

Population Architecture using Genomics and Epidemiology (PAGE)

Ver. 02/25/21

PAGE Manuscript Proposal Template

Submit proposals by email to the PAGE Coordinating Center to Natalie Nudelman
(nmakow@hginj.rutgers.edu)

Before submitting this form to Natalie, please fill out the yellow portion of the

PAGE II & III Ongoing Papers Google Sheet, see,
https://docs.google.com/spreadsheets/d/17E7E5G4u_nYWZEKzqqeRigwRbGxYzVNsUUIVjXCytY/edit#gid=1451131466

Do not exceed 3 pages in length (not including references).

PAGE Ms. Number: 4198 **Submission Date:** 2/14/23 **Approval Date:**

Title of Proposed MS: Epigenome wide association study of BMI, visceral adipose tissue, and subcutaneous adipose tissue including MESA and the Jackson Heart Study

I. INVESTIGATOR INFORMATION:

Name of Lead Author:

Kendra Ferrier

Email Address:

Kendra.ferrier@cuanschutz.edu

Authorship model*:

Junior Investigator?

Name of Corresponding Author (if different): Leslie Lange

Email Address: Leslie.lange@cuanschutz.edu

Names, affiliations and email address of PAGE Investigators proposed as co-authors:

Name	Affiliation in PAGE	Email
Heather Highland	UNC	heather.highland@unc.edu
Misa Graff	UNC	migraff@email.unc.edu
Chris Gignoux	CCPM	chris.gignoux@cuanschutz.edu
Leslie Lange	REGARDS	LESLIE.LANGE@CUANSCHUTZ.EDU
Laura Raffield	JHS	laura_raffield@unc.edu
April P. Carson	JHS	apcarson@umc.edu
Kari North	ARIC	kari_north@unc.edu
Steve Rich	MESA	ssr4n@virginia.edu
Jerome Rotter	MESA	jrotter@lundquist.org

Partner studies collaborating with PAGE in this ms. proposal: JHS

II. SCIENTIFIC RATIONALE (Please be specific and concise)

The global prevalence of obesity, defined as a body mass index (BMI) greater than 30 kg/m², has nearly tripled in the last 50 years, reaching pandemic proportions. Obesity is a significant contributor to an individual's risk of metabolic, cardiovascular, and

musculoskeletal diseases, Alzheimer's disease, depression, and numerous cancers. Furthermore, obesity has surpassed smoking as the highest-ranking modifiable risk factor for premature death. Obesity is a leading cause of reduced quality of life, disability, and early retirement. In the U.S.A., non-Hispanic black adults are disproportionately burdened by obesity (49.6%), followed by Hispanic (44.8%) and non-Hispanic white (42.2%) adults. Genetics has been shown to be a significant contributor to obesity risk, with MZ/DZ twins reared apart compared to MZ/DZ twins reared together showing that MZ twins have higher concordance of BMI than DZ twins, regardless of childhood (reared together/apart) environment (Stunkard AJ, et al., N Engl J Med 1990;322:1483). While both genetic and environmental factors have roles in the etiology of obesity, the impact of "environment" on genetics is not understood. Environment, whether through specific exposures or societal factors, may help explain the observed differences in obesity (BMI) across racial and ethnic groups. DNA methylation is one epigenetic mechanism that is affected by environmental factors, providing a crucial link between genetics, the environment, and disease. DNA methylation can be influenced by diet, hormones, stress, as well as exposure to chemicals or radiation. Few epigenome-wide studies have been conducted on deeply phenotyped longitudinal cohorts, and fewer still in ancestrally diverse populations. Although BMI does not reflect tissue-specific adiposity, it is a widely used measure in genetic studies as it broadly estimates body fat distribution. Currently, BMI is the recommended adiposity-related biomarker for cardiovascular disease, type 2 diabetes, and all-cause mortality, despite its modest correlation with body fat composition and direct measurements of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). In particular, the ratio VAT/SAT (as measured by CT or MRI) provides a metric of the propensity to store fat viscerally versus subcutaneously, measures amenable to genetic and DNA methylation analyses. As VAT and SAT are imaging measures and are limited in large epidemiologic studies, examining VAT and SAT in smaller sample sizes with BMI may provide insights into understanding the genetic epidemiology and epigenetic effects on obesity to motivate strategies for prevention and treatment of obesity worldwide.

III. OBJECTIVES AND PLAN (Please be specific and concise)

a. Study Questions/Hypotheses.

We propose to use methylation panel (Illumina Human EPIC in MESA and JHS; Illumina450K in ARIC and WHI) to perform EWAS analysis for BMI, VAT, SAT, and VAT/SAT. BMI analysis will be cross-sectional (i.e. methylation and BMI both from Visit 1) and VAT(SAT) analyses (MESA and JHS only) will be using methylation from Visit 1 and VAT(SAT) from Visit 2 (for both MESA and JHS).

1. BMI-associated CpGs will be identified in the African American EWAS meta-analysis of JHS and MESA results, and some will replicate in ARIC and WHI African Americans.
2. VAT(SAT)-associated CpGs will be identified in the African American EWAS meta-analysis of JHS and MESA results.
3. Additional BMI-associated CpGs will be identified in the multi-ethnic EWAS meta-analysis in JHS, MESA, ARIC, and WHI.

b. Outcomes, Covariates, OMICs Data Considered.

