Genome-Wide Association Study of Cardiac Structure and Systolic Function in African Americans
The Candidate Gene Association Resource (CARE) Study

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Background—Using data from 4 community-based cohorts of African Americans, we tested the association between genomewide markers (single-nucleotide polymorphisms) and cardiac phenotypes in the Candidate-gene Association Resource study.

Methods and Results—Among 6765 African Americans, we related age, sex, height, and weight-adjusted residuals for 9 cardiac phenotypes (assessed by echocardiogram or magnetic resonance imaging) to 2.5 million single-nucleotide polymorphisms genotyped using Genome-wide Affymetrix Human SNP Array 6.0 (Affy6.0) and the remainder imputed. Within the cohort, genomewide association analysis was conducted, followed by meta-analysis across cohorts using inverse variance weights (genome-wide significance threshold=4.0×10^{-8}). Supplementary pathway analysis was performed. We attempted replication in 3 smaller cohorts of African ancestry and tested lookups in 1 consortium of European ancestry (EchoGEN). Across the 9 phenotypes, variants in 4 genetic loci related genome-wide significance: rs4552931 in UBE2V2 (P=1.43×10^{-7}) for left ventricular mass, rs7213314 in WIP1 (P=1.68×10^{-7}) for left ventricular internal diastolic diameter, rs1571099 in PPAPDC1A (P=2.57×10^{-8}) for interventricular septal wall thickness, and rs9530176 in KLF5 (P=4.02×10^{-5}) for ejection fraction. Associated variants were enriched in 3 signaling pathways involved in cardiac remodeling. None of the 4 loci replicated in cohorts of African ancestry was confirmed in lookups in EchoGEN.

Conclusions—In the largest genome-wide association study of cardiac structure and function to date in African Americans, we identified 4 genetic loci related to left ventricular mass, interventricular septal wall thickness, left ventricular internal diastolic diameter, and ejection fraction, which reached genome-wide significance. Replication results suggest that these loci may be unique to individuals of African ancestry. Additional large-scale studies are warranted for these complex phenotypes. (Circ Cardiovasc Genet. 2013;6:37-46.)

Key Words: echocardiography ■ ethnic ■ genome-wide association studies ■ left atrium genetics ■ left ventricular mass genetics

Although several traditional cardiovascular risk factors contribute substantially to interindividual variation in cardiac structure and systolic function, much of the observed variation in cardiac target organ damage is unexplained by established environmental risk factors and may be attributable to genetic factors. Both animal and human studies support a genetic influence on left ventricular (LV) structure and function. In a relatively recent 100K single-nucleotide polymorphism (SNP) genome-wide association (GWA) study in the Framingham Heart Study, investigators confirmed modest-to-strong heritabilities (estimates, 0.30–0.52) for several echocardiographic traits in white participants of European descent. More recently, Vasan et al conducted a GWA study using 2.5 million SNPs in a combined sample of 12,612 individuals of African ancestry. These authors contributed equally as joint first authors.

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European ancestry from 5 community-based cohorts and identified 5 genetic loci associated with variation in phenotypes of cardiac structure. Data on genetic influences on cardiac structure and function in African Americans are quite limited. Analyses from the Hypertension Genetic Epidemiology Network (HyperGEN) and the Genetic Epidemiology Network of Arteriopathy (GENOA) studies suggest a high heritability of LV mass (estimates ranging from 0.55 to 0.88) and genetic influences on LV geometric remodeling.8

Clinical Perspective on p 46

The GWA method to identify novel SNPs contributing to the underlying risk for complex diseases has been successful.13 Data from the Candidate-gene Association REsource (CARe) Study allowed us to perform the first African American GWA study on cardiac phenotypes assessed by either echocardiography or magnetic resonance imaging (MRI).

Methods

CARe Consortium

Details of the CARe consortium are described elsewhere.14 Briefly, the CARe Study consists of 9 population-based cohort studies sponsored by the National Heart, Lung, and Blood Institute. Within CARe, 4 cohorts with African Americans (the Atherosclerosis Risk In Communities [ARIC], the Coronary Artery Risk Development in Young Adults [CARDIA], the Jackson Heart Study [JHS], and the Multi-Ethnic Study of Atherosclerosis [MESA]) had both echocardiography or MRI and DNA data available to investigate GWAs. These 4 cohorts were used for the discovery phase of this investigation. Guidelines on collaboration, phenotype harmonization, covariate selection, and the analysis plan for both within-cohort GWA and prospective meta-analysis of results across studies were adopted by each study. Also, each CARe cohort obtained approval from the respective institutional review boards for consent procedures, examination and surveillance components, data security measures, and DNA collection and its use for genetic research.

Echocardiographic and MRI Methods

Details on the collection of echocardiographic and MRI data by cohort are discussed in online-only Data Supplement Section 1. In 3 of the cohorts, participants underwent routine transthoracic echocardiography at selected examinations (visit 1 for JHS and ARIC and visit 3 for CARDIA). For MESA, participants underwent cardiac MRI at visit 1. For participants undergoing echocardiography, M-mode measurements of LV internal diastolic and systolic diameter, the thickness at end-diastole of the posterior wall, interventricular septal wall thickness (IVST) diameter, the diameter at end-systole of the aortic root (ARD), and the left atrial diameter were obtained using the American Society of Echocardiography guidelines. LV mass (LVM) was calculated by using the American Society of Echocardiography–corrected formula by Devereux et al:15

$$0.8 \times [0.04 \times ((LVDD+IVS+PW)^{3} - (LVDD)^{3})] + 0.6.$$  

LV systolic dysfunction on echocardiogram was defined as the presence of reduced fractional shortening (<0.29, which corresponds to an ejection fraction of 0.50) on M-mode or a depressed ejection fraction (<0.50) on 2-dimensional echocardiography.

For MESA, LVM and LV ejection fraction were determined by cardiac MRI using 1.5-T magnets. Specifically, LVM was determined by taking the difference between the epicardial and endocardial areas for all slices, multiplying the result by the slice thickness and section gap, and multiplying that result by the specific gravity of myocardium.

