ARIC Manuscript Proposal #3785

PC Reviewed: 2/9/21 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Serum metabolites association with kidney failure and ESKD in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Metabolites and adverse kidney outcomes

2. Writing Group:
Lauren Bernard, Aditya Surapaneni, Linda Zhou, Jingsha Chen, Casey Rebholz, Josef Coresh, Bing Yu, Eric Boerwinkle, Pascal Schlosser, Morgan Grams, others welcome

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

First author: Lauren Bernard
Address:

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).
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3. Timeline: Analysis will begin upon receipt of the data and approval of the manuscript proposal. Manuscript will be written and submitted for ARIC Publications Committee review within one year of manuscript proposal approval.

4. Rationale:

Metabolomics offers an opportunity to discover potential biomarkers of human health. In nephrology, metabolomics has been used to identify novel biomarkers of incident chronic kidney disease (CKD).[i,ii,iii] Bing et. al has explored individual metabolites' association with incident CKD and identified two strongly protective metabolites in ARIC.[i] No ARIC studies have investigated the association between the serum metabolome and hard outcomes such as kidney failure and ESKD. As these renal outcomes are projected to increase in the United States through the next decade, it would be advantageous to better understand their biological underpinnings.[iv]

Further, exploring these endpoints could help identify people at high risk for adverse renal outcomes and possibly aid in targeting therapeutics to prevent these outcomes.

We propose examining the longitudinal associations between individual metabolites and adverse renal outcomes using Cox proportional hazard models. The few previous metabolomic studies of ESKD have examined this association with relatively short follow-up, a low number of events, and in populations that were exclusively or primarily diabetic.[v,vi,vii] Studying these associations in ARIC can overcome these limitations. Additionally, we propose using Netboost, a dimension reduction technique, to create eigenclusters of correlated metabolites to identify cumulative effects of correlated features.[viii] These techniques may identify metabolic pathways that could be potentially targeted to avoid progression of chronic kidney disease to ESKD or kidney failure.

5. Main Hypothesis/Study Questions:

Aim 1: To identify metabolites associated with adverse kidney outcomes (ESKD and kidney failure).
Aim 2: To identify clusters of metabolites, and to relate them to adverse kidney outcomes.

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).

Study Design: This will be a prospective analysis of the ARIC cohort using study visit 1 with follow-up through December 31, 2018 (or the most recent surveillance year).

Study Population: The study population will include all members of the ARIC cohort with available metabolomics data. All analyses will exclude those with prevalent CKD at the study visit.

Exposure: The exposure for aim 1 will be individual metabolites that were measured using baseline fasting serum samples from ARIC visit 1 that have been stored at -80 °C since collection. Metabolon, Inc. has detected and quantified 602 metabolites using untargeted gas-chromatography-mass spectrometry and liquid chromatography-mass spectrometry-based metabolomic quantification protocols. Metabolites will be excluded if more than 80% of the samples have values below the detection limit as has been done in previous metabolomic studies, and we will use similar data cleaning procedures to these studies.[i,ix] Metabolites will be log-transformed.

The exposure for aim 2 will be eigenclusters of correlated metabolites. Similar to aim 1, we will focus on metabolites missing <80% of the time. For metabolites with missing data, we plan to impute the value using the lowest value detected for the metabolite from all samples.[i] For the purposes of analysis, we will use the residuals of the regression of log-transformed metabolite on age, sex, race-center, and eGFR.

Outcomes:

The primary outcome will be incident ESKD. Incident ESKD will be defined as the initiation of renal replacement therapy (either dialysis or transplant) or death from kidney disease, identified through linkage with the USRDS registry last updated in July 2017. Secondary outcomes will be the slope of eGFR decline and kidney failure. GFR will be estimated with the Chronic Kidney Disease Epidemiology equation based on serum creatinine and cystatin C.[x] Kidney failure will be defined as eGFR < 15 mL/min/1.73 m^2, United States Renal Data System (USRDS) registry identification, or through one of the ICD-9-CM/ICD-10-CM codes validated by Rebholz et. al.[xi]

Statistical Analysis:

We will use linear regression for the cross-sectional association of metabolites and eGFR and mixed models to evaluate individual metabolites association with eGFR decline. We will use Cox proportional hazards models for kidney failure and incident ESKD. We will test associations in unadjusted models, demographic- and study-center adjusted models, an extended adjusted model (including systolic blood pressure, antihypertension medications, diabetes status, history of CHD, smoking status, eGFR, HDL), and a final model adjusting for metabolite principal components. We will perform these models in two batches with the first using data only from the Jackson, MS community where all participants were African American and then conduct a second analysis with data all four communities. We plan to then meta-analyze the beta coefficients.

