1/12/17

Dear ARIC P and P Committee

The following authors have contacted me for replication of their BMI EWAS study. We are providing them *in silico* replication results for meta-analysis. This work falls under the approved ms proposal #2106. The following ARIC authors will be included on the manuscript now in preparation (abstract below):
E. Demerath
S. Nguyen
M. Grove
W.Guan
J.Pankow

Thank you,
Ellen Demerath
****

Novel associations between blood DNA methylation and body mass index in middle-aged and older adults

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Abstract

There is increasing evidence of a relationship between blood DNA methylation and body mass index (BMI). We aimed to assess associations of BMI with individual methylation measures (CpGs) through a cross-sectional genome-wide DNA methylation association study and a longitudinal analysis of repeated measurements over time. Using the Illumina Infinium HumanMethylation450 Beadchip, DNA methylation measures were determined in peripheral blood samples from 5,361 adults recruited to the Melbourne Collaborative Cohort Study (MCCS) and selected for nested cancer case-control studies. For a subset of 1,088 controls, these measures were repeated using blood samples collected at wave 2 follow-up, a median of 11 years later; weight was measured at both time points. Associations between BMI and blood DNA methylation were assessed using linear mixed effects regression models adjusted for batch effects and potential confounders. Cross-sectional analysis of data from 2,775 controls identified 66 CpGs associated with BMI ($p<1.0\text{x}10^{-7}$). Highly consistent results were observed using data from 2,586 cases, with no empirical evidence of bias towards false-positives. A meta-analysis of control and case data identified 289 novel BMI-associated CpGs ($P<1.0\text{x}10^{-7}$) and replicated 50 of 83 previously reported associations ($P<1.2\text{x}10^{-3}$). Consistent associations between change in BMI and change in methylation were observed for 34 of these ($P<0.05$), including a CpG in *ABCG1* ($P=2.0\text{x}10^{-4}$), which is involved in cholesterol and phospholipid transport. Together, these findings suggest that BMI is associated with blood DNA methylation at a large number of CpGs across the genome and that methylation in *ABCG1* might be a predictive marker of weight change.

ARIC Manuscript Proposal #

PC Reviewed: ___/___/13 **Status: _____** **Priority: ____**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Epigenome-wide association study of obesity traits in African American adults: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters):

2. Writing Group: ARIC Epigenetics Working Group

Writing group members:

Ellen Demerath
Weihua Guan
Jan Bressler
Myriam Fornage
Chindo Hicks
Tom Mosley
Linda Kao
Eric Boerwinkle
Kari North
James Pankow
Others welcome…

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

Name:
Address:

Phone: Fax:
E-mail:

3. Timeline:

Preliminary analysis is underway (February 22, 2013). Draft ready to submit for Publications Committee review in July, 2013.

4. Rationale:

Epigenetics. Epigenetics is the study of mitotically heritable modifications in chromatin structure (i.e., modifications not involving the underlying DNA sequence), and their impact on the transcriptional control of genes and cellular function. Epigenetic variation includes post-translational modifications of histone proteins, non-coding RNAs, and DNA methylation, the latter primarily occurring at cystosine-guanine dinucleotides (CpGs). Although the placement of epigenetic marks is thought to be largely determined early in development to initiate and maintain cell-type specific gene expression (Armstrong, 2012), DNA methylation and other features of the epigenome are modifiable by environmental factors such as the nutrient content of the diet (Dolinoy et al., 2006), maternal behavior and stress (Weaver et al., 2004), and environmental pollutants (Baccarelli et al., 2009). Understanding epigenetic variation may therefore help to explain, at least in part, the mechanisms by which environmental factors of public health importance influence genetic susceptibility to a variety of diseases.

Available Epigenetic data in ARIC: Of the different forms of epigenetic modification, DNA methylation is the most extensively studies and best understood. Recent technological advances have provided multiple platforms for systematically interrogating DNA methylation variation across the genome (Laird, 2010). This has paved the way for epigenome-wide association studies (EWASs), analogous to genome-wide association studies, to evaluate regions of the genome in which variation in DNA methylation may influence gene expression and ultimately disease risk (Raykan, 2011). In ARIC, the recently released Illumina 450K Infinium Methylation BeadChip has being used to measure DNA methylation in peripheral blood obtained from approximately 3,000 African American participants at visit 2 (and a small number at visit 3). The array includes 485,577 assays and provides coverage of 98.9% of RefSeq genes with a global average of 17.2 probes per gene region (Bibikova, 2011; Dedeurwaerder, 2011). The ARIC epigenetics working group has been working to develop QC procedures, compare different analytic approaches, and identify CpG (cytosine-guanine dinucleotide) sites that are influenced by sex, age, and other potential confounding factors (see ARIC Ms Proposals #1928 and #1929). The present proposal focuses on obesity and adiposity traits, one phenotype class specified in the "umbrella", over-arching ARIC Ms Proposal related to DNA-methylation-phenotype association studies and lead researchers (MS #1928).

