ARIC Manuscript Proposal # 3010

1.a. Full Title: Epigenomic Characterization of Statin Use and Diabetes Association in Multiethnic Populations

b. Abbreviated Title (Length 26 characters): Epigenomics of Statin Use and Diabetes

2. Writing Group:
   Writing group members: Amanda A Seyerle, James Pankow, Ellen Demerath, Myriam Fornage. Other interested investigators welcome

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

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3. Timeline: Analysis to be conducted on study question 1 during 2017-18. Completed manuscript expected by summer 2018

4. Rationale: Over the past decade, the use of prescription drugs has skyrocketed, with nearly half of all American adults taking at least one prescription drug. Despite the considerable increases in drug exposure, variability in drug response, a significant cause
of morbidity and mortality accounting for approximately 100,000 deaths and 2.2 million serious health effects annually,\textsuperscript{2-6} remains poorly understood.\textsuperscript{7} The recent emphasis on precision medicine has prompted an increased need to better understand the relationship between the human genome and variable drug response.\textsuperscript{8}

Statins are a well-established therapy for the prevention and treatment of dyslipidemia. The use of statins by the U.S. population is rapidly increasing, from 21.8 million (17.9\% prevalence) in 2002-2003 up to 39.2 million (27.8\% prevalence) in 2012-2013,\textsuperscript{9} and will likely continue to increase given recent American College of Cardiology/American Heart Association (ACC/AHA) treatment guidelines. The 2013 ACC/AHA guidelines expand the recommended use of statins to all adults with known cardiovascular disease, regardless of low density lipoprotein (LDL) cholesterol levels, as well as increasing the range of LDL cholesterol levels that lead to a recommendation for statin therapy.\textsuperscript{10} Statins are more commonly prescribed to Whites (29.8\%) than Blacks (22.8\%) or Hispanics (18.8\%).\textsuperscript{9} This is concerning given that the Multiethnic Study of Atherosclerosis (MESA) found that dyslipidemia, defined using risk group specific guidelines, to be more prevalent in Blacks and Hispanics than in Whites (29.1\% and 26.8\% compared to 24.4\%, respectively).\textsuperscript{11} Additionally, both adverse reactions and protective characteristics of statin use are not equally distributed among race/ethnicity. For example, statins are more likely to be associated with the development of diabetes mellitus (DM) in Whites than in Blacks (hazard ratio [HR] = 1.49 and 1.18, respectively).\textsuperscript{12} Furthermore, among renal transplant patients, statin use conferred greater protection against mortality among Blacks than Whites.\textsuperscript{13} Additionally, statin myopathy, one of the most common side effects of statin use, is the most likely cause of statin intolerance in Whites, an association which has not been replicated in other race/ethnicities.\textsuperscript{14}

While statin use has clearly demonstrated cardioprotective effects (e.g. anti-inflammatory and anti-atherothrombotic properties),\textsuperscript{15} there are also adverse effects (e.g. DM, acute renal failure, myopathy, rhabdomyolysis).\textsuperscript{16} Given the high prevalence of statins in the U.S. population, adverse reactions are associated with significant cost and morbidity. Because of the large potential benefits, statins are used at a high rate, which also leads to a greater risk of adverse events. Therefore, a great deal of research has been conducted to better characterize statin efficacy and adverse reactions. In particular, pharmacogenomic work in statin use has successfully identified variants in \textit{SLCO1B1} which alter the transport of statins and their metabolites into the liver.\textsuperscript{17} Furthermore, variants in genes encoding OATP1B1 and ABCG2 proteins involved in hepatic uptake and efflux of statins have been associated with increased statin plasma concentrations in multiple race/ethnic groups, particularly in Asian populations.\textsuperscript{18} However, there remains limited work in the pharmacogenomics of statin response in Black or Hispanics. In addition, there is evidence that epigenetic mechanisms may mediate statin action, with cell line evidence that treatment with statins results in changes in histone modification.\textsuperscript{19} However, the role of epigenetic modification in variable response to statins remains unclear. The role of both genetics and epigenetics in human health can vary by genetic ancestry, making it imperative that race/ethnic differences be considered in all future pharmacogenomics and epigenetic research.
5. **Main Hypothesis/Study Questions:** This proposal will identify and characterize the DNA methylation sites that predict the development of DM (including glucose and insulin measures) among future statin users.

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).**

This analysis will be a collaboration between studies with medication use data, methylation data, and diabetes data conducted through the Cohorts for Heart and Aging Research using Genomic Epidemiology (CHARGE).

Analyses will use data from ARIC visits 2, 3, and 4. At visit 2 (i.e. the visit at which methylation was measured [visit 3 for a subset of participants]), participants who are currently on a statin will be excluded. Statin use will be determined from visit 3 data (or visit 4 for the subset of participants with methylation data collected at visit 3). Visit 3 (or visit 4 for a subset) will serve as the baseline for follow-up for incident DM. DM will be ascertained through data collected at follow-up visits and annual follow-up calls. By using methylation data from visit 2/3 and statin use data from visit 3/4, we can ensure statin use does not modify methylation.

For analysis of DM status (dichotomous variable), each cohort will analyze their data using 2 linear mixed-effect Cox proportional hazards models, run separately for each CpG site and separately in African Americans and European Americans. DM will be included as the dependent variable and the interaction beta between methylation and statin use will be the independent variable of interest. Model 1 will adjust for sex, age, blood cell distribution (comprised of the fractions of CD4+ T cells, NK cells, monocyte, and eosinophils measured or estimated using the Houseman et al. method), and technical covariates. Model 2 will adjust for all covariates in Model 1 as well as BMI, SBP, and smoking status. Meta-analyses will be run separately for African American and European American populations using the restricted likelihood criterion in R’s metaphor package. For glucose and insulin levels, the same protocol will be followed using a linear mixed-effect model, rather than a Cox proportional hazards model.

Additionally, each cohort will analyze data using 2 generalized linear mediation models, run separately for each CpG and separately in African Americans and European Americans. DM, glucose levels, or insulin levels will be included as the dependent variable, methylation site as the independent variable, and statin use as the mediator. Model 1 will adjust for sex, age, blood count, and technical covariates. Model 2 will adjust for all covariates in Model 1 as well as BMI, SBP, smoking status, and propensity score of statin use. Meta-analyses will be run separately for African American and European American populations. Results at each stage of analysis will be compared between African American and European American populations using one-way ANOVA.
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

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”?  
__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.csc.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)?

This manuscript proposal is related to the recently submitted manuscript proposal entitled “Pharmacoepigenomics: Human population studies of DNA methylation and statin treatment response” submitted by James Floyd. The main hypothesis of James Floyd’s manuscript proposal is that DNA methylation in the blood is associated with the LDL-cholesterol lowering response to statin therapy, while we hypothesize that DNA methylation is associated with the development of DM among statin users. Both writing groups are in contact with each other as the manuscripts move forward.

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.cscc.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.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.

References

11. Goff DC, Bertoni AG, Kramer H, Bonds D, Blumenthal RS, Tsai MY and Psaty BM. Dyslipidemia Prevalence, Treatment, and Control in the Multi-Ethnic Study of