We will also use Netboost to analyze the residuals of metabolite clusters with the same statistical models described above. For residuals of eigenclusters that are significantly associated with kidney failure or ESKD, we will evaluate genetic associations for potential Mendelian randomization studies and seek replication using other cohorts as available.

Limitations:

GFR will not be directly measured and instead estimated from the CKD-EPI equation.

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.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)?

ARIC Manuscript Proposal #1182: A longitudinal Study of Metabolomics and Kidney Function among African Americans in the Atherosclerosis Risk in Communities (ARIC) Study

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

_X__ A. primarily the result of an ancillary study (list number* 2017.27, 2013.21)

___ 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.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __X__ No.

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[i] Yu B, Zheng Y, Nettleton JA, Alexander D, Coresh J, Boerwinkle E. Serum metabolomic profiling and incident CKD among African Americans. *Clin J Am Soc Nephrol*. 2014;9(8):1410-1417. doi:10.2215/CJN.11971113

[ii] Grams ME, Tin A, Rebholz CM, et al. Metabolomic Alterations Associated with Cause of CKD. *Clin J Am Soc Nephrol*. 2017;12(11):1787-1794. doi:10.2215/CJN.02560317

[iii] Grams ME, Shafi T, Rhee EP. Metabolomics Research in Chronic Kidney Disease. *J Am Soc Nephrol.* 2018;29(6):1588-1590.

[iv] McCullough KP, Morgenstern H, Saran R, Herman WH, Robinson BM. Projecting ESRD Incidence and Prevalence in the United States through 2030. *J Am Soc Nephrol*. 2019;30(1):127-135. doi:10.1681/ASN.2018050531

[v] Niewczas MA, Sirich TL, Mathew AV, et al. Uremic solutes and risk of end-stage renal disease in type 2 diabetes: metabolomic study. *Kidney Int*. 2014;85(5):1214-1224. doi:10.1038/ki.2013.497

[vi] Niewczas MA, Mathew AV, Croall S, et al. Circulating Modified Metabolites and a Risk of ESRD in Patients With Type 1 Diabetes and Chronic Kidney Disease. *Diabetes Care*. 2017;40(3):383-390. doi:10.2337/dc16-0173

[vii] Titan SM, Venturini G, Padilha K, et al. Metabolomics biomarkers and the risk of overall mortality and ESRD in CKD: Results from the Progredir Cohort. *PLoS One*. 2019;14(3):e0213764. Published 2019 Mar 18. doi:10.1371/journal.pone.0213764

[viii] Schlosser P, Knaus J, Schmutz M, et al. Netboost: Boosting-supported Network Analysis Improves High-Dimensional Omics Prediction in Acute Myeloid Leukemia and Huntington's Disease. *IEEE/ACM Trans Comput Biol Bioinform*. 2020;PP:10.1109/TCBB.2020.2983010. doi:10.1109/TCBB.2020.2983010

[ix] Yu B, Heiss G, Alexander D, Grams ME, Boerwinkle E. Associations Between the Serum Metabolome and All-Cause Mortality Among African Americans in the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 2016;183(7):650-656. doi:10.1093/aje/kwv213

[x] Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367(1):20-29. doi:10.1056/NEJMoa1114248

[xi] Rebholz CM, Coresh J, Ballew SH, et al. Kidney Failure and ESRD in the Atherosclerosis Risk in Communities (ARIC) Study: Comparing Ascertainment of Treated and Untreated Kidney Failure in a Cohort Study. *Am J Kidney Dis*. 2015;66(2):231-239. doi:10.1053/j.ajkd.2015.01.016