Genotyping Methods and Imputation

Genotyping and Quality Control

Genotyping of all cohorts was performed at the BROAD Institute of Harvard and MIT using Affymetrix Genome-Wide Human SNP array 6.0 (Affy6.0), which interrogates simultaneously 1.8 million markers for genetic variation (906,600 SNPs and 946,000 copy number variation probes) under the CARe consortium.16 Quality control of genotyped data (SNPs) was performed using the BROAD genetic analysis platform that consists of PLINK17 and Birdseed v1.3318 software. Quality control measures included removal of samples with genotyping success rate <95%, monomorphic SNPs, SNPs that mapped to several loci in the human genome, and SNPs with minor allele frequency <1%. Samples with very low (<4 SDs) heterozygosity, suggesting poor DNA quality, and samples with very high (>4 SDs) heterozygosity, suggesting sample contamination, were also removed. In all cohorts except for JHS, relatedness was identified by computing identical by descent and identical by state scores across the data sets. All pairs that shared ≥5% of their genome were removed, as were samples that did not cluster well when subjected to multidimensional scaling or genome-wide neighbor analysis in PLINK. This was done to eliminate familial correlation. For the family-based subcohort of the JHS, early analytic assessment by CARe investigators found little effect on inflation factor as a result of familial correlation. Other quality control filters included removing SNPs, for which genotype missingness can be predicted by surrounding haplotypes, with mendelian inconsistencies and removing SNPs with significant deviation from Hardy-Weinberg equilibrium. In total, 113,238 SNPs were excluded in ARIC, 69,710 in CARDIA, 40,653 in JHS, and 27,956 in MESA (ie, >99% genotyping success rate).

Genotype Imputation

Genotype imputation performed in CARe has been detailed elsewhere. Briefly, in CARe, imputation was performed using the MACH (http://www.sph.umich.edu/csg/abecasis/MACH/) program with HapMap phase 2 (build 36 release 22) as input. Because the African American population is admixed with the proportion of European ancestry, which is estimated to be 17% to 19%,18,19 an artificial reference panel consisting of equal proportions of the YRI and CEU HapMap phased haplotypes (using only SNPs found in both YRI and CEU panels, ie, ≈2.2M SNPs) was constructed. Hao et al20 suggested that the accuracy of using the mixed panel for African Americans is comparable with the accuracy reported when imputing a population of Nigerians using YRI as a reference panel.

Statistical Methods

Because participants within and between cohorts were unrelated, we used logistic or linear regression (implemented in PLINK genetic software) to investigate the association of SNP alleles with dichotomous or continuous echo trait, respectively, assuming an additive genetic model. Fractional shortening and ejection fraction were the only 2 dichotomous cardiac traits. In these 2 traits, we compared cases with controls while adjusting for age, sex, weight, height, and site (for CARDIA and MESA cohorts only) after excluding participants who had a previous myocardial infarction. For the 7 continuous traits (LVM, thickness at end-diastole of the posterior wall, IVST diameter, LV internal diastolic diameter, LV internal systolic diameter, left atrial diameter, ARD), we used linear regression of log-transformed measures to obtain sex-specific residuals after adjusting for age, weight, and height. The sex-specific residuals were then pooled, and within-cohort linear associations of SNP alleles with each echocardiographic continuous trait were performed. Ten principal components calculated from selected ancestry informative markers were used to account for population stratification common in African Americans because of admixture.

Genomic control correction was applied in each study before the meta-analysis, which ensured that the inflation factor lambda ($\lambda$) is maintained around unity.

Within-cohort GWA results included parameter estimates ($\beta$ regression coefficient and their SEs). Meta-analysis was conducted using METAL software (http://www.sph.umich.edu/csg/abecasis/).
of each SNP, METAL calculated an overall \( \beta \) estimate, \( z \)-statistic, and \( P \) value from the weighted average of individuals’ statistics. No filtering on minor allele frequency was used.

A priori genome-wide statistical significance threshold of \( \leq 4.0 \times 10^{-7} \) was chosen to represent the probability for at least 1 SNP to have a \( P \) value below a stringent threshold. This strategy has been used in GWA studies to reduce false discovery rates.\(^{12,21}\)

**Pathway Analysis**

We assigned the overall association significance of each genetic variant to the cardiac structure equivalent to the most significant \( P \) value among the 9 cardiac traits. We then mapped these genetic variants back to the human genome (NCBI Build 36, 2006) and RefSeq genes. A gene region was defined as between 110 kb upstream and 40 kb downstream of the gene’s most extreme transcript boundaries, which would encompass the majority of its cis-eQTLs (expression quantitative trait loci).\(^{22}\) The lowest \( P \) value of SNPs within the gene region was assigned as the significance score for the gene. Of the 22374 genes evaluated, 1718 reached significance scores \( <1.0 \times 10^{-4} \). These genes were then imported into Ingenuity IPA for pathway analysis (Ingenuity Systems, Redwood, CA). Fisher exact test was used to justify the enrichment significance of each of the canonical pathways.

**Replication Analysis in Cohorts of African and European Ancestry and Reciprocal Lookups of Top Loci in Cohorts of European Ancestry**

Genome-wide significant SNPs discovered in the meta-analysis of the 3 cohorts were subjected to replication analysis in 3 cohorts of African ancestry (GENOA, n=651; HyperGEN, n=1316; Cardiovascular Health Study, n=501) and 1 cohort of European ancestry (Echo Genetics-EchoGEN, n=12612). We adopted a criterion for declaring significance in the replication analysis at significance level \( P < 0.05/number \) of SNPs sent for replication. In addition, we performed a lookup of the top 50 CARe hits in the EchoGEN cohort. Subsequently, we tested the 5 published genome-wide significant SNPs from the EchoGEN cohort analysis in our CARe African American sample.

**Table 1. Study Sample Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis Risk in Communities</th>
<th>Coronary Artery Risk Development in Young Adults Study</th>
<th>Jackson Heart Study</th>
<th>Multi-Ethnic Study of Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Age (mean±SD), y</td>
<td>59±6</td>
<td>59±6</td>
<td>30±4</td>
<td>29±4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163±6</td>
<td>176±7</td>
<td>164±7</td>
<td>177±7</td>
</tr>
<tr>
<td>Weight, lbs</td>
<td>186±41</td>
<td>190±36</td>
<td>167±47</td>
<td>183±40</td>
</tr>
</tbody>
</table>

**Results**

The demographic and clinical characteristics of the 4 populations in the discovery meta-analysis are summarized in Table 1. The age range for most of the participants was comparable, except for CARDIA, which had younger participants (<31 years old) overall. Of the 4 cohorts, only JHS had all 9 echocardiographic phenotypes, and the remaining 3 cohorts measured different subsets of phenotypes. MRI was available in MESA only.

The per-cohort genomic inflation factor (\( \lambda \)) was consistently \(<1.02 \) for all traits studied. The post–meta-analytic \( \lambda \) was also \(<1.02 \), indicating absence of systematic inflation. The meta-analysis quantile-quantile plots of observed against expected \( P \) value distributions are shown in online-only Data Supplement Figure IA–IH.

