ARIC Manuscript Proposal #3961 (revised)

PC Reviewed: 1/11/22	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Plasma Proteomic Analysis on Mid-Life Mechanisms of Late-Life Frailty

b. Abbreviated Title (Length 26 characters): **Mid-life Proteomics of Frailty**

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

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3. Timeline:
This proposal is for dissertation research. We aim to complete analyses and manuscript for Aim 1 by the end of 2022, and complete the analyses and manuscript Aim 2 by April 2024.

4. Rationale:

Frailty is a syndrome of reduced physiological reserve and increased vulnerability to adverse outcomes, resulting from multi-system deregulation.[1,2] The most common measure of frailty in the literature is the physical frailty phenotype developed by Fried and colleagues, which consists

of unintentional weight loss, poor grip strength, slow walking, exhaustion and low physical activity.[2] The presence of 1-2 and 3 or more criteria indicates prefrail and frail stage, respectively. The global prevalence of frailty and prefrailty using these definitions is estimated at 12% and 46%, respectively, in community-dwelling older adults and is projected to rise with the aging of the population.[3] Physical frailty phenotype is predictive of various adverse health outcomes,[4] making prevention of frailty a public health priority.[5] Understanding the biological and physiological etiology of frailty is essential in informing prevention efforts.

Many studies have examined the biological processes involved in the pathogenesis of frailty.[4,6] To date, most of these studies have focused on a single hypothesized pathway of frailty, such as chronic inflammation.[7] Such an approach may not adequately capture the multifactorial nature of frailty.[8] To potentially address this gap, three recent studies utilized a plasma proteomic approach,[7,9,10] which simultaneously explores thousands of proteins of varying functions and may thus be more effective in discovering multiple pathways implicated in frailty. However, several important questions remain unaddressed.

First, majority of the current proteomics studies and targeted protein studies have only examined protein levels in older adults, i.e., individuals aged 65 years and older. Though often thought as a geriatric syndrome, frailty-related biological and physiological changes may start earlier in life. Ferrucci and colleagues discussed in a review three metrics of aging from lower to higher hierarchy: biological aging, phenotypic aging (e.g., body composition and energetics), and functional aging (e.g., frailty). Due to the presence of compensatory mechanisms at biological and physiological levels, changes in the lower two hierarchies will only lead to changes in the functional level when all compensatory mechanisms were exhausted, resulting in a time lag between the trajectories of these metrics.[11] Fried and colleagues also discussed in her review the threshold effects of multisystem dysregulation –physiological systems integrity may be deteriorating above a critical threshold and will only precipitate clinical frailty when the threshold is crossed.[4] To that end, the underlying mechanistic changes of frailty may already be seen in mid-life whereas clinical manifestation is only apparent in older age. Studies on risk factors such as obesity, and physical inactivity during middle age have provided some indirect evidence on the mid-life importance on later-life frailty.[12–14] More empirical evidence for mid-life proteins is needed to provide insights to etiological relevant window of frailty.

Second, none of the current proteomics studies have established the causal links between the identified proteins and frailty. A plasma protein may be associated with frailty because it is a causal effector or because it is changed concurrently with frailty due to common age-related condition. While biomarkers concurrently changed with frailty can be useful in early diagnosis of frailty, it does not provide much information on the etiology of frailty.

The objectives of this proposal are to identify causal pathophysiological mechanisms of frailty for men and women in mid-life. We propose to examine the associations between plasma proteins measured Visit 3 (1993-1995) using a modified aptamer technology (SOMAscan assay) and frailty status (prefrail and frail) measured using five criteria of physical frailty phenotype at Visit 5 through Visit 7.

5. Main Hypothesis/Study Questions:

Aim 1: To identify the plasma proteins in mid-life that are most strongly associated with frailty and prefrailty status in older age.
Aim 2: To investigate the causal link between identified frailty-associated proteins and frailty using one-sample bi-directional Mendelian randomization

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-temporal design between proteomics measures at V3 and frailty measured at V5-7.

Exclusion:
1. Participants who were neither Black nor white. Non-whites in Washington Co. and Minneapolis.
2. Missing proteomic data at Visit 3 or failed QC.
3. Missing frailty status at Visit 5.

Independent variables:
Plasma proteins measured by SOMAscan, a modified aptamer-based platform, at Visit 3 are the main predictors.

Outcome:
Various frailty categories (frail vs prefrail vs robust; frail vs non-frail; non-robust vs robust) at Visit 5-7.

Covariates for Aim 1:
(Unless stated otherwise, assessments at Visit 3 are used for below variables.)
Model 1: Age, sex, race-site, education, socioeconomic status (total household income, occupational standing, and self-reported community standing, Visit 4).
Model 2: Model 1 + smoking status, alcohol use status, eGFR, total cholesterol, and cholesterol-lowering and anti-inflammatory medication use.
Model 3: Model 2 + functional assessment and comorbidities including hypertension, diabetes, CHD, heart failure, cancer, COPD, and chronic inflammatory disease.

Covariates for Aim 2:
Our final covariates for Aim 2 will be directed by analyses in Aim 1. We will perform tests for association within GENESIS relying on a full genetic relationship matrix to accommodate cryptic relationships as well as population stratification. However, we will also evaluate the need added inclusion of principal components for ancestry as covariates if our QQ-plots from the frailty GWAS are inflated.

Statistical analysis:

Plasma proteins associated with frailty. We will use logistic and multinomial regression models to examine the associations between individual protein (log-transformed if needed) and being frail and prefrail. Benjamin-Hochberg false discovery rate (FDR) adjustment and Bonferroni correction will be applied to account for the multiple testing. We will explore whether sex modifies the associations between the proteins and frailty status. To better interpret the biological and functional pathways represented by the identified proteins and the interplay of the proteins, we will conduct pathway and network analyses using QIAGEN's Ingenuity Pathway Analysis (IPA). Identified networks will be used to investigate whether dysregulation of multiple systems (multiple networks) has non-linear effects on the risk of the frailty. As a sensitivity analysis, we will incorporate inverse probability weighting (IPW) of loss to follow up to examine and control the potential bias associated with differential attrition during the long intervening period between Visit 3 and Visit 5. To address the limitation of unknown frailty status at Visit 3 (baseline), we will estimate an E-value that measures how strong the unmeasured confounding need to be to explain away the protein-frailty associations.[15]

Replication of protein-frailty association. We will analyze the associations of proteins at Visit 2 with frailty categories at Visit 5-7 in ARIC as a within-sample replication of the main findings. We also plan to confirm the identified proteins using an external cohort, the Offspring cohort of the Framingham Heart Study (FHS). Plasma proteins were profiled in this cohort in 1991-1995 using SOMAscan platform which assayed 1373 proteins.[16] Frailty was ascertained using Fried's physical frailty criteria in 2005-2008.[17] We are flexible to explore other cohorts if they have larger panels of proteins measured in midlife.

Genetic variants associated with proteins and frailty. To facilitate the bi-directional Mendelian randomization in Aim 2, (1) we plan to test for cis-QTLs for the set of plasma proteins identified as being associated with frailty outcomes in Aim 1, and (2) we plan to run a full GWAS for the frailty outcome itself. We will perform tests for association within GENESIS relying on a full genetic relationship matrix to accommodate cryptic relationships as well as population stratification. We will use QQ-plots to evaluate residual stratification issues given the ancestry representation, and adapt to ancestry stratified followed by meta-analysis if needed. In consideration of the potential power issue for GWAS of frailty, we proposed two alternatives. First, we will use gene and loci identified in unpublished frailty GWAS in ARIC and published GWAS on frailty index, another measurement of frailty, to inform candidate regions for frailty phenotype, and then obtain the IVs from ARIC. Second, we can use the genetic markers in suggestive regions ($p < 10^{-5}$) as the genetic instruments.

Causal relationship between frailty-associated proteins and frailty. We will utilize the genetic instruments identified in the GWAS for bi-directional Mendelian randomization (MR). Genetic variants associated with plasma proteins will be used as instrumental variables to estimate the causal relationship between proteins identified in Aim 1 and frailty status. To examine reverse causation, genetic variants associated with frailty will be used as instrumental variables to estimate the causal relation on the opposite direction. Inverse-variance weighting (IVW) methods will be used meta-analyze multiple genetic instruments.

Secondary analyses on the heterogeneity between causal estimates will be tested using Cochran's heterogeneity test. Weak IVs will be identified as mean F statistics for IVW < 10.

To test potential violations of Mendelian randomization assumptions and the robustness of the main results to such violations, we will perform the several sensitivity analyses. We will compare the consistency of causal estimates using other approaches including Mendelian Randomization-Egger, weighted-median method and CONtamination MIXture89 to the estimates from IVW approach. Bowden I^2 statistics for Mendelian randomization-Egger analysis will be used to assess the 'No Measurement Error' assumption. Egger intercept test and Mendelian Randomization Pleiotropy RESidual Sum and Outlier test will be used to test the no horizontal pleiotropy assumptions. Radial plots will be constructed to detect potentially invalid or influential instruments ('outliers') and we will perform an IVW Mendelian randomization analysis again after excluding outliers. The Mendelian randomization association *p* value will be Bonferroni-adjusted for the number of proteins with IV available.

Limitations:
Frailty status is unknown at Visit 3 when the plasma proteins were measured.

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? __x__ 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"? __x__ 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/aricproposals/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)?

There are no prior proteomics of frailty proposals, but below proposal were relevant in terms of similar methodology.

MP #3327r. A proteomic analysis of incident dementia: The ARIC Study.
MP #3803. Proteomics of diabetes and glycemic biomarkers in the ARIC Study.
MP #2995. Frailty – a genome-wide association study.

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* ___2017.27_____)

___ 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 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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