ARIC Manuscript Proposal #3709 (Revised)

PC Reviewed: 11/10/20	**Status: _____**	**Priority:2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: DNAm markers associated with type 2 diabetes and glycemic biomarkers in African Americans: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): **DNAm and T2D in AA**

2. Writing Group: Sowmya Venkataraghavan, Debashree Ray, Liz Selvin, Josef Coresh, James Pankow, Anna Köttgen, Bing Yu, Eric Boerwinkle

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

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3. Timeline: The manuscript preparation will be done over next 12 - 24 months

4. Rationale:

There is a disproportionately high burden of diabetes among African Americans. African Americans are 1.6 times more likely to be diagnosed with diabetes than non-Hispanic Whites.[1, 2] They are more likely to develop microvascular complications of diabetes such as retinopathy and nephropathy.[3, 4] On the other hand, African Americans have equal or lower incidence of macrovascular complications like cardiovascular disease (CVD) than their European counterparts;[5, 6] yet they are more likely to die from CVD, making even a lower incidence a cause for concern.[5, 6] African Americans with diabetes also have higher rates of mortality than European Americans.[7] Diabetes has 2 major forms - type 1 diabetes (T1D) and type 2 diabetes (T2D). While T1D is an early onset disease with low prevalence,[8] 90-95% of diabetes cases are T2D.[9]

There are racial disparities in prevalence of environmental risk factors of T2D such as obesity and low socio-economic status. However, these disparities do not completely account for the increased risk of T2D among African Americans.[10] There is increased insulin resistance among non-obese African Americans even after adjustment for body weight and fat distribution.[4] A study reported that after adjustment for education level, income level, other socio-economic factors and body mass index (BMI), there was still excess risk for T2D in African American women.[11] Thus, there is a need to search for novel risk factors to further explain the racial disparities in T2D risk. Some have been implicated including serum potassium levels and forced vital capacity.[12] However, DNA methylation markers are yet to be studied as novel risk factors that could explain this disparity in risk.

DNAm markers could help explain disparities in T2D outcomes and may be potential targets of intervention. DNAm is modifiable and can offer targets of intervention.[13] For example, 5-azacytidine/vidaza (AZA) and 5-aza-2′-deoxycytidine/dacogen (DAC) are approved drugs by FDA that target DNAm in cancer.[14] Since DNAm that is studied is located in gene-body regions, it can elucidate biological pathways.[15] One longitudinal study examined DNAm markers and incident T2D in individuals from other populations such as Europeans and Indian Asians.[16] However, till date, DNAm markers have not been examined in African Americans as risk factors for developing T2D. Studying DNAm markers can help us understand how the DNAm markers that confer risk in other populations influence T2D risk in African Americans. Additionally, we will be comparing the effect sizes between African American and European American populations to understand if differences exist between populations in US. Previously, European and Indian Asian populations were compared in a similar manner and greater effect sizes were reported for Indian Asians, a population with higher incidence of T2D.[16] *We will examine whether DNAm markers associated with T2D in Europeans and Indian Asians are associated with incident T2D in African Americans*. We will refer to the subset of DNAm markers that we are testing in this aim as candidate DNAm markers. Our candidate DNAm markers will include DNAm markers found to be associated with T2D in various other populations. In addition, we will expand our set of candidate DNAm markers to include DNAm markers that were identified to be differentially methylated between African Americans and European Americans in previous DNAm studies. [17, 18]

DNAm markers could be associated with levels or changes in levels of glycemic biomarkers. DNAm markers associated with fasting glucose and fasting insulin have previously been studied

as markers of early pathogenesis of T2D in non-diabetic individuals.[19, 20] In addition to fasting glucose, HbA1c is the predominant biomarker used in T2D management.[21-23] It is a reliable indicator of glycemia for the 2-3 month period preceding the test. However, there are several shortcomings of HbA1c test.[24] HbA1c is related to erythrocyte and hemoglobin levels and fails to perform well in anemic patients.[25] HbA1c also differs by age and ethnicity. African Americans have higher levels of HbA1c, although there is a lack of consensus in literature on whether this difference is indicative of higher glycemic levels and T2D risk in African Americans.[26-28] Examining HbA1c levels as a biomarker for T2D risk can help provide us with additional insight on the variation of HbA1c levels determined by T2D DNAm markers.