We identified 4 genome-wide significant loci associated with LV mass, IVST, LV internal diastolic diameter, and LV ejection fraction \(<0.50 \) (Table 2). Genetic effects (\( \beta \)) and SEs, minor and major alleles, minor allele frequency, SNP type, and the nearest genes (within \( \pm 500 \) kb of either site of the SNP) are also shown in Table 2. Figure 1A–1D summarizes the primary findings from meta-analysis and displays the genome-wide \(-\log_{10}P\) values for interrogated SNPs across the 22 autosomal chromosomes separately for the 4 cardiac traits that were significantly associated with the 4 loci. Figure 2A–2D shows the forest plots associated with the top loci. \( \beta \) coefficients (for continuous traits LV mass, IVST, and LV diastolic diameter) and odds ratios (for the dichotomous trait LV ejection fraction \(<0.50 \)) from each cohort analysis and from the meta-analysis are shown. Figure 3A–3D shows the regional plots for the 4 top SNPs. The nearest gene loci to the top SNPs within 500 kb are also shown.

\( ^* \)For the CARDIA study, there were 13 participants with ejection fraction, which was too small to warrant meaningful analysis; at least 22 cases was the minimum for dichotomous trait analyses.

LV indicates left ventricular; and MRI, magnetic resonance imaging.
Online-only Data Supplement Table I lists 7 additional top genetic loci (and the SNP at each locus with the lowest
P value) associated with cardiac traits based on the criterion
5.0 × 10^-7 < P < 9.9 × 10^-7 (arbitrary threshold). In online-only
Data Supplement Figure IIA–IIG, the regional plots of the 7
additional top loci are presented.

Pathway Analysis
We examined the interaction and relationship between the top
GW A study loci. Accumulating evidence suggests that com-
plex diseases and traits usually result from the incremental
effects of many genetic variants.23–25 Pathway analysis pro-
vides a potential route to investigate the collective effects of
multiple genetic variants on biological systems.26–28

A total of 1718 genes were found to be moderately related
to cardiac structure. Ingenuity IPA (Ingenuity Systems,
Redwood, CA) was used to study whether these genes
were significantly enriched in some specific biological
pathways beyond that expected from random distribution.
Our analysis reveals that 3 canonical pathways were most
significantly enriched with cardiac-related genes, including
the sonic hedgehog signaling pathway (6 CARe genes/33
total genes in the pathway [18.2%]; P =1.88 × 10^-5), the
cardiac β-adrenergic signaling pathway (16 CARe genes/151
total genes in the pathway [10.6%]; P =3.22 × 10^-4), and
the oncostatin M signaling pathway (6 CARe genes/35 total
total genes in the pathway [17.1%]; P =3.88 × 10^-4). The results
suggest that the disruption of these signaling pathways might
be the potential mechanisms affecting cardiac structure and
related echocardiographic traits, which are also implicated in
previous studies. A table showing the list of the gene symbols
and names from the CARe data set identified in each of the 3
pathways is shown in online-only Data Supplement Table II.

We applied the same pathway approach to EchoGen data set
and identified 942 cardiac-related genes with
P < 1.0 × 10^-4 (see
Methods section). Only a small proportion of them (97 genes)
were also classified as cardiac-related genes from CARe data
set because of factors such as the sample size and population
stratification. Interestingly, one of the most enriched pathways
from CARe data set, cardiac β-adrenergic signaling path-
way, was also moderately enriched in the EchoGen data set
(P =0.069; online-only Data Supplement Figure III).

Table 2.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Locus</th>
<th>SNP Position (bp)</th>
<th>Minor/Major SNP Type</th>
<th>Nearest Gene</th>
<th>MAF</th>
<th>Effect Size</th>
<th>Meta-Analysis P</th>
<th>Replication Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass</td>
<td>8q11</td>
<td>rs4552930</td>
<td>G/A†</td>
<td>Intergenic</td>
<td>UBE2V2</td>
<td>0.05</td>
<td>-0.036 (0.007)</td>
<td>2.57 × 10^-8</td>
</tr>
<tr>
<td>LV internal diastolic diameter</td>
<td>17q24</td>
<td>rs7213314</td>
<td>T/C†</td>
<td>Intergenic</td>
<td>WIPI1; PRKAR1A; FAM20A; ABCA8</td>
<td>0.17</td>
<td>0.017 (0.003)</td>
<td>0.009 (0.011)</td>
</tr>
<tr>
<td>Interventricular septal wall thickness</td>
<td>10q26</td>
<td>rs1571099</td>
<td>C†/T</td>
<td>Intronic</td>
<td>PPAPDC1A</td>
<td>0.11</td>
<td>-0.003 (0.007)</td>
<td>0.24</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>13q22</td>
<td>rs9530176</td>
<td>A†/T</td>
<td>Intergenic</td>
<td>KLF5; PIBF1</td>
<td>0.07</td>
<td>0.124 (0.240)</td>
<td>4.02 × 10^-7</td>
</tr>
</tbody>
</table>

bp indicates base pairs; CARe, Candidate-gene Association Resource; CHS, Cardiovascular Health Study; GENOA, Genetic Epidemiology Network of Arteriopathy; HyperGEN, Hypertension Genetic Epidemiology Network; LV, left
ventricular; MAF, minor allele frequency; and SNP, single-nucleotide polymorphism.

†Coded allele.
Reciprocal Lookups of Top Loci in Cohorts of European Ancestry

We tested the top 50 CARe SNPs for each trait in the EchoGen consortium (exclusively European ancestry). There was a moderate association between 9 of the CARe SNPs and key phenotypes of cardiac structure in EchoGen. Specifically, rs13241730 (ARD, $P=1.18 \times 10^{-5}$) was associated with systolic dysfunction ($P=0.00925$), rs11187518 (ejection fraction, $P=4.44 \times 10^{-6}$) with LV wall thickness ($P=3.94 \times 10^{-5}$), rs7159121 (fractional shortening [FS], $P=4.67 \times 10^{-6}$) with FS ($P=0.000862$), rs1549850 (internal systolic diameter, $P=1.36 \times 10^{-5}$) with ARD ($P=0.00142$), rs4752424 (interventricular septal wall thickness [IVST], $P=1.95 \times 10^{-5}$) with LVM ($P=0.00088$), rs11758777 (left atrial diameter, $P=9.29 \times 10^{-6}$) with left atrial size ($P=0.00911$), rs9536417 (LVDD, $P=1.19 \times 10^{-5}$) with FS ($P=0.00113$), rs6907666 (LVM, $P=1.45 \times 10^{-5}$) with ARD ($P=0.000566$), and rs33432 (PWT, $P=1.94 \times 10^{-5}$) with LVDD ($P=0.0106$). We tested whether the top 5 hits from EchoGEN replicated in CARe and did not find replication of any of the 5 SNPs.

Discussion

In this largest study assessing the influence of genetic variation on cardiac structure and function in African Americans, we identified 4 genome-wide significant loci associated with LV structure (1 SNP for LV mass, 1 SNP for IVST, and 1 for LV internal diastolic dimension) and 1 significant locus associated with LV systolic dysfunction based on LV ejection fraction <0.50 or fractional shorting <0.29. Findings from the replication analysis of the 4 genome-wide significant loci in European ancestry suggest that these SNPs may represent loci specific to African ancestry. In further analysis, we found that 9 of the top 50 hits were noted to be moderately associated with cardiac structure in a large European ancestry cohort.

All of the genome-wide significant loci and several other top loci identified that were near (but did not reach) the threshold for genome-wide significance were near genes that can be linked to biological pathways implicated in influencing cardiac structure and function. Descriptions of these loci and nearby genes are noted in online-only Data Supplement Sections V and VI.

In a pathway analysis, we noted that top loci were enriched in cardiac genes from 3 signaling pathways (the sonic hedgehog pathway, the cardiac β-adrenergic signaling pathway, and the oncostatin M signaling pathway). Genes in the sonic hedgehog pathway have been identified in the adult heart and probably play a role in normal cardiac homeostasis and function. This pathway is key in the embryonic development of the coronary vasculature. Several genes in the β-adrenergic...
signaling pathway were represented among the top hits. It is established that this pathway is important in the induction and maintenance of cardiac hypertrophy, redistribution of myosin isoforms, and cardiac contractility. Further supporting our finding in the adrenergic pathway is that a similar analysis performed in the EchoGen consortium also revealed genes moderately enriched in this pathway. Finally, the oncostatin M signaling pathway was identified in the supplemental analysis. Oncostatin M is an inflammatory mediator; the signaling pathway involving oncostatin M has been found to induce stromal-derived factor-1 protein secretion in human cardiac cells and play a role in repair and tissue regeneration.

Our results suggest that population stratification may complicate the discovery of genetic variants associated with cardiac structure and function, despite the evidence of shared mechanisms. It is thus necessary to investigate genetic variants specific to African Americans.

Strengths and Limitations
The fact that there was no replication of the top loci in populations of European ancestry suggests that the association of these loci with cardiac structure and function may be unique to African ancestry. One limitation to replication is that the African American community represents an admixed population with smaller linkage equilibrium blocks compared with those of European ancestry. There is significant heterogeneity among individuals within the ethnic group. Therefore, replicating findings of our study population is more challenging compared with those from cohorts of European ancestry, despite the use of ancestral informative markers. Because HyperGEN and GENOA are family studies ascertained on hypertension, and therefore enriched with genes that contribute to elevated blood pressure (assuming blood pressure is genetically determined, which remains the prevailing thought), it is not completely unexpected that our
results did not replicate in these cohorts. These families might have a distinct hypertension-induced phenotype. Another limitation of the current study is that differences in study design and data collection between cohorts may lower our statistical power to detect modest genetic effects in GWA. Using GWA, we are focused on detecting multiple variants with small effects that influence complex diseases; our statistical power in this study to detect rare variants associated with phenotypes of cardiac structure and function is limited. In addition, we acknowledge that we are only able to identify an association between genetic loci and phenotypes of interest; we are not able to establish a cause–effect relationship or identify a mechanism leading to the association. Finally, the cohorts studied were all of African ancestry descent, limiting the generalizability of our findings to individuals of non-African ancestry.

These limitations are balanced against our ability to conduct the largest GWA on African Americans with participants from community-based cohorts (each using standardized methods of M-mode echocardiography or MRI with quality control procedures in individual imaging laboratories) and with harmonization of imputation strategies and analytic methods into a prospective meta-analysis.

Conclusions

Our prospective meta-analysis of cardiac structure and function from more than 6765 participants in 4 community-based cohorts identified 4 loci: rs4552931 in UBE2V2 on chromosome 8 for LVM, rs7213314 in WIP1 on chromosome 17 for LV internal diameter in diastole, rs1571099 in PPAPDC1A on chromosome 10 for IVST, and rs9530176 in KLF5 on chromosome 13 for ejection fraction.

In a pathway analysis, top loci in the meta-analysis were significantly enriched with genes from the sonic hedgehog signaling pathway, the cardiac β-adrenergic signaling pathway, and the oncostatin M signaling pathway.

After testing the top 50 CARe SNPs for each trait in the EchoGen consortium, we observed moderate association between 9 of these SNPs and cardiac structure in EchoGen.

Implications

Identification of genetic variations that contribute to cardiac structure and function through GWA analysis may help us better understand the role that genes play in the development and progression of cardiac end-organ damage in African Americans. This is particularly important given the current racial disparity in LV hypertrophy and dysfunction (both of which are predictors of cardiovascular morbidity and mortality). Findings in this study warrant further investigation, including replication analysis in much larger samples and identification of potential biological mechanisms explaining the association of these variants with phenotypic findings on cardiac imaging.

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Appendix

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References

CLINICAL PERSPECTIVE

We investigated the association between genome-wide markers with cardiac structure and systolic function using data from 4 community-based cohorts of African Americans in the Candidate-gene Association Resource study. Findings from this analysis may help us better understand the role that genes play in the development and progression of cardiac end-organ damage in African Americans. This is particularly important given the current racial disparity in left ventricular hypertrophy and dysfunction (both of which are predictors of cardiovascular morbidity and mortality). Findings in this study warrant further investigation, including replication analysis in much larger samples and identification of potential biological mechanisms explaining the association of these variants with phenotypic findings on cardiac imaging.
Genome-Wide Association Study of Cardiac Structure and Systolic Function in African Americans: The Candidate Gene Association Resource (CARe) Study


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Supplementary Material

The supplementary materials have the following sections in this order:

I. Discovery Populations and Phenotype Characterization and Harmonization

II. Replication Cohorts

III. Acknowledgments

IV. Supplemental Tables and Figures

V. Description of Nearest Genes by Top Identified SNPs

VI. Findings Related to Left Atrial Diameter

VII. References
Supplementary Material

I. Discovery Populations and Phenotype Characterization and Harmonization

Discovery Populations

The Atherosclerosis Risk in Communities (ARIC) Study was initiated in 1987 to investigate the causes and natural history of CVD in four United States communities. At the Jackson, Mississippi, field center, only African-Americans were recruited. During the third ARIC examination (1993 to 1995), a full-scale echocardiographic evaluation was performed. Participants were eligible for the present investigation if their genotyping (as part of CARe) was complete and if they had available echocardiographic phenotypic information (N=3279).

The Coronary Artery Risk Development in Young Adults (CARDIA) study began in 1985 to investigate the development and progression of coronary disease in young adults, with an initial enrollment of 5115 European Americans and African American men and women between ages 18 and 30 years (52% African American and 55% women). The study is multicenter with recruitment in Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Baseline measurements were repeated, and additional measurements performed, at years 2, 5, 7, 10, 15, and 20.

