ARIC Manuscript Proposal #3442

PC Reviewed: 8/13/19	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title:
Associations between gut microbial metabolites and metabolic syndrome related traits

b. Abbreviated Title (Length 26 characters): **metabolites and GMC**

2. Writing Group:
Writing group members: **Cristina Menni, Kristin Young, Kari E. North, Casey M. Rebholz , Elizabeth Selvin, Eric Boerwinkle and Bing Yu**

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __CM___ **[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).
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3. Timeline: All the analyses in the TwinsUK cohort have been completed and the manuscript is drafted. We will start the replication analysis in ARIC as soon as the proposal is approved and anticipate the analysis and paper will be completed in two months.

4. Rationale:
Measures of gut microbial diversity are becoming common markers of good health and are strong prognostics of inflammatory and other disease outcomes [1]. Higher microbiome diversity correlates

with a number of metabolic traits, including a significantly lower long-term risk of weight gain[2], of metabolic syndrome[3], and of type 2 diabetes. Part of this effect is related to the production of beneficial metabolites produced by gut microbes and released into the blood. A missing part of the puzzle is the ability to test the role of gut microbial diversity on long-term outcomes. Such information will inform whether interventions targeting gut microbiome diversity can be expected to influence outcomes such as cardiovascular events, incidence of auto-immune disorders or mortality. The aim of our study is to identify serum metabolites reflective of gut microbiome function that can mediate the effect of gut microbiome composition on cardiometabolic traits. We included 1018 females from the TwinsUK cohort with concurrent metabolomic profiling (592 metabolites) and faecal 16S sequencing (measuring microbiome composition) to identify a panel of six circulating metabolite markers that can predict different measures of gut microbiome composition (Shannon Alpha Diversity, number of OTUs, Simpson index) and explain 27% of the variance in the discovery set and 18.68% in the test set. Moreover, this metabolites score is negatively associated with cardiometabolic phenotypes, including BMI, visceral fat, arterial stiffness and risk of T2D in the TwinsUK cohort and we find that part of the association of microbiome diversity with cardiometabolic traits is mediated by these metabolites. Two of the metabolites that predict higher diversity have already been shown to be linked to lower incidence of diabetes. Those that correlate with lower diversity include metabolites, which are known to have detrimental effects on human health.

These circulating metabolites could be surrogates of both microbiome composition and function simplifying the assessment of these factors in clinical interventions. Moreover, our results suggest that the effect of diversity on cardiometabolic health is mediated by microbial metabolites.

We wish to replicate the associations of the MetaboliteDiversityScore with two cardiometabolic endpoints also measured in the ARIC study (BMI and T2D). This would provide additional insights into the assessment of these factors in clinical interventions.

1. Schwiertz, A. *et al.* Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* **18**, 190-5 (2010).
2. Menni, C. *et al.* Gut microbiome diversity and high fibre intake are related to lower long term weight gain. *Int J Obes (Lond)* (2017).
3. Pallister, T. *et al.* Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship to metabolic syndrome. *Sci Rep* **7**, 13670 (2017).

5. Main Hypothesis/Study Questions: A metabolite alpha diversity score inferring microbiome abundance is associated with cardiometabolic endpoints (i.e. BMI, and risk of T2D).

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 would like to test our metabolite score in European Americans from Wash Co, Forsyth, and Minnesota sites in the ARIC study with BMI and T2D using the models below. We will consider the score to be reproducibly associated it it is negatively correlated with BMI and diabetes with a p-value of 0.05 or lower.

MetaboliteAlphaDiversityScore= -0.0476306+0.1746576×(3-phenylpropionate (hydrocinnamate))) -0.0867652×(imidazole propionate)+ 0.1461903×(cinnamoylglycine))+0.1674953×(5alpha_pregnan_3beta,20alpha_diol monosulfate (2))-0.145269×(glutarate (pentanedioate))+ 0.0683178×(indolepropionate)

Linear regression: BMI=Score+age+gender+center
Cox regression: T2D (incident)=Score+age+gender+center
Logistic regression: T2D (prevalent)=Score+age+gender+center

We will focus on ARIC visit 1European Americans with available metabolite data, covariates, BMI, and T2D information. Prevalent T2D cases (defined using ARIC visit 1 DIABTS03) will be excluded in the incident T2D analysis.

T2D definition in TwinsUK: T2D case subjects (fasting glucose ≥7 mmol/L or physician's letter confirming diagnosis). However as T2D cutoff are slightly different in the US, we would like to use the same definition proposed in ARIC metabolomics T2D project (Rebholz, Diabetologia, 2018):

The incidence of diabetes was ascertained from baseline through to the end of follow-up on 31 December 2015. Incident diabetes was defined as elevated glucose at any of the four subsequent study visits (fasting glucose ≥7.0 mmol/l or non-fasting glucose ≥11.1 mmol/l), self-report of a diabetes diagnosis at a study visit or annual follow-up telephone interview, or self-report of diabetes medication use during a study visit or annual follow-up telephone interview.

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? 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"? ____ 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)?

MS#2756 Serum Metabolomic Profile of Diabetes and Glycemic Biomarkers
MS#3384 The Metabolome of BMI

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* _2014.20_____)

___ 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.