Obesity and Epigenetics. Obesity is a leading cause of United States mortality, morbidity, disability, healthcare utilization and healthcare costs. Obesity and body fat distribution are potent risk factors for type 2 diabetes (Wang et al., 2005; Carey et al., 1997; Sesai et al., 2010) and cardiovascular disease, including coronary heart disease (CHD) (Hubert, 1983; Folsom et al., 1998; Rexrode et al., 1998; Rexrode et al., 2001; Wilson et al., 2002), among other chronic conditions including many cancers (Calle et al., 2003). Current understanding of obesity genetics and the interaction of genetic susceptibility with environmental and behavioral exposures is still only partial, but such knowledge as the important potential to personalize obesity prevention and treatment.

To date there have been only a handful of human studies examining the relationship of obesity-related traits to DNA methylation; most have examined methylation near known obesity candidate genes, but epigenome-wide association analyses are now also beginning to be published (reviewed in Drong et al., 2012). A number of these have demonstrated association of FTO risk alleles with local CpG methylation variation (Almen et al., 2012; Bell et al., 2010; Toperoff et al., 2012). Existing studies are generally small (N<200) and cross-sectional, however, and therefore have not had sufficient statistical power or appropriate study designs to test the complex interactions among genotype, environment, and DNA methylation variation that likely exist. A summary of published studies examining the relationship of obesity traits with DNA methylation is provided in **Table 1** (adapted from Drong et al., 2012). To our knowledge, none of these studies has focused on African ancestry individuals, despite the fact that African ancestry groups tend to carry higher chronic disease risk factor loads, including greater obesity, compared with European ancestry populations (Harris, 1990; Brancati et al., 2000; El-Sayed et la., 2011). For instance, in ARIC, the incidence of diabetes was 2.4-fold greater in African-American women and 1.5-fold greater in men than in their white counterparts, which was strongly predicted by their greater adulthood rate of weight gain (Brancati et al., 2000). Greater waist-hip ratio, a marker of visceral adiposity, was also found for African American women in ARIC (Brancati et al., 2000) and this adverse deposition pattern may be one reason why African American women have lower adiponectin and higher pro-inflammatory adipokine profiles than Caucasian women, even after adjustment for total body fatness (Khan et al., 2012).

Table 1. DNA methylation candidate gene and epigenome-wide association studies for obesity

Candidate Gene or EWAS	Phenotype/s	N (Case Control or Quant. Trait)	Tissue type		Reference
Obesity					
ALOX12, ALPL, BCL2A1, CASP10, CAV1, CCL3, CD9, CDKN1C, DSC2, EPHA1, EVI2A, HLA, IRF5, KRT1, LCN2, MLLT4, MMP9, MPL, NID1, NKX31, PMP22, S100A12, TAL1, VIM	BMI, fat mass, and lean mass	qt: 178	Umbilical cord blood		Relton (2012)
KCNQ1OT1, H19, IGF2, GRB10, MEST, SNRPN, GNAS	BMI (discordance in twins)	c/c: 16/16	Saliva		Souren (2011)
MCHR1	BMI	qt: 49	Whole blood		Stepanow (2011)
POMC	Obesity	c/c: 71/36	Whole blood	c/c: 54/100	Kuehnen (2012)
IL8, NOS3, PIK3CD, RXRA, SOD1	Fat mass and %fat mass	qt: 78	Umbilical cord tissue	qt: 239	Godfrey (2011)
SLC6A4	BMI, weight, and waist circumference	qt: 168	Peripheral blood leukocytes		Zhao (2012)
TACSTD2	Fat mass	qt: 94	Whole blood	qt: 161	Groom (2012)
EWAS	BMI	qt: 64	Lymphocytes		Feinberg (2010)
EWAS	Obesity	c/c: 7/7	Peripheral blood leukocytes	c/c: 46/46	Wang (2010)
EWAS	Obesity	c/c: 23/24	Whole blood		Almen (2012)

Adapted from: Drong et al. *Clinical Pharmacology & Therapeutics* (2012); **92** 6, 707–715.

Preliminary Analyses and Power: The ARIC study has the largest genome-wide DNA methylation database to our knowledge yet assembled for African Americans. There are a total of 2,861 individuals with concurrent DNA methylation and BMI data, and 2,867 with concurrent DNA methylation and waist circumference data. There were a total of 426 individuals (~15%, 50% female) with BMI<25 at the time of their DNA visit, and 165 subsequently had a BMI>=25 at a later visit (=N cases incident overweight). There were a total of 1,338 individuals (~47%, 55% female) who had a BMI <30 at the time of their DNA visit, and 256 of these subsequently had a BMI >30 at a later visit. With this large sample size and the rich longitudinal phenotypic data set, the ARIC study is well-positioned to address the hypothesis that CpG site-specific DNA methylation variation is associated with adiposity, both cross-sectionally and prospectively, and with careful consideration of potential confounders and effect modifiers such as sex, smoking, and physical activity level. Power calculations indicate that we will have excellent power for tests of association between methylation level and quantitative traits such as waist circumference, or change in waist circumference over time. With N > 2,800 individuals, we will have 80% power to detect a contribution to R^2 of at least 0.015 (1.5% variation explained), and 96% power to detect a contribution of 0.020 or greater, for genomewide significant, Bonferonni-correct p <

$2x10^{-6}$ ($p< 0.05/450,000$). Adequate power to detect association has furthermore been established empirically by our preliminary finding of 20 CpG site associations with BMI, at $p < 1 \times 10^{-7}$. In these analyses, each unit increase in BMI (kg/m^2) was associated with 1-3% difference in CpG site beta value, showing that we have power to detect very small differences in CpG methylation.

5. Main Hypothesis/Study Questions:

1) We will test whether methylation variation is associated with obesity traits before or at the time of the DNA collection (BMI at DNA visit, BMI at age 25, weight gain from age 25 to their DNA visit, circumferences, central adiposity ratios (WHR, etc), and skinfolds), independent of potential confounders including sex, age, percent European ancestry, physical activity, socioeconomic status, smoking status and pack-years of smoking, and diabetes at their DNA visit.
    a. Exploratory analyses using a subset of subjects from the ARIC-Jackson Heart Study shared cohort (N~500) having Abdominal CT measures of visceral adipose tissue area (VAT) and subcutaneous adipose tissue area (SAT) will be conducted similarly to assess abdominal adipose tissue deposition variation.

2) We will test whether methylation variation is associated with incident obesity among individuals who were not obese at the time of their DNA visit, and whether methylation variation is associated with subsequent change (slope) for weight, waist, and skinfolds.

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 data from the ARIC visits 1-5.

Inclusions/Exclusions:
- Missing DNA methylation data
- Missing adiposity exposure and outcome
- Missing covariate data

Sample Size estimate:
Preliminary analyses show we will have approximately 2,861 African American adults with concurrent BMI and DNA methylation data.

Identifiers/Demographics
Patient ID, Sex, Date of DNA collection visit (visit 2 or 3), Field center, Age, Education, Household income

Obesity Traits
Aim 1) Cross-sectional analysis; Weight at age 25, Weight, Height, BMI, Waist circumference, Hip circumference, Waist-Hip ratio, Waist-height ratio at same visit as when DNA was collected for methylation analysis are the exposures, and DNA methylation is the outcome/dependent variable.

Exploratory subset: CT measures of VAT and SAT from Jackson visit 2

Aim 2) Prospective analysis; DNA methylation is the exposure variable and incident cases of overweight (at least 1 BMI ≥25.0 in subsequent visits for individuals whose BMI at DNA visit was <25.0); Incident cases of obesity (at least 1 BMI ≥ 30 in subsequent visits for individuals whose BMI at DNA visit was <30); and Change (slope) in BMI, Waist circumference, and skinfolds are the dependent variables.

Methylation values
Methylation level (beta values, ranging from 0 to 1.0) at each of approximately 485,000 CpG sites will be analyzed as continuous variables. The beta value can be interpreted as the percent of the time that the CpG is methylated in a given DNA sample. Although across the genome, most CpG sites are either highly methylated (e.g., mean beta near 0.80) or are not highly methylated (e.g., mean beta near 0.15), nonetheless at a given CpG site, variation approximates normality, allowing standard linear regression approaches to be used. An alternative is to use the M-value, which although less easily interpretable, provides better performance in terms of Detection Rate (DR) and True Positive Rate (TPR) for both highly methylated and unmethylated CpG sites (Du et al., 2010). However, for relatively large sample sizes as in ARIC, test statistics are similar for M and beta-values (Zhuang et al., 2012). We will explore use of M values for our incident obesity and overweight models.