The community-based Jackson Heart Study (JHS) was initiated in 2000 to investigate prospectively the epidemiology and determinants of cardiovascular disease in African Americans. JHS recruited 5,302 participants after completion of data adjustment, representing more than 5% of African Americans 35-84 years old living in the Jackson, MS tri-county area. Of this number, approximately 30% were prior Jackson participants in the Atherosclerosis Risk in Communities Study. Of the remaining, 23% were recruited by random selection from a commercial listing that represents the overall tri-county population and an additional 23% volunteer sample, in which recruitment was distributed among defined demographic cells in proportions designed to mirror those in the overall population. Those who were overlapping ARIC participants and those with previous myocardial infarction were excluded from the JHS
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sample in the present GWAS. The analysis included those with genotyping completed and with echocardiography data available from visit 1.

The Multi-Ethnic Study of Atherosclerosis Study (MESA) Study is a multicenter prospective cohort study initiated to evaluate the development of subclinical cardiovascular disease. A total of 6814 women and men between the age of 45 and 84 years were recruited for the first examination between 2000 and 2002. Participants were recruited in six US cities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan, NY; and St. Paul, MN). Approximately 38% were European Americans and 28% were African Americans. Those with a history of CVD (defined as physician-diagnosed myocardial infarction, angina, heart failure, stroke, transient ischemic attack or history of invasive procedure for CVD) were excluded from participation.5

Phenotypic characterization & Harmonization

High-quality phenotyping is critical for GWAS. In this context, echocardiography and MRI are the most commonly used noninvasive cardiac imaging tools for cardiac structure and function. For echocardiography sources of variability include inter- and intra-reader and sonographer variability, beat-to-beat and day-to-day within subject variability, temporal drift within laboratories, and biases due to missing data. Each of the 5 participating cohorts in the CARe Study has implemented several quality control measures for echocardiography and MRI. Furthermore, the analytical strategy does not involve pooling of individual participant data, but rather within-cohort analyses, and a prospective meta-analysis across the cohorts.

Atherosclerosis Risk in Communities (ARIC) Study

Details of the echocardiographic examination and interpretation are described elsewhere6. Briefly, image acquisition was performed with the Acuson XP128/10c echo machine. Steerable 2-dimensional directed M-mode was used, and the Freeland Systems CineView digital imaging computer provided control of the resolution and timing of image
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acquisition and storage. Serial frames showing cardiac motion throughout the cardiac cycle were available, and full-screen acquisition of M-mode or spectral Doppler data was used for quantitative measurements. The reader was able to select the best available single frame from several full screens that were stored by 2 trained nurses. Quantitative measurements were obtained from the parasternal short-axis view with the M-mode cursor positioned through the center of the ventricle. A cardiologist (T.N.S.) performed all echocardiographic readings. Variability within and between sonographers and within the reader was assessed during the examination period with the use of a 2% random sample. The intrasonographer and intersonographer correlation for LV mass between the first and second scan was 0.94 and 0.82, respectively. The intrareader intraclass correlation coefficient for LV mass was 0.98.

Coronary Artery Risk Development in Young Adults (CARDIA) Study

Transthoracic two-dimensional echocardiography was obtained by trained echocardiographers in all participants at the fourth (2002-2004) examination, using an Acuson Cypress with a 3V2c transducer. A standardized protocol was used, including two-dimensional scanning in the parasternal long axis view, parasternal short axis view, apical view and subcostal view. In addition, M-Mode scanning in the parasternal long axis view was performed (to obtain two-dimension guided M-mode measurements of the LV and aortic root), as well as pulsed wave Doppler scanning in the apical four chamber view. All studies were recorded onto videotape and assessed at the reading center by trained echocardiographers. Assessment of intra-reader and inter-reader agreement was performed during the study.

Jackson Heart Study (JHS)

In the Jackson Heart Study, acquisition was performed with Sono 4500 echo machine. Serial frames showing cardiac motion throughout the cardiac cycle were available, and full-screen acquisition of M-mode or spectral Doppler data was used for quantitative measurements. The reader was able to select the best available single frame from several full screens that were stored by 2 trained sonographers. Quantitative measurements were obtained from the
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parasternal short-axis view with the M-mode cursor positioned through the center of the ventricle. A cardiologist (Tandaw Samdarshi) performed all echocardiographic readings.

**Multi-Ethnic Study of Atherosclerosis (MESA)**

LV mass and volume determination was performed by cardiac MRI using 1.5-T magnets. The endocardial and epicardial myocardial borders were contoured using a semi-automated method (MASS 4.2, Medis, Leiden, the Netherlands). The difference between the epicardial and endocardial areas for all slices was multiplied by the slice thickness and section gap, and then multiplied by the specific gravity of myocardium (1.04 g/ml) to determine the ventricular mass. Papillary muscle mass was included in the LV cavity and excluded from the LV mass. The cardiac MRI protocol, image analysis and inter- and intra-reader reproducibility in MESA have been previously described.  

Il. Replication Cohorts

**Cardiovascular Health Study (CHS)**

All attendees underwent routine transthoracic two-dimensionally-guided M-mode echocardiography at the second (1979-1982), fourth (1987-1990), fifth (1991-1995) and sixth (1996-1998) Offspring cohort examinations. Echocardiographic equipment for image acquisition varied across these examinations: at examination cycle 2, a Hoffrel 201 ultrasound receiver (and Aerotech transducer) was used; at examinations 4 and 5, a Hewlett Packard (model 77020AC) ultrasound machine was used; at examination 6 images were acquired using a Sonos 1000 Hewlett-Packard machine. Two-dimension guided M-mode measurements of the LV and aortic root were obtained. The reproducibility of Echo measurements was systematically assessed at the sixth examination and was very good.

The **Hypertension Genetic Epidemiology Network (HyperGEN)** is a family-based study focused on identifying genetic determinants of hypertension. Investigators recruited hypertensive sibships, along with their normotensive offspring and also had a random sample of approximately 800 subjects. HyperGEN has collected data on 2,471 Caucasian-American
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subjects and 2,300 African-American subjects, from 5 centers in Alabama, Massachusetts, Minnesota, North Carolina, and Utah. For these analyses, only African American subjects were used.

The Genetic Epidemiology Network of Arteriopathy (GENOA) is 1 of 4 networks in the Family Blood Pressure Program. In FBPP, investigators recruited hypertensive African American and non-Hispanic European-ancestry sibships for linkage and family-based association studies to investigate genetic contributions to blood pressure and the cardiac and renal complication of hypertension in multiple racial groups. Recruitment for GENOA (Exam 1, 1995-2000 and Exam 2, 2000-2005) was population-based in 2 geographic locations: Jackson, Mississippi and Rochester, Minnesota. African Americans in this study were located solely at the GENOA Jackson field center. Hypertensive probands were ascertained from the Jackson cohort of the Atherosclerosis Risk in Communities (ARIC) Study if they were in a sibship with ≥ 2 individuals with essential hypertension (systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg on the second and third clinic visit.) diagnosed prior to age 60 and were willing to be recruited. Those index sibpairs with possible secondary hypertension (including sibpairs with previously diagnosed kidney disease (defined by serum creatinine level >2 mg/dL)) were excluded.