Studies have examined trajectories in levels of glycemic biomarkers across time, and have established that while there was an increase in levels of glycemic biomarkers with age, this increase was higher among individuals who developed T2D.[29-31] Previously, a GWAS examinations associations between SNPs and changes in levels of fasting glucose.[32] No study has been conducted till date to study DNAm markers and changes in levels of fasting glucose. Additionally, examining differences in effect sizes of DNAm markers on HbA1c levels in African Americans and European Americans can help examine if DNAm markers explain some of the disparity in HbA1c levels between the two populations.

To overcome some of the challenges posed by HbA1c, novel biomarkers have been proposed to improve management and treatment of T2D. These non-traditional biomarkers, fructosamine, glycate albumin and 1,5- AG, have great potential for clinical utility. For example, glycated albumin is a good indicator of glycemic control in patients with hematological conditions such as anemia.[21, 33] It is widely used in Japan and does not vary based on sex or BMI, as opposed to HbA1c.[34] Another biomarker with potential clinical utility is fructosamine, which is a cheaper and easier test than HbA1c.[33] When used in addition to HbA1c, the regression of HbA1c on fructosamine, a metric called the glycation gap, is an important indicator of T2D associated health outcomes such as macro-vascular disease, micro-vascular disease and treatment related hypoglycemia.[35] Lastly, 1,5 - AG is an additional biomarker that can be potentially used to monitor blood glucose levels. There is higher renal absorption and lower circulating levels of 1,5 - AG in the presence of high levels of glucose implying an inverse relationship between fasting glucose and 1,5 - AG.[33, 36] Till date, only a limited number of studies have been published examining genetic determinants of fructosamine, glycated albumin and 1,5 - AG.[37-39] No studies have examined DNAm markers and its association with levels of non-traditional biomarkers. Comparing and contrasting the effect of DNAm markers on non-traditional glycemic biomarkers with HbA1c and fasting glucose can help us better understand the advantages and shortcomings of their clinical utility and uncover biological pathways in glucose metabolism.

We would like to briefly acknowledge research in the same area that is being carried out by PAGE and CHARGE, large consortia comprising of multi-ethnic populations The CHARGE consortium recently completed an epigenome wide DNAm association analysis of prevalent T2D. Several DNAm markers were implicated. This paper is yet to be published. Similarly, PAGE will be doing an epigenome wide DNAm association analysis with incident T2D to examine whether the relationship between socio-economic status and incident T2D is mediated through DNAm.

The key difference between our study and the ongoing efforts in PAGE and CHARGE is that we are performing a candidate DNAm time-to-event analysis with incident T2D. Our study design is longitudinal and uses temporality to establish causation. While PAGE is also studying the association between DNAm markers and incident T2D, they will be using a cross-sectional study design. Additionally, we will also be examining the association between DNAm markers and changes in levels of fasting glucose across time and the association between DNAm markers and non-traditional glycemic biomarkers such as fructosamine, glycated albumin and 1,5 - AG. PAGE and CHARGE are doing an epigenome wide DNAm association analysis and are well - powered to discover novel DNAm and T2D associations. On the other hand, we aim to study candidate DNAm markers in African Americans to understand whether DNAm markers can help explain some of the disparities in T2D risk across populations. Hence, our study will not detract from ongoing effort in large consortia and we aim to complement the studies conducted by the consortia with our different research questions and approach.

5. Main Hypothesis/Study Questions:

Examine the association of DNAm markers with incident T2D and glycemic biomarkers in African Americans and compare effect sizes with European Americans. We will limit this longitudinal analysis to DNAm markers previously identified as risk factors for T2D in individuals of European and Indian Asian ancestry. DNAm marker is a continuous trait and its measure is referred to as DNAm marker levels. We hypothesize that (a) some of the DNAm markers will be associated with incident T2D in African Americans; (b) the effect size of DNAm marker levels in African Americans will be different from what was previously discovered in European and Indian-Asian populations; (c) the effect sizes will be different in African Americans and European Americans and (d) higher DNAm marker levels will be associated with greater increasing trajectories of glycemic biomarkers across visits.

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).

