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EFFECT OF ANTIHYPERTENSIVE DRUGS ON LEFT VENTRICULAR TRAITS IN AFRICAN AMERICANS

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ABSTRACT

The purpose of this dissertation is to evaluate the association of antihypertensive treatment as well genomic variants with left ventricular (LV) hypertrophy (LVH) -related traits among African Americans (AAs) in five cross-sectional epidemiology cohorts from the United States. AAs especially those with hypertension, are overburdened by left ventricular (LV) hypertrophy (LVH) compared to other ethnic groups. LVH is associated with increased risk for cardiovascular morbidity and mortality. Antihypertensive treatments have been found to improve LVH and related echocardiographic measures (i.e. LV traits) among hypertensive patients. However, the effect of antihypertensive treatments on LVH and related traits in AAs has not been well studied. Furthermore, high heritability of LV traits (e.g. LV mass heritability in AAs is 34%) and variability of the response to antihypertensive treatments suggests genetic factors may be involved.

The first aim evaluated the association of rare variants with LV traits among AAs using exome chip data from the Hypertension Genetic Epidemiology Network study (HyperGEN) and Genetic Epidemiology Network of Atherosclerosis study (GENOA) studies. The second aim evaluated the main effect of the three most common antihypertensive treatments for AAs as well as SNP-by-drug interaction effects on LV traits in five cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium. Antihypertensive treatments considered included thiazide diuretics (TDs), angiotensin converting enzyme inhibitors (ACE-Is), and

dihydropyridine calcium channel blockers (dCCBs). The third aim evaluated the interaction between CpGs and TDs, ACE-Is and dCCBs on LV traits using data from AAs from the HyperGEN and GENOA studies.

We found that TDs may be better for prevention of LVH in comparison to dCCBs in AAs. It also suggests that common variants could modify the association between antihypertensive treatment and LV traits in AAs. In addition, rare protein coding variants could be important contributors to LV structures and LV functions in AAs. A strength of this body of work is that these are some of the first investigations of these exposures and traits among AAs. Future sequencing studies that capture more variants in larger populations of AAs treated for hypertension are needed to expand these findings here.

Keywords: left ventricular hypertrophy, rare variants, antihypertensive treatment, African American.

DEDICATION

To my parents, Thanh D. Do and Anh T. Pham, who gave life to me and cared for me all my life; my husband, Hieu D. Hoang, who care for me until the day I die and I him; my daughter, ThaoVi D. Hoang, who will hopefully become another researcher, and my younger brother Tam D. Do, who always supports and listens to me when I am down. I am also thankful to my teacher Sinh Q. Nguyen, who inspired and encouraged me to study abroad. Next, I want to especially thank my mentors, Dr. Ryan Irvin, Dr. Donna Arnett, and other members in our lab who have given me the most nurturing learning environment, extensive care and advice, and most importantly steered me to the right path throughout my graduate learning journey.

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INTRODUCTION

Cardiovascular (CV) disease is a leading cause of death in the U.S.[1], responsible for one out of fourth deaths in 2015.[2] According to the latest estimates, approximately 24 million Americans is expected to develop the condition by 2030.[3] Chronic and complex CV disease affects racial groups differently with African Americans suffering higher rates of morbidity and mortality.[4] One risk factor, left ventricular (LV) hypertrophy (LVH), is known to increase CV endpoints including stroke and heart failure.[5] LVH is defined as a cardiac condition in which left ventricle wall thickness and/or size increase. In fact, LVH doubles the risk of CV morbidity and mortality across racial, gender, and age groups.[5] LVH is also a better predictor of mortality than coronary artery disease in multiple populations.[6]

Another related progressive CV condition is LV diastolic dysfunction referring to abnormalities and impairments of LV function during diastole.[7] LV diastolic dysfunction is common. Among middle aged Americans randomly selected from Olmsted County, Minnesota 27.4% of 2,042 were affected, and among elder participants of the Framingham Heart Study 36% had the condition.[8, 9] Similar to LVH, LV diastolic dysfunction predicts heart failure and coronary heart disease (CHD) in diverse populations.[10-12] LV diastolic dysfunction is also known to be associated with increased risk of hospitalization and all-cause mortality.[8]

In comparison to Caucasians and Hispanics, African Americans are overburdened by LVH and related conditions. In particular, they have the highest prevalence of LVH, about 43%, two times more than that of Caucasians.[13] They also exhibit greater average LV mass and cardiac wall chamber thickness (both common measures of LVH) compared with Caucasians.[14, 15] Additionally, increased LV mass and cardiac wall chamber thickness are reported to contribute to higher CV risk and adverse prognosis.[15] Similarly, African Americans have slightly higher prevalence of LV diastolic dysfunction than the general population.[16]

The presence of LV hypertrophy and magnitude of LVH-related traits substantially vary between individuals and more recently genetic risk factors associated with LV traits have emerged.[17, 18] Recent genome wide association studies (GWAS) have identified common variants associated with LV traits in African Americans. Several novel genes including ubiquitin-conjugating enzyme E2 variant 2 (*UBE2V2),* WD-repeat-domain phosphoinositide interacting protein 1 *(WIPI1),* phospholipid phosphatase 4 *(PPAPDC1A),* kruppel-like factor 5 (*KLF5)* and neural cell adhesion molecule 1 (*NCAM1)* were found to be associated with LV traits in meta-analyses of observational epidemiology studies conducted in African Americans.[19, 20] However, all common variants identified to date only explain a small proportion of inter-individual variation of LV traits. For example, common variants of *NCAM1* only explained 1.96% of variation in posterior cardiac wall thickness in HyperGEN African Americans.[19] To our knowledge, there have been no studies of rare variants in relation to LV traits or LV diastolic function.

Hypertension tightly correlates with LVH. Hypertension is a well-established risk factor for LVH and other CV outcomes including myocardial infarction and heart failure.[21] Up to 60% hypertensive individuals are inflicted with LVH.[22] Earlier onset and severe hypertension might increase the prevalence of LVH in diverse populations.[15, 23] Hypertension induces LVH by increasing pressure overload and long-term adaptive hypertrophy of vascular and myocardial tissues through different mechanisms.[24] First, hypertension increases constriction in coronary arterioles, and inflammation that might cause hyperplasia of fibroblasts and enlarged vascular smooth muscle layer.[24] Consequently, interstitial collagen and intra-myocardial capillary density increase and arterioles thicken.[24] In other words, LVH is a compensatory process. Moreover, LVH in normotensive participants at baseline were also conversely associated with the development of hypertension after 3-8 years of follow-up.[25-27] However the mechanism of how LVH may lead to hypertension is less clearly understood.

Antihypertensive drugs have been found to decrease LV mass in hypertensive patients in meta-analysis studies however there is a lack of consensus on the best antihypertensive agents for decreasing LV mass and likely there are subgroups of patients who may benefit more from on a specific class of drug. [28-32] Additionally, the effect of antihypertensive agents on LV diastolic function is also controversial.[28-32] Overall, AAs have been underrepresented in previous studies.

Though studies suggest that antihypertensive agents may improve LV mass, data show there are inter-individual variations in treatment response suggesting genetic factors may be at play.[33-35] Previous candidate gene studies have attempted to find pharmacogenetic factors associated with LVH and related traits.[33, 34] However, most previous studies were small in size, considered few variants and results were not

replicated. Recently, candidate gene work has suggested DNA methylation may play a role in LV trait variation in relation to antihypertensive treatment. In particular one study found methylation of the *ADRB1* gene modifies the effect of metoprolol, on cardiomyocytes in rats.[36] That we know of no human studies have investigated whether DNA methylation modifies the association between antihypertensive treatment class and LVH related traits. Genome-wide scans for markers that modify the effect of antihypertensive treatment class on LV traits in a sizable population of AAs can help identify novel pharmacogenetic variants.

In the current body of work, we evaluate how genomic factors affect LV related traits in AAs. First, we examined the relationship between rare variants and LV related traits. Next, we evaluate the relationship between the three most common antihypertensive treatments for AAs (thiazide and related diuretics (TDs), angiotensin-converting enzyme inhibitors (ACE-Is), and dihydropyridine calcium channel blockers (dCCBs))[37] and LV traits. Next, we evaluate how the drug-trait relationship may be modified by genomic markers. We studied quantitative traits related to LVH for three reasons 1) they allow capturing subtle inter-individual variations, 2) they provide more information about the trait distribution in the study population, and 3) they are more powerful to detect genetic exposure association with the outcome in comparison to dichotomous traits.[38]

The overall goal of this project is to help understand genetic risk factors for LVH and which antihypertensive treatment classes are associated with better LV trait profiles. This project benefits from an unprecedented sample size of AAs for the study of LVH related traits, and robust study design using data collected from observational epidemiology studies (including the Coronary Artery Risk Development in Young Adult

Study (CARDIA), Cardiovascular Health Study (CHS), Jackson Heart Study (JHS), Genetic Epidemiology Network of Atherosclerosis study (GENOA), and Hypertension Genetic Epidemiology Network (HyperGEN)).

The specific aims for this study are:

Aim 1. (Manuscript 1) To investigate the effect of rare variants on echocardiographic LV structural and functional traits using data from AAs from the HyperGEN and GENOA studies.

Aim 2a. (Manuscript 2) To investigate the effect of TDs, ACE-Is and dCCBs on echocardiographic LV structural, and functional traits using data from AAs treated for hypertension from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium as well as other observational epidemiology cohorts.

Aim 2b. (Manuscript 2) To investigate the interaction between genetic variants from genome wide association study (GWAS) array data and antihypertensive treatment classes from aim 2a on echocardiographic LV structural and functional traits using data from AAs treated for hypertension from the CHARGE consortium.

Aim 3. (Manuscript 3) To investigate the interaction between DNA methylation and antihypertensive treatment class on echocardiographic LV structural traits using data from AAs treated for hypertension from the HyperGEN and GENOA studies.

Phenotype and Drug exposure

Phenotype

LV traits and diastolic function were collected by using a standardized echocardiogram protocol in all six cohorts.[17, 23, 39-43] Quality assurance was conducted to assure protocol adherence and high-quality scans.[17, 23, 41-43] In all six cohorts, two-

dimensional directed M-mode and Doppler echocardiographic data were collected for LV structure.[17, 23, 41-43] Previous publications in CHARGE have harmonized echocardiographic data across these cohorts for Caucasians and AAs.[20, 44]

LV structure. For the current study, we consider two quantiative traits including LV mass (LVM) and LV relative wall thickness (RWT) for LV structure. LVM is defined as 0.80 x [1.04(IVSDD + PWTD + LVIDD)^3 - LVIDD^3] + 0.6g, and RWT=(2x posterior wall thickness)/LV end diastolic dimension. Better cardiac structure is indicated by lower LVM and RWT (see **Figure 1.**). See **Figure 2.** for an illustration of LV structural measures from echocardiogram which contribute to LVM and RWT.

LV diastolic function. For LV diastolic function, we use early diastolic tissue velocity at the septal mitral annulus (e' velocity) and the ratio of early (E) transmitral flow velocity to early diastolic tissue velocity at the septal mitral annulus (E/e' ratio). e' velocity and E/e' ratio are a measure of LV relaxation and LV diastolic filling pressure, respectively. See **Figure 3.** for an illustration of LV diastolic function. Better cardiac function is indicated by lower E/e' ratio, and low e' velocity indicate worse cardiac function (see **Figure 1**.).

Exploratory Speckle Tracking Trait. A more sensitive method to measure cardiac function is speckle tracking echocardiography. This method measures intrinsic indices of cardiac mechanics including LV strain and tissue velocities to detect subclinical cardiac performance changes.[45] A speckle tracking measure considered in this project is LV global longitudinal strain (GLS), a measure of LV systolic function. GLS is shown to be

strongly associated with cardiovascular mortality in the literature.[45, 46] GLS is available in HyperGEN and CARDIA studies. GLS describes the relative length change of the LV myocardium between end-diastole and end-systole.[47] The low absolute GLS indicates worse cardiac function (see **Figure 1.**).[45]

Drug exposure

Three antihypertensive drug classes are compared in a pairwise manner.

Model 1: ACE-I use vs. TD use (reference = TD use) where ACE-I exposure is use of an ACE-I in a single or combination preparation without concomitant use of a TD versus TD exposure without ACE-I.

Model 2: dCCB use vs. TD use (reference = TD use) where dCCB exposure is use of a dCCB in a single or combination preparation without concomitant use of a TD versus TD exposure without dCCB.

Model 3: dCCB use vs. ACE-I use (reference =ACE-I use) where dCCB exposure is use of a dCCB in a single or combination preparation without concomitant use of an ACE-I versus ACE-I exposure without dCCB.

Using this approach, a study participant taking more than one medication class may contribute data to more than model (see **Appendix 1 List 1** for a medication inventory of eligible antihypertensive treatments for each model and **Appendix 1 List 2** for excluded treatments)). Note: this proposal focuses on thiazide and thiazide-like diuretics and dihydropyridine CCBs only.

Exclusions

We exclude individuals meeting the following criteria: 1) Non-consenters; 2) Not treated for hypertension; 3) Using any combination preparation of three antihypertensive treatments including TD, ACE –I, and dihydropyridine CCB (**List 2**); 4) Missing LV phenotype of interest; 5) Urinary albumin-to-creatinin >300 mg/g.

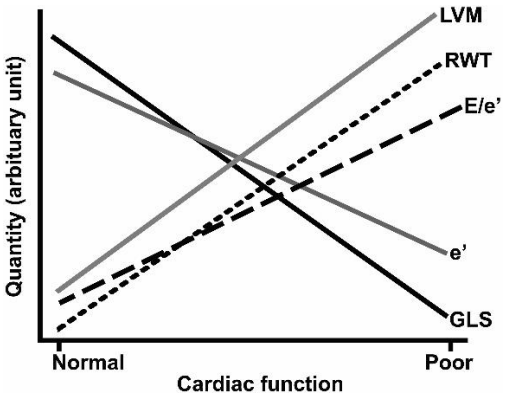

Figure 1. ***The relationship between LVH related traits and cardiac function.***

LVM, left ventricular mass; RWT, relative wall thickness; e', early diastolic tissue velocity at the septal mitral annulus; E/e', the ratio of early (E)

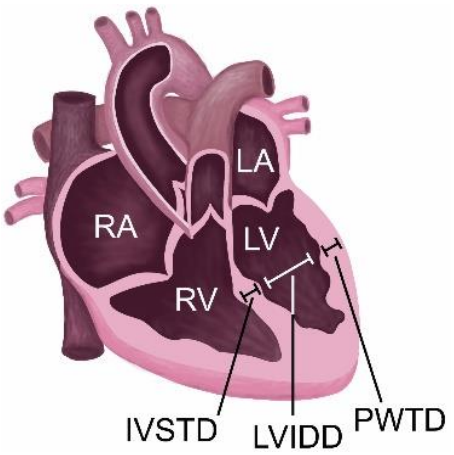

Figure 2. ***LV structure.***

RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; IVSTD, intraventricular internal dimension in diastole; LIVDD, LV internal dimension-diastolic; PWTD, posterior wall thickness in diastolic.

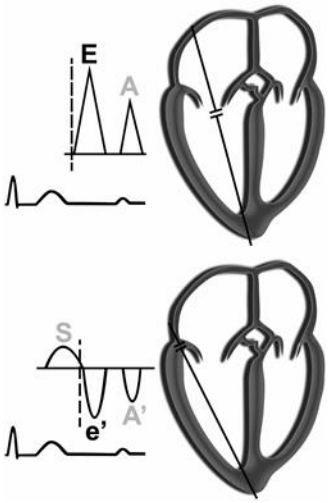

Figure 3. ***LV diastolic function.***

e', early diastolic tissue velocity at the septal mitral annulus; E/e', the ratio of early (E) transmitral flow velocity to early diastolic tissue velocity at the septal mitral annulus.

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MANUSCRIPT 1

WHOLE EXOME ANALYSES TO EXAMINE THE IMPACT OF RARE VARIANTS ON LEFT VENTRICULAR TRAITS IN AFRICAN AMERICAN PARTICIPANTS FROM THE HYPERGEN AND GENOA STUDIES

by
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ABSTRACT

Objective The aim of this study is to investigate the association between rare variants and left ventricular (LV) traits in African Americans.

Methods LV structural and functional traits and genotypes (exome chip) from 1,934 African American participants in the family-based Hypertension Genetic Epidemiology Network (HyperGEN) study were analyzed. The four LV structural traits included LV mass, LV internal dimension-diastole (LVIDD), relative wall thickness, and left atrial dimension (LAD). The two LV functional traits included fractional shortening (FS) and the ratio of LV early-to-late transmitral velocity (E/A ratio). Single-variant and gene-based analyses were used to investigate the association between 86,927 variants and LV traits using the RAREMETAL Software. Only rare variants (MAF <1% and <5%) were considered in gene-based analyses. Significant HyperGEN results were replicated using comparable data from 1,090 African American participants from the Genetic Epidemiology Network of Arteriopathy (GENOA) study.

Results In gene-based analyses, we found a statistically significant association between potassium voltage-gated channel subfamily H member 4 (*KCNH4*) and E/A ratio ($P=8.7*10^{-8}$ using a burden test). Endonuclease G (*ENDOG*) was associated with LAD using the Madsen Browning weighted burden (MB) test ($P=1.4*10^{-7}$). Neither gene result was replicated in GENOA, but the direction of effect of single variants in common was comparable. G protein-coupled receptor 55 (*GPR55)* was marginally associated withLAD in HyperGEN ($P=3.2*10^{-5}$ using the MB test) and E/A ratio in GENOA, but with opposing directions of association for variants in common (P=0.03 for the MB test). No

single variant was statistically significantly associated with any trait after correcting for multiple testing.

Conclusions The findings in this study highlight the potential cumulative contributions of rare variants to LV traits which, if validated, could improve our understanding of heart failure in African Americans. Continued investigation of the top gene-based findings are needed since many variants observed in HyperGEN were available in GENOA.

INTRODUCTION

Heart disease is the leading cause of death in the United States [1]. Heart failure (HF) is an often lethal form of heart disease that may be preceded by left ventricular (LV) hypertrophy and diastolic dysfunction [2]. Arterial hypertension is the most common risk factor for this disease process which is thought to precipitate changes in the structure and function of the left ventricle leading to changes in relaxation and filling [3]. Therefore, studies of LV structure and function may shed light on underlying HF pathogenesis.

It is important to understand risk factors for these traits in African Americans as they have a higher risk of heart failure compared to Caucasians [4]. African Americans also have the highest prevalence of LV hypertrophy (43%), twice that of Caucasians, and they exhibit higher measures of LV mass and relative wall thickness compared to Caucasians [5, 6]. Additionally, studies report African Americans exhibit worse diastolic function. For instance, in the Cardiac Abnormalities and Brain Lesions study African Americans had lower mitral annulus early diastolic velocities (e') in comparison to their Caucasian counterparts and in the Anglo-Scandinavian Cardiac Outcomes Trial African Caribbeans showed lower e' and higher ratio of transmitral early (E) velocity to e' compared to Caucasians [7, 8].

There is evidence that racial differences in LV mass and related traits may have genetic underpinnings [9-12]. A study from the Hypertension Genetic Epidemiology Network (HyperGEN) cohort indicated that the gender-paired sibling correlation coefficient for LV mass is twice as large in African Americans (r=0.44) compared to Caucasians (r=0.22) [13]. Recent genome wide association studies (GWAS) have identified common variants associated with LV traits in African Americans [10, 11].

However, all common variants identified to date only explain a small proportion of inter-individual variation of LV traits [10]. To our knowledge, there have been no studies of rare variants in relation to LV traits. To fill this knowledge gap, we investigated the association between exonic variants and six LV traits among 1,934 African Americans from the HyperGEN study using exome chip data. We sought replication of the top results among the African American participants of the Genetic Epidemiology Network of Arteriopathy (GENOA) study.

METHODS

Discovery study

The HyperGEN study is one of the four networks in the Family Blood Pressure Program (FBPP) supported by the National Heart, Lung, and Blood Institute to identify genetic contributors to hypertension [14]. HyperGEN is a family based study with a sib-pair design. Hypertensive African American sibships were recruited from population-based cohorts in Forsyth County, NC, and from the community-at-large in Birmingham, AL, from 1995 to 2000. Sibling pairs with onset of hypertension before age 60 were recruited in the first phase. The study was later extended to other siblings and the offspring of the hypertensive probands who were unmedicated adults. Hypertension was defined as having an average systolic blood pressure ≥ 140 mmHg and/or average diastolic blood pressure ≥ 90 mmHg at two separate clinic visits or taking any antihypertensive medication [15]. This analysis included 1,934 self-reported African Americans with relevant echocardiographic measurements and genetic data. The study

was approved by the Institutional Review Boards of the participating organizations. All HyperGEN participants provided informed consent for use of samples and data for subsequent analyses.