The EchoGEn Consortium is made up participants of European ancestry from 7 cohorts. Three of the cohorts were part of the CHARGE consortium (Cardiovascular Health Study, the Rotterdam Study and the Framingham Heart Study). The other 2 cohorts were 1) the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease study, which was continued since 1996 in the Southern German region of Augsburg centered on studying cardiovascular risk factors in a population in Bavaria, Germany and 2) The Gutenberg Heart Study, Mainz, Germany, that was initiated in 2006 to determine a sex-specific cardiovascular risk score. All studies had both genome-wide variation data and echocardiographic measurements to perform GWAS. All participating studies approved guidelines for collaboration, and arrived at a consensus not only on phenotype definitions including
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harmonization, covariate selection and analytic plans for within-study analyses, but also on a prospective meta-analysis of results.
Supplementary Material

III. Acknowledgments

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Atherosclerotic Risk in Communities (ARIC): University of North Carolina at Chapel Hill (N01-HC-55015), Baylor Medical College (N01-HC-55016), University of Mississippi Medical Center (N01-HC-55021), University of Minnesota (N01-HC-55019), Johns Hopkins University (N01-HC-55020), University of Texas, Houston (N01-HC-55017), University of North Carolina, Forsyth County (N01-HC-55018); Cardiovascular Health Study (CHS): University of Washington (N01-HC-85079), Wake Forest University (N01-HC-85080), Johns Hopkins University (N01-HC-85081), University of Pittsburgh (N01-HC-85082), University of California, Davis (N01-HC-85083), University of California, Irvine (N01-HC-85084), New England Medical Center (N01-HC-85085), University of Vermont (N01-HC-85086), Georgetown University (N01-HC-35129), Johns Hopkins University (N01 HC-15103), University of Wisconsin (N01-HC-75150), Geisinger Clinic (N01-HC-45133), University of Washington (N01 HC-55222, U01 HL080295); Cleveland Family Study (CFS): Case Western Reserve University (RO1 HL46380-01-16); Cooperative Study of Sickle Cell Disease (CSSCD): University of Illinois (N01-HB-72982, N01-HB-97062), Howard University (N01-HB-72991, N01-HB-97061), University of Miami (N01-HB-72992, N01-HB-97064), Duke University (N01-HB-72993), George Washington University (N01-HB-72994), University of Tennessee (N01-HB-72995, N01-HB-97070), Yale University (N01-HB-72996, N01-HB-97072), Children’s Hospital-Philadelphia (N01-HB-72997, N01-HB-97056), University of Chicago (N01-HB-72998, N01-HB-97053), Medical College of Georgia (N01-HB-73000, N01-HB-97060), Washington University (N01-HB-73001, N01-HB-97071), Jewish Hospital and Medical Center of Brooklyn (N01-HB-73002), Trustees of Health and Hospitals of the City of Boston, Inc., (N01-HB-73003), Children’s Hospital-Oakland (N01-HB-73004, N01-HB-97054), University of Mississippi (N01-HB-73005), St. Luke’s Hospital-New York (N01-HB-73006), Alta Bates-Herrick Hospital (N01-HB-97051),
Columbia University (N01-HB-97058), St. Jude’s Children’s Research Hospital (N01-HB-97066), Research Foundation, State University of New York-Albany (N01-HB-97068, N01-HB-97069), New England Research Institute (N01-HB-97073), Interfaith Medical Center-Brooklyn (N01-HB-97085); **Coronary Artery Risk in Young Adults (CARDIA)**: University of Alabama at Birmingham (N01-HC-48047), University of Minnesota (N01-HC-48048), Northwestern University (N01-HC-48049), Kaiser Foundation Research Institute (N01-HC-48050), University of Alabama at Birmingham (N01-HC-95095), Tufts-New England Medical Center (N01-HC-45204), Wake Forest University (N01-HC-45205), Harbor-UCLA Research and Education Institute (N01-HC-05187), University of California, Irvine (N01-HC-45134, N01-HC-95100); **Framingham Heart Study (FHS)**: Boston University (N01-HC-25195); **Jackson Heart Study (JHS)**: Jackson State University (N01-HC-95170), University of Mississippi (N01-HC-95171), Tougaloo College (N01-HC-95172); **Multi-Ethnic Study of Atherosclerosis (MESA)**: University of Washington (N01-HC-95159), Regents of the University of California (N01-HC-95160), Columbia University (N01-HC-95161), Johns Hopkins University (N01-HC-95162), University of Minnesota (N01-HC-95163), Northwestern University (N01-HC-95164), Wake Forest University (N01-HC-95165), University of Vermont (N01-HC-95166), New England Medical Center (N01-HC-95167), Johns Hopkins University (N01-HC-95168), Harbor-UCLA Research and Education Institute (N01-HC-95169); **Sleep Heart Health Study (SHHS)**: Johns Hopkins University (U01 HL064360), Case Western University (U01 HL063463), University of California, Davis (U01 HL053916), University of Arizona (U01 HL053938), University of Minnesota (relocating in 2006 to University Arizona) (U01 HL053934), University of Pittsburgh (U01 HL077813), Boston University (U01 HL053941), MedStar Research Institute (U01 HL063429), Johns Hopkins University (U01 HL053937).

**Replication Cohorts**

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Lung, Blood Institute, and MD002249 from National Institute on Minority Health and Health Disparities.” Kristin Meyers received additional funding through the National Center for Advancing Translation Sciences (NCATS) grant 9U54TR000021.

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IV. Supplemental Tables and Figures

Figure 1 (Panels A - H): Metanalysis Q-Q plots of Observed against Expected p Value

A. Left Ventricular Mass
B. Interventricular Septal Wall Thickness
C. Posterior Wall Thickness
D. Left Ventricular Internal Diastolic Diameter
E. Left Atrial Diameter
F. Aortic Root Diameter
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G. Left Ventricular Ejection Fraction
H. Fractional Shortening
Figure 2 (Panels A - G): Regional Plots of Seven Additional Top Genetic Loci with SNPs Associated with Cardiac Traits $5.0 \times 10^{-7} < P < 9.9 \times 10^{-7}$

A. SNP and Locus identified for Left Ventricular Internal Diameter

rs2700294 (YRI)
B. SNP and Locus identified for Left Atrial Diameter
C. SNP and Locus identified for Left Atrial Diameter
Supplementary Material

D. SNP and Locus identified for Interventricular Septal Wall Thickness
Supplementary Material

E. SNP and Locus identified for Interventricular Septal Wall Thickness

rs562490 (YRI)

Observed [logP]

Recombination rate (cM/Mb)

Chromosome 5 position (hg18) (kb)

rs562490

P=8.939e-07
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F. SNP and Locus identified for Ejection Fraction

![Graph showing SNP and Locus identified for Ejection Fraction]
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G. SNP and Locus identified for Ejection Fraction

