ARIC Manuscript Proposal #3774 (Revised)

PC Reviewed: 5/11/21	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Replication Request for: Omic signatures of vitamins C and E

b. Abbreviated Title (Length 26 characters): Omic signatures of vit C & E

2. Writing Group:

Writing group members: Kristin Young, Misa Graff, Anne Justice (member of the ARIC epigenetics working group), Roby Joehanes, Jiantao Ma, Michael Mendelson, DH Lee, Joshua Keefe, Shih-Jen Hwang, Paul Jacques, Dan Levy, other investigators welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __KLY___ **[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 (this must be an ARIC investigator).
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3. Timeline:

1.5 years (July 2021 – Sept 2022)

Replication cohort data QC/statistical analyses: July-August 2021

Interpretation and meta-analyses: August 2021 – Dec 2021

Manuscript preparation: Jan-July 2022 Manuscript submission: Sept 2022

4. Rationale: Many older adults suffer from chronic conditions such as chronic obstructive pulmonary disease and chronic kidney disease, and the need for treatments has a significant impact on the US health care budget.[1] Developing a preventative approach has the potential to mitigate this rise in healthcare costs, but this relies on understanding biological mechanisms related to healthy lifespans and identifying novel treatment targets that can lengthen "healthspan." One such mechanism that might underlie the aging process is DNA methylation, which has been shown to affect many physiological processes such as gene expression.[2] In fact, DNA methylation has been highlighted as a contributor to the **molecular aging clock** (MAC)[3,4], which is a composite score calculated from levels of DNA methylation. In addition to predicting mortality[4-6], there is some evidence that DNA methylation is predictive of accelerated aging in infants[7-9], children[9,10], and adolescents[10], thus affording it potential prognostic utility for predicting healthy aging.
Quantitative metrics such as the MAC also offer the possibility of testing whether two common antioxidant vitamins, vitamins C and E, affect healthy aging. Vitamins C and E have been implicated in multiple aging pathways,[11–14] and have been shown to interact with each other in the human body.[15,16] Recent in vitro studies have implicated vitamin C in DNA demethylation[17,18], which in turn affects downstream gene expression.[19] However, it remains unknown how the effects of vitamins C and E on DNA demethylation might be involved in well-known aging pathways related to healthy aging. This knowledge may hold the key to identifying potential biomarkers and therapeutic targets for healthy aging.

5. Main Hypothesis/Study Questions: To investigate DNA methylation and gene expression signatures of vitamin C and E intake. (NOTE: ARIC will only be conducting replication for the methylation data, as gene expression data are not available)

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 analyze the association between vitamin C and E intake (with or without supplements) and visit 1 DNA methylation. We will use linear-mixed models for DNA methylation-phenotype associations in ARIC accounting for the following covariates:

Model 1: sex, age, BMI, differential cell counts[20,21], caloric intake, potential batch effects and family relatedness.
Model 2: Model 1 + dichotomous smoking status

We will calculate effect size, standard error, t-statistic, R-squared, and p-value for each CpG, and use false discovery rate or Bonferroni correction to account for multiple comparisons. We will join meta-analysis efforts with other CHARGE cohorts.

We will also perform gene set enrichment analysis (GSEA) to identify pathways implicated by the CpGs and/or transcripts arising from meta-analysis results. We will identify relevant phenotypes from GWAS databases arising from the CpGs and/or transcripts.

Vitamin C & E Analysis Plan

Phenotype:
- Vitamin C & E intake from FFQ (including supplement use, see notes below)
- No transformation

Intake category	Vitamin C	Vitamin E
Low	0mg – 75mg (females) 0mg – 90mg (males)	0mg – 15mg
Medium	75mg – 300mg (females) 90mg – 300mg (males)	15mg – 22.5 mg
High	> 300mg	> 22.5 mg

Outlier removal: Remove all phenotypes outside ± 4 SD.

Deliverables:
1) Visit 1 DNA methylation: Results for all CpG sites that passed QC.

Scale: Methylation: beta proportion (range: 0 to 1), using your favorite normalization method. Framingham uses DASEN of WateRmelon Package of R

Models:
For each model below, please collect effect values (beta coefficients), standard error, P-value, Partial R2, FDR, and overall FDR for every CpG site. You will have one set of values for the medium intake category and one set of values for the high intake category:

CpG/RNA transcript	β med.	SE, med.	P-value, med.	Partial R^2, med.	FDR, med.	β, high	SE, high	P-value, high	Partial R^2, high	FDR, high	P-value, overall	FDR, overall

Model 1
Methylation beta = phenotype + sex + age + caloric intake + BMI + CD8T + CD4T + NK + Bcell + mono + technical covariates

Model 2
Methylation beta = Model 1 + trichotomous smoking status (current/former/never)

Model 3
Methylation beta = Model 2 + Mediterranean diet score + physical activity index

Definitions:
- Phenotype: either Vitamin C intake or Vitamin E intake, categorical as defined above
- Caloric intake: daily calories consumed, continuous
- Smoking, categorical
    - Current smokers: Anyone who has smoked at least one cigarette per day within the last 12 months.

    - Former smokers: Anyone who has previously smoked at least one cigarette a day, but has stopped for at least 12 months.
    - Never smokers: Anyone who has never smoked.
- CD8T, CD4T, NK, Bcell, Mono: estimated blood cell counts from Houseman et al. NOTE: including Gran will make the equation nearly collinear, and so it is not included in this or any model. If you have directly measured cell counts or better imputation methods, feel free to use them. If your sample source came from single-type cell source (e.g., monocytes), then omit these terms.
- Technical covariates are cohort specific. For Framingham's DNA methylation dataset, we use Row, Column, Chip, and a few PCs. So, feel free to add your technical covariates as necessary.

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/aric/mantrack/maintain/search/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)? none

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 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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