Genotyping

Genotyping was performed on 2,147 African Americans in HyperGEN using the Illumina exome array HumanExome-12v1-2. Genotype calling was performed by Illumina's clustering algorithm in Genome Studio [16]. A total of 19 blind duplicate samples, 17 samples with poor quality (e.g. gender mismatches), and 23 samples from individuals with misreported familial relationship were excluded, leaving 2,088 individuals with genotype data. We removed monomorphic markers, insertion/deletion variants, and single nucleotide polymorphisms (SNPs) with missing rate >5% or Mendelian errors. The number of autosomal SNPs after quality control and exclusion was 100,994. Those SNPs were annotated with the corresponding gene name and SNP function (e.g. amino acid change) by using human genome assembly hg19 and FASTA sequences as reference in ANNOVAR [17]. After annotation, 86,927 exonic SNPs (including nonsynonymous, stop-gain, and stop-loss variants) were included in the analysis. Additionally, Combined Annotation Dependent Depletion (CADD) was used to predict the level of protein damage due to the amino acid change of significant SNPs from gene-based analysis [18].

Study outcome

Left ventricular traits were assessed by two-dimensional (2D) guided M-mode and Doppler echocardiography at the Birmingham and Forsyth County field centers following a standardized protocol. All instruments were calibrated against a standard phantom at installation and were validated regularly [19]. Certificated sonographers from each center were trained at the echocardiography reading center at New York Hospital-Weill Cornell Medical Center, where measurements were computerized, calibrated, and quantified using a review station with digitizing tablet and monitor overlay [20].

The study assessed LV structural and functional phenotypes. LV structural outcomes included LV mass (LVM), LV internal dimension-diastole (LVIDD), relative wall thickness (RWT), and left atrial dimension (LAD). LV mass was calculated with the following formula: LV mass = $0.8 \times 1.04 \times [(\text{IVS} + \text{LVIDD} + \text{PWT})^3 - \text{LVIDD}^3] + 0.6$ g, in which IVS is the interventricular septum thickness, LVIDD is the LV internal dimension-diastole, and PWT is the posterior wall thickness. IVS, LVIDD, and PWT were measured by M-mode or 2D echocardiography according to the American Society of Echocardiography recommendations [21]. RWT was calculated as twice the PWT divided by the LVIDD [22]. LAD was measured from the parasternal long-axis view and normalized for the linear measure of height [23]. Reproducibility of LV measurements between separate echocardiograms by the reading center was reported in a previous study (e.g., intra class correlation coefficient is 0.93 for LV mass) [19]. LV functional outcomes included fractional shortening (FS) and the ratio of LV transmitral early (E) velocity to late/atrial (A) velocity (E/A ratio). FS measures the degree of shortening of the LV diameter between end-diastole and end-systole, and is therefore indicative of the

contractibility of the heart [24]. E and A velocity were measured with pulse-wave Doppler at the mitral leaflet tips in apical 4-chamber view during diastole [19]. Approximately 65% of participants in HyperGEN had available E and A velocities. The rest of participants had those measurements only at the annulus. When mitral leaflet tip measurement was missing, diastolic filling parameters at the mitral annulus were used to calculate E and A velocities using the following equations: E velocity at the tips= 0.84*E velocity at the annulus +23.3; and A velocity at the tips=0.76*A velocity at the annulus + 28.9 [12, 25].

Statistical analysis

To achieve normality of residuals, LVM, RWT, LVIDD, and E/A ratio were ln-transformed and LAD was square root-transformed. We excluded participants with extreme LV trait values, defined as exceeding the distance of 4.5 standard deviations from the mean of a trait. Twelve individuals with extreme values of FS and three individuals with extreme values of E/A ratio were excluded from the analysis after transforming.

We performed single-variant analyses and genome-wide gene-based analyses using RAREMETALWORKER (RMW) and RAREMETAL, respectively [26]. For single-variant analyses, we considered an additive genetic model and included common (minor allele frequency (MAF) $\geq$ 5%) and rare (MAF <5%) exonic variants, excluding very rare variants with MAF <0.5%. We considered two MAF threshold cutoffs (<1% and <5%) for the gene-based analysis. RMW reports four different statistical tests

including a simple burden test, the Madsen-Browning weighted burden (MB) test, the sequence kernel association test (SKAT) and the variable frequency threshold (VT) test [27]. Extremely rare (MAF <0.5%) variants, including singletons and doubletons, were part of all gene-based analyses. In cases where only one variant contributed to a gene-based test we did not report the result. To account for phenotypic correlations among related individuals, a kinship matrix built from the pedigree structure was input to RAREMETAL. LV traits were modeled as a continuous outcome. The models were adjusted for potential confounders including age, gender, body-mass index (BMI), field center, and population substructure via the top ten principal components (PCs). The PCs were estimated with the EIGENSOFT package from 30,164 common (MAF ≥5%) SNPs [28]. The genomic inflation factor (λ) was calculated as the ratio of the observed to the expected median χ^2 values. A Bonferroni correction was used to adjust for multiple testing. The Bonferroni-corrected threshold for single-variant analysis was $1.2*10^{-7}$ (α=0.05/(86,927*6) where 6 is the number of LV traits). The gene-based analysis with MAF cutoffs of 1% and 5% included 10,460 genes and 11,554 genes yielding Bonferroni-corrected thresholds of $8.0*10^{-7}$ and $7.2*10^{-7}$, respectively. Gene-based test results that were at least marginally significant ($P<4.0*10^{-5}$) were considered for replication. We performed sensitivity analysis with heart rate, systolic and diastolic blood pressure, and antihypertensive treatment as additional covariates in the model.

Replication

GENOA, another family-based FBPP study [14], served as the replication population. GENOA consists of hypertensive sibships that were recruited for linkage and

association studies in order to identify genes that influence blood pressure and related target organ damage. In the initial phase of the GENOA study (Phase I: 1996-2001), all members of sibships containing ≥ 2 individuals with essential hypertension clinically diagnosed before age 60 were invited to participate, including both hypertensive and normotensive siblings. GENOA participants include African Americans from Jackson, MS (N=1,854 at phase I) [29]. A second study visit was completed from 2001 to 2005 (Phase II)[30]. During phase II, LV traits were measured on African American participants using M-mode, 2D echocardiograms and read in the New York Hospital-Weill Cornell Medical Center similarly as for HyperGEN [10].

The Illumina Human Exome Beadchip v1.1 was used to genotype 1,429 individuals [31]. A total of 37 samples were excluded (15 intended duplicates, 13 unexpected duplicates, 5 gender mismatches, 2 with sex chromosome abnormalities, 1 sample with low concordance with genome-wide genotype data, 1 outlier on principal component analysis). All samples had a call rate >98%. Genotype calling was performed using Genome Studio. A total of 1,392 individuals remained after quality control [31]. Among these, 1,090 individuals with echocardiographic measures were included in this study. SNPs with call rate <95% or ≥1 HapMap replicate errors were excluded [31]. Similar to HyperGEN, ANNOVAR with human genome assembly hg19 was used to retrieve gene name and SNP function, and 91,101 exonic SNPs were included in the analyses. Similarly to HyperGEN the natural log of LVM, RWT, LVIDD, and E/A ratio and the square root of LAD were used for analysis. Extreme outliers for each trait were removed similarly to HyperGEN. Single variant and gene-based tests were carried out using RMW and RAREMETAL and the models included covariates comparable to

HyperGEN, including age, gender, body-mass index (BMI), field center, and first ten PCs.

RESULTS

Table 1 summarizes the general characteristics of the study populations. HyperGEN participants were on average sixteen years younger than GENOA participants. Participants were more likely to be female in both studies. LV traits were different across the two studies ($P<0.05$) with higher LVM, RWT, FS and E/A ratio in HyperGEN. The range of E/A ratio in HyperGEN was from 0.3 to 14.3 (25th percentile=0.9, median=1.1, and 75^{th} percentile=1.4). The range of E/A ratio in GENOA was from 0.13 to 3.9 (25^{th} percentile =0.83, median=1.1, and 75^{th} percentile=1.3).

We performed a single-variant analysis using 86,927 exonic SNPs, including 85,726 nonsynonymous, 1,092 stop-gain, and 109 stop-loss SNPs in HyperGEN. Supplemental Table 1 presents the genomic inflation factors λ, which were generally close to 1 for different LV traits and statistical tests. Table 2 presents results of the ten top SNPs with MAF $\geq 0.5\%$ from our single-variant analysis. This included seven common SNPs with MAF >5%. No single variant was statistically significantly associated with any of the six LV traits. However, rs45470697 and rs45519432 in peroxisome proliferator-activated receptor gamma, coactivator 1 beta (*PPARGC1B)* were marginally associated with LAD ($P=5.6*10^{-7}$ and $5.7*10^{-7}$, respectively). These two SNPs did not replicate in GENOA.

Table 3 shows the top results from gene-based analyses for the six LV traits across four statistical tests at each MAF threshold with P $<4.0*10^{-5}$. Three genes, namely potassium voltage-gated channel, subfamily H member 4 (*KCNH4*), endonuclease G (*ENDOG*) and receptor activity modifying protein 1 (*RAMP1*), passed the Bonferroni-corrected statistical significance thresholds for both the MAF 1% and 5% cutoffs of E/A ratio, LAD and LVIDD, respectively. The models showed the minor allele at two SNPs in *KCNH4* (rs139161684 and rs147730487) were associated with increased E/A ratio (Burden test effect size=0.55 in the natural log scale (1.7 after back transformation)). Two SNPs in *ENDOG* (rs145739062 (MAF=0.21%) and rs186499200 (MAF=0.03%)) were statistically significantly ($P=1.4*10^{-7}$) associated with LAD for the MB test at MAF $\leq$ 5% and $\leq$ 1% (effect size=0.007, and 0.08 after back transformation). Three SNPs in *RAMP1* (rs142335491 (MAF=0.05%), rs61758799 (MAF=0.05%) and rs142757790 (MAF=0.3%)) were statistically significantly ($P=2.6*10^{-7}$) associated with the LVIDD for the MB test. Other marginally significant results from Table 3 include phosphorylase kinase regulatory subunit beta (*PHKB*), and G protein-coupled receptor 55 (*GPR55*) which were associated with E/A ratio for the MB test ($P=2.1*10^{-6}$), and with LAD for MB test ($P=3.2*10^{-5}$), respectively. In a sensitivity analysis, further adjustment for heart rate, systolic and diastolic blood pressure, and antihypertensive treatment did not substantially change the results presented in Table 3 (see Supplemental Table 2). The results for *KCNH4* and *PHKB* were consistent when the analysis was restricted to participants with directly measured E/A ratio (Supplemental Table 3).

Supplemental Table 4 presents functional annotation of each SNP contributing to gene-based tests from Table 3. Each variant in *KCNH4*, *ENDOG*, *RAMP1*, *PHKB* and

GPR55 represented in Table 3 is a missense variant. Further *KCNH4* rs139161684, *ENDOG* rs145739062 and rs186499200, *RAMP1* rs61758799, and *PHKB* rs56257827, rs150683838, rs79509460, rs34667348, rs142381554, and rs12918964 were predicted to have damaging effects on protein function according to CADD. Other SNPs were not predicted to have damaging effects on protein function.

Subsequently, we sought to replicate top findings from Table 3 in GENOA. The Bonferroni-corrected threshold for gene-based analysis was 0.01 (α=0.05/5, where 5 is the number of genes for replication). Both *KCNH4* variants (rs147730487 and rs139161684) and one *ENDOG* variant (rs186499200) variant overlapped between the studies, but the gene-based tests were not significant in GENOA (Table 4). Still, the direction of effect of *KCNH4* and *ENDOG* SNPs in common were the same between studies. *PHKB* was also not significant in Table 4 for E/A ratio. Eight of eleven variants from HyperGEN were observed in GENOA and three of them (rs56257827, rs79509460 and rs12918964) had the same direction of association with E/A ratio. No variants in *RAMP1* were observed after QC in GENOA, therefore, we could not replicate that finding. *GPR55* was marginally associated with LVM and E/A ratio at the MAF cutoff of 5%. However, *GPR55* was not associated with LAD as it was in HyperGEN. Among three SNPs contributing to the *GPR55* gene-based test in GENOA, two of them (rs34229723 and rs141404889) overlap with variants found in HyperGEN. However, the direction of effect in GENOA for LAD for the two SNPs was opposite that of HyperGEN.

DISCUSSION

We report the results of an exome chip association analysis of six LV traits among African American participants from the HyperGEN and GENOA studies. To our knowledge, this study represents the first association analysis of rare variants with LV traits in African Americans. No single variant was statistically significantly associated with any trait in HyperGEN and the top SNP findings did not replicate in GENOA. Gene-based results provide preliminary evidence of association between *KCNH4*, *ENDOG*, and *RAMP1* and E/A ratio, LAD, and LVIDD, respectively.

Our most statistically significant gene-based test result in HyperGEN was the association of *KCNH4* with E/A ratio. *KCNH4* encodes a pore-forming (alpha) subunit of the potassium channel, voltage-gated, subfamily H. The gene is highly expressed in neural tissue [32]. Though potassium transport is important to cardiovascular function [33], *KCNH4* was not replicated in GENOA and is not expressed in cardiac tissue according to public databases. The biological relevance of *KCNH4* in the context of cardiac function is unknown at the present time.

Our second most statistically significant gene finding, *ENDOG*, is biologically plausible. *ENDOG* encodes a mitochondrial endonuclease. Interestingly, the gene was identified as a mediator of blood-pressure-independent cardiac hypertrophy in rats [34]. In that study, a loss-of-function mutation was associated with increased LVM and impaired cardiac function [34]. Additionally, inhibition of *ENDOG* in cultured cardiomyocytes leads to increased cardiomyocyte size and upregulation of biomarkers indicative of hypertrophy [34]. Unfortunately, the gene was not replicated in GENOA. This could be due to differences in the variants observed after QC (rs145739062 was not

observed in GENOA) or differences in the study populations (e.g. the comparatively older age of GENOA participants). Notably, the direction of effect of rs186499200 was the same between the two studies.

RAMP1 encodes a G protein-coupled receptor belonging to the receptor (calcitonin) activity modifying proteins (RAMPs) family. *RAMP1* was reported to regulate blood pressure in animals [35, 36]. Overexpression of *RAMP1* is associated with lower mean arterial pressure change among hypertensive mice caused by angiotensin II [35]. Moreover, a loss-of-function mutation in *RAMP1* was found to be associated with higher blood pressure in mice [36]. Unfortunately, sequencing information for this gene was not available in GENOA.

PHKB encodes the regulatory subunit beta of phosphorylase kinase. The gene plays an important role in glycogen breakdown and cell growth [37]. A mutation in *PHKB* is associated with autosomal phosphorylase kinase deficiency in muscle and liver [38]. The transcript level of *PHKB* is higher in the right ventricular cells among hypoplastic left heart syndrome patients [39]. Three of eight variants in common between HyperGEN and GENOA had the same direction of effect on E/A ratio, but given there was not complete overlap of variants between the studies this gene also warrants additional study.

GPR55 was marginally associated with LAD in HyperGEN. *GPR55* encodes an orphan G-protein coupled receptor weakly expressed in cardiac tissue [37]. In a mouse neonatal ventricular myocyte model, GPR55 was activated exogenously by a lyso-phospholipid and induced intracellular Ca^{2+} increase, membrane depolarization, and consequently cardiomyocyte contractility [38]. A single loss-of-function mutation in

GRP55 was associated with reduced LV wall thickness, increased collagen deposition, and reduced load-dependent systolic function (e.g. ejection fraction, end-systolic pressure-volume relationship, and Emax) among mature mice in another study and is worthy of continued investigation in humans in relation to calcium regulation and left ventricular structure [39]. Two *GRP55* SNPs (rs34229723 and rs141404889) were found overlapping between HyperGEN and GENOA. The gene was marginally associated with other LV traits in GENOA, however, the gene was not associated with LAD and SNPs in common were associated in opposing directions across studies. Therefore, the findings in GENOA cannot be considered a replication of the discovery finding.

Our study has several strengths. This study is the first to examine the association between rare protein coding variants and LV traits in African Americans, a group with a high burden of cardiovascular morbidity and mortality. Second, the family-based study design in HyperGEN and GENOA potentially increases the power to observe rare variants by (1) reducing potential population stratification, (2) improving data quality by genotyping error checking, and (3) enriching for rare variants in the extremes of quantitative traits [40]. Finally, HyperGEN and GENOA had identical echocardiography protocols, and utilized the same reading centers.

Our study findings should also be interpreted in context of some limitations. First, generalization of these findings is difficult because the HyperGEN study population has a high prevalence (~60%) of hypertension. Therefore, applying these results to other populations with lower prevalence of hypertension (e.g. athletes) might not be appropriate. Second, the echocardiography estimates could be biased by the effect of anti-hypertensive medications used by most of the hypertensive participants in our study.

Third, our study did not capture information on heart failure. Instead, we studied earlier phenotypes (LV traits) which could have relevance for preventing heart failure. Furthermore, exome chip is biased toward rare variants specific to Caucasians. Therefore, we might fail to capture important rare variants in African Americans. We recognize that the associations of our top genes (*KCNH4* and *ENDOG*) are driven by very rare (MAF<0.5%) single variants. However, given the biological plausibility of the findings, they merit reporting. Larger datasets will be needed in the future to better understand the relationship of these genes and variants to left ventricular structure and function. Finally, HyperGEN did not include more contemporary measures of diastolic function such as tissue Doppler imaging. Therefore, we could not examine the association between exome chip data and more comprehensive indicators of diastolic function, such as grade of diastolic dysfunction.

In this study, we report an association between *KCNH4*, *ENDOG, RAMP1,* and *PHKB* with LV traits in African Americans from the HyperGEN study. These genes were not validated in African Americans from GENOA (a sister study in the Family Blood Pressure Program), however many variants from HyperGEN were not observed in GENOA. Future sequencing studies that capture more rare variants in larger populations are needed to expand these findings. Importantly, this study suggests rare protein coding variants could be important contributors to left ventricular structure in African Americans and have the potential to shed new insights to disease pathophysiology.

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Conflicts of interest

The authors declare no conflicts of interest.

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Table 1 ***Demographics and echocardiographic characteristics of the study populations***

	HyperGEN (N= 1,934)	**GENOA (N= 1,090)**	**P***
Age, years	46.3 ± 13.2	61.8 ± 10.1	<0.0001
Female, %	63.1%	71.3%	<0.0001
Body mass index, kg/m^2	32.0 ± 7.7	31.6 ± 6.6	0.13
Centers in Alabama, %	75.2%	0%	<0.0001
Hypertension, %	65.7%	82.3%	<0.0001
Diabetes, %	20.1%	30.5%	<0.0001
Left ventricular traits			
Left ventricular mass, g	170.2 ± 49.4	160.0 ± 44.6	<0.0001
Left ventricular internal dimension-diastole, cm	5.2 ± 0.5	5.2 ± 0.5	0.99
Relative wall thickness	0.34 ± 0.06	0.32 ± 0.05	0.003
Fractional shortening, %	33.3 ± 5.5	32.9 ± 4.6	0.003
The ratio of left ventricular transmitral early velocity to late/atrial velocity	1.2 ± 0.5	1.1 ± 0.38	<0.001
Left atrial dimension, cm	3.4 ± 0.5	3.6 ± 0.5	<0.001

*Numeric variables were presented as mean ± SD, categorical variables were presented as percentages. Significance determined using Chi-square test for categorical and two independent sample t-test for continuous.

Table 2 ***The ten top variants from the single-variant analysis with minor allele frequency ≥ 0.5% in HyperGEN (N=1,934)***

Left ventricular traits	SNP	Chr	Position	Gene	SNP function	MAF	Beta	p-value
LAD	rs45470697	5	149212624	*PPARGC1B*	R330W	0.06	-0.04	$5.6*10^{-7}$
LAD	rs45519432	5	149212802	*PPARGC1B*	S389Y	0.06	-0.04	$5.7*10^{-7}$
E/A ratio	rs8176919	16	3706697	*DNASE1*	G127R	0.06	0.08	$4.7*10^{-6}$
E/A ratio	rs41277210	1	216144049	*USH2A*	R2292H	0.01	0.23	$8.4*10^{-6}$
LVIDD	rs35892492	20	62333521	*ARFRP1*	W105R	0.01	0.09	$9.8*10^{-6}$
RWT	rs769929111	1	55075452	*FAM151A*	A416V	0.23	0.03	$1.3*10^{-5}$
FS	rs11555566	20	43255220	*ADA*	K80R	0.07	1.54	$1.4*10^{-5}$
FS	rs36009281	5	70798541	*BDP1*	K722E	0.01	-3.58	$1.7*10^{-5}$
E/A ratio	rs1801270	6	36651971	*CDKN1A*	S31R	0.27	0.04	$2.1*10^{-5}$
E/A ratio	rs9911502	17	73518203	*TSEN54*	K347N	0.20	0.04	$3.0*10^{-5}$

* The models were adjusted for age, gender, center, body mass index and first 10 principal components. LAD: left atrial dimension. E/A ratio, the ratio of left ventricular transmitral early velocity to late/atrial velocity. LVIDD, left ventricular internal dimension-diastole. RWT, relative wall thickness. FS, fractional shortening. LAD was square-rooted. E/A ratio, LVIDD, and RWT were natural log-transformed. MAF: minor allele frequency.

