ARIC Manuscript Proposal #3461

1.a. Full Title:
Metabolite Profiling of Serum Urate and Gout Risk in ARIC

b. Abbreviated Title (Length 26 characters):
Metabolite, serum urate, and gout

2. Writing Group:
Writing group members:
Adrienne Tin, Peggy Sekula, Bing Yu, Megan Grove, Jan Bressler, Christine Ladd-Acosta, Morgan Grams, Casey Rebholz, Allan Gelber, Eric Boerwinkle, Joe Coresh and Anna Kottgen. Others are welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _AT___ [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:
Analysis will begin immediately. The manuscript is expected to be completed in 6 months.
4. **Rationale:**

Gout is the most common form of inflammatory arthritis and affects ~8 million U.S. adults.\(^1\), \(^2\) Emergency department visits for gout have been increasing and the annual gout hospitalization rate has doubled to 8.8 per 100,000 over the last two decades.\(^3\), \(^4\) Discovering novel risk factors and improving our understanding of mechanisms underlying gout pathogenesis may contribute to its prevention, treatment, and management.\(^5\)

Gout attacks result from an inflammatory response to the deposition of monosodium urate crystals in articular tissues, including the synovial lining, causing rapidly intensifying painful and swollen joint(s). Thus, hyperuricemia has long been recognized as a major risk factor for gout.\(^6\) However, most individuals with hyperuricemia do not develop gout.\(^1\) As such, what renders some hyperuricemic individuals susceptible to developing gout is a major unanswered question. The immune response is known to be involved in gout flares, including activation of the inflammasome.\(^7\), \(^8\) The circulating metabolites may contain markers related to gout susceptibility, such as markers of the immune system, or markers that relate to protective factors against the development of gout such as levels of plasma ascorbic acid (vitamin C).\(^9\)

High levels of serum urate is an established causal factor for gout.\(^10\) Metabolite profiling of serum urate may increase our knowledge of urate metabolism. A study of the metabolite profiling of serum urate in KORA, a cohort of European ancestry, suggests dipeptides, amino acids, and steroid hormones may play a role in urate regulation.\(^11\) It is unknown whether similar association of serum urate may exist in African Americans.

The ARIC study has serum metabolites measured in ~2000 African American and ~1000 European Americans at visit 1 (1987-89).\(^12\) These metabolite measures are valuable resources for discovering novel pathways for serum urate regulation, risk factors for gout to improve our understanding of gout pathogenesis.

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

- Some circulating metabolites will be associated with incident gout.
- Some circulating metabolites will be associated with serum urate in a cross-sectional analysis

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:** Longitudinal prospective study for incident gout and cross-sectional study for serum urate

**Inclusion criteria:** Participants with serum metabolites, outcome, and covariate data.

**Exclusion criteria:** Participants who only consented for CVD research. For the analysis of serum urate, participants who were on urate lowering medications will be excluded.
Outcome:

**Primary outcome for gout.** Incident self-reported gout collected at visit 4 (1996-98), excluding those missing age of gout or with an age of gout that preceded visit 1. This analysis relates serum metabolites to incident gout occurring within 9 years of the measurement of metabolites.

**Secondary outcome for gout.** Incident self-reported gout collected at visit 4 (1996-98) and visit 5 (2011-13). With this outcome, some incident gout cases would occur between 9 to 24 years after the measurement of the serum metabolites (post-visit 4 to visit 5). Metabolite levels may be less associated with incident gout occurring long after the measurement of the metabolites. Therefore, this is a secondary outcome.

Exposure for gout:
Serum metabolite levels measured at visit 1 using mass spectrometry by Metabolon, Inc. We will investigate appropriate transformations, such as log, inverse normal transformation, or winsorization. We will investigate whether missing values represent low values that are undetectable and devise appropriate imputation methods.

**Independent and dependent variables for serum urate analysis:**
Independent variable: serum metabolite levels measured at visit 1 using mass spectrometry by Metabolon, Inc.
Dependent variable: serum urate at visit 1

Covariates:
Known risk or protective factors of gout that are available in ARIC: age, sex, race-center, serum urate, HDL-C, triglycerides, body mass index (BMI), current drinking, systolic blood pressure (SBP), diabetes, diuretic use, estimated glomerular filtration rate (eGFR) from serum creatinine, alcohol consumption and other dietary risk factors of gout, such as seafood, meat, and beverages sweetened with fruit sugar (fructose).

Primary statistical analysis for incident gout:
We will evaluate the association between serum metabolites and incident gout using Cox regression in three models:
Model 1: age, sex, race-center
Model 2: Model 1 + HDL-C, triglycerides, BMI, current drinking, SBP, diabetes, diuretic use, alcohol consumption, eGFR.
Model 3: Model 2 + alcohol consumption and dietary risk factors of gout
Model 3: Model 2 + serum urate.

Models 1, 2 and 3 aim to discover metabolites that are associated with incident gout but may be correlated with known risk factors, except for serum urate
Model 4 aims to discover metabolites associated with gout independently of serum urate levels and other known risk factors.
Primary statistical analysis for serum urate:
Model 1: age + sex + race-center + HDL-C, triglycerides, BMI, SBP, diabetes, diuretic use, eGFR + serum urate.
Model 2: Model 1 + alcohol consumption + dietary risk factor of gout + serum urate

Statistical significance threshold for primary analysis will be determined using the Bonferroni method adjusting for the number of metabolites tested.

Secondary analyses
For significant metabolites, we will conduct:
1) race-stratified analyses to evaluate whether the effect sizes are similar between African and European Americans given that African Americans have a higher risk of developing gout after adjusting for risk factors.13
2) sex-stratified analyses to evaluate whether the effect sizes are similar between men and women. Men have higher serum urate levels and higher risk of gout.10 Substantial difference between the sexes in metabolite associations have been reported.11, 14

We will investigate appropriate metabolite pathway databases and analysis methods to apply to the association results to enhance their interpretation, including MetaCyc15 and Gaussian graphic modeling.16

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes    ___ 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? __X__ 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? _____ Yes  __X__ 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.escc.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)?

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* AS2017.27_)

  ___   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.
References: