ARIC Manuscript Proposal # 3338

PC Reviewed: 1/8/19  Status: _____  Priority: 2
SC Reviewed: __________  Status: _____  Priority: ____

1.a. Full Title: DNA methylation associated with glycemic traits and type 2 diabetes in multi-ethnic analyses: CHARGE consortium

b. Abbreviated Title (Length 26 characters): DNAm & diabetes traits

2. Writing Group:
   Writing group members:

This paper will present meta-analysis results from the CHARGE consortium. Participating cohorts include ARIC, BLSA, CHS, FHS, GENOA, GOLDN, HYPERGEN, InCHIANTI, KORA, LBC1921, Rotterdam Study, TwinsUK, and WHI. Author representatives from each cohort and authorship order are still being determined.

Co-first authors on the paper are Samantha Lent, Department of Biostatistics, Boston University, (lent@bu.edu) and Alexia Cardona, MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine (Alexia.Cardona@mrc-epid.cam.ac.uk).

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

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**Name:**
**Address:**
3. **Timeline:** Submission for publication in spring 2019

4. **Rationale:**

Fasting glucose, fasting insulin, and hemoglobin A1c are important glycemic biomarkers and elevated levels of these markers predict future glycemic dysfunction and type 2 diabetes (T2D). Candidate gene studies and genome-wide association studies (GWAS) have identified a number of sequence variants that can explain some of the interindividual variation in the susceptibility for T2D, however large component of heritable T2D risk remains poorly understood. This unexplained heritability could be partly explained by epigenetic modifications, DNA alterations that lead to changes in gene expression without changes in the DNA sequence. Epigenetic changes such as DNA methylation might be responsible for the inter-individual variability in T2D susceptibility. It is also possible that elevated glycemia or related traits influence DNA methylation levels which may reflect hyperglycemia or might be part of pathways towards downstream complications.

To date, observational epigenetic studies have provided evidence of positive associations between DNA methylation and of glycemic traits/T2D. These studies, inherently by design, are subject to bias and confounding. It is therefore often unclear whether epigenetic modifications cause perturbations in glycemic biomarkers and/or T2D, or whether epigenetic modifications are a result of T2D pathogenesis.

5. **Main Hypothesis/Study Questions:**

The present study will leverage epigenetic data from over 17,000 individuals from the CHARGE Consortium. The overall objective is to determine whether DNA methylation is associated with fasting glucose and insulin, hemoglobin A1c, and prevalent type 2 diabetes.

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 conduct an epigenome-wide fixed effects meta-analysis for 3 glycemic traits (fasting glucose, log-transformed fasting insulin, and HbA1c) and prevalent T2D in individuals of African (N=4,961), European (N=11,629), and Hispanic (N=711) ancestry across 17 CHARGE cohorts. We aim to identify CpG sites at which DNA methylation levels in peripheral blood were associated with glycemic traits and T2D across multiple ancestries and test for causal relationships at these loci. Methylation beta values will be modeled as the outcome in all analyses. All analyses will be stratified by ancestry and adjusted for age, sex, smoking status,
BMI, and technical covariates. Adjustment techniques for technical covariates, including cell type proportions and batch effects, will vary cohort.

ARIC will contribute results for the fasting glucose, hemoglobin A1c, and prevalent diabetes meta-analyses, using phenotypic data from the same visit at which DNA was collected (either visit 2 or visit 3). Data will be restricted to African Americans (N=2774 for prevalent T2D, 1875 for fasting glucose, and 1603 for hemoglobin A1c).

A bidirectional Mendelian randomization (MR) approach will be used to investigate the direction of causality at all CpG sites associated with glycemic traits. Analyses will be run using the ‘TwoSampleMR’ R package. Forward-MR analysis will be conducted to predict the causal effect of methylation on glycemic traits and prevalent T2D. Methylation quantitative trait loci (methQTLs) associated with the CpGs significant in the meta-analysis will be identified in the GoDMC database. To test whether the changes in methylation identified in this study are a consequence of (rather than cause of) metabolic differences, reverse-MR will also be performed. SNPs significant in the genetic associations for fasting glucose, fasting insulin and HbA1c from the MAGIC consortium and T2DM in the DIAGRAM consortium will be tested for association with methylation at CpG sites identified in the CHARGE EWAS meta-analysis.

Ingenuity Pathway Analysis software (http://www.ingenuity.com/) will be used to determine potential canonical pathways and networks our DNA methylation top hits in an agnostic way.

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 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

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/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)?

Diabetes is listed as one of the phenotypic domains in MS1928 (Genome-wide methylation analyses of cardiovascular disease and its risk factors). MS1928 is an “umbrella” proposal that has served as a placeholder until domain-specific proposals such as this one are developed.

The ARIC Study contributed look-up results for 15 CpG sites for MS3208 (Novel DNA methylation sites of glucose and insulin homeostasis: an integrative cross-omics analysis). The author of that paper is aware of the current proposal, and sees little or no overlap.

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 https://www2.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.