

ARIC Manuscript Proposal #2802

PC Reviewed: 7/12/16

Status: A

Priority: 2

SC Reviewed: _____

Status: _____

Priority: _____

1.a. Full Title: Epigenetic Factors Influencing Central Adiposity: The ARIC Study

b. Abbreviated Title (Length 26 characters): EWAS of Central Adiposity

2. Writing Group:

Writing group members: Anne Justice, Ellen Demerath, Jan Bressler, Myriam Fornage, Megan Grove, Kari North, Weihua Guan, Lindsay Fernandez-Rhodes, Eric Boerwinkle and other investigators welcome

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

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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 year

- Study-level statistical analyses: May-June 2016
- Interpretation and meta-analyses: July-August 2016
- Manuscript preparation: September-December 2016
- Manuscript submission: February 2017

4. Rationale: Elevated central adiposity is a recognized risk factor for cardiometabolic disease (CMD)¹⁻³, however, rates of obesity, and particularly central obesity, have more than doubled in the U.S. over the past three decades⁴⁻⁶. Further, there are stark differences

in obesity risk among minorities⁴⁻⁷ in body fat distribution and its genetic predisposition^{8,9}. While the genetic influence of central adiposity is well-established¹⁰, there are multiple critical questions that remain unanswered, which, if answered, could lead to important discoveries about potentially preventable contributors to obesity. One such priority is the importance of epigenetic factors in the pathogenesis of obesity as highlighted in the 2011 NIH Strategic plan for obesity. DNA methylation is an important epigenetic mechanism that links genotypes, the environment, and obesity, but methylation studies have primarily been conducted in small studies of only European descent (EUD) subjects, with limited consideration of the possible role of clinically relevant environmental influences. Also, there is a dearth of epigenetic studies of central adiposity¹¹⁻¹³. The identification of epigenetic factors that influence the pathogenesis of central obesity may allow for the identification of biomarkers for risk and/or progression, and thus new public health interventions¹⁴.

Epigenetics is creating a paradigm shift in health sciences, providing a dynamic mechanistic framework for identifying the molecular pathways linking environmental insults to disease risk¹⁴. Total methylation density is a known risk factor for many chronic diseases, and varies strongly with age and sex; however DNA methylation and other features of the epigenome are also modifiable by environmental factors such as smoking^{15,16}, which also has a strong influence on central adiposity.

The discovery of epigenetic variation influencing adiposity-related traits has the potential to identify important pathways for disease prediction and treatment¹⁷. Yet, the bulk of this research has focused on homogeneous populations, with very few genetic studies on ancestrally diverse, admixed populations¹⁸. In the U.S., individuals of African American (AA) ancestry have elevated burden of hypertension, obesity, insulin resistance, impaired glucose metabolism, and ensuing cardiometabolic diseases (CMD), compared to other U.S. populations^{3,5,6}. Yet, epigenetics has just begun to address more diverse populations^{13,19}. Studies that interrogate obesity epigenetics across a range of ethnic groups are critical to gain a comprehensive understanding of the genomic architecture of these traits and to allow this information to be used to improve public health.

5. Main Hypothesis/Study Questions:

Aim: Conduct an epigenome wide association analysis (EWAS) of central adiposity with direct comparison of effects across race/ethnicity, using extant phenotypic and Illumina HumanMethylation 450K Beadchip (HM450K) data in 2,861 AA and 939 European descent (EUD) participants in the Atherosclerosis Risk in Communities (ARIC) study. A) Identify methylation quantitative trait loci (meQTL) associated with central adiposity (waist circumference [WC], WC to hip ratio [WHR], and WC to height ratio [WHtR] adjusted for BMI). B) Replicate findings in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

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 analysis using data taken from **visit 2 or 3** (dependent on concordantly measured methylation data) in the ARIC population-based cohort.

Inclusion:

- Adults ≥ 18 years of age
- European and African American Ancestry

Exclusion:

- Individuals < 18 years of age
- Pregnant women
- Missing epigenetic data
- Missing or unrealistic values ($\pm 4SD$ from the mean) for outcome (waist circumference, hip circumference, waist-to-hip ratio, height) data
- Missing covariate (BMI, age, PCs, study center, smoking status, and sex) data

Outcomes: WC, WHR ratio, and WHtR ratio, all adjusted and unadjusted for BMI.

Genotype data:

- HM450 array

Summary data analysis:

Discovery Analyses. To determine if site specific β values of methylation probes are associated with central adiposity, measured as WC and WHR both adjusted for smoking, and WtHR, we will employ linear mixed models (LMM) in R with methylation β values as the independent variable, central adiposity as the dependent variable, and with chip array specified as a random

effect. Additionally, the following variables will be tested for inclusion as potential fixed effects: 10 principal components scores (PCs) from the HM450 array to account for potential confounding by genetic ancestry, study center, WBC count, sex, age, study center, education, household income, current smoking status, current alcohol consumption, and physical activity. The final choice of covariates will be

based on the Bayesian model averaging (BMA) algorithm for linear regression models to choose the best fit model for central adiposity without meQTL. BMA will be implemented using the R package BMS v0.3.0. Additionally, all analyses will be conducted stratified and meta-analyzed across self-identified race/ethnicity.

Table 4. Replication cohorts available through CHARGE Consortium.

Cohort Name	Ethnicity(ies)	Smoking	Sample Available Size
EGCUT	European Descent	Y	165
FHS/FHSgen2	European Descent	Y	2,846
GOLDN	European Descent	Y	993
InChianti	European Descent	Y	472
KORA	European Descent	Y	499
RS	European Descent	Y	503
TwinsUK	European Descent	Y	318
WHI	African- and European- American	Y	406

Replication Analyses. MeQTL sites with association p values $< 1.03 \times 10^{-7}$ (chip-wide significance [CWS] corrected for number of CpG variants tested) for each trait in

ancestry-specific and/or across ancestry meta-analysis will be carried forward for replication in participating CHARGE cohorts (**Table 1**). Given the different analytic strategies and cell types used among the replication studies, I will conduct a sample size-weighted meta-analysis implemented in METAL. Replication will be defined as consistent direction of the beta coefficient, and a CWS meta-analysis p value. As in the discovery analysis, all analyses will be conducted stratified and meta-analyzed across self-identified race/ethnicity.

Limitations/challenges: The methods discussed herein may be supplemented or altered as newly established methods develop. I will discuss my preferred analytical method with an understanding of the limitations and propose an alternative should such limitations arise.

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.c.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)?

-“Genome-wide methylation analyses of cardiovascular disease (CVD) and its risk factors” #1928

-“ Epigenome-wide association study of obesity traits in African American adults: The Atherosclerosis Risk in Communities (ARIC) Study” #2106

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.csc.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.csc.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.

Bibliography

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