ARIC Manuscript Proposal #1784

PC Reviewed: 5/10/11          Status: A          Priority: 2
SC Reviewed: _________          Status: _____          Priority: ____

1.a. **Full Title:** Is the effect of the genetic urate score on the risk of gout fully mediated by serum uric acid levels in the Atherosclerosis Risk in the Communities Study

b. Abbreviated Title (Length 26 characters): genetic urate score and gout

2. **Writing Group:**

   Writing group members: Mara McAdams DeMarco, Janet Maynard, Linda Kao and Josef Coresh. Others are welcome.

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

First author: Mara McAdams DeMarco, MS

Address: 2024 E. Monument St, Ste. 2-604

        Baltimore, MD 21287

        Phone: (973) 943-1967        Fax: none

        E-mail: mmcadams@jhsph.edu
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).

Name: Josef Coresh, MD, PhD
Address: Welch Center for Prevention, Epidemiology & Clinical Research
2024 E. Monument St., Suite 2-600
Baltimore, MD 21287
Phone: (410) 245-0495     Fax: (410) 955-0476
E-mail: coresh@jhu.edu

3. **Timeline:** Data analysis to start after approval of this manuscript proposal, first draft available by June, 2011

4. **Rationale:**

Hyperuricemia is the strongest risk factor for gout and previous studies have identified genes influencing urate metabolism. A recent meta-analysis of genetic studies has identified 8 loci that are associated with serum urate levels [(SLC22A11, GCKR, R3HDM2-INHBC region, RREB1, PDZK1, SLC2A9, ABCG2, and SLC17A) and 3 additional loci were identified in a separate GWAS (SLC16A9, LRRC16, and SLC22A11)]. The cumulative effects of the loci identified from the meta-analysis have been summed in a genetic urate score, which explains 6% of the variance of serum urate levels. However, only SLC2A9 and ABCG2 were associated with gout in the genome wide association study (GWAS). A 1.68 mg/dl change in the genetic urate risk score was associated with a 12-fold increase in the odds of gout (95% CI: 8.5, 18.0) after adjustment for common risk factors such as age, sex, BMI, alcohol consumption, and
However, when uric acid was included in these models, the genetic urate score remained associated with incident gout (data unpublished), suggesting partial mediation. This suggests that the effect of the genetic urate score was not fully mediated by a single measure of uric acid. These urate genes are thought to have no direct effect on the risk of gout above and beyond the regulation of serum uric acid levels. Therefore, it is unclear whether the observed association of the genetic urate score and gout is due to biological variation in uric acid. One measure of serum urate may not be enough to capture this variation uric acid.

We will test whether the presence of the renal urate transporter genes (SLC2A9, ABCG2, PDZK1, SLC22A11 and SLC17A1) and the other loci associated with urate levels (GCKR, R3HDM2-INHBC region, and RREB1) are associated with the development of gout after accounting for measurement error in serum uric acid levels. Thus, we will be testing whether a corrected measure of uric acid fully mediates the genetic urate score on the development of gout.

We strive to quantify the effect of the genetic urate score on incident gout after adjusting for a corrected measure of uric acid. We will be using the existing and valuable research infrastructure of a long-term prospective cohort: Atherosclerosis Risk in the Communities Study (ARIC). We hypothesize that after accounting for biological variation in uric acid, uric acid fully mediates the association of urate handling genes on incident gout:

Specific Aim 1: Evaluate whether genetic urate score is fully mediated by hyperuricemia after controlling for measurement error in uric acid
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).

*Population:* We will restrict our analyses to those participants who self-reported gout at visit 4 and were free of gout before visit 1. Additionally, we will limit the population to those who were white because the SNPs were identified among cohorts of European and white participants. Participants with missing genetic data on the eight SNPs will be excluded. Finally, we will be using complete case analysis and limit the population to those without missing data on any of the confounders.

*Study design:* Cohort study. We will utilize the longitudinal cohort aspect of this data for the development of gout through visit 4.

*Exposure:* We will use the previously identified eight serum urate loci as exposures (SLC22A11, GCKR, R3HDM2-INHBC region, RREB1, PDZK1, SLC2A9, ABCG2 and SLC17A1). The minor allele frequencies from the CHARGE cohort\(^1\) are listed below.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>Gene</th>
<th>MAF</th>
<th>Minor (Major) Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs13129697</td>
<td>Chr 4: 9536065</td>
<td>SLC2A9</td>
<td>0.27</td>
<td>G (T)</td>
</tr>
<tr>
<td>rs2199936</td>
<td>Chr 4: 89264355</td>
<td>ABCG2</td>
<td>0.11</td>
<td>A (G)</td>
</tr>
<tr>
<td>rs2078267</td>
<td>Chr 11: 64090690</td>
<td>SLC22A11</td>
<td>0.46</td>
<td>C (T)</td>
</tr>
<tr>
<td>rs1165196</td>
<td>Chr 6: 25921129</td>
<td>SLC17A1</td>
<td>0.46</td>
<td>G (A)</td>
</tr>
<tr>
<td>rs780093</td>
<td>Chr 2: 27596107</td>
<td>GCKR</td>
<td>0.40</td>
<td>T (C)</td>
</tr>
<tr>
<td>rs1106766</td>
<td>Chr 12: 56095723</td>
<td>INHBC</td>
<td>0.23</td>
<td>T (C)</td>
</tr>
</tbody>
</table>
We will calculate the cumulative effect of the eight loci using the previously published genetic urate score as the exposure of interest. Briefly, the score is calculated by multiplying the number of minor alleles for each locus that a person carries by the beta coefficient from the published meta-analysis. The results are summed to create the genetic urate score as is listed below:

