**ARIC Manuscript Proposal #2719**

**PC Reviewed: 3/8/15**  
**Status: A**  
**Priority: 2**

**SC Reviewed: __________**  
**Status: _____**  
**Priority: ____**

1.a. **Full Title**: Effect of antihypertensive drugs on left ventricular traits in African Americans

b. **Abbreviated Title (Length 26 characters)**: antihypertensive drugs, left ventricular traits, African Americans

2. **Writing Group**:
   Writing group members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Contact Information</th>
<th>Responsibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anh Do</td>
<td><a href="mailto:anhdo85@uab.edu">anhdo85@uab.edu</a></td>
<td>Data analysis, manuscript writing</td>
</tr>
<tr>
<td>Ryan Irvin</td>
<td><a href="mailto:irvinr@uab.edu">irvinr@uab.edu</a></td>
<td>Supervising data analysis, supervising manuscript writing</td>
</tr>
<tr>
<td>Sanjiv Shah</td>
<td><a href="mailto:sanjiv.shah@northwestern.edu">sanjiv.shah@northwestern.edu</a></td>
<td>Phenotype interpretation</td>
</tr>
<tr>
<td>Degui Zhi</td>
<td><a href="mailto:dzhi@uab.edu">dzhi@uab.edu</a></td>
<td>Advised biostatistics method</td>
</tr>
<tr>
<td>Thomas Mosley</td>
<td><a href="mailto:tmosley@umc.edu">tmosley@umc.edu</a></td>
<td>Conducted/supervised Genotyping/Phenotyping in ARIC</td>
</tr>
<tr>
<td>Ervin Fox</td>
<td><a href="mailto:efox@umc.edu">efox@umc.edu</a></td>
<td>Conducted/supervised Genotyping/Phenotyping in ARIC</td>
</tr>
<tr>
<td>Amil Shah</td>
<td><a href="mailto:ashah11@partners.org">ashah11@partners.org</a></td>
<td>Critical review of data and manuscript</td>
</tr>
</tbody>
</table>

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

**First author: Anh N. Do**
Address: University of Alabama at Birmingham, School of Public Health. Department of Epidemiology, 1665 University Blvd. Ryals Building Room 220, Birmingham, AL 35294-0022.
Telephone: (205) 975-7669  
Fax: (205) 934-8665  
Email: anhdo85@uab.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: **Thomas Mosley and Ervin Fox**
Address: The University of Mississippi Medical Center, School of Health Related Professions, 2500 N. State Street, Jackson, MS 39216-4505.

Phone: (601) 984-2763  
Fax: (601) 815-3422  
E-mail: tmosley@umc.edu

3. **Timeline**: December/2016

4. **Rationale**:
   Left ventricular hypertrophy (LVH) is the thickening of the myocardium (muscle) of the left ventricle of the heart. LVH is common in the general population (16% of Caucasians, and up to 43% of AAs)\(^1\) and even more common among individuals with hypertension (up to 60%).\(^2-4\) It is
recognized as an independent risk factor for several cardiovascular-related outcomes including stroke, and heart failure as well as all-cause mortality.\textsuperscript{5-8} Research shows LVH may be a better predictor of mortality than coronary artery disease in many populations.\textsuperscript{10} The prevalence of LVH in AAs is twice that of Caucasians and poses greater cardiovascular risk as compared to other ethnic groups.\textsuperscript{1,11,12} Genetic factors have been widely accepted to influence LVH and other structural and functional cardiac phenotypes. Heritability estimates as large as 0.59 have been reported for left ventricular (LV) mass.\textsuperscript{13} Notably, heritability of LV mass was reported higher in AAs compared to that in Caucasians.\textsuperscript{13} Additionally, linkage and genome-wide association studies (GWAS) have identified biologically plausible genetic loci for LVH, and other structural/functional echocardiographic phenotypes.\textsuperscript{14-19}

Antihypertensive drugs have been found to decrease LV mass in hypertensive patients.\textsuperscript{2,20-23} In an 80-study meta-analysis representing data on over 3,767 persons treated for hypertension, Klingbeil et al. found a significant difference in LV mass regression by antihypertensive treatment class.\textsuperscript{21} Specifically, LV mass index was improved the most for calcium channel blocker (CCB) treatment (average 11% decrease), followed by angiotensin-converting enzyme inhibitor (ACE-I) treatment (10% average decrease), and least for diuretic treatment (average by 8%) compared to LV mass at baseline.\textsuperscript{21} Another meta-analysis concluded that LV mass was decreased by antihypertensive drug treatment in general, with no significant differences between treatment classes.\textsuperscript{23} Therefore, there is a lack of consensus regarding the best antihypertensive agents for LVH prevention. The effect of antihypertensive agents on diastolic function is also controversial.\textsuperscript{2,20-23} Overall, AAs have been underrepresented in previous studies.

Though studies suggest that antihypertensive agents may improve LV mass, data show there are large inter-individual variations in treatment response suggesting genomic factors may be at play.\textsuperscript{24-26} Previous candidate gene studies have attempted to find pharmacogenetic factors associated with LVH and related traits.\textsuperscript{24,25} However, most previous studies were small in size, considered few variants and results were not replicated. Additionally, DNA methylation may alter drug response via affecting gene expression and downstream functions of coded biomolecules (proteins or RNAs) that antihypertensive agents target.\textsuperscript{41,42}

To evaluate the relationship between the three most common antihypertensive treatments for AAs (diuretics, ACE-Is, and CCBs)\textsuperscript{28} and continuous traits related to LVH including LV mass (LVM), LV internal dimension-diastole (LVIDD), relative wall thickness (RWT), fractional shortening (FS) as well as modification of those relationships by genomic markers, we propose to use echocardiography data from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) EchoGEN consortium. The overall goal of this project is to help understand which antihypertensive treatment classes are best for LVH prevention and to promote future personalized medicine research for antihypertensive treatment in this race group. This project will benefit from an unprecedented sample size of AAs for the study of LVH related traits, and robust study design using data collected from the CHARGE Consortium.

5. Main Hypothesis/Study Questions:

**Hypothesis 1.** ACEI, CCB, and diuretic have different effects on LV traits and diastolic function in AAs.

**Aim 1.** We will investigate the effect of diuretic, ACE-I and CCB on echocardiographic LV structural, and functional traits using data from ~6000 AAs treated for hypertension from the CHARGE consortium.
Hypothesis 2. Common genetic variants modify the association between ACEI, CCB, and diuretic use on LV traits and diastolic function in AAs.

Aim 2. We will investigate the interaction between genetic variants and antihypertensive treatment class from aim 1 on echocardiographic LV structural and functional traits using data from ~6,000 AAs treated for hypertension from the CHARGE consortium.

Hypothesis 3. DNA methylation modifies the association between ACE-I, CCB, and diuretic use on LV traits and diastolic function in AAs.

Aim 3. We will investigate the interaction between CpGs and antihypertensive treatment class on echocardiographic LV structural and functional traits using data from ~3,000 AAs treated for hypertension from the CHARGE consortium including ARIC, CHS, BHS, GENOA, and HyperGEN cohorts.

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).

6.1. Data

1. Phenotype used in analysis

We will investigate four quantitative traits related to LVH and diastolic function including LV mass indexed by height$^{2.7}$ (LVM, g/m$^{2.7}$), LV internal dimension-diastole (LVIDD, cm), LV relative wall thickness (RWT), fractional shortening (FS, %). We will use the echocardiography data from visit 3 for all aims.

2. Drug Exposure definition for each of two drug classes

Three antihypertensive drug classes will be compared in a pairwise manner. The first model will compare any ACE-I use vs. diuretic use (reference = diuretic use) where ACE-I exposure will be use of an ACE-I in a single or combination preparation without concomitant use of a diuretic versus diuretic exposure without ACE-I. The second model will compare CCB use vs. diuretic use (reference = diuretic use) where CCB exposure will be use of a CCB in a single or combination preparation without concomitant use of a diuretic versus diuretic exposure without CCB. Similarly, the third model will be CCB use vs. ACE-I use (reference = ACE-I use) where CCB exposure will be use of a CCB in a single or combination preparation without concomitant use of an ACE-I versus ACE-I exposure without CCB. Using this approach a study participant taking more than one medication class may contribute data to more than model.

3. Gene Exposure definition

GWAS data from cohorts will be imputed using hg18 as the reference representing ~2.5 million SNPs available in each dataset. We will use additive genetic model for all analysis.

4. DNA methylation data preparation

DNA methylation data can be prepared independently in each cohort. Please correct for color channel difference, background values, and probe type differences. It is preferred to use commonly used normalization methods in R packages including minfi, BMIQ, ChAMP and others. If Combat is used, please make sure to adjust for the different probe types in addition to Combat.

QC or exclusion criteria for DNA methylation data

- Set a DNA methylation level (of a probe of a sample) with detection p-value > 0.01 as missing.
- Exclude probes with detection p-values > 0.01 in > 5% of samples.
- Exclude samples with detection p-values > 0.01 for > 5% of probes.
Please use beta-score of DNA methylation levels.

5. Exclusions
- Non-consenters
- Not treated for hypertension
- Use any combination preparation of three antihypertensive treatments including diuretic, ACE –I, and CCB.
- Missing LV phenotype of interest
- Overlapping with the GENOA or JHS cohorts.

6. Covariates
- Age, sex (reference=male), body mass index, number of antihypertensive agents used, chronic kidney disease (CKD) (Yes/No, reference=No), type 2 diabetes (T2D) (Yes/No, reference=No). CKD is defined by physician diagnosed or glomerular filtration rate (GFR) <60 mls/min/1.73m² or albumin-to-creatinin >30 mg/g. DM is defined by physician diagnosed or using at least one of anti-diabetic treatments or having fasting plasma glucose ≥ 7.0 mmol/l (or 126mg/dL).

6.2. Brief Statistical Analysis Plan and Methods: (Including power calculations, if necessary.)

1. Hypothesis 1: Cross-sectional
The effect of diuretics, ACE-I, and CCBs on LV traits, and diastolic function will be assessed as outlined below:

Analysis by ARIC analyst
a. Baseline characteristics and the four LV traits will be described among users of diuretics, ACE-I, and CCBs.
Data from visit 3 will be considered cross-sectionally.
b. Primary outcomes for regression models will be LVM, RWT, LVIDD, FS. Exclude extreme values (>5 standard deviations of its mean) for each echocardiographic measure. Natural log-transformations will be made for LVM, LVIDD, and RWT to satisfy model distributional assumptions.
c. Use linear regression to test the main effect of antihypertensive treatment class (ACE-I vs. Diuretic; CCB vs. Diuretic; CCB vs. ACE-I, as described in section Drug Exposure) on each of the four LV traits separately. Each model will be adjusted for age, sex, body mass index, and count of antihypertensive treatment classes, CKD, and T2D. Study site and/or other study specific variables should be included as covariates as needed.
Analysis at UAB by Anh Do
d. Inverse-variance weighted fixed-effects meta-analyses will be carried out using the software package meta (R repositories). Statistical heterogeneity will be evaluated using Cochrane's χ² test (Q-test). P-values < 0.0042 will indicate significant results (α=0.05/3*4 for 3 models and 4 traits).

2. Hypothesis 2: Cross-sectional
The effect of the interaction between genetic variants and ACE-I vs. diuretic, CCB vs. diuretic, and CCB vs. ACE-I exposure on LV structural, and functional traits among AAs will be assessed as outlined below:

Analysis by ARIC analyst
a. Outcomes: The four primary outcomes and a speckle tracking measurement (gls) are the same as those listed for Hypothesis 1. Exclude extreme values (>5 standard deviations of its mean) for
each echocardiographic measure. Natural log-trait transformations will be made for LVM, LVIDD, and RWT to satisfy model distributional assumptions.
b. Use linear regression to test the interaction between antihypertensive treatment class exposure (pairwise comparison, see section Drug Exposure) and each SNP (under an additive model) on the outcomes.

Each model will be adjusted for age, sex, body mass index, count of antihypertensive treatment classes, CKD, T2D and principal components for ancestry. Study site and/or other study specific variables should be included as covariates as needed. The models for a speckle tracking measure will also be adjusted for institution, reader, and image quality as covariates beside the above listed covariates.

Analysis at UAB by Anh Do
c. We will follow meta-analytic guidelines from the CHARGE Consortium working group where fixed effects inverse variance weighted meta-analysis will be used, unless early departure of test statistics from the null distribution occurs; then a t-distribution approach will be used. Briefly, p-values will be recalculated by applying a t-reference distribution to the drug-SNP estimates of $\beta$ (standard error), and then meta-analyzed using a weighted Z-statistic, with weights based on the SNP imputation quality multiplied by the estimated number of independent observations exposed to the drug (Nexposed is the non-reference drug). The cohort- and SNP-specific degrees of freedom (DF) for the t-reference distribution will be estimated using Satterthwaite’s method.
d. To control for multiple testing, we will use the Bonferroni correction where $\alpha$ will be set to $0.05/(2.5 \text{ million SNPs}*4*3)=2*10^{-9}$ where 3 is the number of treatment comparisons, 4 is the number of outcomes in our primary analyses.

3. Hypothesis 3: Cross-sectional
The effect of the interaction between CpGs and ACE-I vs. diuretic, CCB vs. diuretic, and CCB vs. ACE-I exposure on LV structural, and functional traits among AAs will be assessed as outlined below:
Analysis by ARIC analyst
a. Outcomes: The four primary outcomes and a speckle tracking measurement (gls) are the same as those listed for Hypothesis 1 and 2. Exclude extreme values (>5 standard deviations of its mean) for each echocardiographic measure. Natural log-transformations will be made for LVM, LVIDD, and RWT to satisfy model distributional assumptions.
b. Use linear regression to test the interaction between antihypertensive treatment class exposure (pairwise comparison, see section Drug Exposure) and each CpG on each of the four LV traits and a speckle tracking measurement, separately.

Each model will be adjusted for age, sex (reference=male), body mass index, and number of antihypertensive agents used, chronic kidney disease (CKD) (Yes/No, reference=No), type 2 diabetes (T2D) (Yes/No, reference=No), principal components from genotypes (for ancestry), and proportion of white blood cell counts (WBC) as fixed effects, and random effects for plate, row, and column. The models for a speckle tracking measure will also be adjusted for institution, reader, and image quality as fixed effects beside the above listed covariates.

Model: Echocardiographic measures ~ Antihypertensive drug exposure + CpG + Antihypertensive drug exposure *CpG + age + sex + BMI + number of antihypertensive treatment use + CKD + DM + SNP PC1-4 + WBC + (1| plate) + (1|row) +(1|column) + study site.

The models for a speckle tracking measure will also be adjusted for institution, reader, and image quality as fixed effects beside the above listed covariates.
Analysis at UAB by Anh Do

c. We will follow meta-analytic guidelines from the CHARGE Consortium working group where fixed effects inverse variance weighted meta-analysis will be used. To control for multiple testing, we will use the Bonferroni correction where \( \alpha \) will be conservatively set to \( \frac{0.05}{(450k \text{ CpGs} \times 4 \text{ traits} \times 3 \text{ drug comparisons})} = 9 \times 10^{-9} \) for all analyses.

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? __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.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)?


11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes    __x__ No

b. If yes, is the proposal

| A. primarily the result of an ancillary study (list number* ____________) |
| 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.cscc.unc.edu/aric/forms/
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.cscce.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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript _____ Yes __x__ No.

References

9. Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Annals of internal medicine.* 1991;114(5):345-352.


