1. Full Title: The interaction of diuretic use and serum urate handling genes on the risk of gout in the Atherosclerosis Risk in the Communities Study (ARIC)

b. Abbreviated Title (Length 26 characters): Diuretics, genetics and gout

2. Writing Group:

Writing group members: Mara McAdams DeMarco, Janet Maynard, Alan Baer, Anna Köttgen, 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 May, 2011
4. Rationale:

Gout is the leading cause of inflammatory arthritis in adults. In 2005, the estimated prevalence of gout in the United States was 3 million cases, and had increased significantly from 2.1 million in 1995.\(^1\) It is important to identify whether environmental and genetic risk factors interact to increase the risk of gout. Hyperuricemia is the strongest risk factor for gout and previous studies have identified genes influencing urate metabolism.\(^2\)\(^-\)\(^4\) A recent meta-analysis of genetic studies has identified eight loci that are associated with serum urate levels [(SLC22A11, GCKR, R3HDM2-INHBC region, RREB1, PDZK1, SLC2A9, ABCG2, and SLC17A1) and three additional loci were identified in a separate GWAS (SLC16A9, LRRC16, and SLC22A11)].\(^2\)\(^,\)\(^5\) 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).\(^2\)

Though there is a genetic component to hyperuricemia and gout, there are clear environmental risk factors for gout, including, hypertension, purine-rich foods and diuretic use.\(^6\) Our previous work has suggested that use of any diuretic, thiazide diuretics and/or loop diuretics, is independently associated with incident gout in hypertensive participants in ARIC (McAdams DeMarco, submitted to Archives of Internal Medicine 1/2011). Additionally, we found that diuretic use increases serum urate, which mediates the association of diuretic use and gout.

Of the eight loci that were associated with serum urate levels in the CHARGE consortium, five are renal urate transporters or regulators thereof (SLC2A9, ABCG2, PDZK1, SLC22A11 and SLC17A1); the biological mechanism relating the other three (GCKR, R3HDM2-INHBC, and RREB1) to serum urate levels is unknown. Decreased renal excretion of serum urate is thought to be the main cause of hyperuricemia and gout. Urate handling genes encode renal urate transport proteins that are targets of some diuretics. For example, a previous study has found that loop diuretics interact with the human sodium phosphate transporter 4 (SLC17A3, which is in same gene cluster as SLC17A1) to block the transport proteins and is thus a common secretion route for both diuretics and urate.\(^7\) Thus there is biological evidence that a shared pathway may lead to diuretic-induced hyperuricemia and gout. However, there have not been population-based studies to quantify the association of the diuretic by gene interaction and the development of gout. We know that both elevated urate levels and diuretic use increase gout risk. Therefore, we will test whether there is an interaction such that genetically susceptible individuals are prone to gout when on a diuretic.
We will test whether the presence of the renal urate transporter genes (SLC2A9, ABCG2, PDZK1, SLC22A11 and SLC17A1) and the other loci (GCKR, R3HDM2-INHBC region, and RREB1) that are associated with serum urate levels are differentially associated with gout risk among individuals using a diuretic.

We strive to understand whether diuretic use modulates the association between the urate handling genes and gout using the existing and valuable research infrastructure of a long-term prospective cohort: Atherosclerosis Risk in the Communities Study (ARIC). We hypothesize that the association between risk variants and incident gout is higher in individuals additionally using diuretics. Through a candidate gene study of urate handling genes, we will test our hypotheses with the following specific aims:

**Specific Aim 1:** Test whether the association of diuretic intake and incident gout differs by the presence of genes that influence urate renal excretion (SLC22A11, GCKR, R3HDM2-INHBC region, RREB1, PDZK1, SLC2A9, ABCG2 and SLC17A1).

**Specific Aim 2:** Test whether the association of diuretics with incident gout differ by a serum urate risk score.

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 limit the population to those with hypertension at any visit.

**Study design:** Candidate gene study of urate handling genes, diuretics and gout. Both aims will utilize the longitudinal cohort aspect of this data for the development of gout.

