1.a. Full Title: Diuretic-gene interactions affecting lipid traits

b. Abbreviated Title (Length 26 characters): Diuretic-gene lipid effects

2. Writing Group:
   Writing group members: Evan L. Busch, Eric A. Whitsel, Christy L. Avery, Til Stürmer, Eric Boerwinkle, Jorge L. Del-Aguila, other members of the CHARGE Drug-Gene GWAS consortium, other members of ARIC

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

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3. **Timeline:** Analyses: March 29, 2013 for all 3 lipid traits

4. **Rationale:** Hypertension (HTN) is a significant risk factor for cardiovascular morbidity and mortality. In 2006, approximately one in three U.S. adults had hypertension, with African Americans at especially high risk.\(^1\) Although diuretics are recommended as first-line therapy for the treatment of HTN\(^2\), several reports have suggested adverse effects associated with treatment.\(^3\)-\(^5\) For example, a meta-analysis of 474 randomized controlled trials of predominantly European descent participants reported that in addition to lowering blood pressure, diuretic therapy also was associated with increases in mean concentrations of triglycerides (TG, 31 mg/dL) and low-density lipoprotein cholesterol (LDL-C, 9 mg/dL), as well as decreases in high-density lipoprotein cholesterol concentration (HDL-C, 1 mg/dL).

Pharmacogenomic mechanisms may influence these diuretic-related changes in LDL-C, TG and HDL-C concentrations. For example, several genes have been identified that influence the anti-hypertensive efficacy of thiazide diuretics.\(^6\) Genome-wide association studies (GWAS) also have identified numerous genes that influence lipid concentrations.\(^7,8\) However, few studies have examined how common genetic variants modify the association between diuretic treatment and lipid concentrations on a genome-wide scale.

5. **Main Hypothesis/Study Questions:** To identify genetic variants that modify the effects of thiazide or thiazide-like and loop diuretics on lipid (TG, LDL-C, and HDL-C) concentrations in participants of European American (EA) and African American (AA) descent.

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).**

**Overview**

Our goal is to use ARIC data to examine interactions between use of diuretic medications and genes as they relate to blood lipid concentrations. Included participants will have used a thiazide or thiazide-like diuretic (without a loop diuretic) OR used a loop diuretic (without a thiazide or thiazide-like diuretic) during at least one ARIC visit. We will use ARIC genotyping data to identify genetic modifiers of the association between diuretic exposure and fasting concentrations of three lipids: TG, LDL-C, and HDL-C.

This proposal is nested within the CHARGE pharmacogenomics working group (PWG), a consortium of >70,000 participants predominantly of EA and AA descent.\(^9\) Following the CHARGE PWG meta-analysis protocol, we will conduct within-study,
race/ethnicity-stratified longitudinal analyses (harnessing repeated drug and lipid measures) and meta-analytically combine them within race-ethnicity across cohorts.

**Validity of Pharmacogenomics Research Using Non-Experimental Data**

Non-experimental data from ARIC are well-suited for the investigation of pharmacogenomic effects when compared with clinical trials, which can have a run-in phase to exclude participants experiencing early manifestations of drug intolerance prior to randomization. Despite these benefits, we note that the drug-SNP interaction models used to evaluate genetic susceptibility to medications in the context of non-experimental studies may be prone to bias and error, which remain incompletely characterized. In previous simulations, we found that confounding by indication does not appreciably affect cross-sectional non-experimental pharmacogenomic studies. However, effects on longitudinal models and the influence of measurement error, duration of use, bias, type I error, and statistical power have yet to be quantified. Therefore, we are extending the simulations to accommodate repeated assessments of exposure and outcome. Results from these simulations will inform interpretation of results and selection of the most valid statistical approach.

**Study Design**

Repeated observations, cross-sectional GWAS consortium study

**Medication Exposure Definition**

1. Use of a thiazide or thiazide-like diuretic (thiazides: yes/no) without use of a loop diuretic
2. Use of a loop diuretic (yes/no) without use of a thiazide or thiazide-like diuretic

Medication use (yes/no) is defined for each clinic visit as the use of any medication in the medication class, regardless of dosage, and with or without the use of a potassium supplement, as assessed by medication inventory at that clinic visit.

Our common comparison group will be participants who used other antihypertensive medications, such as beta-blockers, calcium-channel blockers, or angiotensin converting-enzyme inhibitors.

**Phenotype Outcome Definition (excluding participants who are not fasting)**

1. TG (mg/dL) – natural-log transformed
2. LDL-C (mg/dL)
3. HDL-C (mg/dL)

**Inclusions**

Consenting EA and AA men and women with GWAS data, medication data, and at least one fasting lipid concentration.

**Visit-Specific Exclusions**

- Exclude participants with missing data for any of the medications and/or covariates
- For analysis of each diuretic exposure pattern, exclude participants taking other diuretics:
  1. For thiazides, exclude participants taking loop diuretics.
  2. For loop diuretics, exclude participants taking thiazides.
• For analysis of each lipid concentration, exclude participants with missing fasting status or fasting <9-12 hours after previous caloric intake. Note: participants with a missing value for one lipid concentration will be used for analysis of another non-missing concentration.

• Since LDL-C concentrations in ARIC are derived using the Friedewald equation, visit-specific LDL-C concentrations will be excluded when TG > 400 mg/dL.

• All first-degree relatives are excluded, as are overlapping participants in the Jackson Heart Study.

**Statistical Model**

Additive genetic model:

\[ Y_{ij} = \beta_0 + \beta_E I_{ij} + \beta_G SNP_i + \beta_{G:E} SNP_i I_{ij} + \beta_C C_i, \]

where

- \( Y_i \) is the fasting lipid concentration for the \( i^{th} \) participant
- \( \beta_0 \) is the intercept
- \( I_{ij} \) is the indicator for drug use at each visit
- \( SNP_i \) is the dosage of the genetic variant
- \( C_i \) is the vector of covariates: age, sex, BMI, and ten principal components

The initial strategy is to longitudinally model the average of repeated outcomes and thereby facilitate estimation of effects by increasing power using methods we have identified, tested and applied to large-scale genomic data in the CHARGE PWG over the last two years under ARIC AS#2009.10. The strategy relies on conventional generalized estimation equations (GEE). Although other structures can be accommodated, an independence correlation structure will be used in this context to ensure consistency of the GEE estimates in the presence of time-varying covariates, and protect against potential bias related to the putative effects of past lipid concentrations on future medication use.\(^\text{11,12}\) Pan and Wall's small-sample GEE extension\(^\text{13}\) of Satterthwaite’s method of approximating the degrees of freedom\(^\text{14}\) associated with the \( t \) reference distribution will be implemented in R using the bossWithdf package.

**Meta-analysis**

Summary results will be meta-analyzed by race/ethnicity. Fixed effects inverse variance weighted meta-analysis will be used unless early departure of test statistics from the null distribution occurs.

**Genome-Wide Significance Level**

\[ P < 5 \times 10^{-8} \]

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 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.csc.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)?


There is substantial overlap between the authors of the above proposals and the present proposal, indicating that the authors of the present proposal have good experience working on this topic.

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* 2009.10__)

______ 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.csc.unc.edu/aric/forms/

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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