![Graph showing SNP and locus identified for Ejection Fraction](image-url)
Figure 3. The most significantly enriched canonical pathway identified by the Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com). A data set containing 1718 cardiac related genes was uploaded and overlaid onto the molecular networks developed from information contained in the Ingenuity Knowledge Base. Red nodes are cardiac related genes as described in the text.
### Table I. Remaining Ten Genetic Loci with SNPs associated with cardiac traits $5.0 \times 10^{-7} < P < 9.9 \times 10^{-07}$

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP</th>
<th>Locus</th>
<th>SNP type</th>
<th>Nearest gene</th>
<th>MAF</th>
<th>Effect size (standard error)</th>
<th>Meta-analysis p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV diastolic diameter</td>
<td>rs2700294</td>
<td>7p15</td>
<td>intergenic</td>
<td>SP4; DNAH11</td>
<td>0.342</td>
<td>-0.0116 (0.0024)</td>
<td>8.02x10^{-7}</td>
</tr>
<tr>
<td>Left atrial diameter</td>
<td>rs13016354</td>
<td>2q32</td>
<td>intronic</td>
<td>DNAH7; STK17B; HECW2</td>
<td>0.027</td>
<td>-0.0508 (0.0103)</td>
<td>7.92x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>rs11648047</td>
<td>16p13</td>
<td>intronic</td>
<td>A2BP1</td>
<td>0.355</td>
<td>0.0164 (0.0033)</td>
<td>9.85x10^{-7}</td>
</tr>
<tr>
<td>Interventricular septal wall thickness</td>
<td>rs11848255</td>
<td>14q24</td>
<td>intronic</td>
<td>FOS; JDP2; BATF; FLVCR2; C14orf1; TTLL5</td>
<td>0.166</td>
<td>0.0325 (0.0066)</td>
<td>6.81x10^{-7}</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>rs16991189</td>
<td>20p12</td>
<td>intergenic</td>
<td>RP5-022P6.2; C20orf196; CHGB</td>
<td>0.944</td>
<td>-1.40 (0.28)</td>
<td>5.95x10^{-7}</td>
</tr>
<tr>
<td></td>
<td>rs2404490</td>
<td>20p11</td>
<td>intergenic</td>
<td>FOXA2</td>
<td>0.038</td>
<td>1.32 (0.27)</td>
<td>9.28x10^{-7}</td>
</tr>
</tbody>
</table>

### Table II. CARe Genes Identified Among All Cardiac-Related Genes In Pathway Analysis
### Supplementary Material

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sonic Hedgehoge Pathway</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADRBK1</td>
<td>Adrenergic, beta, receptor kinase 1</td>
<td></td>
</tr>
<tr>
<td>DYRK1A</td>
<td>Dual specificity tyrosine-phosphorylation-regulated kinase 1A</td>
<td></td>
</tr>
<tr>
<td>GLI3</td>
<td>GLI family zinc finger 3</td>
<td></td>
</tr>
<tr>
<td>GSK3B</td>
<td>Glycogen synthase kinase 3 beta</td>
<td></td>
</tr>
<tr>
<td>HHIP</td>
<td>Hedgehog-interacting protein</td>
<td></td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>cAMP-dependent protein kinase type I-alpha regulatory subunit</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiac β-adrenergic Pathway</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADRB1</td>
<td>Beta-1 adrenergic receptor</td>
<td></td>
</tr>
<tr>
<td>ADRBK1</td>
<td>Adrenergic, beta, receptor kinase 1</td>
<td></td>
</tr>
<tr>
<td>AKAP3</td>
<td>A-kinase anchor protein 3</td>
<td></td>
</tr>
<tr>
<td>AKAP12</td>
<td>A-kinase anchor protein 12</td>
<td></td>
</tr>
<tr>
<td>CACNA1C</td>
<td>Calcium channel, voltage-dependent, L type, alpha 1C subunit</td>
<td></td>
</tr>
<tr>
<td>PDE11A</td>
<td>Dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11A</td>
<td></td>
</tr>
<tr>
<td>PDE3A</td>
<td>cGMP-inhibited phosphodiesterase 3A</td>
<td></td>
</tr>
<tr>
<td>PDE4D</td>
<td>cAMP-specific 3',5'-cyclic phosphodiesterase 4D</td>
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</tr>
<tr>
<td>PDE6C</td>
<td>Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha</td>
<td></td>
</tr>
<tr>
<td>PPP1CA</td>
<td>Serine/threonine-protein phosphatase PP1-alpha catalytic subunit</td>
<td></td>
</tr>
<tr>
<td>PPP1R12A</td>
<td>Protein phosphatase 1 regulatory subunit 12A</td>
<td></td>
</tr>
<tr>
<td>PPP2CB</td>
<td>Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform</td>
<td></td>
</tr>
<tr>
<td>PPP2R2B</td>
<td>Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B beta isoform</td>
<td></td>
</tr>
<tr>
<td>PPP2R5D</td>
<td>Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform</td>
<td></td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>cAMP-dependent protein kinase type I-alpha regulatory subunit</td>
<td></td>
</tr>
<tr>
<td><strong>Oncostatin M Signaling Pathway</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP1</td>
<td>Matrix metalloproteinase-1</td>
<td></td>
</tr>
<tr>
<td>MMP3</td>
<td>Matrix metalloproteinase-3</td>
<td></td>
</tr>
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<td>MMP13</td>
<td>Matrix metalloproteinase-13</td>
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</tr>
<tr>
<td>MT2A</td>
<td>Metallothionein 2A</td>
<td></td>
</tr>
<tr>
<td>RRAS</td>
<td>Ras-related protein R-Ras</td>
<td></td>
</tr>
<tr>
<td>SOS1</td>
<td>Son of sevenless homolog 1</td>
<td></td>
</tr>
</tbody>
</table>

CARe, Candidate-Gene Association Resource Study
V. Description of Nearest Genes of Top SNPs Identified for LV Structure and Function

For LV Mass, a SNP on chromosome 8, rs4552931 reached genome-wide significance (p=1.43E-07) in AA in CARe. The nearest gene at this locus is UBE2V2 (ubiquitin-conjugating enzyme E2 variant 2). Ubiquitin-conjugating enzyme E2 variant proteins constitute a distinct subfamily within the E2 protein family and is involved in protein degradation. The robust increase in protein synthesis during cardiac myocyte hypertrophy is accompanied by moderate increases in protein degradation, resulting in higher protein turnover and net accumulation of proteins. The ubiquitin-proteasome pathway plays the major role in proteolytic degradation of misfolded or damaged proteins, turnover of short-lived proteins, and also of sarcomeric proteins\(^\text{10}\). The ubiquitin-proteasome pathway appears to be a target for upregulation by fibronectin. It has been suggested by some that inhibition of this pathway may modify the hypertrophic phenotype.\(^\text{10}\)

