aim 2: Cox proportional hazards regression will be used to evaluate the association between GDF3 categories and risk of incident hospitalized infection. Person-time will accrue from the baseline (visit 2 or visit 5, respectively) until hospitalization with an incident infection, loss-to-follow-up, death, or administrative censoring.

- The proportional hazards assumption will be tested by evaluating the interaction between GDF3 categories and the natural log of person-time, and by visual inspection of graphs of the survival function vs survival time stratified by GDF3 categories.
- Model 1 will adjust for age, sex, and race/center. Model 2 will further adjust for BMI. Lastly, we will explore whether adjustment for systemic inflammation via CRP attenuates the association.
- Multiplicative interactions by age (median split), sex, and BMI category will be tested by including cross-product terms in the models. Stratified results will be reported regardless, given inherent interest

e) Any anticipated methodologic limitations or challenges if present

GDF3 is measured by SomaScan. The correlation with gold-standard assays is unknown.

f) Will the author need Limited data to complete the proposed manuscript? ☐ Yes, Limited data is needed (Provide a brief (2-3 sentences) justification for requesting PHI data) . ☒ No, De-identified data will be sufficient.

*Please note, Limited dataset access is strict and rarely provided. Limited data includes identifiable information such as dates (birthdays, visit dates, etc.). CMS, Genomic, Geocoded, Proteomics/Somalogic, and other -omic data all fall under the limited data category. De-identified data does not include dates. All dates are date adjusted to "Days since Visit 1".

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? (Non-ARIC analysis means that the authors are not regarded as ARIC investigators and the "ARIC author" is essentially just a facilitator rather than an integral part of the writing group.) ☐ Yes ☒ 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 is distributed to ARIC PIs annually, 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

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 or the "sponsoring" ARIC 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 website at: https://aric.cscc.unc.edu/aric9/proposalsearch [ARIC Website□Publications□Proposal Search]

☒ 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)?

No papers have been proposed exploring GDF3, there are a few exploring GDF-15, but that is a different biomarker.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or does it use current [or ongoing] ancillary study data (this includes ACHIEVE)? ☐ **Yes** ☒ **No → Skip to question 12**

11.b. If yes to 11.a., 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)**

11.c. If yes to 11.a., list number* __

*ancillary studies are listed by number https://aric.cscc.unc.edu/aric9/researchers/ancillary_studies/approved_ancillary_studies [ARIC Website□Ancillary Studies□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 https://aric.cscc.unc.edu/aric9/publications/policies_forms_and_guidelines [ARIC Website□Publications□Publication Policies, Forms, and Guidelines].

http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

References: ____

To view publications materials, click "Log in" at the top right of the ARIC website. Click "Forgot Password" if you are experiencing issues with logging in.

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