ARIC Manuscript Proposal #2827

PC Reviewed: 09/13/15   Status: _____   Priority: 2
SC Reviewed: _____   Status: _____   Priority: ____

1.a. Full Title: A prospective study of DNA methylation age acceleration and incidence of coronary heart disease, heart failure, and peripheral arterial disease in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters):

2. Writing Group:
Writing group members:

Nicholas Roetker
Jim Pankow
Jan Bressler
Alanna Morrison
Eric Boerwinkle
Other interested investigators are welcome, including the Epigenetics Working Group

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

F**irst author**:   **Nicholas S. Roetker**
Address: Division of Epidemiology and Community Health
                  University of Minnesota
                  1300 South Second Street, Suite 300
                  Minneapolis, MN 55454

          Phone: 612-624-9261   Fax:
          E-mail:

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
          Name: **Jim Pankow**
          Address: Division of Epidemiology and Community Health
                  University of Minnesota
                  1300 South Second Street, Suite 300
                  Minneapolis, MN 55454

Phone: 612-624-2883   Fax: 612-624-0315
E-mail: pankow@umn.edu

3. Timeline:

Analysis will begin upon approval. We anticipate a draft ready to submit for Publications Committee Review in winter 2015-16.

4. Rationale:

Changes in DNA methylation patterns occur in response to external and internal stimuli over the life course, and it may be possible to characterize these changes as a proxy for the rate of aging (i.e., slowed or accelerated). Recent studies have reported sets of cytosine-guanine dinucleotides (CpGs) that can be used to accurately predict chronological age (1,2). Using methylation data from 82 publicly available datasets consistent of 51 healthy tissue and cell types, Horvath (1) developed a model based on 353 CpG sites to predict age. Correlations between chronological and predicted age using blood DNA were strong (r=0.95) in datasets of subjects ranging widely in age from infants to elderly and modest (r=0.6 to 0.8) in datasets restricted to middle- or older-aged adults. Using DNA from whole blood of 482 individuals aged 19 to 101 years, Hannum et al. (2) developed a similar age predictor consisting of 71 CpG sites and demonstrated a high correlation with chronological age (r=0.9) in 174 independent samples. The Horvath and Hannum et al. age predictors share only 6 CpG sites in common.

Age acceleration, defined as the difference between DNA methylation age and chronological age, predicts all-cause mortality (3). One cross-sectional study compared levels of DNA methylation age among different vascular tissues in patients undergoing surgery for severe coronary artery stenosis (4), but to our knowledge no studies have assessed whether methylation age predicts incident coronary heart disease (CHD), heart failure (HF), or peripheral arterial disease (PAD) events.

5. Main Hypothesis/Study Questions:

Higher age acceleration (DNA methylation age – chronological age) will be associated with greater risk of incident CHD, HF, and PAD and greater prevalence and extent of subclinical atherosclerotic disease.

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).

Design: prospective; cross-sectional

Endpoints:
- Incident event endpoints: CHD incidence (defined as hospitalized myocardial infarction or fatal CHD); HF incidence; PAD incidence [defined as ABI<0.9 at Visits 3 or 4 or from hospital discharge diagnoses (ICD codes: 440.21, 440.22, 440.23, 440.24, 443.9, 785.4, 84.11, 84.12, 84.15, 84.17, 38.18, 39.25, 39.29, 39.50, 39.90, 00.55)]
- Cross-sectional subclinical endpoint: carotid intimal medial thickness (IMT)

Exposure: DNA methylation age acceleration

Covariates: chronological age, sex, study center, education, smoking (status and pack-years), alcohol intake, physical activity, body mass index, systolic blood pressure, use of antihypertensive medication, diabetes, total cholesterol, HDL cholesterol, use of cholesterol medication, and white blood cell type proportions (as estimated from DNA methylation levels by the Horvath online calculator (1)).

DNA methylation age: Methylation status was measured from DNA extracted from whole blood white cells using the Illumina HM450 chip. Degree of methylation was determined using Illumina GenomeStudio 2011.1, Methylation module 1.9.0 software. The methylation score for each CpG was represented as a beta (β) value calculated by dividing the fluorescence intensity of the methylated allele by the sum of the intensities of the methylated allele and unmethylated allele. Background subtraction was conducted with the GenomeStudio software using built-in negative control bead types on the array. An average normalization was applied to minimize scanner-to-scanner variation. We will perform additional normalization and imputation for missing beta values epigenetic clock software by Horvath (1). The main exposure variables will be the DNA methylation age acceleration measures "BioAge4HOAdjAge" and "BioAge4HAAdjAge," which are described further in the online tutorial (1).

Inclusion: 2,786 African American participants with DNA methylation measured at Visit 2 or Visit 3 meeting DNA methylation QC criteria (listed in exclusions).

Exclusions: Prevalent CHD/HF/PAD prior to the date of DNA collection and those whose DNA methylation has integrity issues (failed bisulfite conversion, pass rate <95%, possible gender mismatches).

Data analysis:
For the incident event endpoints, we will run separate Cox proportional hazards regression models with incident CHD, HF, and PAD as the events of interest and DNA methylation age acceleration (separate models for Horvath and Hannum et al. predictors) as the exposure of interest. As a sensitivity analysis, we will also run separate models for CHD subcomponent events (myocardial infarction and fatal CHD). Follow-up time will be defined as the time from the date of DNA collection to date of incident disease, death, loss to follow-up, or December 31, 2012, whichever comes first. We will examine the shape of the age acceleration—outcome relationships using restricted cubic spline models to decide whether to model age acceleration continuously or in categories for the

regression models. Models will be adjusted for the potential confounding factors listed above.

For subclinical outcomes, we will assess the association of age acceleration with carotid IMT using linear regression models, again modeling age acceleration either continuously or in categories depending on the shape of association. Models will be adjusted for the potential confounding factors listed above.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ 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?
    __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 file ICTDER03 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/ARIC/search.php

    ___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)?

Note: the present manuscript proposal is using specific sets of methylation markers that predict age as an exposure to predict cardiovascular disease outcomes, making its focus different than the genome-wide methylation proposals by Bressler et al.

MS#1928 Bressler J et al. Genome-wide methylation analysis of cardiovascular disease (CVD) and its risk factors

MS#2399 Bressler J et al. Genome-wide analysis of DNA methylation and coronary heart disease (CHD): the Atherosclerosis Risk in Communities (ARIC) Study

MS#2345 Roetker N et al. A prospective study of the association of DNA methylation age with lung function and type 2 diabetes in the Atherosclerosis Risk in Communities Study

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 (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/

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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __X__ No.

References

1. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115.

2. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 49(2):359–67.

3. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16:25.

4. Nazarenko MS, Markov AV, Lebedev IN, Freidin MB, Sleptcov AA, Koroleva IA, et al. A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. PloS One. 2015;10(4):e0122601.