Two other genes located at this loci are Fos (FBJ murine osteosarcoma viral oncogene homolog) and JDP2 (Jun dimerization protein 2) gene.\(^\text{11-13}\). The JDP2 gene has been shown to interact with activating transcription factor 2 (AP-2)\(^\text{14}\) and appear to play an important role in cardiac development and function. Overexpression of JDP2 has been suggested to inhibit hypertrophy and apoptosis in ventricular cardiomyocytes. The activator protein 1 (AP-1) transcriptional complex, contains both Jun and Fos proteins, and is involved in regulating many cellular processes such as proliferation and differentiation. Cardiomyocytes in rats that are treated with endothelin 1 (ET) and phenylephrine (PE) induce myocardial cell hypertrophy by enhancing AP-1 DNA binding activities. Adenovirus carrying dominant negative mutation of c-Jun (DNJun) prevent the transcriptional activation of the AP-1 by ET and PE thereby inhibits cardiomyocyte hypertrophy through inhibition of AP-1 transcriptional activity.\(^\text{15}\) So, (ET)-1, that is partially synthesized and secreted by cardiomyocytes appears to induce hypertrophy of cardiomyocytes to some extent by the upregulation of c-Fos and c-Jun.\(^\text{16}\)
BATF (Basic leucine zipper transcription factor, ATF-like protein) encodes for a protein that is a nuclear basic leucine zipper (bZIP) protein that belongs to the AP-1/ATF superfamily of transcription factors. Mice without BATF (BATF knockout mice) lack a type of inflammatory immune cell (Th17) and are resistant to conditions that normally induces an autoimmune condition similar to multiple sclerosis.\textsuperscript{17-20}

Finally, PTPRK (receptor-type tyrosine-protein phosphatase kappa) gene is located at this locus and encodes for a protein that is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. We identified a SNP that reached genome-wide significance for LVIDD on chromosome 17 (p=1.68E-07). Two genes of interest are located at this locus. PRKAR1A ((protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)) is a signaling molecule important for a variety of cellular functions. cAMP exerts its effects by activating the cAMP-dependent protein kinase, which transduces the signal through phosphorylation of different target proteins. The PKA system has long been known to play a key role in cardiac function and has been studied in mice by the use of cardiac-targeted transgenes such as β-adrenergic receptors or the PKA-C subunit. However, a role in cardiac development has not previously been appreciated.\textsuperscript{21} Recently, it has been shown that Prkar1a-CKO mutant embryos develop thinned and disordered myocardium. The depletion of Prkar1a causes excess PKA activity, with resultant downregulation of the transcriptional activity of cardiac transcription factors and their downstream targets, including key cardiac structural proteins and proteins involved in calcium handling. PKA is a key mediator of the deleterious effects of chronic βAR signaling and that exacerbated PKA activity selectively results in dilated cardiomyopathy suggesting that chronic phosphorylation of PKA substrates including RyR2 is associated with dilation and failure.\textsuperscript{22}
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For interventricular septal wall thickness in diastole, a SNP on chromosome 10 (rs1571099) reached genome-wide significance (p= 2.57x10^{-87}). Several genes at this locus can be linked to cardiomyocyte hypertrophy. One gene encodes for the enzyme PPAPDC1A (phosphatidic acid phosphatase type 2 domain containing 1A). This protein helps to form a novel type of Mg2+-independent and NEM-sensitive mammalian phosphatidate phosphatase (PAP) with PPAD1B. PPAPDC1A is preferentially expressed in endothelial cells and studies of PPAPDC1A and PAP activity suggest that they may play a role in angiogenesis. It has been shown that overexpression of PPAPDC3 (that is also in the same superfamily of phosphatases) in myoblasts repress myogenesis while knockdown by RNA interference promoted differentiation indicating its part in the regulatory mechanism for myogenesis.

Another gene near the loci for LVIDD is SP4 (Sp4 transcription factor). Sp family transcription factor, HF1b/Sp4 is misregulated in hearts of Lmna<sup>N195K/N195K</sup> mice. The nuclear lamina is a ~10 nm thick proteinaceous layer underlying the inner nuclear membrane. The A-type laminins, nuclear intermediate filament proteins encoded by the LMNA gene, are basic components of the nuclear lamina. Mutations within the LMNA gene cause a dilated cardiomyopathy by disrupting the internal organization of the cardiomyocyte and/or altering the expression of transcription factors essential to normal cardiac development, aging or function. Many forms of hypertrophic and dilated cardiomyopathies are caused by mutations affecting sarcomeric proteins and the cytoskeleton, such as myosin heavy chain, actin, dystrophin and desmin. LMNA mutations identify another subcellular component important for cardiac development and maintenance.

We found a SNP reaching genome-wide significance on chromosome 13 (rs9530176, p=4.02E-07) for LV systolic dysfunction. Chromogranin B (CHGB) is a gene of interest located at this loci. CHGB is the most abundant core protein in human catecholamine secretory vesicles, playing an important role in their biogenesis. Variation at the CHGB locus may contribute to phenotypic variation in CHGB and catecholamine secretion, autonomic stability of
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circulation, or blood pressure (BP) in the population. Common genetic variation at the CHGB locus, especially in the proximal promoter, influences CHGB expression and later catecholamine secretion and the early heritable responses to environmental stress, eventuating in changes in resting/basal BP in the population. Both the early (gene expression) and late (population BP) consequences of CHGB variation are sex dependent. Neuroendocrine activation also occurs in patients with congestive heart failure, and the plasma chromogranin A level rises as a result of this activation. Moreover, the chromogranin A level is directly correlated with the severity of the disease.

Another gene forkhead box A2 (FOXA2) is located at the locus on chromosome 13 associated with EF. FOXA2 encodes a member of the forkhead class of DNA-binding proteins. This gene has been linked to sporadic cases of maturity-onset diabetes of the young. FOXA2 controls multiple genes implicated in metabolism-secretion coupling of glucose-induced insulin release. Hyperinsulinemia may result in an increase in mitogen-activated protein kinase activity and influence myocyte hypertrophy that over time may affect ejection fraction.
VI. Findings Related to Left Atrial Diameter

One of the top SNPs for LA diameter (rs11648047) in CARe was found to be significant in the HyperGen study. The closest gene near this SNP is ataxin-2 binding protein gene (\textit{A2BP}). This gene is a highly conserved among RNA binding proteins suggesting that it has an important role in function and development. A polyglutamine repeat in \textit{A2BP} has been implicated in spinocerebellar ataxia. Significant for our finding, though the protein has been identified by immunocytochemistry in the CNS and connective tissue in mouse embryo and been studied in relation to its impact on nerve conduction, it has as well been identified in the mouse heart at all stages. Its effect in the heart is less studied.
Supplementary Material

References


Supplementary Material