- Genetic data: genetic ancestry principal components

- Omics data: Methylation array data
- Outcome data: methylation M-values, BMI, visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT)
- Covariates: Age, centered-mean-age-squared, sex, smoking status (never/past/current), race/ethnicity, study, center, estimated blood cell type proportions, and technical covariates.

c. Analytical Approach

BMI, VAT, SAT, and VAT/SAT will be used as the main predictors and Methylation M-values as the outcome in the linear regression models. Ideally, the M-values will be adjusted for technical covariates (batch, plate, well, etc.) using the ComBat function in the sva R package prior to analysis. If this is not possible then the technical covariates can be added as covariates. Other covariates will include age, centered-mean-age-squared ($\text{age} - \text{mean}(\text{age})^2$), sex, smoking (never = 0, past = 1, current = 2), the first ten genetic ancestry principal components, and estimated blood cell proportions using the Houseman method (Houseman et al., 2012). The general linear regression model that will be used is: Methylation (M value) ~ adiposity measure (BMI, VAT, SAT, or VAT/SAT ratio) + covariates. After performing linear regression analyses, the results will be adjusted for bias and inflation using the BACON R package. The significant p-value threshold will be Bonferroni adjusted to account for multiple testing $\alpha = (0.05 / (\text{number of CpG sites tested}))$. Meta-analyses of JHS and MESA results will be performed using the inverse-variance weighted method (METAL). For any significant CpGs identified near established BMI- or obesity-related genetic loci, we will assess whether the CpG(s) mediate the association between the genetic variant and the adiposity outcome (BMI, VAT, SAT, VAT/SAT ratio). Because methylation was measured at visit 1 but VAT and SAT were measured at visit 2, and there is variable time between visits 1 and 2 for participants, we will perform a sensitivity analysis that includes time since visit 1, VAT (or SAT), and an interaction term for time since visit 1 – by - VAT (or SAT) to assess whether any observed significant associations between methylation and VAT (SAT) are modified by time since visit 1 for these measures. Finally, For any significant CpGs identified near established BMI- or obesity-related genetic loci, we will assess whether the CpG(s) mediate the association between the genetic variant and the adiposity outcome. We will conduct the mediation analysis as based on the methods outlined by Baron and Kenny.

d. Anticipated date of draft manuscript to P&P: Spring 2023

e. What manuscript proposals listed on www.pagestudy.org/index.php/manuscripts/ are most related to the work proposed here? Approved PAGE ms. numbers: _____

– If any: Have the lead authors of these proposals been contacted for comments and/or collaboration? Yes__ No__

IV. SOURCE OF DATA TO BE USED (Check all that apply)

Study Name	Included	Analysis 1	Analysis 2	Analysis 3
ARIC	x	yes	no	yes
JHS	x	yes	yes	yes
WHI	x	yes	no	yes
MESA	x	Yes	Yes	yes

I, _KRF_, affirm that this proposal has been reviewed and approved by all listed investigators.

V. REFERENCES

1. World Health Organization. *Obesity and overweight*. 2020 1 Apr [cited 2020 9 Jun]; Available from: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>.
2. Blüher, M., *Obesity: global epidemiology and pathogenesis*. Nature Reviews Endocrinology, 2019. **15**(5): p. 288.
3. Dhana, K., et al., *An epigenome-wide association study of obesity-related traits*. American journal of epidemiology, 2018. **187**(8): p. 1662-1669.
4. Hales, C.M., et al., *Prevalence of obesity and severe obesity among adults: United States, 2017–2018*. 2020.
5. Campanella, G., et al., *Epigenome-wide association study of adiposity and future risk of obesity-related diseases*. International Journal of Obesity, 2018. **42**(12): p. 2022-2035.
6. Demerath, E.W., et al., *Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci*. Human molecular genetics, 2015. **24**(15): p. 4464-4479.
7. Sayols-Baixeras, S., et al., *DNA methylation and obesity traits: An epigenome-wide association study. The REGICOR study*. Epigenetics, 2017. **12**(10): p. 909-916.
8. Wahl, S., et al., *Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity*. Nature, 2017. **541**(7635): p. 81-86.
9. Hoffmann, T.J., et al., *A large multiethnic genome-wide association study of adult body mass index identifies novel loci*. Genetics, 2018. **210**(2): p. 499-515.
10. Meeks, K.A., et al., *An epigenome-wide association study in whole blood of measures of adiposity among Ghanaians: the RODAM study*. Clinical epigenetics, 2017. **9**(1): p. 103.
11. Carroll, J.F., et al., *Visceral fat, waist circumference, and BMI: impact of race/ethnicity*. Obesity, 2008. **16**(3): p. 600-607.
12. Neeland, I.J. and J.A. de Lemos, *Time to retire the BMI?: evaluating abdominal adipose tissue imaging as novel cardiovascular risk biomarker*. 2016, Journal of the American College of Cardiology.
13. Fox, C.S., et al., *Genome-wide association for abdominal subcutaneous and visceral adipose reveals a novel locus for visceral fat in women*. PLoS genetics, 2012. **8**(5).
14. Kaess, B., et al., *The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk*. Diabetologia, 2012. **55**(10): p. 2622-2630.