Exposure measurements:

DNAm markers: DNAm of participants was collected from whole blood samples at ARIC Visit 2 (n=2504) or Visit 3 (n=441) and analyzed using Illumina Infinium Human Methylation 450K BeadChip (Illumina Inc.).[40] DNAm was quantified at 485,577 sites. DNAm marker is measured as a continuous trait and defined as beta (β) values of fluorescent intensity ratio ranging from 0 (completely unmethylated) to 1 (completely methylated) at each site. The β value approximates percentage of methylation at a site. Quality control measurements at a SNP and sample level will be applied.[41]

Outcome measurements:

Glycemic biomarkers:

Incident T2D: In ARIC, incident diabetes is determined during one of the follow-up visits as blood glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, self-report physician diagnosis of diabetes or taking medication for diabetes or during annual phone interviews as self-report physician diagnosis of diabetes or glucose lowering medications.[42] We do not have sufficient data to demarcate between T2D and T1D in ARIC. However, T1D is an early-onset disease, and we are prospectively following individuals aged 45-65 who do not have diabetes at baseline. It is safe to assume that all our incident diabetes cases are T2D cases. To maintain consistency, we will be referring to our outcome as T2D throughout the proposal even though ARIC did not make this distinction.

Study design, inclusion/exclusion, outcome and other variables of interest:

Exposures: Candidate DNAm markers implicated to be associated with T2D in previous studies including but not restricted to *TXNIP, PROC, C7orf29, SREBF1, PHOSPHO1, SOCS3* and *ABCG1*.

(i) **Study design:** Longitudinal cohort
Outcome: Incident T2D
Inclusion criteria: Cohort contains African Americans in ARIC (~2500) with genotyping data and DNAm data available from Visit 2 and Visit 3.

(ii) **Study design:** Longitudinal cohort
Outcome: fasting glucose measured at all visits
Inclusion criteria: All African Americans in ARIC cohort (~2500) with genotyping data and DNAm data available from Visit 2 and Visit 3 who have multiple measurements of glycemic biomarkers across visits.

(iii) **Study design:** cross-sectional
Outcome: fasting glucose, HbA1c, 1,5 - AG, fructosamine, glycated albumin, HOMA-IR. Measurements for these biomarkers were obtained at Visit 2.
Inclusion criteria: African Americans in ARIC with genotyping data and DNA methylation data available from Visit 2.

Comparison study with European Americans: We will repeat analyses (i) & (ii) in European Americans participants for whom DNAm marker data is available (~1000). DNAm data is available in a subset of Europeans Americans who underwent a Brain MRI at Visit 3. Since DNAm data was collected at Visit 3, we will not be able to replicate analysis (iii).

Exclusion criteria: Considerations for missing data, missing visits and low quality data.

Statistical analysis plan:

(i) To examine the association of DNAm markers and incident T2D, we will use cox regression models.

(ii) To examine the association between DNAm markers and changes in levels of glycemic biomarkers, we will fit a linear mixed-effects model with random intercept to account for variation in levels of glycemic biomarkers at baseline. Additionally, we will also include time as a covariate in the model, and interaction term for time and DNAm marker level.

(iii) To cross-sectionally examine the association between DNAm markers and glycemic biomarkers, we will fit a linear model with biomarker levels as outcome. In all the above models, DNAm marker levels will be a continuous trait and we will fit separate models for each DNAm marker.

Overall limitations:

- There is a large gap in follow up between Visit 4 and Visit 5 (12 years). T2D outcome identified at Visit 5 may be delayed from time of onset and could potentially bias results. Additionally, if we had many incident T2D cases between Visit 4 and Visit 5 and many loss-to-follow up events, our rare disease assumption might not be met
- We are underpowered to detect associations at a genome-wide Bonferroni corrected significance level. However, using a candidate DNAm marker approach can help us lower our p-value significance threshold.
- African Americans from ARIC cohort with DNAm data are heavily sampled from Jackson, MS and are not representative of all African Americans.
- European Americans with DNAm data available are sampled from Forsyth County, NC and are not representative of all European Americans.

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)?
ARIC NO: 3208 Novel DNA methylation sites of glucose and insulin homeostasis and their integrative cross-omics analysis - James Pankow, Jan Bressler (Part of CHARGE)

ARIC NO: 3338 DNA methylation associated with glycemic traits and type 2 diabetes in multiethnic analyses: CHARGE consortium -(James Pankow, Jan Bressler, Eric Boerwinkle, Weihua Guan, Chong W, Liz Selvin)

PAGE Ms NO: 3696. Identifying DNA methylation biomarkers that are potentially causally associated with type 2 diabetes and its risk factors among multiethnic populations -- Lead Author: Lang Wu

PAGE Ms NO: 3695. Methylome-wide association study of socioeconomic adversity, type 2 diabetes, and CVD events among 3 large NIH cohorts -- Lead Author: Kari North

.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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