Table 3 *The top results from gene-based analysis across traits in HyperGEN N=1,934 (the smallest p-value for each gene is shown in bold)*

Left ventricular traits	Gene name	Gene-based association p-values								MAF cumulative (Total # of carriers)	Top single-variant signal		
		MAF < 1%				MAF < 5%					SNP	MAF	p-value
		Burden	MB	SKAT	VT	Burden	MB	SKAT	VT				
E/A ratio	*KCNH4*	**$8.7*10^{-8}$** (++)	**$8.7*10^{-8}$** (++)	$5.2*10^{-6}$ (++)	**$8.7*10^{-8}$** (++)	**$8.7*10^{-8}$** (++)	**$8.7*10^{-8}$** (++)	$5.2*10^{-6}$ (++)	**$8.7*10^{-8}$** (++)	$1.6*10^{-3}$ (6)	rs139161684	$8.1*10^{-4}$	$2.0*10^{-10}$
LAD	*ENDOG*	0.003 (++)	**$1.4*10^{-7}$** (++)	0.01 (++)	N/A	0.003 (++)	**$1.4*10^{-7}$** (++)	0.01 (++)	N/A	$2.4*10^{-3}$ (9)	rs186499200	$2.6*10^{-4}$	$2.2*10^{-11}$
LVIDD	*RAMP1*	$3.4*10^{-5}$ (+++)	**$2.6*10^{-7}$** (+++)	0.005 (+++)	$2.2*10^{-6}$ (++)	$3.4*10^{-5}$ (+++)	**$2.6*10^{-7}$** (+++)	0.005 (+++)	$1.9*10^{-6}$ (++)	$4.2*10^{-3}$ (16)	rs142335491	$5.3*10^{-4}$	$4.6*10^{-5}$
E/A ratio	*PHKB*	$5.7*10^{-5}$ (+++++++ ++-)	$8.4*10^{-6}$ (++++++ +++-)	0.008 (++++++ +++-)	N/A	$4.9*10^{-5}$ (++++++++ ++-)	**$2.1*10^{-6}$** (+++++++ +++-)	0.02 (++++++ ++++-)	N/A	0.04 (142)	rs117218785	$5.4*10^{-4}$	$5.8*10^{-5}$
LAD	*GPR55*	N/A	N/A	N/A	N/A	0.14 (++)	**$3.2*10^{-5}$** (++)	0.08 **(++)**	N/A	0.02 (93)	rs141404889	$7.9*10^{-4}$	$2.1*10^{-7}$

* The models were adjusted for age, gender, center, body mass index and first 10 principal components. Direction of SNPs contributing gene-based analysis inside parentheses. Plus means increase, minus means decrease compared to common alleles (additive model). E/A ratio, the ratio of left ventricular transmitral early velocity to late/atrial velocity. LAD, left atrial dimension. LVIDD, left ventricular internal dimension-diastole. E/A ratio and LVIDD were natural log-transformed. LAD was square-rooted. MB, the Madsen-Browning weighted burden test. SKAT, the sequence kernel association test. VT, the variable frequency threshold test. MAF, minor allele frequency. MAF cumulative and total number of carriers for the most significant test.

Table 4 ***Replicated results from gene-based analysis in GENOA N=1,090***

Left ventricular traits	Gene name	Gene-based association p-values							
		MAF < 1%				MAF < 5%			
		Burden	MB	SKAT	VT	Burden	MB	SKAT	VT
E/A ratio	*KCNH4*	0.13 (++)	0.13 (++)	0.18 (++)	0.21 (++)	0.13 (++)	0.13 (++)	0.18 (++)	0.21 (++)
LAD	*ENDOG*	0.39 (+)	0.39 (+)	0.39 (+)	0.39 (+)	0.39 (+)	0.39 (+)	0.39 (+)	0.39 (+)
LVIDD	*RAMP1*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
E/A ratio	*PHKB*	0.41 (++++--)	0.45 (+++ +--)	0.74 (+++ +--)	0.60 (+++--)	0.63 (++++----)	0.55 (++++ ----)	0.86 (++++ ----)	0.64 (++ +--)
LAD	*GPR55*	0.68 (--)	0.57 (--)	0.28 (--)	0.21 (-)	0.71 (---)	0.91 (---)	0.54 (---)	0.30 (-)
LVM	*GPR55*	0.98 (--)	0.99 (--)	0.99 (--)	0.99 (--)	0.03 (---)	0.15 (---)	**0.02 (---)**	0.08 (---)
E/A ratio	*GPR55*	0.44 (--)	0.46 (--)	0.66 (--)	0.63 (-)	**0.03 (---)**	0.07 (---)	0.05 (---)	0.07 (---)

* The models were adjusted for age, gender, center, body mass index and first 10 principal components. Direction of SNPs contributing gene-based analysis inside parentheses. Plus means increase, minus means decrease compared to common alleles (additive model). E/A ratio, the ratio of left ventricular transmitral early velocity to late/atrial velocity. LAD, left atrial dimension. LVIDD, left ventricular internal dimension-diastole. LVM, left ventricular mass. E/A ratio, LVIDD and LVM were natural log-transformed. LAD was square-rooted. MB, the Madsen-Browning weighted burden test. SKAT, the sequence kernel association test. VT, the variable frequency threshold test. MAF, minor allele frequency.

SUPPLEMENTAL MATERIALS

Supplemental Table 1 ***Genomic inflation values (λ) for analyses of left ventricular traits in HyperGEN(N=1,934)***

Left ventricular traits	Single-variant analysis	MAF < 1%				MAF < 5%			
		Burden	MB	SKAT	VT	Burden	MB	SKAT	VT
LVM	1.0	0.99	0.97	1.0	0.98	1.0	0.99	1.1	0.99
LVIDD	1.0	0.97	0.98	0.99	0.94	0.99	0.98	1.0	0.94
RWT	1.0	1.0	1.0	0.97	0.94	1.0	1.0	1.0	0.97
FS	1.0	0.96	0.94	0.91	0.82	1.0	0.94	0.97	0.85
E/A ratio	1.0	0.97	0.97	0.98	0.90	0.95	0.95	0.99	0.89
LAD	1.0	0.97	0.95	0.98	0.94	0.97	0.98	1.0	0.95

*LVM, left ventricular mass. LVIDD, left ventricular internal dimension-diastole. RWT, relative wall thickness. FS, fractional shortening. E/A ratio, the ratio of left ventricular transmitral early velocity to late/atrial velocity. LAD, left atrial dimension. LVM, LVIDD, RWT, and E/A ratio were natural log-transformed. LAD was square-rooted. MB: the Madsen-Browning weighted burden test. SKAT: the sequence kernel association test. VT: the variable frequency threshold test.

Supplemental Table 2 ***The top results from gene-based analysis across five traits in HyperGEN N=1,934 from sensitivity analysis (the smallest p-value for each gene is shown in bold)***

LV traits	Gene name	Gene-based association p-values								MAF cumulative (Total # of carriers)	Top single-variant signal		
		MAF < 1%				MAF < 5%					SNP	MAF	p-value
		Burden	MB	SKAT	VT	Burden	MB	SKAT	VT				
E/A ratio	*KCNH4*	**$1.2*10^{-10}$** (++)	**$1.2*10^{-10}$** (++)	$5.1*10^{-6}$ (++)	**$1.2*10^{-10}$** (++)	**$1.2*10^{-10}$** (++)	**$1.2*10^{-10}$** (++)	$5.1*10^{-6}$ (++)	**$1.2*10^{-10}$** (++)	$1.6*10^{-3}$ (6)	rs139161684	$8.1*10^{-4}$	$2.0*10^{-10}$
LAD	*ENDOG*	0.003 (++)	**$1.0*10^{-7}$** (++)	0.009 (++)	N/A	0.003 (++)	**$1.0*10^{-7}$** (++)	0.009 (++)	N/A	$2.4*10^{-3}$ (9)	rs186499200	$2.6*10^{-4}$	$2.2*10^{-11}$
LVIDD	*RAMP1*	$7.1*10^{-5}$ (+++)	**$5.5*10^{-7}$** (+++)	0.006 (+++)	$1.7*10^{-6}$ (++)	$7.1*10^{-5}$ (+++)	**$5.5*10^{-7}$** (+++)	0.006 (+++)	$1.5*10^{-6}$ (++)	$4.2*10^{-3}$ (16)	rs142335491	$5.3*10^{-4}$	$4.6*10^{-5}$
E/A ratio	*PHKB*	$6.6*10^{-5}$ (++++++ +++-)	$1.3*10^{-5}$ (++++++ +++-)	0.005 (++++++ +++-)	NA	$1.8*10^{-4}$ (++++++ ++++-)	**$6.0*10^{-6}$** (++++++ ++++-)	0.02 (++++++ ++++-)	NA	0.04 (142)	rs117218785	$5.4*10^{-4}$	$5.8*10^{-5}$
LAD	*GPR55*	NA	NA	NA	NA	0.16 (++)	**$4.1*10^{-5}$** (++)	0.07 **(++)**	NA	0.02 (93)	rs141404889	$7.9*10^{-4}$	$2.1*10^{-7}$

The model was adjusted for age, gender, center, body mass index, heart rate, systolic blood pressure, diastolic blood pressure, using anti-hypertensive medication (Yes/No), and first 10 principal components. Direction of SNPs contributing gene-based analysis inside parentheses. Plus means increase, minus means decrease compared to common alleles (additive model). LV, left ventricular. E/A ratio, the ratio of left ventricular transmitral early velocity to late/atrial velocity. LAD, left atrial dimension. LVIDD, left ventricular internal dimension-diastole. E/A ratio and LVIDD were natural log-transformed. LAD was square-rooted. MB, the Madsen-Browning weighted burden test. SKAT, the sequence kernel association test. VT, the variable frequency threshold test. MAF, minor allele frequency. MAF cumulative and total number of carriers for the most significant test.

Supplemental Table 3 ***The top results from gene-based analysis of E/A ratio from subjects with transmitral annulus velocities fully recoded (N=1,221) in HyperGEN (the smallest p-value for each gene is shown in bold)***

LV traits	Gene name	Gene-based association p-values								MAF cumulative (Total # of carriers)	Top single-variant signal		
		MAF < 1%				MAF < 5%					SNP	MAF	p-value
		Burden	MB	SKAT	VT	Burden	MB	SKAT	VT				
E/A ratio	*KCNH4*	**$2.8*10^{-6}$** (++)	**$2.8*10^{-6}$** (++)	$6.2*10^{-6}$ (++)	**$2.8*10^{-6}$** (++)	**$2.8*10^{-6}$** (++)	**$2.8*10^{-6}$** (++)	$6.2*10^{-6}$ (++)	**$2.8*10^{-6}$** (++)	$2.5*10^{-3}$ (6)	rs139161684	$1.2*10^{-3}$	$2.2*10^{-8}$
E/A ratio	*PHKB*	$4.4*10^{-4}$ (+++++ ++---)	$7.8*10^{-5}$ (+++++ ++---)	0.004 (+++++ ++---)	$4.4*10^{-4}$ (+++++ +--)	$8.1*10^{-4}$ (+++++++ +---)	**$4.2*10^{-5}$** (++++++ ++---)	0.02 (+++++ +++---)	$4.8*10^{-4}$ (++++++--)	0.04 (102)	rs117218785	$8.2*10^{-4}$	$7.4*10^{-5}$

* The models were adjusted for age, gender, center, body mass index and first 10 principal components. Direction of SNPs contributing gene-based analysis inside parentheses. Plus means increase, minus means decrease compared to common alleles (additive model). LV, left ventricular. E/A ratio, the ratio of left ventricular transmitral early velocity to late/atrial velocity. E/A ratio was natural log-transformed. MB, the Madsen-Browning weighted burden test. SKAT, the sequence kernel association test. VT, the variable frequency threshold test. MAF, minor allele frequency. MAF cumulative and total number of carriers for the most significant test.

Supplemental Table 4 *Annotation of single nucleotide polymorphisms contributing to gene-based analysis of KCNH4, ENDOG, RAMP1, PHKB and GPR55 in HyperGEN*

Gene name	SNP name	Amino acid change	Beta coeff.	P-value	MAF in African Americans in HyperGEN	MAF in Africans from ExAC database	SNP impact on protein predicted by PolyPhen	PolyPhen value	SNP impact on protein predicted by SIFT	SIFT score	Test contribute to							
											MAF<1%				MAF<5%			
											Burden	MB	SKAT	VT	Burden	MB	SKAT	VT
KCNH4	rs147730487	R386Q	0.16	0.27	$8.1*10^{-4}$	$9.7*10^{-4}$	benign	0.003	tolerated	0.42	x	x	x	x	x	x	x	x
	rs139161684	R699C	0.91	$2.0*10^{-10}$	$8.1*10^{-4}$	$1.1*10^{-3}$	possibly damaging	0.89	deleterious	0.01	x	x	x	x	x	x	x	x
ENDOG	rs145739062	R184H	0.03	0.46	$2.1*10^{-3}$	$2.0*10^{-3}$	probably damaging	0.99	deleterious	0	x	x	x		x	x	x	
	rs186499200	I270T	0.82	$2.2*10^{-11}$	$2.7*10^{-4}$	$3.1*10^{-4}$	probably damaging	0.97	deleterious	0	x	x	x	x	x	x	x	x
RAMP1	rs142335491	R37Q	0.27	$4.6*10^{-5}$	$5.3*10^{-4}$	0	benign	0.001	tolerated	1	x	x	x	x	x	x	x	x
	rs61758799	T134M	0.18	$2.9*10^{-3}$	$5.3*10^{-4}$	$8.1*10^{-4}$	probably damaging	0.99	deleterious	0	x	x	x	x	x	x	x	x
PHKB	rs142757790	T144S	0.05	0.06	$3.2*10^{-3}$	$2.2*10^{-3}$	benign	0.05	tolerated	0.62	x	x	x		x	x	x	
	rs146558295	T139A	0.20	0.03	$2.2*10^{-3}$	$2.1*10^{-3}$	benign	0.001	tolerated	0.67	x	x	x		x	x	x	
	rs56257827	M178I	0.16	0.04	$3.0*10^{-3}$	$1.7*10^{-3}$	possibly damaging	0.685	deleterious	0.05	x	x	x		x	x	x	
	rs117218785	I185V	0.81	$6.8*10^{-6}$	$5.4*10^{-4}$	$9.7*10^{-5}$	benign	0.277	tolerated	0.43	x	x	x	x	x	x	x	x
	rs141379798	R422Q	0.22	0.13	$8.1*10^{-4}$	$1.9*10^{-3}$	benign	0.229	tolerated	0.31	x	x	x		x	x	x	
	rs150683838	D456H	0.16	0.14	$1.6*10^{-3}$	$1.4*10^{-3}$	probably damaging	0.943	deleterious	0	x	x	x		x	x	x	
	rs79509460	P518H	0.10	0.12	$4.3*10^{-3}$	$5.7*10^{-3}$	probably damaging	0.998	deleterious	0	x	x	x		x	x	x	
	rs34667348	Q650K	-0.03	0.78	$1.1*10^{-3}$	$1.3*10^{-3}$	probably damaging	0.999	deleterious	0.03	x	x	x		x	x	x	
	rs142381554	K685R	0.01	0.92	$3.3*10^{-3}$	$3.5*10^{-3}$	possibly damaging	0.557	tolerated	0.42	x	x	x		x	x	x	
	rs56010117	V755I	0.08	0.50	$1.4*10^{-3}$	$2.0*10^{-3}$	benign	0.013	tolerated	0.22	x	x	x		x	x	x	
	rs9934849	E813V	0.05	0.11	0.02	0.02	benign	0.062	tolerated	1					x	x	x	
	rs12918964	R1034W	0.10	0.39	$1.4*10^{-3}$	$1.7*10^{-3}$	possibly damaging	0.453	deleterious	0.02	x	x	x		x	x	x	
GPR55	rs34229723	T215N	0.01	0.61	0.02	0.02	benign	0.006	tolerated	0.48					x	x	x	
	rs141404889	M105T	0.39	$1.1*10^{-7}$	$7.9*10^{-4}$	$1.2*10^{-3}$	benign	0.07	tolerated	0.24	x	x	x	x	x	x	x	x

MANUSCRIPT 2

GENOME-WIDE META-ANALYSIS OF SNP AND ANTIHYPERTENSIVE MEDICATION INTERACTION ON LEFT VENTRICULAR TRAITS IN AFRICAN AMERICANS IN CHARGE

by
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ABSTRACT

Left ventricular (LV) hypertrophy (or LVH) affects up to 43% of African Americans. It is commonly comorbid with hypertension and associated with increased risk for cardiovascular morbidity and mortality. Antihypertensive treatments have been found to reduce LV mass (LVM). However, inter-individual variation in LV traits during antihypertensive treatments exists. We hypothesized genetic markers may modify the association of treatment class with left ventricular traits measured by echocardiography. We evaluated the main effects of the three most common antihypertensive treatments for African Americans as well as the SNP-by-drug interaction on LVM and relative wall thickness (RWT) in five cohorts with a total of 2,068 participants on these treatments, including thiazide diuretics (TDs), angiotensin converting enzyme inhibitors (ACE-Is), and dihydropyridine calcium channel blockers (dCCBs). TDs, ACE-Is, and dCCBs were compared in a pairwise manner. Diastolic functions including e' velocity, E/e' ratio and global longitudinal strain were considered as secondary outcomes. Models were adjusted for age, sex, height, weight, count of antihypertensive treatment classes, estimated glomerular filtration rate, type 2 diabetes. Principal components for ancestry at the cohort level were also included for models of SNP-by-drug interaction. We used an estimated degrees of freedom filter that incorporated SNP imputation quality, minor allele frequency (MAF), and the number of exposed participants. We performed fixed effects inverse variance weighted meta-analyses of 2.5 million SNP-by-drug interaction estimates. We found that dCCBs vs. TDs were associated with higher LVM after adjusting for covariates (P=0.001). We also found 3 SNPs at a single locus associated with RWT when comparing dCCBs to ACE-Is with consistent direction of effect across

four cohorts (smallest P=4.7*10^{-8}, MAF range 0.09-0.12). The SNPs are located on chromosome 20 between long intergenic non-protein coding RNA 687 (*LINC00687*) and long non-coding RNA (*LOC339593*). Ten SNPs in *U80770* located on chromosome 2 were associated with LVM when comparing dCCBs vs. ACE-Is (smallest P=1.2*10^{-7}, MAF range 0.17-0.22). *U80770* is expressed the left ventricle but otherwise not been related to CVD. In conclusion, genome-wide SNP-by-drug interaction analyses for LV traits may give clues toward optimizing antihypertensive treatment for African Americans. Despite this being the largest SNP*antihypertensive treatment class study of LV traits in this race group to date, additional data is needed to expand these findings.

INTRODUCTION

Left ventricular (LV) 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 African Americans) and even more common among individuals with hypertension (up to 60%).[1-4] It is recognized as an independent risk factor for several cardiovascular-related outcomes including stroke, and heart failure as well as all-cause mortality.[5-7] Research shows LVH may be a better predictor of mortality than coronary artery disease in many populations.[8] The prevalence of LVH in African Americans is two times that of Caucasians and poses greater cardiovascular risk as compared to other ethnic groups.[9, 10]

Antihypertensive treatments have been found to decrease LV mass (LVM) independent of blood pressure change in participants treated for hypertension.[11-13] However, there is a lack of consensus on the best antihypertensive agents for decreasing LVM.[13-15] There are likely subgroups of patients who may benefit more on a specific class of drug. Additionally, the effect of antihypertensive agents on LV diastolic function is also controversial.[14-16] Overall, African Americans have been underrepresented in previous studies.

Though studies suggest that antihypertensive agents may improve LVM, follow-up studies showed there are inter-individual variations in treatment response suggesting genetic factors may be at play.[17-19] Previous candidate gene studies have attempted to find pharmacogenetic factors associated with LVH and related traits.[18, 19] However, most previous studies were small in size, considered few variants and results were not

replicated. Genome-wide scans for markers that modify the effect of antihypertensive treatment class on LV traits in a sizable population of African Americans can help identify novel pharmacogenetic variants.