\[
\text{genetic urate score}=\text{rs1967017(T)} \times 3.3 + \text{rs780093(T)} \times 5.2 - \text{rs13129697(G)} \times 22.2 + \text{rs2199936(A)} \times 18.1 + \text{rs675209(T)} \times 4.4 - \text{rs1165196(G)} \times 6.2 + \text{rs2078267(C)} \times 6.8 - \text{rs1106766(T)} \times 5.2
\]

Note: the parentheses following the SNP ID contain the minor allele and will be substituted by the number of copies of the allele that each participants carries. The subsequent number represents the beta coefficient (effect size) per one copy of the allele.

**Mediator:** Serum urate concentrations were measured with the uricase method at visit 1 and 2 in mg/dL. The reliability coefficient of serum urate was 0.91, and the coefficient of variation was 7.2% in a sample of 40 individuals with repeated measures taken at least 1 week apart. In the complete ARIC cohort, the mean serum urate levels were 0.36 mg/dL higher at visit 2 due to lab drift compared with visit 1 after adjustment for age at the visit. Therefore, we will subtracted 0.36 mg/dL from the visit 2 serum urate levels to make them comparable to visit 1 values to correct for lab drift.
**Outcome:** At ARIC visit 4, participants were asked, “Has a doctor ever told you that you had gout?” Participants who answered, “Yes” to the gout query then reported the age of gout diagnosis. The outcome of interest is incident gout based on self-reported onset after visit 1. Our previous research suggests that self-report of a physician diagnosis of gout is a sensitive and reliable measure of gout.$^6$

**Potential confounders:** We will identify confounders of association of genetic urate score, serum uric acid levels and gout. Though no factors are thought to effect genetic status, risk factors for gout may also be associated with serum urate level. We will consider baseline (1989) age, sex, blood pressure, alcohol intake (grams/week), hypertension, diuretic use and body mass index as potential confounders. Additionally, we will use serum creatinine, measured using a modified kinetic Jaffé reaction, to calculate the estimated glomerular filtration rate (GFR) by using the CKD-EPI equation.$^7$ We will categorize eGFR as being less than 60 ml/min/1.73m$^2$, 60-90 ml/min/1.73m$^2$, and greater than 90 ml/min/1.73m$^2$.

**Analysis:** We will calculate the hazard rate ratio (HR) for incident gout by continuous genetic urate score, adjusted for uric acid level (at visit 2 to represent mediation) using Cox Proportional Hazards Model with age as the timescale. The model will be adjusted for sex blood pressure, alcohol intake (grams/week), hypertension, diuretic use and body mass index as potential confounders. Next, using regression calibration,$^8$ we will correct for measurement error in uric acid level at visit 2 using visit 1 uric acid. The corrected uric acid level will be included in both adjusted models. We will conclude that there is
full mediation by the corrected measure of uric acid if the Wald p-value for the genetic urate score is greater than 0.05 and thus not associated with the development of gout.

**Limitations:** Gout was only ascertained at visit 4. Therefore, our sample size is limited to those participants who attended visit 4. Additionally, our study will be subject to all the statistical issues related to detection of statistical interactions, such as type I and type II error and confounding.

7.a. Will the data be used for non-CVD analysis in this manuscript? ___ 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? ___ Yes ___ No

(This file ICTDER03 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.cscu.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)?

# 1343: Stage II of a Genome-Wide Association Study for Genetic Variants Associated with Uric Acid Levels and Gout

# 1748: “The interaction of diuretic use and serum urate handling genes on the risk of gout in the Atherosclerosis Risk in the Communities Study (ARIC)”

This work will build off the findings of proposal #1343. Both # 1748 and this proposal are part of the thesis work of Mara McAdams DeMarco.

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* AS #2006.16)
B. primarily based on ARIC data with ancillary data playing a minor role
(usually control variables; list number(s)* albuminuria, AS#_2002.02_)

*ancillary studies are listed by number at [http://www.cscc.unc.edu/aric/forms/](http://www.cscc.unc.edu/aric/forms/)

12. 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.

Works cited:
