1.a. Full Title: An Epigenome-Wide Association Study of Frailty and Physical Function in Older Adults: The Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Epigenomics of Frailty

2. Writing Group:
   Writing group members: Steve Nguyen, James Pankow, Ellen Demerath, Weihua Guan. Additional interested investigators welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal.  

3. Timeline: Analysis 1 during 2017-18. Completed manuscript expected by fall 2018

4. Rationale:

   Frailty, the age-related decline in lean body mass, strength, exhaustion, walking speed, and physical activity, is an emerging public health challenge in developed nations as the older adult population grows in size [1]. Although individuals experience decreases in physical function as they age, frailty is associated with an increased risk of adverse
health outcomes such as falls, disability, and mortality as frail individuals have a decreased resistance to stressors as shown in the Atherosclerosis Risk in Communities (ARIC) study [2]. The prevalence of frailty in community-dwelling individuals aged 65 or older is estimated to be 10.7 percent and is more common in women than men [3]. Given the magnitude of frailty and declines in physical function, there is a need for an increased understanding of frailty in terms of risk factors and etiology throughout the life course to help develop interventions.

Risk factors for frailty include a sedentary lifestyle, insufficient nutrition, and clinical biomarkers such as LDL cholesterol, hemoglobin A1C, white blood cell count, and C-reactive protein [2, 4]. Frailty itself is a risk factor for cardiovascular disease. It is theorized that dysregulation of inflammation and catabolism may be involved in frailty, which could overlap with other conditions such as sarcopenia [5]. While research on establishing and operationalizing the frailty phenotype and its association with adverse health outcomes is extensive, knowledge on the various pathways involved in frailty pathogenesis are unclear.

A genome-wide association study (GWAS) meta-analysis in 195,180 individuals for hand grip strength identified loci in genes related to skeletal muscle function (ACTG1), signaling (PEX14, TGFA, SYT1), and proliferation (LRPPRC, KANSL1) [6]. A GWAS in 38,292 individuals for lean body mass identified loci in genes involved in signaling (VCAN), skeletal muscle (HSD17B11), adiposity (FTO), and insulin signaling and growth (IRS1) [7]. While genetic studies yield insight into genes and pathways involved in frailty, there remains the potential issue of missing heritability where only a proportion of variation in phenotypes, which could extend to frailty, can be explained by genetic factors [8]. Theories for explaining the missing heritability include rare variants, common variants with small effects, residual environmental confounding, and recently epigenetic factors [9].

Epigenetics, the study of heritable changes in gene expression not involving DNA sequence changes, may be involved in frailty pathogenesis. Epigenetic modifications can be influenced by both genetic and environmental factors, suggesting a potential pathways between environment and changes in gene expression [10]. DNA methylation, the most studied form of epigenetic modification, involves the addition of a methyl group to the cytosine base of a cytosine-guanine dinucleotide, known as CpG sites, is involved in normal human development and gene expression regulation. Methylome-wide association studies (MWAS) have identified CpG sites associated with adiposity, blood lipids, blood pressure, and incident type 2 diabetes [11-14]. For example, DNA methylation in ABCG1, involved in lipid transport and insulin secretion, is associated with BMI, blood lipids, and diabetes. DNA methylation has also been shown to be associated with accelerated aging and predicted mortality [15]. Currently the association between DNA methylation and frailty and its components are unknown. The identification and characterization of the frailty-DNA methylation association could yield more insight in genes and pathways involved in frailty and chronic diseases and have potential applications in risk stratification.

5. **Main Hypothesis/Study Questions:** This proposal will identify and characterize DNA methylation markers associated with frailty and its components: weight loss, weakness, exhaustion, slow walking speed, and low physical activity.
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 sectional for the association between CpG methylation measured at visit 2 or 3 and frailty and its components measured at visit 5.

Inclusion criteria: African American and European American participants with HM450K DNA methylation data that passed quality control who have complete covariate data.

Exclusion criteria: participants with prevalent MI, heart failure, stroke, or cancer at visit, 2 or 3, which DNA methylation was measured. Missing frailty phenotype.

Outcome of interest: CpG methylation at ~480,000 CpG sites measured at visit 2, or visit 3 for a subset of participants

Predictor(s) of interest: frailty, definitions 1, measured in visit 5 as well as its components modeled as dichotomous variables:
- Weakness (low grip strength defined as the lowest 20\textsuperscript{th} percentile of the distribution of gender- and BMI-specific grip strength)
- Slowness (slowest walking speed defined as the 20\textsuperscript{th} percentile of the distribution for gender- and height-adjusted time in seconds used to walk 4 meters)
- Exhaustion (responded “some of the time” or “most of the time” to the questions “I felt everything I did was an effort” or “I could not get going”).
- Weight loss (10\% of weight lost from V4 to V5 OR BMI <18.5 kg/m\textsuperscript{2} at V5))
- Low physical activity (gender- and height-adjusted 20\textsuperscript{th} percentile rank of the Baecke leisure sports activity index)

Additionally, grip strength (kg), walking speed (time in seconds to walk 4 meters), weight loss from visit 4 (kg), and physical activity (moderate-vigorous physical activity minutes per week) will be modeled as continuous measures.

Covariates: race, education, and income from visit 1. Age, sex, study center, smoking status, alcohol use, hypertension status, diabetes status, physical activity, body mass index, white blood cell count, imputed white blood cell type proportions, blood lipids top 10 principal components from Affymetrix 6.0 genotype data to control for genetic ancestry, and technical variables to control for batch confounding/variation in HM450 data, measured at visit 2 or 3.

Data analysis: Linear regression models will be used to test the association between DNA methylation and frailty and its components. DNA methylation will be set as the dependent variable with frailty or its components set as the independent variables. Model 1 will contain frailty or its components, age, sex, study center, education, income, physical activity, body mass index, white blood cell count, Affymetrix principal
components, and HM450 technical variables. Model 2 will additionally contain diabetes status, hypertension status, and imputed white blood cell type proportions. There will be separate models for each CpG, and for each outcome (frailty phenotype, dichotomous frailty phenotype components, continuous frailty phenotype components). Sensitivity analyses, using linear regression with repeated measures (for BMI, physical activity at visits 1 or 3 and 5, lipids, and so on where available) will also be performed.

7.a. 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

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”?  ____ 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)?

MP#2577 Epigenome-wide association study of mitochondrial genetic variation, DNA copy number, and heteroplasmy.

In MP#2577, CpGs identified to be significantly associated with mitochondrial DNA copy number, mitochondrial DNA SNPs, and mitochondrial DNA heteroplasmy in a cross sectional design and analysis will be taken forward in a prospective cohort study design and analysis to test for an association with frailty whereas the present proposal involves a cross sectional study design and analysis to test the association of some ~480,000 CpGs measured at visit 2 or 3 with frailty and its components measured at visit 5.
11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  

   ___ Yes   ___ No

11.b. If yes, is the proposal
   ___  A. primarily the result of an ancillary study (list number* __________)
   ___  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/](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/](http://publicaccess.nih.gov/) are posted in [http://www.cscc.unc.edu/aric/index.php](http://www.cscc.unc.edu/aric/index.php), under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit_process_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to Pubmed central.

References