To fill those research gaps, we evaluated the relationship between the three most common antihypertensive treatments for African Americans (thiazide and related diuretics (TDs), angiotensin-converting enzyme inhibitors (ACE-Is), and dihydropyridine calcium channel blockers (dCCBs)) and quantitative traits related to LVH measured by echocardiography under a cross-sectional design. Next, we examined how the drug-trait relationship may be modified by genomic markers using data collected from five observational epidemiology studies from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium.

METHODS

Study Population

Our analyses were performed within the CHARGE Consortium. Details of the CHARGE consortium were described elsewhere.[20] Within CHARGE, five studies with African Americans including Coronary Artery Risk Development in Young Adult Study (CARDIA), Cardiovascular Health Study (CHS), Jackson Heart Study (JHS), Genetic Epidemiology Network of Atherosclerosis Study (GENOA), and Hypertension Genetic Epidemiology Network (HyperGEN)) had both echocardiographic and genome-wide association (GWA) data. These five cohorts were used for the discovery phase of this study. Guidelines on collaboration, phenotype harmonization, covariate selection, and the analysis plan for both within-cohort GWA and meta-analysis of results across studies

were adopted by each cohort. Additionally, each cohort obtained approval from the respective institutional review boards for consent procedures, examination and surveillance components, and data security measures. We included 2,068 African Americans treated for hypertension with available echocardiographic and GWA data from five cohorts. More details of study population are provided in **SUPPLEMENTAL MATERIALS.**

Echocardiographic Phenotypes

The study assessed two LV structures as primary outcomes including LVM and relative wall thickness (RWT). In all five studies, LVM was calculated by using the American Society of Echocardiography corrected formula by Devereux: 0.80 x 1.04 x $[(IVSDD + PWTD + LVIDD)^3 - LVIDD^3] + 0.6g$ in which IVSDD is the interventricular septum thickness, LVIDD is the LV internal dimension-diastole, and PWTD is the thickness at end-diastole of the posterior wall.[21] RWT was calculated as twice the PWTD divided by the LVIDD.[22] Better cardiac function is indicated by lower LVM and RWT. Both LVM and RWT are available in all five studies.

The secondary outcomes in the study were two LV diastolic functions and a speckle tracking trait. LV diastolic functions included early diastolic tissue velocity at the septal mitral annulus (e' velocity) and the ratio of early (E) transmitral flow velocity to early diastolic tissue velocity at the septal mitral annulus (E/e' ratio). e' velocity and E/e' ratio are a measure of LV relaxation and LV diastolic filling pressure, respectively. Better cardiac function is indicated by low E/e' ratio and high e' velocity. LV global longitudinal strain (GLS) is a measure of LV systolic function assessed by speckle

tracking. GLS has been strongly associated with cardiovascular mortality.[23, 24] GLS describes the relative length change of the LV myocardium between end-diastole and end-systole.[21] High absolute GLS indicates better cardiac function.[21] Each of e' velocity, E/e' ratio and GLS are available in CARDIA and HyperGEN studies.

Definition of Drug Exposure

We examined three antihypertensive classes of drugs ACE-Is, dCCBs, and TDs. Three antihypertensive drug classes were compared in a pairwise manner in the following three models. Model 1: ACE-I use vs. TD use (reference = TD use) where ACE-I exposure was defined as the use of an ACE-I in a single or combination preparation without concomitant use of a TD versus TD exposure without ACE-I. Model 2: dCCB use vs. TD use (reference = TD use) where dCCB exposure was defined as the use of a dCCB in a single or combination preparation without concomitant use of a TD versus TD exposure without dCCB. Model 3: dCCB use vs. ACE-I use (reference =ACE-I use) where dCCB exposure was defined as the use of a dCCB in a single or combination preparation without concomitant use of an ACE-I versus ACE-I exposure without dCCB. Drug groupings were based on manually-curated lists that were reviewed by experts from each study to include all relevant drugs from the United States. Drug exposure was assessed by self-report in each of the five studies (see **Supplemental Material**). In our approach, participants taking more than one medication class may contribute data to more than one model. Details of medication inventory of eligible antihypertensive treatments for each model and excluded treatments are provided in **Supplemental Material List 1** and **List 2**, respectively.

Genotyping and Imputation

Genome-wide SNP genotyping was performed within each study using Illumina or Affymetrix genotyping arrays. SNP quality control (QC) was performed using PLINK, Birdseed v1.33, or Illumina GenomeStudio. QC measures removed 1) samples with genotyping success rate <95%, 2) SNPs failing genotyping call rate thresholds, typically between 95% and 99%, 3) monomorphic SNPs; 4) SNPs that mapped to several loci in the human genome, and 5) SNPs with minor allele frequency (MAF) <1%. Other QC filters included removing SNPs 1) with Mendelian inconsistencies (for cohorts with family data), and 2) those with significant deviation from Hardy-Weinberg equilibrium. To increase coverage and facilitate evaluation of the same SNPs across cohorts, SNPs passing quality control were used to for imputation. A combined YRI and CEU reference panel from HapMap phase 2 (build 36 release 22) was used for imputation in each of the five cohorts because African-American population is admixed with ~17–19% proportion of European ancestry.[25] More details of genotyping, QC, and imputation for each study are provided in **Supplemental Table 1**.

Statistical analysis for main effect of antihypertensive medication on LV traits

Statistical analysis within studies

Each study independently implemented a pre-defined analysis plan. All five cohorts excluded extreme values (>5 standard deviations of its mean) for each echocardiographic measure. Natural log-transformations were made for LVM, RWT, and E/e' ratio in all cohorts to satisfy model distributional assumptions. Linear regression for

cohorts of unrelated individuals including CARDIA, CHS, and JHS and mixed effects models (MEM) for cohorts with related individuals including HyperGEN and GENOA were used. The models tested the main effect of antihypertensive treatment class (ACE-I vs. TD; dCCB vs. TD; dCCB vs. ACE-I, as described in **Definition of drug exposure**) on each of the LV traits separately. Each model was adjusted for age, sex, weight, height, count of antihypertensive treatment classes, estimated glomerular filtration rate (eGFR), and type 2 diabetes (T2D). Study site and/or other study specific variables were included as covariates as needed by the individual cohorts (e.g. center in HyperGEN). Family information was used as a random effect in HyperGEN and GENOA. The models for e' velocity, E/e' ratio, and GLS, speckle tracking measures were additionally adjusted for institution, reader, and image quality to control for inter-observer variability.

Discovery meta-analysis for antihypertensive treatment main effects

We used fixed effects inverse variance weighted meta-analysis in the software METASOFT where the weights were calculated as the reciprocal of estimated variance (SE) of the effect size (β) from each study. In random effects model, between-study variance of heterogeneity was used to weigh for the random effect. If heterogeneity was observed (P-value of Cochran's Q statistic <0.05) we reported results of a random effect model. A P-value <0.0083 was considered significant ($\alpha=0.05/3*2$ in which 3 is the number of drug pairwise and 2 is the number of LV traits).

Statistical analysis for pharmacogenetic effect of antihypertensive medication on LV traits

Statistical analysis within studies

Each study independently implemented a pre-defined GWAS analysis plan. The models were identical to those described above for the main effect of antihypertensive treatment class on LV traits except that SNPs (under an additive model) and SNP-by-drug interaction terms were added. Likewise the covariates were the same except we added the first ten principal components for ancestry. Study site and/or other study specific variables were included as covariates as needed by the individual cohorts. Family information was used as a random effect in HyperGEN and GENOA. The models for e' velocity, E/e' ratio, and GLS, speckle tracking measures were additionally adjusted for institution, reader, and image quality to control for inter-observer variability.

Discovery meta-analysis

Prior to the meta-analysis, we verified strand alignment across studies by comparing each SNP in each study to the same SNP in 1000 Genome phase 3. The GWAS data was aligned to the positive strand in each study. We then calculated SNP-specific filter degree of freedom (DF) for each cohort as the product of the number of drug-exposed participants, the number of non-reference drug exposure, the SNP imputation quality (range: 0, 1), and the minor allele frequency (MAF) (range: 0, 0.50). [26] The SNP-specific degree of freedom helps control inflation for poorly-calibrated test for less common variants among less common drug exposures. We excluded cohort-specific results for SNPs with degree of freedom less than 10. We applied 'genomic control' to

studies whose genomic inflation factor was greater than 1.00 and other studies for analytic consistency. [27] To conduct meta-analysis, we restricted to autosomal SNPs that were available in at least two studies. We used study-specific interaction estimates (β) and "corrected" standard errors (SE) in fixed effects inverse variance weighted meta-analysis using METAL software. To obtain "corrected" SE, p-values were recalculated by applying a t reference distribution for the ratio of the SNP-by-drug estimates (β) to its SE; then corrected SEs were the SE values that would give the t-distribution-based p-values when assuming a normal distribution for the ratio of the SNP-by-drug estimates (β) to its corrected SE. Such correction was necessary due to known underestimation of SEs by robust methods when any SNP-treatment stratum is small. The cohort specific DF for the t reference distribution were estimated using Satterthwaite's method in cohorts with unrelated participants. In HyperGEN and GENOA, DF was estimated as the filter DF described above. The genome-wide threshold for significant SNP-by-drug interaction was $P < 5\times10^{-8}$.

Replication analysis

The top hit results will be replicated in the Multi-Ethnic Study of Atherosclerosis (MESA) study and the Atherosclerosis Risk in Communities Study (ARIC) study.

RESULTS

Characteristics of study population

Characteristics of 2,068 participants are shown in **Table 1**. On average, participants were predominantly female, middle-aged (mean age range=50-74 years), and

non-diabetic. LVM slightly varied across studies (mean range= 158.8-185.5 g), but RWT was similar among the five studies.

The association between antihypertensive medication and LV traits

We performed meta-analysis across 2,068 African Americans from five studies to examine whether LVM and RWT are different among individuals using different antihypertensive medication classes including ACE-I, dCCB, and TD. Results are presented in **Table 2**. dCCBs vs. TDs were associated with higher LVM after adjusting for covariates (P=0.001, effect size =0.052 in the natural log scale (1.05 after back transformation) from fixed effect model). The directions of effect size were consistent across the five studies. ACE-I vs TD and dCCB vs. ACE-I exposure was not associated with LVM or RWT.

Similar to LV traits, meta-analysis of data from 935 African Americans belonging to the CARDIA and HyperGEN studies was conducted for e' velocity, E/e' ratio, and GLS (**Table 3**). dCCBs vs. TDs were associated with higher lateral E/e' (P=0.006, effect size=0.147 (1.16 after back transformation)) from a random effects model. The directions of effect were positive in both studies. Similarly, dCCBs vs. TDs were associated with lower GLS (P=0.042, effect size=-0.684 from a fixed effects model).

The pharmacogenetic effect of antihypertensive medication on LV traits and functions

Q-Q plots based on meta-analyses for SNP-by-drug interaction parameters are presented in **Supplemental Figure 1**. Lamdas ranged 0.978-1.021 (**Supplemental Table 2**). We detected a genome-wide significant SNP-by-drug interaction ($P<5*10^{-8}$) on RWT

when comparing dCCBs to ACE-Is. Three SNPs within a 20kb locus on chromosome 20 were associated with RWT when comparing dCCBs to ACE-Is. The directions of effect are consistent across four cohorts with available data (smallest $P=4.74*10^{-8}$) (see **Table 4**). The SNPs are located between long intergenic non-protein coding RNA 687 (*LINC00687*) and long non-coding RNA (*LOC339593*) (**Figure 1** and **Figure 2**). They are all common SNPs with MAF range of 0.09-0.12.

Marginally significant SNP-by-drug findings include ten SNPs near *U80770* on chromosome 2 for LVM when comparing dCCBs vs. ACE-Is (smallest $P=1.21*10^{-7}$, MAF range of 0.17-0.22) **(Figure 1**). Ten SNPs near ubiquitin-like 3 (*UBL3)* located on chromosome 13 modified the association between dCCBs vs. TD for LVM ((smallest $P=5.20*10^{-7}$, MAF range of 0.37-0.47). *BICD1* rs326641 modified the association of dCCBs vs. TDs with LVM ($P=1.04*10^{-7}$, MAF=0.15). *THRB* rs2217884 was associated with RWT when comparing dCCBs vs ACE-Is ($P=1.03*10^{-7}$, MAF=0.47).

Similar to primary outcomes, genome-wide association analyses were performed using data from 935 African Americans belonging to the CARDIA and HyperGEN studies for e' velocity, E/e' ratio, and GLS. Q-Q plots based on meta-analyses of the cohort-specific, SNP-by-drug interaction parameters showed p-values for the interaction terms followed expected trends with lambdas close to 1 for all models (**Supplemental Figure 2** and **Supplemental Table 3**).

No genome-wide significant interactions ($P<5*10^{-8}$) for any of the three drug comparisons on secondary outcomes were detected (**Supplemental Figure 3**). However, we found several marginally significant SNPs. Two SNPs (rs11744698 and rs6898102) near poly(ADP-Ribose) polymerase family member 8 (*PARP8*) gene modified the

association of dCCB vs. TD exposure for GLS ($P=7.59*10^{-8}$ and $8.18*10^{-8}$, respectively; both MAF=0.29). We also found 7 SNPs within 300kb of protein phosphatase 2 regulatory subunit b-alpha (*PPP2R3A*) which modified the association dCCB vs TD treatment with GLS (smallest $P=1.25*10^{-7}$, MAF range of 0.235-0.239). Fourteen SNPs within a 7kb locus on chromosome 8 between ST3 beta-galactoside alpha-2,3-sialyltransferase 1 (*ST3GAL1*) and zinc finger and AT-Hook domain containing (*ZFAT*) were associated with average E/e' when comparing ACE-Is to dCCBs (smallest $P=1.77*10^{-7}$, MAF range of 0.26-0.28). Finally, interactions between five intronic SNPs of coiled-coil domain containing 3 (*CCDC3*) and dCCB vs. TD treatment were associated with septal E/e' (smallest $P=2.7*10^{-7}$, MAF range of 0.158-0.21).

DISCUSSION

In this study, we combined the sample size of 2,068 African Americans from five observational epidemiology studies to evaluate the association between antihypertensive medication class and LV traits as well as the association between SNP-by-drug interaction and LV traits among participants treated for hypertension. To our knowledge, this study represents the first GWAS that evaluates SNP-by-drug interaction effect on LV traits (including those measured by speckle tracking data) in African Americans. We found dCCB exposure was associated with increased LVM in comparison to TD exposure. We also observed trends for worse diastolic function when comparing dCCB to TD exposure including lower GLS and higher E/e' ratio. We observed one genome-wide significant SNP-by-drug interaction effect on RWT among dCCB exposed vs. ACE-I

exposed African Americans. We also reported several marginally significant associations that provide preliminary evidence of SNP-by-drug interactions for further studies.

We found dCCBs vs. TDs were associated with worse cardiac structure and function including higher LVM, higher lateral E/e', and lower GLS. In an 80-study meta-analysis representing data on over 3,767 individuals treated for hypertension, Klingbeil et al. found LVM index decreased more with CCB treatment (average 11% decrease) compared to diuretic treatment (average 8% decrease).[13] These results differ from our own, but that study considered prospective changes in LVM index it was not stratified by ethnicity and did not restrict to the dihydropyridine CCB subclass nor the thiazide diuretic subclass. Similar to our findings, a randomized clinical trial of 53 hypertensive Japanese participants reported hydrochlorothiazide treatment in combination with angiotensin II receptor blocker treatment (ARB) was associated with greater improvement in LVM index in comparison to a dCCB/ARB treatment combination. [28] Overall, these results suggest it is important to consider drug subclass when making these comparisons and that TDs may be associated with better LV structure and function in comparison to dCCBs in African Americans treated for hypertension.

Our top statistically significant SNP-by-drug interaction findings lie between two long non-coding RNA genes. Long non-coding RNAs regulate the expression of genes in the nucleus by directly interacting with DNA recruiting chromatin modifying complexes and various transcriptional regulators.[29] Additionally, they can be involved in epigenetic and transcriptional regulation of neighboring loci in cis or distal genes in trans.[29] Circulating levels of other long non-coding RNAs are linked with acute heart failure, LV remodeling and other cardiovascular related outcomes.[29] A *LOC339593* variant,

rs2207418 (a different variant than highlighted by our study) was associated with cardiac hypertrophy among 1,610 unrelated Caucasian cases and 463 unrelated Caucasian controls ($P=8*10^{-6}$) in a candidate gene study.[30] Therefore, the findings here provide preliminary evidence of non-coding genes that could modify the association of antihypertensive medication effects on LV traits.

Other marginally significant findings for our primary outcomes include *U80770* which is not well characterized but is highly expressed in testis. *BICD1* functions to affect telomere length in humans which is important for regulating DNA replication, cellular proliferation, and has been linked to aging.[31, 32] Recently, *BICD1* was reported to directly modulate protease-activated receptor-1 (PAR1), a G protein-coupled receptor that plays an important role in cardiomyocyte contractility.[32] Previous studies reported *BICD1* variants have a main effect association with ejection fraction in Caucasians and association with LVM among Caribbean Hispanics with high waist circumference. [33 34]

An interaction between *THRB* rs2217884 and dCCB vs ACE-I treatment was associated with RWT. The gene encodes the beta subunit of nuclear thyroid hormone receptor known to mediate the effect of its ligand on metabolism and heart rate.[35]Mutations in the gene reduce thyroid hormone signaling and cause a compensatory increase in T3 and T4 thyroid hormones. Additionally, higher circulating levels of T3 and T4 were reported correlated with higher LVM index among 293 hypertensive Japanese patients.[36] Higher circulating level of T3 was correlated with higher RWT among 2078 middle-aged Caucasians untreated for hypertension.[37] Interestingly, daily use of nifedipine, a dCCB was reported to decrease T3 and T4 circulating levels on male albino rabbits during three months of treatment. Additionally, thyroid hormone disorders can

affect the synthesis and secretion of renin-angiotensin system (RAS) components. Increased circulating levels of T3 and T4 were reported to increase plasma renin activity and plasma renin concentration in adult rats.[38-40] In addition increased circulating levels of T3 and T4 have been correlated with increased plasma ACE concentration in rats and humans.[39, 41] Another study showed ACE activity and expression in kidneys were increased in rats injected with T3 or T4.[42] Moreover, ACE-Is directly affect the RAS through blocking the conversion of angiotensin I to angiotensin II.[43] Overall there is strong biological plausibility for the SNP-by-drug interaction observed for RWT in the current study, future studies should continue to investigate if *THRB* variants modify the association of dCCBs vs ACE-Is with RWT.

Among genes marginally associated with secondary outcomes (e' velocity, E/e' ratio and GLS), *PPP2R3A* encodes a regulatory subunit of the protein phosphatase 2 that is involved in negative control of cell growth and division. The gene is expressed in cardiomyocytes and has been associated with fibrinogen in a GWAS meta-analysis of over 120,000 Caucasians ($P=2*10^{-27}$) and triglycerides in a GWAS meta-analysis of over 62,000 Caucasians ($P=8*10^{-9}$).[44] Another interesting gene is *ZFAT* that encodes a zinc finger transcription factor involved in apoptosis and cell survival. *ZFAT* rare variants have been associated with hypertension in two difference case-control studies of Caucasians.[45, 46] *CCDC3* encodes a favine protein involved in fat metabolism and lipid accumulation. [47] We hope to continue to follow-up these genes in additional cohorts with relevant data.

Our study has several strengths. First, phenotypes were well-measured using standardized methods of M-mode echocardiography in all five cohorts. Second, our study

focused on a specific subgroup of diuretic, TD and a specific subgroup of CCB, dCCB which helps avoid heterogeneous effects caused by different subclasses of antihypertensive medication with different mechanisms of actions. Finally, our study focuses on African Americans, a group with high burden of cardiovascular morbidity and mortality.

Our study findings should also be interpreted in context of some limitations. First, this study used a cross-sectional design that cannot establish temporality on the association between antihypertensive treatment and LV traits. Selection bias, missing data, confounding from unmeasured and unknown factors could affect our results. Second, this study was designed to test for modest-to-large interaction effect sizes for common variants. Therefore, our study could not assess rare variants and other types of variants that were not well covered in our GWAS.

In this study, we report variants near/in *LINC00687*, *LOC339593, U80770*, *BICD1* and *THRB* might interact with antihypertensive medications to modulate LV traits in African Americans from five cohorts in CHARGE consortium. These genes need to be validated in other African American populations. Future sequencing studies that capture more common and rare variants in larger populations are needed to expand these findings. Importantly, this study suggests common variants could modify the association between antihypertensive treatment and LV traits in African Americans. The findings here might help optimize the benefit of antihypertensive treatment in improving LV traits in African Americans by matching individual genotypes with treatment classes.

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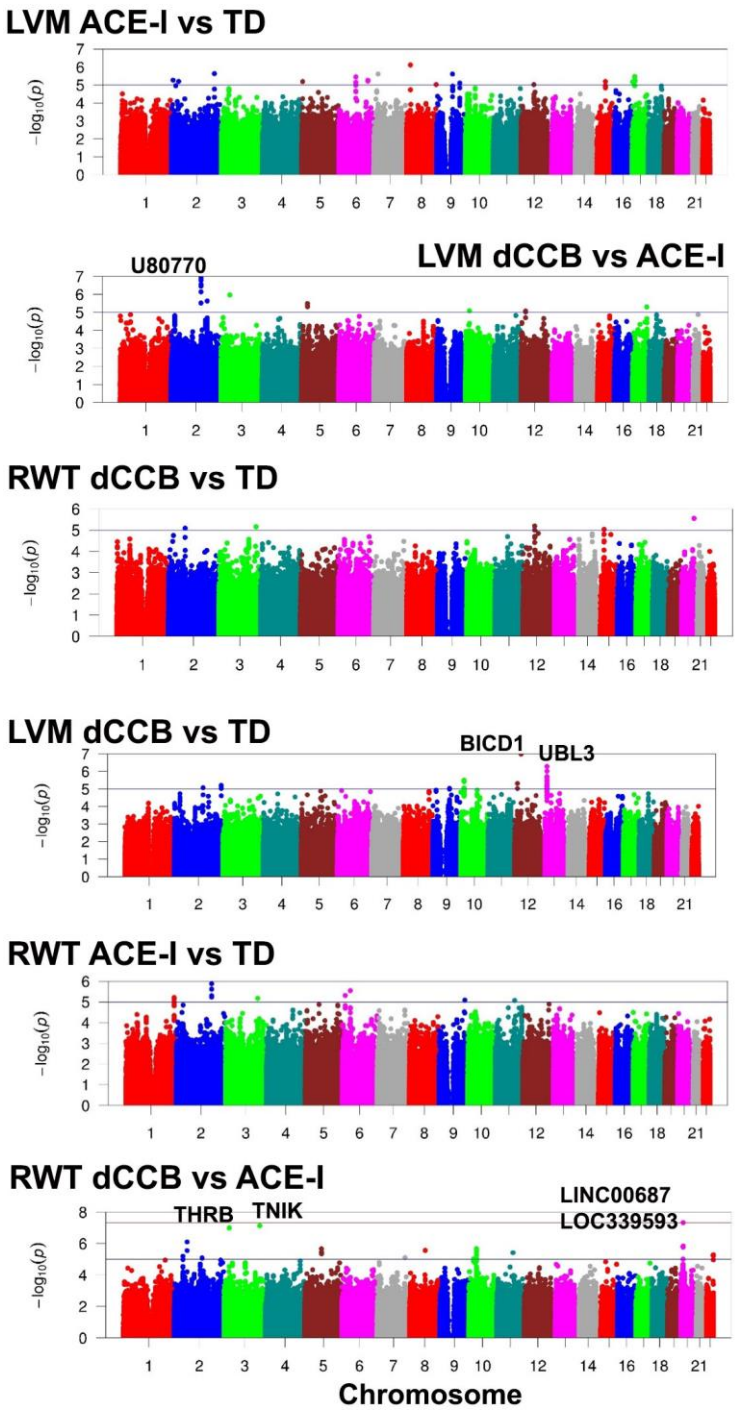


Figure 1. ***Plots show the individual interaction p-values based on discovery meta-analysis against their genomic position for left ventricular mass (LVM) and relative wall thickness (RWT) for three anti-hypertensive medication comparisons.***

ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. Within each chromosome, shown on the x-axis, the results are plotted left to right from the p-terminal end. The nearest genes are indicated for variants with an interaction p-values less than $2*10^{-6}$ in the discovery meta-analysis.

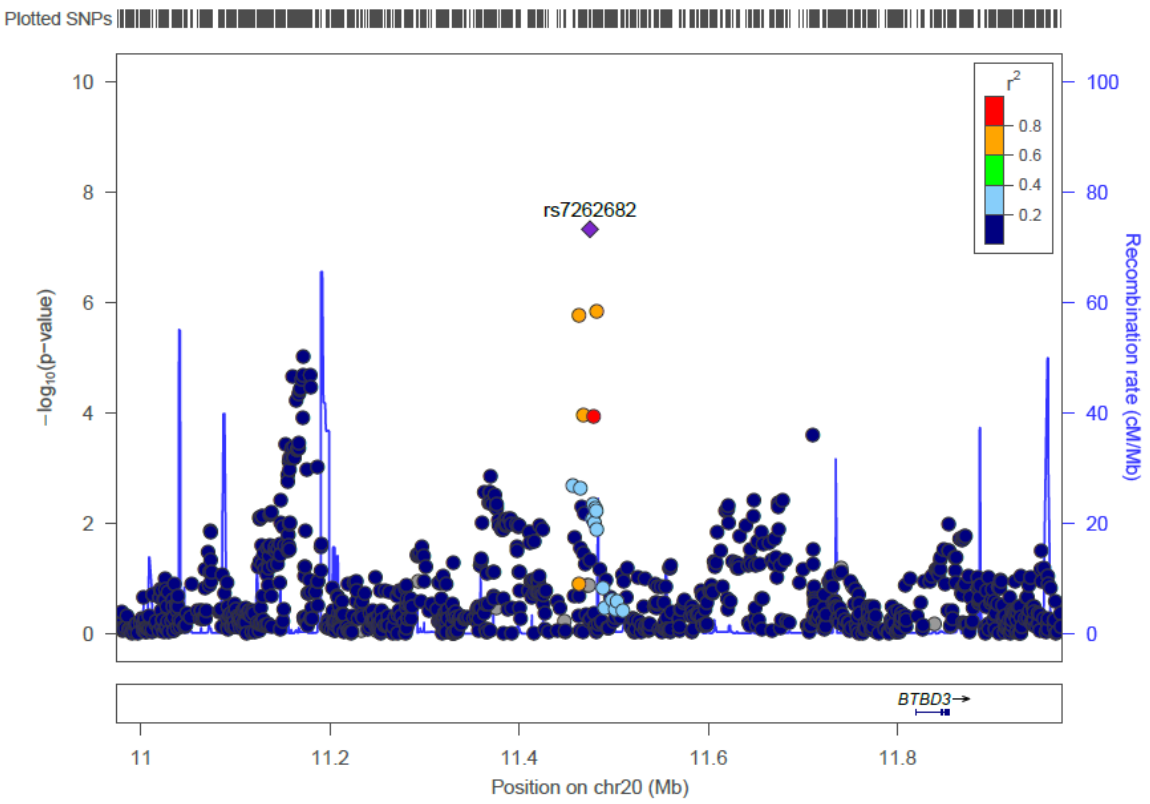


Figure 2. ***Genome-wide association findings in region surrounding rs7262682 for relative wall thickness for dCCB vs. ACE-I.***

ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. Points are collared according to the level of linkage disequilibrium of the each SNP with the rs7262682; r^2 estimates generated using 1000 Genomes June 2010 Yoruban data. Hatch marks at the top reflect SNP density. Bottom, gene location, exons are indicated with a wider band. The figure is based on output generated by LocusZoom.

Table 1. ***Characteristics of study participants (N=2,068).***

	CARDIA	CHS	JHS	GENOA	HyperGEN
Sample size	**251**	**290**	**571**	**280**	**676**
Age, year (SD) (year)	50.3 (3.6)	74.3 (5.2)	54.2 (10.6)	62.7(9.56)	51.5 (10.4)
Female, N (%)	173 (68.9)	203 (70.0)	379 (66.4)	195 (69.6)	490 (72.5)
Height, m, mean (SD)	1.7 (0.1)	1.6 (0.09)	1.7 (0.095)	1.68 (0.09)	1.7 (0.08)
Weight, kg, mean (SD)	96.9 (25.6)	78.0 (14.0)	95.6 (22.3)	90.8 (19.5)	91.9 (22.7)
eGFR, ml/min/1.73m^2, mean (SD)	101.0 (25.2)	80.1 (20.5)	92.4 (22.3)	87.8 (21.03)	89.9 (21.2)
Type 2 diabetes, N (%)	73 (29.3)	81 (27.9)	183 (32.1)	93 (33.2)	173 (25.6)
Echocardiographic measure					
LVM, g, mean (SD)	185.5 (61.5)	161.8 (60.1)	158.8 (43.1)	163.4 (44.8)	177.4 (49.4)
RWT, cm, mean (SD)	0.4 (0.09)	0.4 (0.08)	0.4 (0.06)	0.3 (0.05)	0.4 (0.05)
Drug exposure					
TDs, N (%)	123 (49.0)	45 (15.5)	239 (41.9)	161 (57.5)	172 (25.4)
monotherapy, N (%)	16 (13.1)	17 (37.8)	30 (12.6)	24 (14.9)	68 (39.5)
avg number of ATH, mean (SD)	2.3 (0.9)	1.7 (0.7)	2.3 (0.9)	2.2 (0.8)	1.8 (0.7)
ACE-Is, N (%)	109 (43.4)	53 (18.3)	223 (39.1)	138 (49.2)	208 (30.8)
monotherapy, N (%)	35 (32.11)	11 (20.8)	44 (19.7)	33 (23.9)	47 (22.6)
avg number of ATH, mean (SD)	2.1 (1.0)	2.2 (0.8)	2.3 (1.0)	2.1 (0.9)	2.1 (0.8)
dCCBs, N (%)	70 (27.9)	89 (30.7)	133 (23.3)	74 (26.4)	185 (27.4)
monotherapy, N (%)	11 (15.7)	44 (49.4)	34 (25.6)	24 (32.4)	88 (47.6)
avg number of ATH, mean (SD)	2.8 (1.2)	1.7 (0.8)	2.4 (1.1)	1.9 (0.8)	1.8 (0.9)

*ATH, antihypertensive. eGFR, estimated glomerular filtration rate. LVM, left ventricular mass. RWT, relative wall thickness. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic.

Table 2. ***Meta-analysis results of the main effect of anti-hypertensive medications on left ventricular traits among African Americans across 5 studies (N=2,068)***

	Drug exposure								
Primary outcomes	**Model 1: ACE-I vs. TD (TD=ref)**			**Model 2: dCCB vs. TD (TD=ref)**			**Model 3: dCCB vs. ACE-I (ACE-I=ref)**		
	Beta	**SE**	**P-value**	**Beta**	**SE**	**P-value**	**Beta**	**SE**	**P-value**
LVM	0.030	0.018	0.104	0.052	0.016	**0.001**	0.017	0.016	0.292
RWT	-0.004	0.011	0.745	-0.003	0.011	0.770	0.016	0.010	0.104

LVM, left ventricular mass. RWT, relative wall thickness. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. Results of fixed effects model were reported for all models

Table 3. ***Meta-analysis results of the main effect of anti-hypertensive medications on left ventricular functions among African Americans across CARDIA and HyperGEN studies (N=935)***

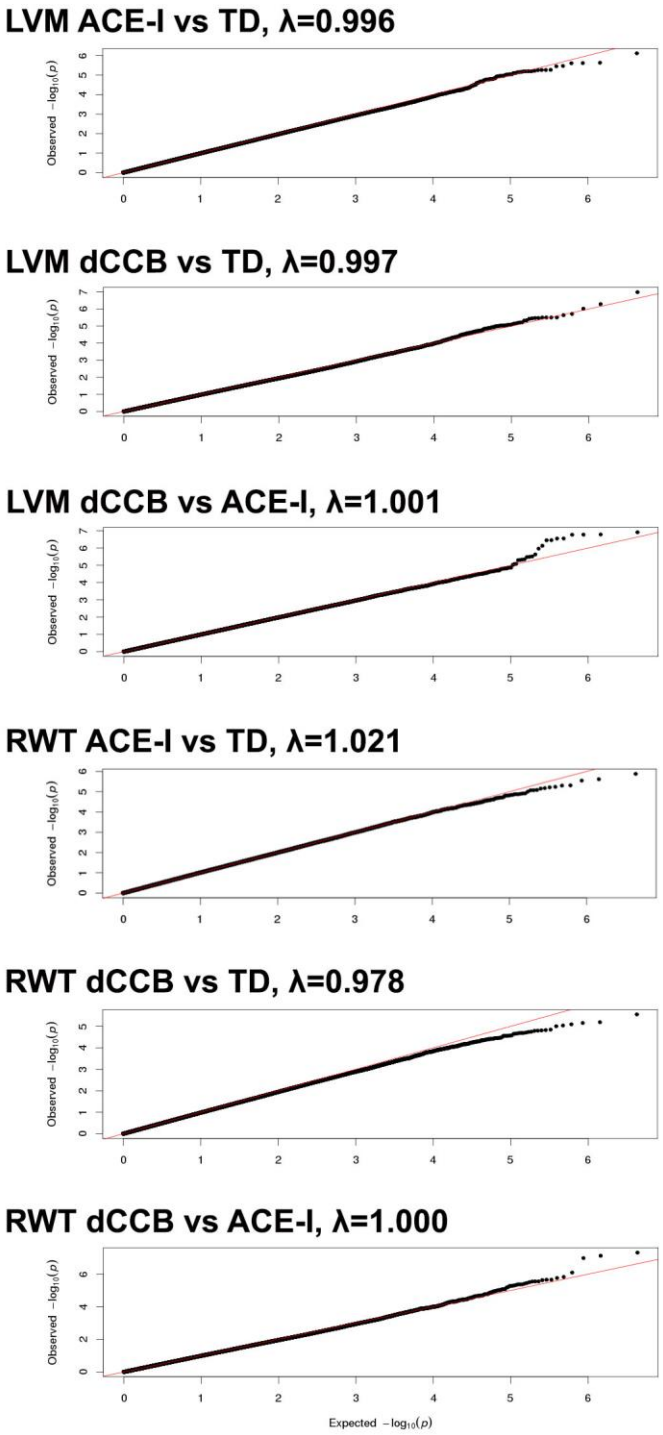
	Drug exposure								
Secondary Outcomes	**Model 1: ACE-I vs. TD (TD=ref)**			**Model 2: dCCB vs. TD (TD=ref)**			**Model 3: dCCB vs. ACE-I (ACE-I=ref)**		
	Beta	**SE**	**P-value**	**Beta**	**SE**	**P-value**	**Beta**	**SE**	**P-value**
septal e'	-0.111	0.151	0.463	0.128	0.133	0.338	0.102	0.134	0.448
lateral e'	-0.219	0.173	0.205	-0.197	0.148	0.183	0.008	0.151	0.959
average e'	-0.136	0.149	0.360	-0.002	0.125	0.989	0.029	0.127	0.821
septal E/e'	0.098	0.052	0.059	0.037	0.043	0.391	0.043	0.047	0.361
lateral E/e'	0.119*	0.076*	0.118*	0.147*	0.053*	**0.006***	0.048	0.053	0.371
average E/e'	0.101*	0.052*	0.052*	0.081	0.045	0.072	0.045	0.046	0.335
GLS	-0.590	0.383	0.123	-0.684	0.336	**0.042**	0.274	0.318	0.389

ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. Results of fixed effects model were reported for all models except those with * (E/e' lateral model 1 and 2, and E/e' average model 1).

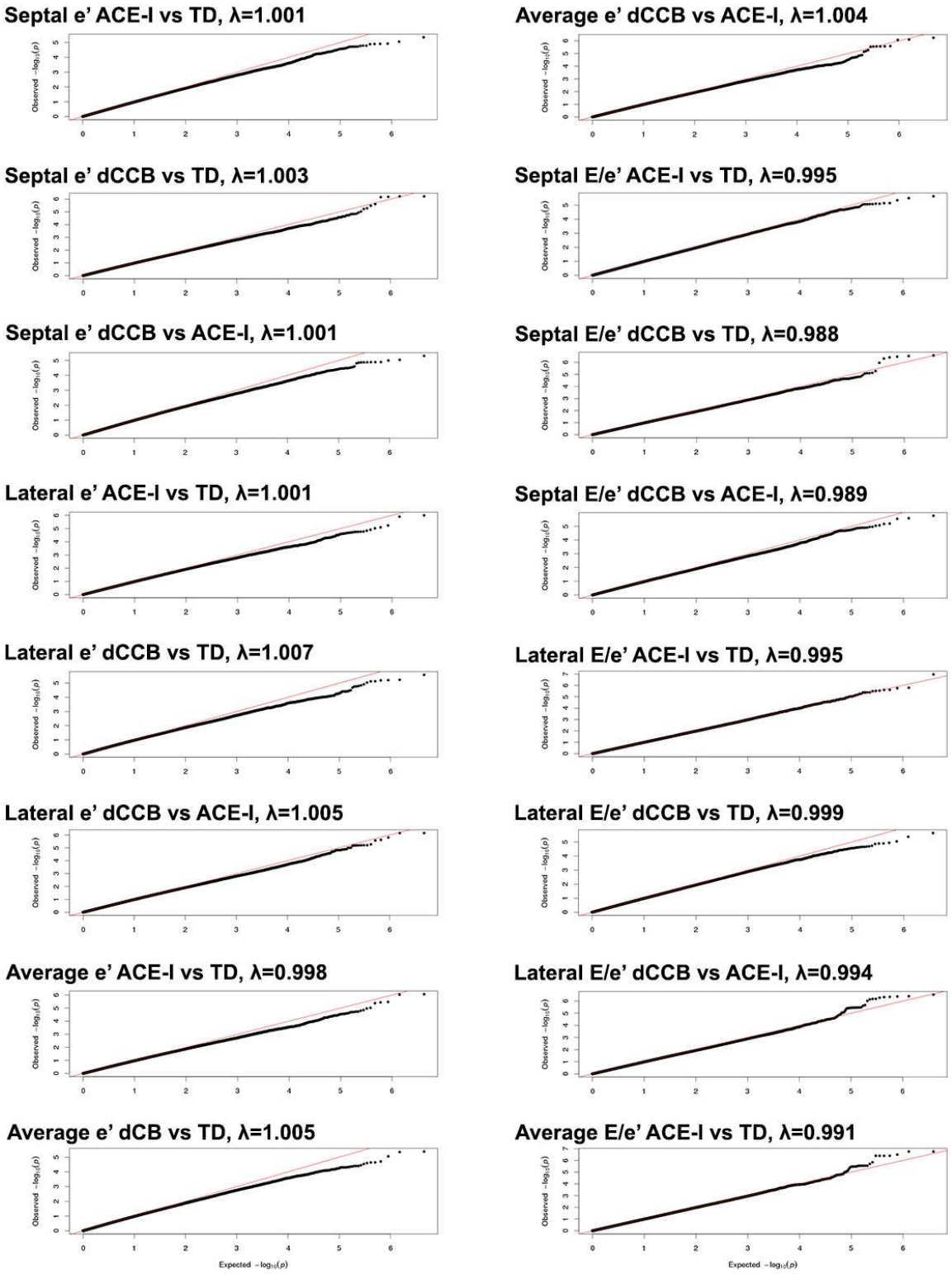
Table 4. ***Top-hits interaction results of meta-analysis for left ventricular mas and relative wall thickness for three antihypertensive medication comparisons.***

rs#	Chr:BP	A1/A2	AF	Effect (SE)	Direction	P-value	Location	Gene	Model
rs7262682	20:11474929	T/C	0.12	0.141 (0.026)	?++++	$4.74*10^{-8}$	intergenic	*LINC00687, LOC339593*	RWT dCCB vs ACE
rs11906708	20:11482244	A/G	0.09	0.163 (0.034)	?++?+	$1.48*10^{-6}$	intergenic	*LINC00687, LOC339593*	RWT dCCB vs ACE
rs11906016	20:11463593	A/G	0.91	-0.159 (0.033)	?--?-	$1.71*10^{-6}$	intergenic	*LINC00687, LOC339593*	RWT dCCB vs ACE
rs10176318	2:145709071	A/G	0.19	0.172 (0.033)	?++++	$1.21*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs7581822	2:145697961	A/G	0.82	-0.172 (0.033)	?----	$1.62*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs13412736	2:145714109	T/C	0.20	0.162 (0.031)	-++++	$1.66*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs10193147	2:145714377	A/C	0.20	0.162 (0.031)	-++++	$1.68*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs10200130	2:145706040	T/C	0.20	0.158 (0.031)	-++++	$2.78*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs16824670	2:145706608	T/G	0.20	0.158 (0.031)	-++++	$2.81*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs10179372	2:145709987	A/C	0.20	0.157 (0.031)	-++++	$3.47*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs10204792	2:145713584	T/C	0.80	-0.157 (0.031)	+----	$3.54*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs10496975	2:145704568	T/G	0.78	-0.152 (0.031)	+----	$7.28*10^{-7}$	intergenic	*U80770*	LVM dCCB vs ACE
rs10172711	2:145707848	T/G	0.18	0.165 (0.035)	?++++	$3.02*10^{-6}$	intergenic	*U80770*	LVM dCCB vs ACE
rs9314972	13:29225969	A/G	0.55	-0.135 (0.027)	-----	$5.20*10^{-7}$	intergenic	*UBL3*	LVM dCCB vs TD
rs9314974	13:29230437	T/C	0.40	0.127 (0.026)	+++++	$9.67*10^{-7}$	intergenic	*UBL3*	LVM dCCB vs TD
rs7995666	13:29258195	C/G	0.44	-0.126 (0.027)	-----	$1.95*10^{-6}$	intronic	*UBL3*	LVM dCCB vs TD
rs9314973	13:29230070	T/G	0.37	-0.13 (0.027)	-----	$2.29*10^{-6}$	intergenic	*UBL3*	LVM dCCB vs TD
rs1854176	13:29231262	T/G	0.46	0.122 (0.026)	+++++	$3.16*10^{-6}$	intergenic	*UBL3*	LVM dCCB vs TD
rs9551739	13:29233311	T/C	0.54	-0.121 (0.026)	-----	$3.56*10^{-6}$	intergenic	*UBL3*	LVM dCCB vs TD
rs4769772	13:29234006	T/G	0.53	-0.12 (0.026)	-----	$4.61*10^{-6}$	intergenic	*UBL3*	LVM dCCB vs TD
rs7330356	13:29261981	A/G	0.43	-0.118 (0.026)	-----	$6.15*10^{-6}$	intronic	*UBL3*	LVM dCCB vs TD
rs9508554	13:29258860	T/C	0.45	0.118 (0.026)	+++++	$6.53*10^{-6}$	intronic	*UBL3*	LVM dCCB vs TD
rs1410110	13:29263017	T/C	0.45	0.118 (0.026)	+++++	$7.05*10^{-6}$	intronic	*UBL3*	LVM dCCB vs TD
rs326641	12:32291136	T/G	0.15	0.208 (0.039)	?++++	$1.04*10^{-7}$	intronic	*BICD1*	LVM dCCB vs TD
rs2217884	3:24442206	T/C	0.47	0.09 (0.017)	+++++	$1.03*10^{-7}$	intronic	*THRB*	RWT dCCB vs ACE

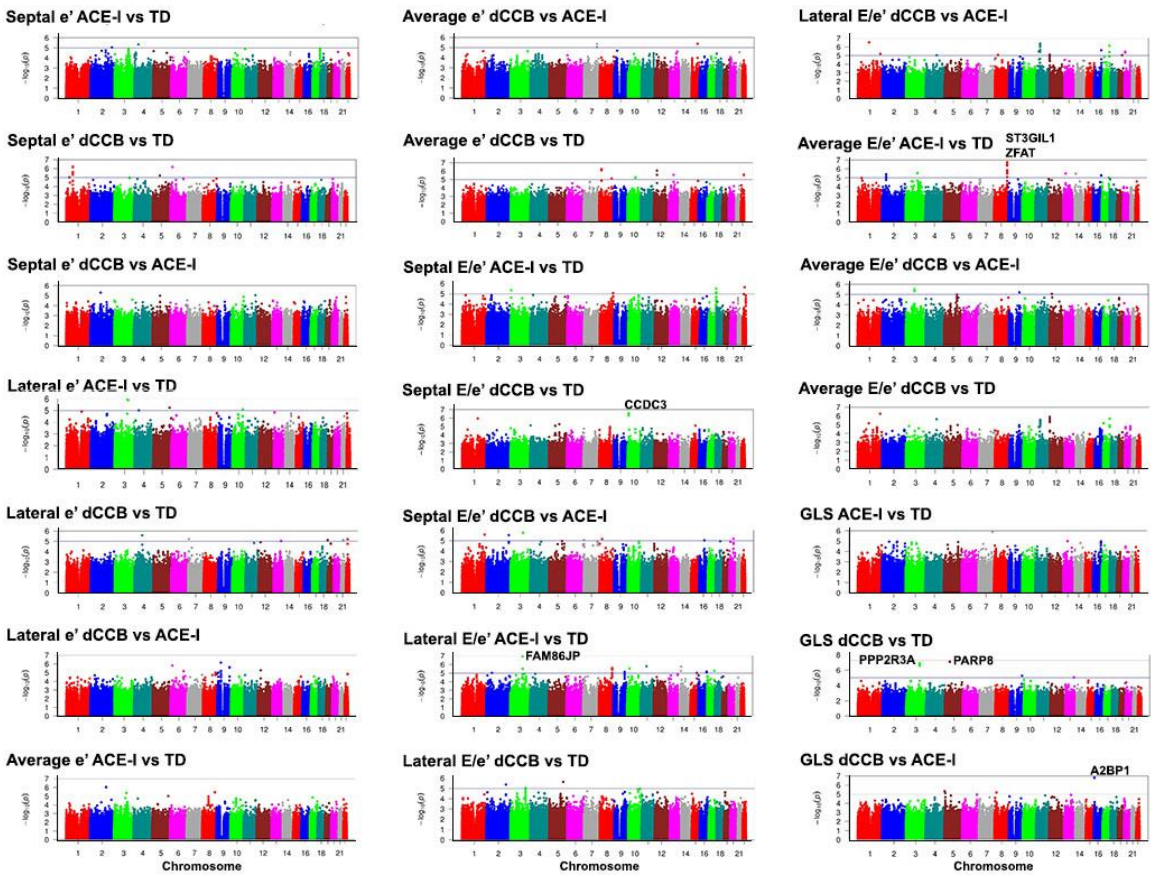
AF, allele frequency. BP, base-pair position. Chr, chromosome. SE, standard error. LVM, left ventricular mass. RWT, relative wall thickness. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic.



Supplemental Figure 1. ***Q-Q plots for discovery meta-analyses for left ventricular mass (LVM) and relative wall thickness (RWT) for three anti-hypertensive medication comparisons***. ACE-I, angiotensin-coverting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. λ indicates genomic inflation factor.



Supplemental Figure 2. ***Q-Q plots for discovery meta-analyses for left ventricular function for three anti-hypertensive medication comparisons*.** ACE-I, angiotensin-coverting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. λ indicates genomic inflation factor. GLS, global longitudinal strain.



Supplemental Figure 3. ***Plots show the individual interaction p-values based on discovery meta-analysis against their genomic position for left ventricular function for three anti-hypertensive medication comparisons.*** ACE-I, angiotensin-coverting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. Within each chromosome, shown on the x-axis, the results are plotted left to right from the p-terminal end. The nearest genes are indicated for variants with an interaction p-values less than $3*10^{-7}$ in the discovery meta-analysis. GLS, global longitudinal strain.

Supplemental Table 1. ***Genotype description by cohorts***

	CARDIA	CHS	JHS	GENOA	HyperGEN
Country	USA	USA	USA	USA	USA
Collection type	Community-based	Community-based	Community and family based	Family-Based	Family-Based
Special exclusions					Participants with type 1 diabetes or advanced renal disease (defined as serum creatinine level > 2 mg/dL) were excluded from the original study since these two conditions can cause secondary hypertension and the goal of HyperGEN was to identify novel essential hypertension loci.
Sample	fasting serum	fasting serum	fasting serum	fasting serum	fasting serum
Collection method	venous	venous	venous	venous	venous
Genotyping platform and SNP panel	Affymetrix 6.0	Illumina Human1M-Duo	Affymetrix 6.0	Affymetrix 6.0	86% with the Affy 6.0 and 14% with Affy 5.0
Genotyping calling algorithm	Plink/ Birdseed	Illumina GenomeStudio	Birdseed		Birdseed
Call rate [filter detail / N individuals excluded]	<95%	< 95%	< 95%	<95%	95%
Other sample QC		Sex discrepancy Discordant with prior genotyping	Sample duplicates, contaminated samples, excess heterozygosity, cryptic relatedness, sample outliers	Sex discrepancy, duplicate samples, samples with low identity-by-state	contaminated samples, blood sample mix-ups
SNP Call Freq		< 97%	< 90%	< 95%	< 95%

Other SNP QC filters applied?	MAF<1%	HWE P < 10^{-5} > 2 DE/MeI hz frequency = 0, not in HapMap	MAF < 0.01 HWE p < 10^{-6}, MeI , mapping to several genomic locations	MAF<1%	mendelian errors, MAF <1%, HWE P<10^{-6}
SNP number in QC'd dataset		940,567	868,969	906,602	846,813
Imputation software	MACH	MACH	MACH	MACH	MACH 1.0.16
Imputation Reference Panel	HapMap P2.r22.b36, 1:1 CEU:YRI	HapMap P2.r22.b36, (CEU, YRI) & HapMap Phase 3 (YRI, ASW, CEU)	HapMap P2.r22.b36, CEU+YRI	HapMap haplotypes, release 22, build 36 (CEU)	HapMap 2, CEU and YRI
Imputation quality metrics		effective allele count(EAC) >10, EAC=allele count*r2 imputation quality	rsq>0.3		r2hat > 0.3
Number of SNPs in analysis	2,657,132	2,662,536	2,655,620	2,203,610	2,846,152
Adjustments	age, sex, height, weight, t2d status, number of anti-hypertensive ttreatment classes, eGFR, 4pcs	age, sex, height, weight, t2d status, number of anti-hypertensive ttreatment classes, eGFR, 10pcs	age, sex, weight, height, number of hypertension medications, diabetes, eGFR (CKD-EPI equation), 10 PCs		age, sex, center, height, weight, count of antihypertensvie treatment classes, type 2 diabetes, eGFR, 10PCs
Analysis method	Linear regression	Linear regression	Linear model, first degree relatives excluded		Linear mixed effect models
Software for analysis	ProABEL	R	ProbAbel		LMEKIN

Supplemental Table 2. ***Number of SNPs and genomic inflation factors for discovery meta-analyses of left ventricular traits***

Outcome	Model	Number of SNPs	lambda
LVM	Model 1: ACE-I vs. TD (TD=ref)	2,158,834	0.996
LVM	Model 2: dCCB vs. TD (TD=ref)	2,131,686	0.997
LVM	Model 3: dCCB vs. ACE-I (ACE-I=ref)	2,191,657	1.001
RWT	Model 1: ACE-I vs. TD (TD=ref)	2,159,038	1.021
RWT	Model 2: dCCB vs. TD (TD=ref)	2,131,688	0.978
RWT	Model 3: dCCB vs. ACE-I (ACE-I=ref)	2,191,661	1.000

Table shows the number of SNPs available for analysis after QC parameters were applied at the level of the individual studies and across all studies contributing to the discovery meta-analysis. Lambda indicates the genomic inflation factor. LVM, left ventricular mass. RWT, relative wall thickness. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic.

Supplemental Table 3. ***Number of SNPs and genomic inflation factors for discovery meta-analyses of left ventricular functions***

Outcome	Model	Number of SNPs	lambda
septal e'	Model 1: ACE-I vs. TD (TD=ref)	1,856,615	1.001
septal e'	Model 2: dCCB vs. TD (TD=ref)	1,532,209	1.003
septal e'	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,541,650	1.001
lateral e'	Model 1: ACE-I vs. TD (TD=ref)	1,866,188	1.001
lateral e'	Model 2: dCCB vs. TD (TD=ref)	1,528,944	1.007
lateral e'	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,536,564	1.005
average e'	Model 1: ACE-I vs. TD (TD=ref)	1,855,715	0.998
average e'	Model 2: dCCB vs. TD (TD=ref)	1,529,874	1.005
average e'	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,541,592	1.004
septal E/e'	Model 1: ACE-I vs. TD (TD=ref)	1,455,135	0.995
septal E/e'	Model 2: dCCB vs. TD (TD=ref)	1,524,051	0.988
septal E/e'	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,527,316	0.989
lateral E/e'	Model 1: ACE-I vs. TD (TD=ref)	1,455,346	0.995
lateral E/e'	Model 2: dCCB vs. TD (TD=ref)	1,524,246	0.999
lateral E/e'	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,538,186	0.994
average E/e'	Model 1: ACE-I vs. TD (TD=ref)	1,455,201	0.991
average E/e'	Model 2: dCCB vs. TD (TD=ref)	1,523,486	0.983
average E/e'	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,529,948	0.994
GLS	Model 1: ACE-I vs. TD (TD=ref)	1,777,048	0.990
GLS	Model 2: dCCB vs. TD (TD=ref)	1,389,060	0.996
GLS	Model 3: dCCB vs. ACE-I (ACE-I=ref)	1,439,601	0.998

Table shows the number of SNPs available for analysis after QC parameters were applied at the level of the individual studies and across all studies contributing to the discovery meta-analysis. Lambda indicates the genomic inflation factor. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic.

MANUSCRIPT 2 SUPPLEMENTAL MATERIAL

Section 1. Discovery Populations and Phenotype Characterization and Harmonization

Discovery Populations

Coronary Artery Risk Development in Young Adult Study (CARDIA)

CARDIA is a prospective study of risk factors for coronary heart disease in young adults from 18 to 30 years of age recruited during 1985-1986.[1] It was conducted across four field centers including Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA.[1] Medical information including current treatment and history were ascertained through questionnaires.[2] At baseline, participants brought in a list of all medications used.[2] At later exams, participants were asked to physically bring in their medications.[2] Echocardiographic data and antihypertensive medication data was collected on ~2,000 AAs at year 25 (or exam 8).[3] Among them, 251 AAs with relevant drug exposures, GWAS data and echocardiography measurement at year 25 were included in this study.

Cardiovascular Health Study (CHS)

CHS is a population-based cohort study of risk factors for CHD and stroke in adults age 65 years and older recruited during 1989-1990.[4] It was conducted across four field centers including Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA.[4] Study participants were asked to bring all medications to the yearly in-person interview, and medication use was assessed at that time. Echocardiographic data on 510 AAs and antihypertensive treatment data was collected at

visit 6.[5] Among them, 290 AAs with relevant drug exposures, GWAS data and echocardiography measurement were included in this study.

Jackson Heart Study (JHS)

JHS is a longitudinal, community-based, cohort study initiated in 2000 in the Jackson, Mississippi area.[6, 7] The study aims to investigate risk factors and causes of CV diseases in adult AAs (21–84 years).[6] The study has an about 31% overlap with the ARIC study (all ARIC study participants from Jackson MS).[6] Three exam cycles include 2000-2004, 2005-2008, 2009-2012. Participants presented all of the medications used within 2 weeks whether prescriptions, over the counter or herbal preparations during the clinical visit.[8] The Medi-Span® therapeutic classification system was used to identify medications. A registered pharmacist resolved and adjudicated any automated coding that led to indeterminate results. Echocardiography measurements on 2,000 AAs and antihypertensive treatment data were collected at visit 1 excluding overlapping participants from ARIC.[5] Among them, 571 AAs with relevant drug exposures, GWAS data and echocardiography measurement were included into this study.

Genetic Epidemiology Network of Atherosclerosis study (GENOA)

GENOA is a family-based study participating in the Family Blood Pressure Program (FBPP).[9]GENOA consists of hypertensive sibships that were recruited for linkage and association studies in order to identify genes that influence blood pressure and its target organ damage. In the initial phase of the GENOA study (Phase I: 1996-2001), all members of sibships containing ≥ 2 individuals with essential hypertension clinically diagnosed before age 60 were invited to participate, including both

hypertensive and normotensive siblings. GENOA participants include African Americans from Jackson, MS (N=1,854 at phase I).[10] A second study visit was completed from 2001 to 2005 (Phase II).[11] During phase II, LV traits were measured on 1,090 African American participants. During the examinations at each phase, prescription medications taken by the participant during the previous month are recorded.[9] The Medi-Span® therapeutic classification system was used to identify medications.[9, 10] Each of prescription antihypertensive medications was assigned a 6-digit code number that categorizes antihypertensive medications into groups with similar modes of action.[9] A total of 280 AAs with relevant drug exposures, GWAS data and echocardiography measurement were included in this study.

Hypertension Genetic Epidemiology Network study (HyperGEN)

HyperGEN is a family based study with a sib-pair design. Hypertensive African American sibships were recruited from population-based cohorts in Forsyth County, NC, and from the community-at-large in Birmingham, AL, from 1995 to 2000. Sibling pairs with onset of hypertension before age 60 were recruited in the first phase. The study was later extended to other siblings and the offspring of the hypertensive probands who were unmedicated adults.[9, 12] Information on race, demographics, height, weight, current medications (via self-report) and co-morbid conditions as well as clinical measures (fasting serum chemistries and urine chemistries) was collected.[12] Participants with type 1 diabetes or advanced renal disease (defined as serum creatinine level > 2 mg/dL) were excluded from the original study since these two conditions can cause secondary hypertension and the goal of HyperGEN was to identify novel essential hypertension

loci.[12] A total of 676 AAs treated for hypertension from HyperGEN had relevant data and were included in this study.

Phenotypic characterization and Harmonization

CARDIA

At year 25, echocardiography was performed using an Artida cardiac ultrasound scanner (Toshiba Medical Systems, Otawara, Japan).[13] LV traits were assessed by two-dimensional (2D) guided M-mode and Doppler echocardiography at all field centers by trained sonographers following a standardized protocol.[14] At the Johns Hopkins University reading center, experienced sonographers made measurements from digitized images using a standard software offline image-analysis system.[14] Assessment of intra-reader and inter-reader agreement was performed during each study. LVM was calculated by using Devereux formula.[13] IVSDD, LVIDD, and PWTD were measured from 2-dimensional (2D)-guided M-mode echocardiograms obtained from optimized parasternal short-axis views.[13] Relative wall thickness (RWT) was calculated as twice the PWTD divided by the LVIDD.

Early peak diastolic mitral annular velocity (e') were measured from pulsed-Doppler echocardiographic recordings of transmitral flow.[14] Using tissue Doppler imaging, e' was measured at the septal and lateral mitral annulus. Then, e’ was calculated from the average of the septal and lateral mitral annular velocities.[14]

CHS

At visit 6, all participants underwent two-dimensional (2D) guided M-mode echocardiography by trained sonographers following a standardized protocol.[15]

Videotapes were then displayed and digitalized at the echocardiography reading center at the University of California, Irvine.[15] Next, LV measurements were made from digitized images using an offline image-analysis system equipped with customized computer algorithms.[3] Quality control was performed at all center fields including standardized training of echo technicians and readers, periodic technician observation by a trained echocardiographer, blind duplicate readings to establish inter-reader and intra-reader measurement variabilities, periodic reader review sessions, phantom studies on the ultrasound equipment, and quality-control audits.[3] The reproducibility of echocardiographic measurements was systematically assessed at visit six that yielded 17% mean difference and 14% median difference for inter-reader and 10% mean difference and 7% median difference for intra-reader of LV mass.[15] Similar to CARDIA, LVM in CHS was calculated using Devereux formula. IVSDD, LVIDD, and PWTD were measured from 2-dimensional (2D)-guided M-mode echocardiograms.[16] Relative wall thickness (RWT) was calculated as the ratio of sum of the posterior wall thickness and interventricular septal thickness by the internal LV diameter.[16]

JHS

Left ventricular traits were measured by 2-dimensional (2D) transthoracic echocardiograms (Sonos-4500, Philips Medical Systems) using standardized protocols.[17] A cardiologist performed all echocardiographic readings. Echocardiograms were then reviewed for clinical interpretation and analytical measurements by experienced cardiologists on networked image workstations.[17] LVM in JHS was calculated by Devereux formula similar to CARDIA and CHS. IVSDD, LVIDD, and PWTD were measured from 2-dimensional (2D) method from parasternal short-axis view with the M-

mode cursor positioned through the center of ventricle.[17, 18] Relative wall thickness (RWT) was calculated as twice the PWTD divided by the LVIDD.[17]

GENOA

LV traits were performed by two-dimensional (2D) guided M-mode and Doppler M-mode using an Acuson 128XP echo machine (Acuson, Mountain View, CA).[19] Readings were performed at the New York Presbyterian Hospital–Weill Cornell Medical Center and verified by a single highly experienced cardiologist following a standardized protocol.[19] Correct orientation of planes for imaging and Doppler recordings was verified using standardized protocols.[11] Measurements were made using a computerized review station equipped with digitizing tablet and monitor screen overlay for calibration and performance of each measurement.[11] M-mode and 2D eachocardiograms via the parasternal acoustic window were used to recode LV structures for ≥10 beats.[11] LV measurements between separate echocardiograms are highly reliable (e.g., the correlation between repeated measures of LVM was 0.93 between paired echocardiograms in hypertensive adults).[11] Similar to other studies, LVM was calculated by Devereux formula.[20 21]IVSDD, LVIDD, and PWTD were measured by M-mode or 2D echocardiography.[21] RWT was calculated as twice the PWTD divided by the LVIDD.[21]

HyperGEN

LV traits were assessed by two-dimensional (2D) guided M-mode and Doppler echocardiography at the Birmingham and Forsyth County field centers following a standardized protocol. All instruments were calibrated against a standard phantom at installation and were validated regularly.[22] Certificated sonographers from each center

were also trained at the echocardiography reading center at New York Hospital-Weill Cornell Medical Center. At the reading center, measurements were computerized, calibrated, and quantified using a review station with digitizing tablet and monitor overlay.[23] LV measurements between separate echocardiograms by the reading center were reliable (e.g., intra class correlation coefficient is 0.93 for LV mass) [22].

Similar to other studies, LVM was calculated by Devereux formula.[20, 21] IVSDD, LVIDD, and PWTD were measured by M-mode or 2D echocardiography.[21] RWT was calculated as twice the PWTD divided by the LVIDD.[21]

Section 2. List of medication inventory and medication exclusions for individual model

List 1. ***Medication inventory***

1. Thiazide and Related Diuretics

- Bendroflumethiazide
- Chlorothiazide
- Chlorthalidone
- Hydrochlorothiazide
- Indapamide
- Methyclothiazide
- Metolazone
- Trichlormethiazide
- Polythiazide
- Quinethazone
- Benzthiazide
- Hydroflumethiazide
- Cyclothiazide
- Benzthiazide
- Indapamidum

2. ACE-I

- Benazepril
- Catopril
- Enalapril
- Fosinopril
- Lisinopril
- Moexipril
- Perindopril
- Quinapril
- Ramipril
- Trandolapril
- Spirapril
- Cilazapril
- Delapril
- Zofenopril
- Imidapril

3. Calcium Channel Blockers (dihydropyridine class)

- Amlodipine
- Aranidipine
- Azelnidipine
- Barnidipine
- Benidipine
- Cilnidipine
- Clevidipine
- Isradipine
- Efonidipine
- Felodipine
- Lacidipine
- Lercanidipine
- Manidipine
- Nicardipine
- Nifedipine
- Nilvadipine
- Nimodipine
- Nisoldipine
- Nitrendipine
- Pranidipine

4. Miscellaneous Thiazide Combinations

- Clonidine and Chlorthalidone
- Hydralazine and Hydrochlorothiazide
- Methyldopa and Hydrochlorothiazide

5. dCCB and ACE-I Combination Preparations

- Benazepril and Amlodipine
- Enalapril and Felodipine
- Enalapril and Lercanidipine
- Lisinopril and Amlodipine
- Perindopril and Amlodipine
- Ramipril and Felodipine
- Enalapril and Nitrendipine
- Ramipril and Amlodipine
- Delapril and Manidipine

6. ACE and Thiazide Combination Preparation

- Benazepril /Hydrochlorothiazide
- Captopril/Hydrochlorothiazide
- Enalapril/Hydrochlorothiazide
- Fosinopril/Hydrochlorothiazide
- Lisinopril/Hydrochlorothiazide
- Moexipril/Hydrochlorothiazide
- Quinapril/Hydrochlorothiazide
- Trandolapril/Hydrochlorothiazide

7. Thiazide with Alpha Blockers

- Prazosin and Polythiazide (Minizide)

8. Thiazide with Beta Blockers

- Atenolol and Chlorthalidone
- Bisoprolol and Hydrochlorothiazide
- Metoprolol and Hydrochlorothiazide
- Nadolol and Bendroflumethazide
- Propanolol and Hydrochlorothiazide
- Timolol and Hydrochlorothiazide

*Model 1: ACE-I use (group 2 and 5) vs. TD use (group 1, 4, 7, and 8).
Model 2: dCCB use (group 3 and 5) vs. TD use (group 1, 4, 6, 7, and 8).
Model 3: dCCB use (group 3) vs. ACE-I use (group 2 and 6).

List 2. *Medication Exclusions for individual models*

Loop Diuretics (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Bumetanide
- Ethacrynic Acid
- Furosemide
- Torsemide
- Piretanide
- Tripamide

Potassium-Sparing Diuretics (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Amiloride
- Triamterene

Thiazide and K-Sparing Combination Preparation (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Amiloride/Hctz (Moduretic)
- Spironolactone/Hctz (Aldactazide)
- Triamterene/Hctz (Dyazide/Maxide)

Aldosterone Antagonist/Potassium Sparing Diuretics (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Eplerenone
- Spironolactone
- Canrenoate Potassium
- Prorenoate Potassium
- Mexrenoate Potassium

ARB (exclude from model 1 and 3, ok if in combination with dCCB or TD for model 2)

- Candesartan
- Eprosartan
- Irbesartan
- Losartan
- Olmesartan
- Telmisartan
- Valsartan
- Azilsartan Medoxomil

ARB and Thiazide Combination Preparation (exclude from model 1 and 3, ok in model 2)

- Irbesartan/Hydrochlorothiazide
- Losartan Potassium/Hydrochlorothiazide
- Valsartan/Hydrochlorothiazide
- Candesartan/Hydrochlorothiazide
- Eprosartan/Hydrochlorothiazide
- Olmesartan Medoxomil/Hydrochlorothiazide
- Telmisartan/Hydrochlorothiazide

Non-dihydropyridine CCB and ACE Combination Preparations (exclude from model 2 and 3, ok in model 1)

- Diltiazem and Enalapril
- Verapamil and Trandolapril

Non-dihydropyridine CCBs (exclude from model 2 and 3, ok in combination with ACE-I or TD in model 1)

- Mibefradil
- Bepridil
- Diltiazem
- Verapamil
- Gallopamil

CCB and ARB Combination Preparations (exclude from model 1 and 3, ok in model 2)

- Amlodipine and Olmesartan
- Amlodipine and Valsartan
- Amlodipine and Telmisartan

Thiazide, ACE-I and CCB Combination Preparations (exclude from all models)

- Perindopril, Amlodipine, and Indapamide

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MANUSCRIPT 3

THE ASSOCIATION OF DNA METHYLATION AND ANTIHYPERTENSIVE TREATMENTS WITH LEFT VENTRICULAR STRUCTURE IN AFRICAN AMERICANS FROM THE HYPERGEN AND GENOA STUDIES

by
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ABSTRACT

Cardiovascular disease (CVD) is a leading cause of death in the United States. It disproportionally burdens African Americans. Left ventricular (LV) hypertrophy is an important predictor of CVD. Antihypertensive drugs have been found to decrease LV mass (LVM). However, inter-individual variations in treatment response suggests that inherent differences e.g. genetic background may be at play. Epigenetics has recently been found to contribute to CVD related traits including antihypertensive treatment response, yet to our knowledge, no pharmaco-epigenetic studies of LV hypertrophy have been published. To fill the gap, we investigated the interaction between CpGs and antihypertensive treatment class on LV traits using data from 536 African Americans from the Hypertension Genetic Epidemiology Network study (HyperGEN) and the Genetic Epidemiology Network of Atherosclerosis study (GENOA) under a cross-sectional design. Antihypertensive treatments considered included angiotensin-converting enzyme (ACE) inhibitors, dihydropyridine calcium channel blockers (dCCB), and thiazide diuretics (TD), the three most commonly used antihypertensive agents in African Americans. TDs, ACE-Is, and dCCBs were compared in a pairwise manner. Methylation at cytosine-phosphate-guanine (CpG) sites was measured using the Illumina Infinium HumanMethylation450 array in HyperGEN and the Illumina Infinium HumanMethylation27 array in GENOA. Models were adjusted for age, sex, height, weight, count of antihypertensive treatment classes, estimated glomerular filtration rate, type 2 diabetes, white blood cell count proportions and principal components for ancestry at the cohort level. A total of ~25,000 CpGs overlapped between the two studies and we performed fixed effects inverse variance weighted meta-analysis unless heterogeneity

across studies was detected then random effects models were used. We found that cg22284302 in cytochrome C oxidase assembly factor 7 (*COA7*) is significantly associated with RWT when comparing ACE-Is vs. TDs ($P=1.15*10^{-7}$). The directions of effect for the interaction term were different between two studies. Other marginally significant findings included eukaryotic translation elongation factor 2 (*EEF2)* and cryptochrome circadian clock 1(*CRY1)* which have been linked to CV-related outcomes giving the findings biological plausibility. However, additional studies with larger sample size and better genome-wide CpG site coverage are required to confirm these results.

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death in the U.S.[1], responsible for one out of four deaths in 2015.[2] Approximately 24 million Americans are expected to develop the condition by 2030.[3] Chronic and complex CVD affects racial groups differently with African Americans suffering higher rates of morbidity and mortality.[4] One risk factor, left ventricular (LV) hypertrophy (LVH), is known to increase CV endpoints including stroke and heart failure.[5] LVH is defined as a cardiac condition in which left ventricle wall thickness and/or size increases. In fact, LVH doubles the risk of CVD morbidity and mortality across racial, gender, and age groups.[5] LVH is also a better predictor of mortality than coronary artery disease in multiple populations.[6]

Antihypertensive drugs have been found to decrease LV mass (LVM) independent of blood pressure effect in hypertensive patients.[7, 8] However, there is a lack of consensus on the best antihypertensive agents for decreasing LVM.[9, 10] There are likely subgroups of patients who may benefit more from a specific class of drug. Given inter-individual variation in LVM on different antihypertensive classes we hypothesize genomic factors may be at play.

Epigenetics (e.g. DNA methylation) has recently been shown to account for some of the heritability of CVD-related outcomes.[11-14] Animal model and cell culture studies have reported associations between epigenetic markers and cardiac structure and function.[15-17] However, the effect of DNA methylation on LV phenotypes in humans is understudied. One study reported that decreased methylation of the T-box 5 (*TBX5)* and heart and neural crest derivatives expressed 1 (*HAND1)* genes was associated with dilated cardiomyopathy in humans as well as increased gene expression of both genes.[18]

Importantly, DNA methylation can alter drug response via affecting gene expression and downstream function of proteins that antihypertensive agents target.[19, 20] Recently, candidate gene work has suggested DNA methylation may contribute to antihypertensive treatment response.[21] Methylation of the *ADRB1* gene modifies the effect of metoprolol, on cardiomyocytes in rats.[21] However, to our knowledge, no human pharmaco-epigenetic studies of LVH or LVH related traits (LV traits) have been published. Motivated by these findings and on the availability of new epigenotypes and extensive antihypertensive treatment data as well as echocardiography data in two well characterized observational epidemiology cohorts, we performed the first pharmaco-epigenetic study of LV traits in African Americans. To do that, we investigated the interaction between CpGs and antihypertensive treatment class including angiotensin-converting enzyme (ACE) inhibitors, dihydropyridine calcium channel blockers (dCCB), and thiazide diuretics (TD) on LV traits. Relevant phenotype, antihypertensive treatment and epigenotype data from 536 African Americans from the Hypertension Genetic Epidemiology Network study (HyperGEN) and the Genetic Epidemiology Network of Atherosclerosis study (GENOA) were available for this study of how DNA methylation may modify the relationship between antihypertensive treatment class and LV traits.

METHODS

Study population

The Hypertension Genetic Epidemiology Network (HyperGEN) study is a population-based study of hypertensive sibships initially recruited between 1996-1999 (N~2100 African Americans with echocardiography data). In 2015, an ancillary

epigenetic study was conducted among the initial African-American cohort participants, restricted to 636 participants selected from extremes of the distribution of LVM to identify CpG sites associated with echocardiography traits. The current project included 328 participants treated for hypertension with echocardiographic measures and epigenotype data.

The Genetic Epidemiology Network of Atherosclerosis study (GENOA) is a community-based study of hypertensive sibships that was designed to investigate the genetics of hypertension and its arteriosclerotic target-organ damage in African Americans from Jackson, Mississippi.[22] All members of sibships containing ≥ 2 individuals with essential hypertension clinically diagnosed before age 60 were invited to participate, including both hypertensive and normotensive siblings during phase I (1996-2001).[22] Eighty percent of African Americans (1,482 subjects) from phase I returned for the second examination (Phase II: 2001-2005).[22] This current project included ~200 African Americans treated for hypertension with echocardiographic measures and methylation data.

Echocardiographic measures

Echocardiographic measures were collected using the same protocol in HyperGEN and GENOA since both studies were part of the Family Blood Pressure Program (FBPP).[23] Images were read for both studies at the same echocardiography reading center (New York Hospital –Cornell Medical center).[24] Measures were performed using Doppler two-dimensional (2D) and M-mode (2D-guided) echocardiograms following a standardized protocol.[24] This project assessed two LV

structures including LVM and relative wall thickness (RWT). In both studies, LVM was calculated by using the American Society of Echocardiography corrected formula by Devereux: 0.80 x 1.04 x [(IVSDD + PWTD + LVIDD)^3 - LVIDD^3] + 0.6g in which IVSDD is the interventricular septum thickness, LVIDD is the LV internal dimension-diastole, and PWTD is the thickness at end-diastole of the posterior wall.[25] RWT was calculated as twice the PWTD divided by the LVIDD in both studies.[26] Better cardiac structure is indicated by lower LVM and RWT.

Definition of drug exposure

We examined three antihypertensive classes of drugs: angiotensin-converting enzyme (ACE) inhibitors, dihydropyridine calcium channel blockers (dCCB), and thiazide diuretics (TD). Three antihypertensive drug classes were compared in a pairwise manner in the following three models. Model 1: ACE-I use vs. TD use (reference = TD use) where ACE-I exposure was defined as the use of an ACE-I in a single or combination preparation without concomitant use of a TD versus TD exposure without ACE-I. Model 2: dCCB use vs. TD use (reference = TD use) where dCCB exposure was defined as the use of a dCCB in a single or combination preparation without concomitant use of a TD versus TD exposure without dCCB. Model 3: dCCB use vs. ACE-I use (reference =ACE-I use) where dCCB exposure was defined as the use of a dCCB in a single or combination preparation without concomitant use of an ACE-I versus ACE-I exposure without dCCB. Drug groupings were based on manually-curated lists that were reviewed by experts from each study to include all relevant drugs from the United States. Drug exposure was assessed by self-report in each study. In our approach, participants

taking more than one medication class may contribute data to more than one model. Details of medication inventory of eligible antihypertensive treatments for each model and excluded treatments were provided in **Supplemental Material** List 1 and List 2, respectively.

Epigenomic analysis

DNA was extracted from all 636 HyperGEN participants and assayed on Illumina Infinium HumanMethylation450 (methyl450) array covering over 480,000 CpG sites. Briefly, 500ng of buffy coat DNA was hybridized to the methyl450 array after bisulfite conversion with EZ DNA kits (Zymo Research, Irvine, CA). Subsequently, β scores and detection p-values were estimated using the GenomeStudio software provided by the manufacturer. β scores were defined as the proportion of total signal from the methylation-specific probe or color channel. Detection p-values were defined as the probability that the total intensity for a given probe falls within the background signal intensity. For quality control (QC), we set β scores of a CpG site to missing if its association detection p-value greater than 0.01. Subsequently, we excluded samples with more than 1.5% missing data points, and any CpG probe where more than 5% of samples fail to yield adequate intensity. After filtering, we normalized the resulting β scores using the Subset-quantile Within Array Normalization (SWAN) method in *minfi* package.[27] A total of 484,366 CpG sites and all 636 samples remained after QC.

For GENOA, the Illumina Infinium HumanMethylation27 array covering over 27,000 CpG sites was used for analysis of DNA extracted from buffy coat on ~1,000 African Americans with blood samples from phase II. Seven samples were removed due

to poor bisulfite conversion control efficiency, and another twenty-nine samples were removed due to extreme control probe values. That yielded 972 African Americans after sample QC.[28] Subsequently, DNA methylation QC was performed including 1) removing CpG sites with control probe values greater than 4 standard deviation from their mean values; 2) reducing batch and chip effect by linearly regressing methylated and non-methylated intensity signals onto control probes; 3) excluding CpG sites located on X and Y chromosomes; 4) using p-value<0.001 on either the methylated or non-methylated signal intensities to take into account modality of CpG sites; 5) flagging 2,984 CpG sites having non-specific binding probes and 875 CpG sites having probes overlapping with SNPs reported in dbSNP.[28] A total of 26,449 CpG sites remained in GENOA after QC. Approximately 25,000 CpG sites overlapping between HyperGEN and GENOA were used for meta-analysis. Top hit CpGs were compared and excluded if they were cross-reactive probes and CpGs sites with SNPs on the probe as reported by Barfield et al.[29]

Statistical analysis

Linear mixed effect models were used to test the interaction between antihypertensive treatment class exposure (pairwise comparison, see section **Definition of drug exposure**) and each CpG site on each of the two LV traits, separately. Each model was adjusted for age, sex, height, weight, count of antihypertensive treatment classes, estimated glomerular filtration rate (eGFR), type 2 diabetes (T2D) (Yes/No), the top four principal components from genotypes (for ancestry), and five estimated cell proportions of white blood cell counts (WBC) as fixed effects. A kinship correlation matrix was used as a random effect in both studies to adjust for relatedness. Study site

and/or other study specific variables were included as covariates as needed by the individual cohorts (e.g. center in HyperGEN). T2D was defined by physician diagnosis or using at least one anti-diabetic treatment or having fasting plasma glucose ≥ 7.0 mmol/l (or 126mg/dL).[30] Five estimated cell-type proportions (CD8 T lymphocytes, CD4 T lymphocytes, natural killer cells, B cells, and monocytes) were imputed using the algorithm developed by Houseman, which predicts underlying cellular composition of each sample from DNA methylation patterns.[31]

Meta-analysis

To conduct meta-analysis, we restricted our analysis to autosomal CpGs that were available in both studies. We used study-specific estimates (β) and standard errors (SE) in both fixed effects or random effects models using METASOFT software. Briefly, we used fixed effect inverse variance weighted meta-analysis unless we found heterogeneity between studies to exist (P-value of Cochran's Q statistic >0.05) and in that case the results from the new random effects model proposed by Han and Eskin was reported.[32] Genomic control was performed for all models to control for inflation. We applied statistical significance criteria of $0.05/25{,}000 = 2*10^{-6}$ for this discovery effort.

RESULTS

Table 1 summarizes the general characteristics of the study populations. HyperGEN participants were slightly younger than GENOA participants. Participants were more likely to be female in both studies. LV traits were slightly different across the two studies with higher LVM, RWT in HyperGEN.

Q-Q plots based on meta-analyses of the cohort-specific, drug-SNPs interaction showed lambda values were close to 1 after genomic control (see **Supplemental Figure 1**). We detected an epigenome-wide significant interaction ($P<2*10^{-6}$) on RWT when comparing ACE-Is vs TDs (see **Figure 1**). Methylation at cg22284302 in cytochrome c oxidase assembly factor 7 (putative) (*COA7*) modified the association of ACE-I vs TD treatment with RWT ($P=1.15*10^{-6}$). The direction of effect for the interaction term was different between the two studies (**Table 2**). Methylation at cg16142977 in eukaryotic translation elongation factor 2 (*EEF2*) modified the association of ACE-I vs TD treatment with RWT ($P=2.56*10^{-6}$). The direction of effect for the interaction term was consistent across the two studies (**Table 2**). Another CpG, cg10126874, in cryptochrome circadian clock 1 (*CRY1*) modified the association of ACE-I vs TD treatment with LVM ($P=9.4*10^{-6}$) (**Table 2**). There were no significant or marginally significant interaction findings for LVM or RWT when comparing dCCBs to ACE-Is or dCCBs to TDs.

DISCUSSION

The evidence in support of epigenetic regulation of complex traits such as LV hypertrophy is growing at a fast pace. However, prior to our study, there are no genome-wide investigations of epigenetic sites that modify the effect of antihypertensive treatments on LV traits. In the current study we take advantage of the advent of high-resolution epigenetic arrays enabling epigenome-wide profiling and report for the first time on genes that may modify the relationship between common antihypertensive treatments for African Americans and LV traits.

The *COA7* gene is involved in assembly of mitochondrial respiratory chain complex I and complex IV. The gene is wide expressed across tissues, but lowly expressed in the left ventricle. However, the biological relevance of *COA7* in the context of cardiac function is unknown at the present time.[33] The protein encoded by *EEF2* gene is an essential regulator of protein synthesis which mediates the translocation step of peptide-chain elongation. The gene has been linked cardiomyocyte enlargement in an animal model report.[34] Another study showed that activated AMP-activated protein kinase (AMPK) indirectly downregulates EEF2 protein resulting in decreased energy expenditure and preservation of myocardial energy charge during ischemia in a mouse model.[35, 36] Ours is the first, that we know of, to link this gene to a CV trait in humans, however the available data in mice lends support for a potential link of EEF2 with pathophysiology of LV traits warranting its further study.

