**ARIC Manuscript Proposal #2337**

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| **PC Reviewed: 5/13/14** | **Status: A** | **Priority: 2** |
| **SC Reviewed: _____** | **Status: _____** | **Priority: ____** |

**1.a. Full Title**: DNA methylation-derived age predicts changes in brain morphology and cognitive decline

**b. Abbreviated Title (Length 26 characters)**: DNA methylation and brain aging

**2. Writing Group**:

Writing group members: Myriam Fornage; Jan Bressler; Li An Lin; Alden Gross; Megan Grove; Yun Li; Tom Mosley; Eric Boerwinkle

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


F**irst author**: **Myriam Fornage, PhD**
Address: University of Texas Health Science Center at Houston
1825 Pressler Street
Houston, TX77030

Phone: 713-500-2463 Fax: 713-500-2424
E-mail: myriam.fornage@uth.tmc.edu


**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: **Tom Mosley, PhD**
Address: University of Mississippi Medical center
2500 N. State Street
Jackson MS, 39216

Phone: Fax:
E-mail: tmosley@umc.edu


**3. Timeline**: Upon acceptance of the proposal

**4. Rationale**:

Age has a significant effect on brain morphology and age-related morphological changes in the brain accompany age-related cognitive decline. Age, when measured chronologically, may not be a reliable indicator of an individual's rate of decline or physiological changes related to the aging process. Therefore, there has been a long-standing interest in the development of blood-based biomarkers of aging that could be used to predict changes in brain morphology and/or cognitive decline. Several authors have recently developed a novel biomarker of aging based on DNA methylation levels. In particular, using 82 Illumina DNA methylation array data sets involving 51 healthy tissues and cell types, Horvath developed a multi-tissue predictor of age which allows one to estimate the DNA methylation (DNAm) age of most tissues and cell types (2013).

Here we will test the hypothesis that this novel biomarker of aging (applied to blood) is more significantly associated with MRI-defined changes in brain morphology and cognitive decline than chronological age.

**5. Main Hypothesis/Study Questions**:
We hypothesize that aging effects measured by DNA methylation in blood tissue predict both morphological changes in the brain (as measured on MRI) as well as cognitive decline.

**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).**

We will use the previously-collected DNA methylation data on ~2900 African-Americans. Methylation at 485,577 CpGs was assessed using the GenomeStudio Methylation Module on the intensity files (.idat) produced by the Illumina iSCAN system. Methylation fraction values with detection p-values>0.01 were set to missing and quality control (QC) analyses were performed on the processed data. Sample-level QC analyses excluded sample data with >1% missing values across all probes; probe-level QC analyses excluded probes with >1% missing values across all samples.
While we have used the Subset-quantile Within Array Normalization (SWAN) method for data normalization, in order to make our data comparable to that of Horvath, we will apply the same normalization method (BMIQ). While normalization alleviates the batch effect problem known to occur with high throughput data, it does not sufficiently control for unwanted variability in the data stemming from experimental or other sources. Principal variance component analysis was used to determine which sources of variability are most prominent. Using the eigenvalues associated with their corresponding eigenvectors as weights, associated variations of all factors are standardized and the magnitude of each source of variability is presented as a proportion of total variance. We identified Plate Number, ChipID, Chip Row, and Visit as strongest sources of unwanted variation, which will be corrected for in the analyses.

We will use the 353 CpG identified by Horvath (2013) to define DNA methylation age. R scripts are available to calculate DNAm age based on the coefficient values of an elastic net regression model in a training set developed by Horvath and will be applied here.

(1) We will first evaluate the correlation between DNA methylation age and chronological age in the ARIC sample.
(2) We will evaluate whether age acceleration (defined as either the difference between DNAm age and chronological age or the regression value of the DNAm age on the chronological age) is associated with brain MRI phenotypes, including brain volume, hippocampal volume, lobar volumes (frontal, occipital, temporal and parietal), white matter volume and integrity, and grey matter volume.
(3) We will evaluate whether age acceleration (defined as either the difference between DNAm age and chronological age or the regression value of the DNAm age on the chronological age) is associated with longitudinal change in brain MRI phenotypes between V3 and ARIC NCS.
(4) We will evaluate whether age acceleration (defined as either the difference between DNAm age and chronological age or the regression value of the DNAm age on the chronological age) is associated with cognitive decline (between V2 and NCS) and dementia status (at NCS visit). Cognitive function will be measured by three standard test, including delayed word recall (DWRT), Digit Symbol Substitution (DSST), and word fluency test (WFT).
(5) We will repeat the analyses above DNAm age and chronological age in the models to examine whether DNAm age is associated with changes in brain MRI phenotypes and cognitive decline above and beyond chronological age.

Linear (or logistic) regression models modeling brain MRI phenotypes (or dementia status) in relation to age acceleration (defined as either the difference between DNAm age and chronological age or the regression value of the DNAm age on the chronological age), adjusting for sex, field center, blood pressure, and DNAm technical covariates will be used to estimate cross-sectional associations. Statistical analyses will be performed incorporating sampling weights (derived by the ARIC coordinating center) to account for the ARIC NCS brain MRI selection process that was designed to oversample cognitively impaired individuals.

To evaluate the association of age acceleration with measures of cognitive decline, we will use generalized estimating equations (GEE), which take into account the intra-individual correlation of scores on repeated cognitive tests. We will assume an unstructured correlation. The models will include age acceleration, follow-up time, and their interaction term, and sex, smoking, diabetes, hypertension, APOE genotype, education. Persons with substantial cognitive impairment are more likely to drop out of the study or the die before the next study visit. Therefore, we will consider inverse probability of attrition weighting (IPAW) to account for differential dropout.

**Reference**

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

**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? __X__ 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)?**

Jim Pankow's proposal "Validation of DNA methylation age predictors and association with long-term survival: the Atherosclerosis Risk in Communities Study". This work will be performed in collaboration with Dr. Pankow's project.

**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* _____)**
**__X__ 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.