Statistical Analysis:
We will utilize standard regression techniques implemented in R.

Covariates
Smoking status, packyears of smoking from visit 1, alcohol consumption, physical activity (Baecke questionnaire leisure and sport indices), education, household income, White blood cell count (to account for variation in cell type distribution in each sample for a subset of ARIC ids with this information), batch effects (e.g., plate#, chip #, chip location).

General linear regression model for Aim 1: Methylation beta value = Obesity Trait (continuous) + covars (for each of ~487,000 CpG sites)

General logistic regression model for Aim 2: Incident Overweight or Obesity = Methylation Beta Value + covars (for each of ~487,000 CpG sites)

a priori threshold for significance = 2×10^{-6}

For example, to evaluate associations between DNA methylation and BMI at age 25 years, we will use linear regression to regress percent methylation (beta, 0.0 to 1.0) at each CpG site on BMI at 25, and summarize results across sites through q-q plots, volcano plots, Manhattan plots, or other techniques. We will describe the genomic context of the CpG's with the strongest evidence of association in terms of location relative to CpG islands and shores, proximity to Hypermethylated sites, and other features. Analyses may require different approaches to account for the unique features of Illumina 450K Infinium Methylation BeadChip data, including variance-stabilizing techniques such as using the M value (Du, 2010) and weighting site-specific analyses by probe-specific detection p-values across samples (Kuan, 2010), CpG site reliability (ICC) across replicates, and others. For example, for BMI at DNA visit, we will test the following cross-sectional models, progressing from minimally to fully adjusted models:

1) Model 1 (unadjusted): Beta values = BMI
2) Model 2 (minimally adjusted): Beta values = BMI + (Age + sex + visit + center) + (batch effects)
3) Model 3 (fully adjusted): Beta values = BMI + (Age + visit + center + smoking status + alcohol + education + income + Leisure visit 1 + European%) + (batch effects)
4) Model 4 (assess confounding by diabetes): Beta values = BMI + (Age + visit + center + smoking status + alcohol + education + income + Leisure visit 1 + European% + diabetes) + (batch effects)

Batch effects include plate, chip, and location on chip, as well as white blood cell (WBC) type distribution. WBC distribution was measured in only 180 of the ARIC DNA methylation samples, but we are working on a

algorithm to infer WBC type distribution for all subjects, using characteristic DNA methylation marks that are reliably associated with lymphocyte, monocyte, and eosinophil proportions (W. Guan, unpublished).

In secondary analyses, we will stratify by sex, smoking status (current vs others), packyears (top 50% vs bottom 50% of smokers), and physical activity (low physical activity= bottom 20% of sex specific Baecke sport index) to examine, in an exploratory fashion, effect modification by sex, smoking, and physical activity level.

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 ICTDER03 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 Limited to ancestry information obtained from AIMs or GWAS markers

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.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)?

Genome-wide DNA methylation profiling in peripheral blood: quality control and association with demographic characteristics (MS1929) Pankow, J et al..
Genome-wide methylation analyses of cardiovascular disease (CVD) and its risk factors (MS1928) Bressler, J. et al

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)* _____ _____ _____)

2007.02 (CARe, genotyping in African Americans)

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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ARIC ATHEROSCLEROSIS RISK IN COMMUNITIES STUDY

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April 17, 2013

Ellen Demerath
University of Minnesota
1300 S. 2nd Street
Minneapolis, MN 55454

Dear Dr. Demerath,

On behalf of the Publications Committee, I am notifying you that ARIC manuscript proposal #2106, "Epigenome-wide association study of obesity traits in African American adults: The Atherosclerosis Risk in Communities (ARIC) Study" has been approved.

We thought this is an important topic. It will be useful to include analysis of continuous BMI in additional to categories.

Note that manuscript proposals are approved for a three year period. If at the end of this time a paper has not been submitted, the authors will be notified and the manuscript proposal will be considered withdrawn. Three years was chosen as a period in which most papers can be completed. However, authors can request an extension.

Keep in mind that lead authors are responsible for circulating each draft of their manuscript to all co-authors for review, comments, and suggestions as the paper progresses. Policies for ARIC paper and meeting abstract submissions are posted at http://www.cscc.unc.edu/aric/policy/ .

Best wishes to you and your writing group in the preparation of this paper.

Sincerely yours,
Josef Coresh
Josef Coresh, M.D., Ph.D.
ARIC Publications Committee

JC/js

cc: ARIC Publications Committee
ARIC Coordinating Center