The *CRY1* gene encodes a circadian clock protein that is important for regulation blood pressure and cardiovascular function.[37-39] In humans, the onset of non-Q-wave angina, unstable angina, myocardial infarction, and sudden cardiac death all show striking elevations in the occurrence between the hours of 6:00 am and 12:00 pm, compared with any other time of day.[40] Hypertensive patients with less than 10% in blood pressure diurnal variation, referred as non-dippers have higher risk of heart failure and stroke.[41] The relationship between *CRY1* methylation, antihypertensive treatment class (ACE-I vs. TD) and LVM could be related to salt sensitivity. *CRY1* and *CRY2* knockout mice exhibits salt-sensitive hypertension due to abnormal high synthesis of aldosterone by the adrenal glands.[37] *CRY1* is also known to interact with clock circadian regulator(*CLOCK)* - aryl hydrocarbon receptor nuclear translocator like (*BMAL1)* activity

to repress transcription targets that might be involved in heart development. *BMAL1* knockout mice have larger heart, higher ratio of heart weight to body weight, increased LV wall thickness, and decreased systolic contractility.[38] Given the biological plausibility of this finding this gene is worthy of future investigation for its potential relationship with antihypertensive treatment class and LVM.

Our study has some strengths. This study is the first to examine the interaction between DNA methylation and antihypertensive treatments on LV traits in African Americans, a group with a high burden of cardiovascular morbidity and mortality. Second, our study had harmonized phenotypes in which the HyperGEN and GENOA studies had identical echocardiography protocols, and utilized the same reading centers. Our study is limited by moderate sample size for investigation of gene-environment interactions. Second, the study had a cross-sectional design so we are uncertain if methylation changes may be the cause or consequence of antihypertensive treatment or LV structure. Third, the two studies used different methylation assays and data in this study only represents the overlap of CpG sites for those assays.

In summary, we have shown that a CpG site in *COA7*, *EEF2*, and *CRY1* may modify the association of ACE-I vs. TD treatment with LV traits among African Americans treated for hypertension. The result needs to be replicated in external populations. Additional efforts to assemble a larger sample size and to interrogate more epigenome data are required. We hope in the future efforts such as this one will help optimize antihypertensive treatment in African Americans for improvement in LV structure.

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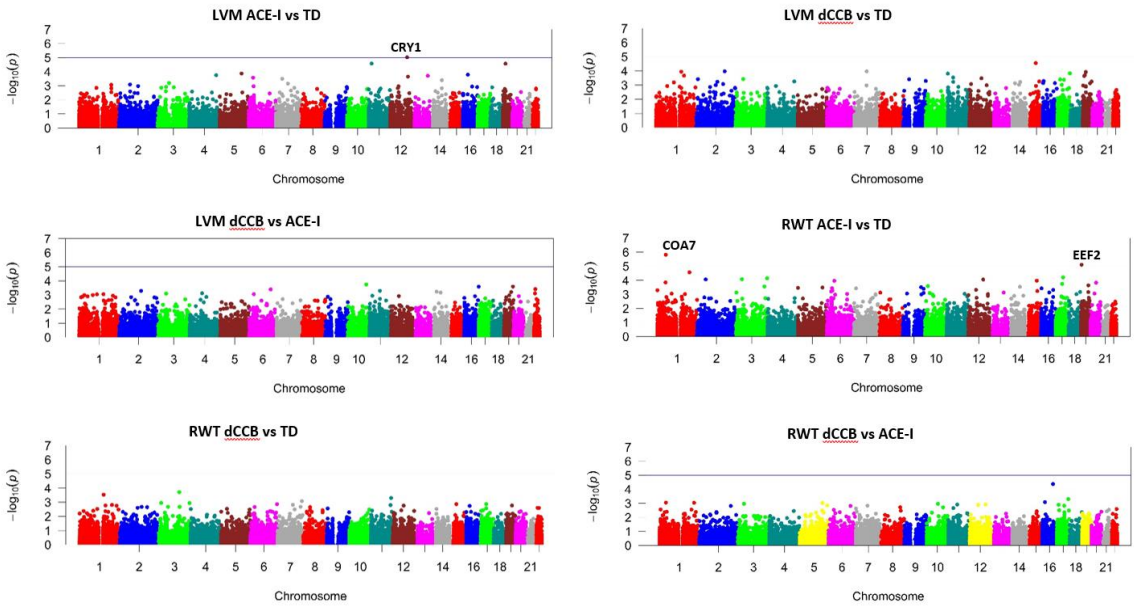


Figure 1: ***Plots show the individual interaction p-values based on discovery meta-analysis against their genomic position for left ventricular mass (LVM) and relative wall thickness (RWT) for three anti-hypertensive medication comparisons.*** ACE-I, angiotensin-coverting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. Within each chromosome, shown on the x-axis, the results are plotted left to right from the p-terminal end. The nearest genes are indicated for variants with an interaction p-values less than 10-5 in the discovery meta-analysis.

Table 1. ***Characteristics of study participants (N=536).***

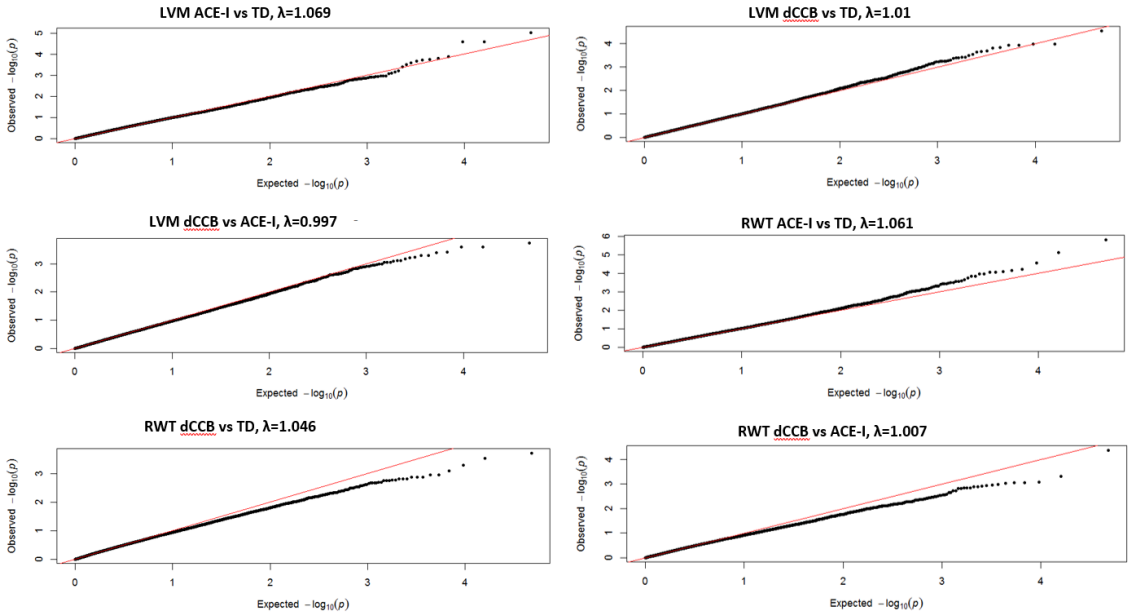
	GENOA	HyperGEN
Sample size	208	328
Age, year (SD) (year)	66.2 (8.03)	51.9 (10.7)
Female, N (%)	143 (68.8%)	234 (71.3)
Height, m, mean (SD)	168.4 (9.05)	1.7 (0.1)
Weight, kg, mean (SD)	90.1 (19.11)	92.8 (24.5)
Number of ATH treatment classes use, mean (SD)	1.97 (0.85)	1.6 (0.7)
eGFR, ml/min/1.73m^2, mean (SD)	84.8 (20.9)	90.6 (22.0)
Type 2 diabetes, N (%)	69 (33.2)	96 (29.3)
Echocardiographic measure		
LVM, g, mean (SD)	164.8 (41.8)	186.8 (64.2)
RWT, cm, mean (SD)	0.3 (0.05)	0.4 (0.06)
Drug exposure		
TDs, N (%)	119 (57.2)	78 (23.8)
monotherapy, N (%)	19 (16.0)	28 (35.9)
avg number of ATH, mean (SD)	2.2 (0.79)	1.8 (0.7)
ACE-Is, N (%)	101 (48.6)	113 (34.5)
monotherapy, N (%)	27 (26.7)	30 (26.6)
avg number of ATH, mean (SD)	2.1 (0.93)	2.0 (0.8)
dCCBs, N (%)	59 (28.4)	102 (31.1)
monotherapy, N (%)	17 (28.8)	49 (48.0)
avg number of ATH, mean (SD)	2.0 (0.9)	1.7 (0.9)

*ATH, antihypertensive. eGFR, estimated glomerular filtration rate. LVM, left ventricular mass. RWT, relative wall thickness. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic.

Table 2. *Top-hit results of meta-analysis from GENOA and HyperGEN studies (N=536).*

CpG name	Chr	Gene name	Beta meta-analysis	SE meta-analysis	P meta-analysis	P of Q statistics	Model	Study	Beta	SE	P	N
cg22284302	1	*COA7*	20.5631	1.49845	$1.15*10^{-6}$	0.01	**RWT ACE-I vs. TD**	**GENOA**	-2.95	0.57	$4.92*10^{-6}$	101
								HyperGEN	0.0039	1.06	0.99	102
cg16142977	19	*EEF2*	10.0674	2.32743	$2.56*10^{-6}$	0.11	**RWT ACE-I vs. TD**	**GENOA**	12.57	2.81	$2.87*10^{-5}$	101
								HyperGEN	4.61	4.15	0.27	102
cg10126874	12	*CRY1*	-29.629	5.976	$9.4*10^{-6}$	0.26	**LVM ACE-I vs. TD**	**GENOA**	-34.16	7.21	$9.81*10^{-6}$	101
								HyperGEN	-19.65	10.69	0.0697	102

Chr, chromosome. SE, standard error. LVM, left ventricular mass. RWT, relative wall thickness. ACE-I, angiotensin-converting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic.



Supplemental Figure 1: ***Q-Q plots for discovery meta-analyses for left ventricular mass (LVM) and relative wall thickness (RWT) for three anti-hypertensive medication comparisons.*** ACE-I, angiotensin-coverting enzyme inhibitor. dCCB, dihydropyridine calcium channel blocker. TD, thiazide diuretic. λ indicates genomic inflation factor after genomic control.

SUPPLEMENTAL MATERIAL

List 1. ***Medication inventory***

1. Thiazide and Related Diuretics
- Bendroflumethiazide
- Chlorothiazide
- Chlorthalidone
- Hydrochlorothiazide
- Indapamide
- Methyclothiazide
- Metolazone
- Trichlormethiazide
- Polythiazide
- Quinethazone
- Benzthiazide
- Hydroflumethiazide
- Cyclothiazide
- Benzthiazide
- Indapamidum

2. ACE-I
- Benazepril
- Catopril
- Enalapril
- Fosinopril
- Lisinopril
- Moexipril
- Perindopril
- Quinapril
- Ramipril
- Trandolapril
- Spirapril
- Cilazapril
- Delapril
- Zofenopril
- Imidapril

3. Calcium Channel Blockers (dihydropyridine class)
- Amlodipine
- Aranidipine
- Azelnidipine
- Barnidipine
- Benidipine
- Cilnidipine
- Clevidipine
- Isradipine
- Efonidipine
- Felodipine
- Lacidipine
- Lercanidipine
- Manidipine
- Nicardipine
- Nifedipine
- Nilvadipine
- Nimodipine
- Nisoldipine
- Nitrendipine
- Pranidipine

4. Miscellaneous Thiazide Combinations
- Clonidine and Chlorthalidone
- Hydralazine and Hydrochlorothiazide
- Methyldopa and Hydrochlorothiazide

5. dCCB and ACE-I Combination Preparations
- Benazepril and Amlodipine
- Enalapril and Felodipine
- Enalapril and Lercanidipine
- Lisinopril and Amlodipine
- Perindopril and Amlodipine
- Ramipril and Felodipine
- Enalapril and Nitrendipine
- Ramipril and Amlodipine
- Delapril and Manidipine

6. ACE and Thiazide Combination Preparation
- Benazepril /Hydrochlorothiazide
- Captopril/Hydrochlorothiazide
- Enalapril/Hydrochlorothiazide
- Fosinopril/Hydrochlorothiazide
- Lisinopril/Hydrochlorothiazide
- Moexipril/Hydrochlorothiazide
- Quinapril/Hydrochlorothiazide
- Trandolapril/Hydrochlorothiazide

7. Thiazide with Alpha Blockers
- Prazosin and Polythiazide (Minizide)

8. Thiazide with Beta Blockers
- Atenolol and Chlorthalidone
- Bisoprolol and Hydrochlorothiazide
- Metoprolol and Hydrochlorothiazide
- Nadolol and Bendroflumethazide
- Propanolol and Hydrochlorothiazide
- Timolol and Hydrochlorothiazide

*Model 1: ACE-I use (group 2 and 5) vs. TD use (group 1, 4, 7, and 8).
Model 2: dCCB use (group 3 and 5) vs. TD use (group 1, 4, 6, 7, and 8).
Model 3: dCCB use (group 3) vs. ACE-I use (group 2 and 6).

List 2. *Medication Exclusions for individual models*

Loop Diuretics (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Bumetanide
- Ethacrynic Acid
- Furosemide
- Torsemide
- Piretanide
- Tripamide

Potassium-Sparing Diuretics (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Amiloride
- Triamterene

Thiazide and K-Sparing Combination Preparation (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Amiloride/Hctz (Moduretic)
- Spironolactone/Hctz (Aldactazide)
- Triamterene/Hctz (Dyazide/Maxide)

Aldosterone Antagonist/Potassium Sparing Diuretics (exclude from model 1 and 2, ok if in combination with dCCB or ACE for model 3)

- Eplerenone
- Spironolactone
- Canrenoate Potassium
- Prorenoate Potassium
- Mexrenoate Potassium

ARB (exclude from model 1 and 3, ok if in combination with dCCB or TD for model 2)

- Candesartan
- Eprosartan
- Irbesartan
- Losartan
- Olmesartan
- Telmisartan
- Valsartan
- Azilsartan Medoxomil

ARB and Thiazide Combination Preparation (exclude from model 1 and 3, ok in model 2)

- Irbesartan/Hydrochlorothiazide
- Losartan Potassium/Hydrochlorothiazide
- Valsartan/Hydrochlorothiazide
- Candesartan/Hydrochlorothiazide
- Eprosartan/Hydrochlorothiazide
- Olmesartan Medoxomil/Hydrochlorothiazide
- Telmisartan/Hydrochlorothiazide

Non-dihydropyridine CCB and ACE Combination Preparations (exclude from model 2 and 3, ok in model 1)

- Diltiazem and Enalapril
- Verapamil and Trandolapril

Non-dihydropyridine CCBs (exclude from model 2 and 3, ok in combination with ACE-I or TD in model 1)

- Mibefradil
- Bepridil
- Diltiazem
- Verapamil
- Gallopamil

CCB and ARB Combination Preparations (exclude from model 1 and 3, ok in model 2)

- Amlodipine and Olmesartan
- Amlodipine and Valsartan
- Amlodipine and Telmisartan

Thiazide, ACE-I and CCB Combination Preparations (exclude from all models)

- Perindopril, Amlodipine, and Indapamide

CONCLUSION

This dissertation investigated the association of antihypertensive treatment as well as genomic variants with left ventricular (LV) hypertrophy (LVH) -related traits among African Americans from different epidemiological cohorts. The findings from this dissertation are presented as three manuscripts, each based on one of three aims described in the introduction section. All three manuscripts focus on quantitative phenotypes (LV structural and functional traits) and use association testing methods for single variants or gene regions. In addition, all three manuscripts use a cross-sectional design making it difficult to establish the temporal relationship between the exposures of interest (specifically CpGs and antihypertensive treatments) and LV traits in the projects two and three. However, this dissertation has several potentially important contributions to the body of literature on LV traits, antihypertensive treatment and genomics. The first project is one of the earliest to examine the association between rare protein coding variants and LV traits in African Americans, a group with a high burden of cardiovascular morbidity and mortality. Few studies have considered the association of antihypertensive treatment class with LVH related traits in African Americans. Our results suggest thiazide diuretics (TDs) may be more beneficial in this race group over dihydropyridine calcium channel blockers (dCCBs) and that it is important to study specific subclasses of these antihypertensive treatments. We also found a SNP in an interesting long noncoding RNA (lncRNA) that modified the association of dCCB vs. angiotensin-converting enzyme inhibitor (ACE-I) treatment with relative wall thickness. This gene has been linked to

LVH in a prior study of a Caucasian population. Finally, though the sample size was small to examine CpG-by-treatment interaction we highlight interesting genes for future study of methylation variation and LV traits during antihypertensive treatment in this race group. Overall, the results from this dissertation require replication but are some of the first studies which could help develop new treatment strategies to reduce the burden of LVH in the African American population with an ultimate aim to reduce racial disparities in CVD outcomes.

APPENDIX

IRB IDENTIFICATION AND CERTIFICATION OF RESEARCH PROJECCTS INVOLVING HUMAN SUBJECTS

UAB

Project Revision/Amendment Form

irb

Form version: June 26, 2012

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

- **Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for Investigators for additional information.**
- **Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.**

21989

1. Today's Date	3.21.2016

2. Principal Investigator (PI)

Name (with degree)	Marguerite Ryan Irvin PhD	Blazer ID	Irvinr
Department	Epidemiology	Division (if applicable)	
Office Address	RPHB220	Office Phone	57672
E-mail	irvinr@uab.edu	Fax Number	934-8665

Contact person who should receive copies of IRB correspondence (Optional)

Name	Kristie Williams	E-Mail	kdidcoct@uab.edu
Phone	57687	Fax Number	4.8665
Office Address (if different from PI)			

3. UAB IRB Protocol Identification

3.a. Protocol Number	X040826012
3.b. Protocol Title	"HyperGEN – Genetic Epidemiology of Left Ventricular Hypertrophy(Hypergen: Genetics of Left Ventricular Hypertrophy)"

3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- ☐ Study has not yet begun — No participants, data, or specimens have been entered.
- ☐ In progress, open to accrual — Number of participants, data, or specimens entered:
- ☐ Enrollment temporarily suspended by sponsor
- ☐ Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)
  - Date closed:
  - Number of participants receiving interventions:
  - Number of participants in long-term follow-up only:
- ☒ Closed to accrual, and only data analysis continues
  - Date closed: N/A no pt recruit at UAB
  - Total number of participants entered: 3483

4. Types of Change

Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- ☒ **Protocol revision (change in the IRB-approved protocol)**
  In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Protocol amendment (addition to the IRB-approved protocol)**
  In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.
- ☒ **Add or remove personnel**
  In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.
  - ☒ **Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication**
    In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).
- ☐ **Change in source of funding; change or add funding**
  In Item 5.c., describe the change or addition in detail, include the applicable OSP proposal number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.

☐ **Add or remove performance sites**
In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

☐ **Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)**
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

☐ **Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)**
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

☐ **Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)**
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

☐ **Revise or amend consent, assent form(s)**
Complete Item 5.d.

☐ **Addendum (new) consent form**
Complete Item 5.d.

☐ **Add or revise recruitment materials**
Complete Item 5.d.

☐ **Other (e.g., investigator brochure)**
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable.
Include a copy of all affected documents, with revisions highlighted as applicable.

▶

5. Description and Rationale

In Item 5.a. and 5.b, check Yes **or** No **and see instructions for** Yes **responses.**
In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.

☐Yes ☒No **5.a. Are any of the participants enrolled as normal, healthy controls?**
If yes, describe in detail in Item 5.c. how this change will affect those participants.

☐Yes ☒No **5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?**
If yes, FAP-designated units complete a FAP submission and send to fap@uab.edu. Identify the FAP-designated unit in Item 5.c.
For more details on the UAB FAP, see www.uab.edu/cto.

5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol. ✓ Added in SIRB.

▶ We would like to add Anh Do to the protocol. She is a graduate student in the Department of Epidemiology. She will work with HyperGEN data in relation to her dissertation "Effects of antihypertensive drugs on left ventricular traits in African Americans". Her dissertation work does not differ from the scope of HyperGEN. Anh will be receiving GWAS results to conduct meta-analysis of de-identified results sets, from the CHARGE consortium, in which HyperGEN is a part. There are no individual level data only results sets from CHARGE cohorts.

5.d. Consent and Recruitment Changes: In the space below,
(a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them;
(b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and
(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" changes**
- **a revised copy for the IRB approval stamp.**

▶

Signature of Principal Investigator M Ryan Irwin Date 3.22.2016

FOR IRB USE ONLY

☐ Received & Noted ☑ Approved Expedited* ☐ To Convened IRB

Maureen Dass 4-1-14

Signature (Chair, Vice-Chair, Designee) Date

DOLA 4/14/16

Change to Expedited Category **Y / N / NA**

*No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111