**Exposure:** We will consider each of the previously identified eight serum urate loci as individual exposures of interest (SLC22A11, GCKR, R3HDM2-INHBC region, RREB1, PDZK1, SLC2A9, ABCG2 and SLC17A1). The minor allele frequencies from the CHARGE cohort\(^2\) are listed
below. We will consider genetic exposure to a single gene to be present when there is at least one copy of the minor allele and absent when there are no minor alleles. As an alternative, we will consider the genetic exposure to be the number of copies of the minor allele (0, 1, 2).

<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>
<tr>
<td>rs675209</td>
<td>Chr 6: 7047083</td>
<td>RREB1</td>
<td>0.26</td>
<td>T (C)</td>
</tr>
<tr>
<td>rs1967017</td>
<td>Chr 1: 144435002</td>
<td>PDZK1</td>
<td>0.47</td>
<td>T (C)</td>
</tr>
</tbody>
</table>

Additionally, we will calculate the cumulative effect of the eight loci using the previously published genetic urate score.² 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:

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

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. The results are estimated in $\mu$mol/l.

**Diuretic use**

Trained interviewers collected information on the medications that participants used in the two weeks prior to the visit. Participants then reported whether they used a medication to treat hypertension. Our exposure will be report of diuretic use at visit 1, 2, or 3. We will not include diuretic use at visit 4 in our analysis because we want to ensure that use of this medication occurred prior to gout onset (reported at visit 4). Participants who report diuretic use after the onset of gout will be considered not exposed to a diuretic. We will parameterize diuretic use as
binary (present, absent). Additionally, we will consider thiazide and loop diuretics as separate classes of diuretics.

**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.\(^8\)

**Potential confounders:** We will identify confounders of the diuretic by gene interaction and gout association. Though no factors are thought to effect genetic status, risk factors for gout may also be associated with diuretic use and thus confounders. We will consider baseline (1989) age, sex, race, blood pressure, alcohol intake (grams/week), diabetes 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.\(^9\) 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:** For each gene we will test whether the development of gout is associated with each genetic locus among hypertensive participants who used a diuretic and among those who did not use a diuretic. We will test for an interaction in a single model with the gene x diuretic use term using the Wald test. Using multiple logistic regression, we will calculate the odds ratio (OR) adjusted for potential confounders of the interaction including, sex, race, BMI, alcohol intake, and categorical estimated glomerular filtration rate. We will test for these interactions with a Wald test and a p-value <0.05 to be considered significant.

Using a multiple logistic regression model, we will estimate the odds ratio (OR) of incident gout by diuretic use and urate handling genes and the interaction of these two exposures. The genotype exposure will be parameterized both as the presence of at least one high risk allele (See table) and as the number of high risk alleles (See explanation in the exposure section). We will also use the genetic urate score and the interaction of this genetic urate score and diuretic use. We will adjust both models for possible confounders of the interaction and gout such as sex, race, BMI, alcohol intake, and categorical estimated glomerular filtration rate. We will test for these interactions with a Wald test and a p-value <0.05 to be considered significant.

Table
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diuretic use</th>
<th>Gout case</th>
<th>Non-case</th>
<th>OR</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>a</td>
<td>b</td>
<td>A=ah/bg</td>
<td>Joint genotype, diuretic vs neither</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>c</td>
<td>d</td>
<td>B=ch/dg</td>
<td>Genotype alone vs neither</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>e</td>
<td>f</td>
<td>C=eh/fg</td>
<td>Diuretic alone vs neither</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>g</td>
<td>h</td>
<td>-</td>
<td>Reference</td>
</tr>
</tbody>
</table>

*Multiplicative Interaction comparison: A vs. B*C*

**Secondary analysis**

We will also test whether sex additionally interacts with the gene by diuretic interaction (sex x diuretic x gene) because the previous work has found that there were sex-specific effects of the SNPs in *ABCG2* and *SLC2A9*. We may also test whether there is an additional interaction with race (race x diuretic x gene) and with hypertension (hypertension x diuretic x gene) in the whole study population. Even though most of the minor alleles are common, we realize that power for this analysis will be very limited.

**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. We recognize that the power for all interaction may be limited.

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 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

     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)?

# 1343: Stage II of a Genome-Wide Association Study for Genetic Variants Associated with Uric Acid Levels and Gout
This work will build off the findings of proposal #1343.

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/

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: