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

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Abstract

Background—Using data from four community-based cohorts of African Americans (AA), we tested the association between genome-wide markers (SNPs) and cardiac phenotypes in the Candidate-gene Association REsource (CARE) study.

Methods and Results—Among 6,765 AA, we related age, sex, height and weight-adjusted residuals for nine cardiac phenotypes (assessed by echocardiogram or MRI) to 2.5 million SNPs genotyped using Genome-Wide Affymetrix Human SNP Array 6.0 (Affy6.0) and the remainder imputed. Within cohort genome-wide association analysis was conducted followed by meta-analysis across cohorts using inverse variance weights (genome-wide significance threshold= 4.0×10^{-07}). Supplementary pathway analysis was performed. We attempted replication in 3 smaller cohorts of African ancestry and tested look-ups in one consortium of European ancestry (EchoGEN). Across the 9 phenotypes, variants in 4 genetic loci reached genome-wide significance: rs4552931 in *UBE2V2* ($p=1.43 \times 10^{-07}$) for left ventricular mass (LVM); rs7213314 in *WIP1I* ($p=1.68 \times 10^{-07}$) for LV internal diastolic diameter (LVIDD); rs1571099 in *PPAPDC1A* ($p=2.57 \times 10^{-08}$) for interventricular septal wall thickness (IVST); and rs9530176 in *KLF5* ($p=4.02 \times 10^{-07}$) for ejection fraction (EF). Associated variants were enriched in three signaling pathways involved in cardiac remodeling. None of the 4 loci replicated in cohorts of African ancestry were confirmed in look-ups in EchoGEN.

Conclusions—In the largest GWAS of cardiac structure and function to date in AA, we identified 4 genetic loci related to LVM, IVST, LVIDD and EF that reached genome-wide

significance. Replication results suggest that these loci may represent unique to individuals of African ancestry. Additional large-scale studies are warranted for these complex phenotypes.

Keywords

echocardiography; ethnic; genome-wide association studies; Left atrium genetics; left ventricular mass genetics

Introduction

Although a number of 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.¹ Both animal^{2–4} and human^{5–10} studies support a genetic influence on left ventricular (LV) structure and function. In a relatively recent 100K SNP genome-wide association study (GWAS) 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.¹¹ More recently, Vasan et al. conducted a GWAS using 2.5 million single nucleotide polymorphisms (SNPs) in a combined sample of 12,612 individuals of European ancestry from 5 community-based cohorts and identified 5 genetic loci associated with variation in phenotypes of cardiac structure.¹² 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 0.55–0.88), and genetic influences on LV geometric remodeling.⁸

The genome-wide association method to identify novel SNPs contributing to the underlying risk for complex diseases has been successful.¹³ African American data from the Candidate-gene Association REsource (CARE) Study allowed us to perform the first AA GWAS 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 data, and DNA data available to investigate genome-wide associations (GWA). 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 Appendix Section I. In 3 of the cohorts, participants underwent routine transthoracic

echocardiography at selected examinations (visit1 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 (LVDD and LVSD), the thickness at end-diastole of the posterior wall (PWTD) and interventricular septum (IVSTD), and the diameter at end-systole of the aortic root (ARD) and the LA diameter (LAD) were obtained using the American Society of Echocardiography (ASE) guidelines. LVM was calculated by using the ASE corrected formula by Devereux:

$$0.8 [1.04\{(LVDD+IVS+PW)^3-(LVDD)^3\}]+0.6.$$

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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 two-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 (GAP) that consists of PLINK¹⁷ and Birdseed v1.33¹⁶ 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 (MAF) <1%. Samples with very low (< 4 standard deviation units, SD) heterozygosity suggesting poor DNA quality and samples with very high (> 4 SD) heterozygosity suggesting sample contamination were also removed. In all cohorts except for JHS, relatedness was identified by computing identical by descent (IBD) and identical by state (IBS) scores across the datasets. All pairs that shared 5% of their genome were removed, as were samples that did not cluster well when subjected to multidimensional scaling (MDS) or genome-wide “neighbor” analysis in PLINK. This was done to eliminate familial correlation. For the family-based sub cohort of the JHS, early analytical assessment by CARE investigators found little effect on inflation factor due to familial correlation. Other quality control filters included removing SNPs: for which genotype missingness can be predicted by surrounding haplotypes; with Mendelian inconsistencies and those 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 i.e. >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. Since the African-

American population is admixed with the proportion of European ancestry estimated to be ~17–19%,^{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, i.e. ~2.2M SNPs) was constructed. Hao et al. suggested that the accuracy of using the mixed panel for African-Americans is comparable to the accuracy reported when imputing a population of Nigerians using YRI as a reference panel.²⁰

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 to 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, PWTD, IVSTD, LVIDD, LVISD, LAD, 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 due to admixture.

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

Within-cohort genome-wide association results included parameter estimates (beta regression coefficient and their standard errors). Meta-analysis was conducted using METAL software (<http://www.sph.umich.edu/csg/abecasis/metal/>). For each SNP, METAL calculated an overall beta estimate, z-statistic and p-value from the weighted average of individual's statistic. No filtering on minor allele frequency was used.

A priori genome-wide statistical significance threshold of 4.0×10^{-07} was chosen to represent the probability for at least one SNP to have a p-value below a very stringent threshold. This strategy has been employed 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 nine 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 110kb upstream and 40kb downstream of the gene's most extreme transcript boundaries, which would encompass the majority of its cis-eQTLs.²² The lowest p-value of SNPs within the gene region was assigned as the significance score for the gene. Of the 22,374 genes evaluated, 1718 reached significance scores less than 1.0×10^{-4} . These genes were then imported into Ingenuity IPA for pathway analysis (Ingenuity Systems, Redwood, CA). Fisher's 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 Look-ups of Top Loci in Cohorts of European Ancestry

Genome-wide significant SNPs discovered in the meta-analysis of the three cohorts were subjected to replication analysis in three cohorts of African ancestry (GENOA, N=651;

HyperGEN, N=1316; CHS, N=501) and one cohort of European ancestry (Echo Genetics-EchoGEN; N=12,612). We adopted a criterion for declaring significance in the replication analysis at significance level $p = 0.05/\text{number of SNPs sent for replication}$. Additionally, we performed a look-up of the top 50 CARE hits in the EchoGEN cohort. Subsequently, we tested the five published genome-wide significant SNPs from the EchoGEN cohort analysis in our CARE AA sample.

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 that had younger participants (<31 years old) overall. Of the 4 cohorts, only JHS had all 9 echocardiographic phenotypes, the remaining 3 cohorts measured different subsets of phenotypes. Magnetic resonance imaging was available in MESA only.

The per-cohort genomic inflation factor (λ) was consistently below 1.02 for all traits studied. The post-meta-analytic λ was also below 1.02, indicating absence of systematic inflation. The meta-analysis quantile-quantile (Q-Q) plots of observed against expected p-value distributions are shown in Appendix, Section IV, Supplementary Figure 1 (Panels A–H).

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 (β) and standard errors (SE), minor and major alleles, minor allele frequency, SNP type and the nearest genes (within ~500 kbp of either site of the SNP) are also shown in Table 2. Figure 1 (Panels A–D) 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 four cardiac traits that were significantly associated with the four loci. Figure 2 (Panel A–D) 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 3 (Panels A–D) shows the regional plots for the four top SNPs. The nearest gene loci to the top SNPs within 500kb are also shown.

Appendix, Section IV, Supplementary 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 $5.0 \times 10^{-07} < p < 9.9 \times 10^{-07}$ (arbitrary threshold). In Appendix, Section IV, Supplementary Figure 2 (Panels A–G), the regional plots of the 7 additional top loci are presented.

Pathway analysis

We examined the interaction and relationship between the top GWAS loci. Accumulating evidence suggests that complex diseases and traits usually result from the incremental effects of many genetic variants.^{23–25} Pathway analysis provides a potential route to investigate the collective effects of multiple genetic variants on biological systems.^{26–28}

A total of 1718 genes were found moderately related to cardiac structure. Ingenuity IPA (Ingenuity Systems, Redwood, CA) was used to study if these genes were significantly enriched in some specific biological pathways beyond that expected from random distribution. Our analysis reveals that three 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%), $p=1.88 \times 10^{-2}$], the cardiac β -adrenergic

signaling pathway [16 CARE genes/151 total genes in the pathway (10.6%), $p=3.22\times 10^{-2}$], and the oncostatin M signaling [6 CARE genes/35 total genes in the pathway (17.1%), $p=3.88\times 10^{-2}$]. 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 dataset identified in each of the three pathways is shown in Appendix, Section IV, Supplementary Table II.

We applied the same pathway approach to EchoGen dataset, and identified 942 cardiac-related genes with $p < 1.0\times 10^{-4}$ (see Methods). Only a small proportion of them (97 genes) were also classified as cardiac related genes from CARE dataset due to factors such as the sample size and population stratification. Interestingly, one of the most enriched pathways from CARE dataset, cardiac β -adrenergic signaling pathway, was also moderately enriched in the EchoGen dataset ($p=0.069$). (Appendix, Section IV, Supplementary Figure 3)

Independent replication of top CARE SNPs in cohorts of African and European Ancestry

Replication cohorts for the study are described in detail in Appendix Section II, Supplementary. The four top genome-wide significant SNPs in the CARE analyses were submitted for lookup in four AA cohorts the GENOA study ($n=651$), the HyperGEN study ($n=1316$), and CHS ($n=501$). Additionally top SNPs were submitted for lookup in one large cohort of EA individuals [EchoGEN ($n=12,612$)]. None of the top SNPs met the *a priori* criteria for replication in the meta-analysis of AA cohorts after correcting for multiple comparisons. None of three SNPs available in EchoGEN (rs4552931, rs7213314, and rs9530176) replicated.

Reciprocal Look-ups 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 nine 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 (EF, $p=4.44\times 10^{-6}$) with LV wall thickness ($p=3.94\times 10^{-5}$); rs7159121 (FS, $p=4.67\times 10^{-6}$) with FS ($p=0.000862$); rs1549850 (ISD, $p=1.36\times 10^{-5}$) with ARD ($p=0.00142$); rs4752424 (LVSTD, $p=1.95\times 10^{-5}$) with LVM ($p=0.00088$); rs11758777 (LAD, $p=9.29\times 10^{-6}$) 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 African American study assessing the influence of genetic variation on cardiac structure and function, 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 shortening < 0.29 . Findings from the replication analysis of the four genome-wide significant loci in European ancestry suggest that these SNPs may represent loci specific to African ancestry. In further analysis, we found that nine 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 a number of the 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 Appendix, Section V and Section VI.

In a pathway analysis we noted top loci were enriched in cardiac genes from three 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 plays 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, in the redistribution of myosin isoforms, and in 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 AA.

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 LD blocks compared to 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 to 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 to phenotypes of cardiac structure and function is limited. Additionally, 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 relation or to 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 the 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 analytical methods into a prospective meta-analysis.

Conclusions

Our prospective meta-analysis of cardiac structure and function from over 6,765 participants in four community-based cohorts identified four loci rs4552931 in *UBE2V2* on chromosome 8 for left ventricular mass, rs7213314 in *WIP1I* on chromosome 17 for left ventricular internal diameter in diastole, rs1571099 in *PPAPDC1A* on chromosome 10 for interventricular septal wall thickness; 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 nine of these SNPs with 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 genes play in 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 to phenotypic findings on cardiac imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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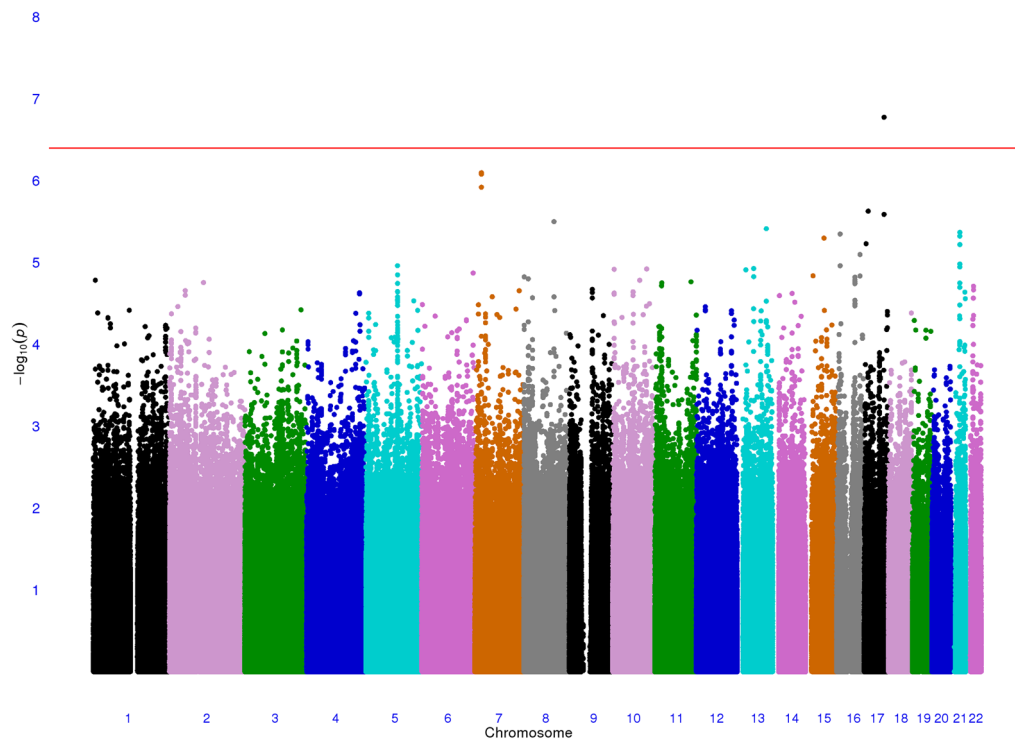
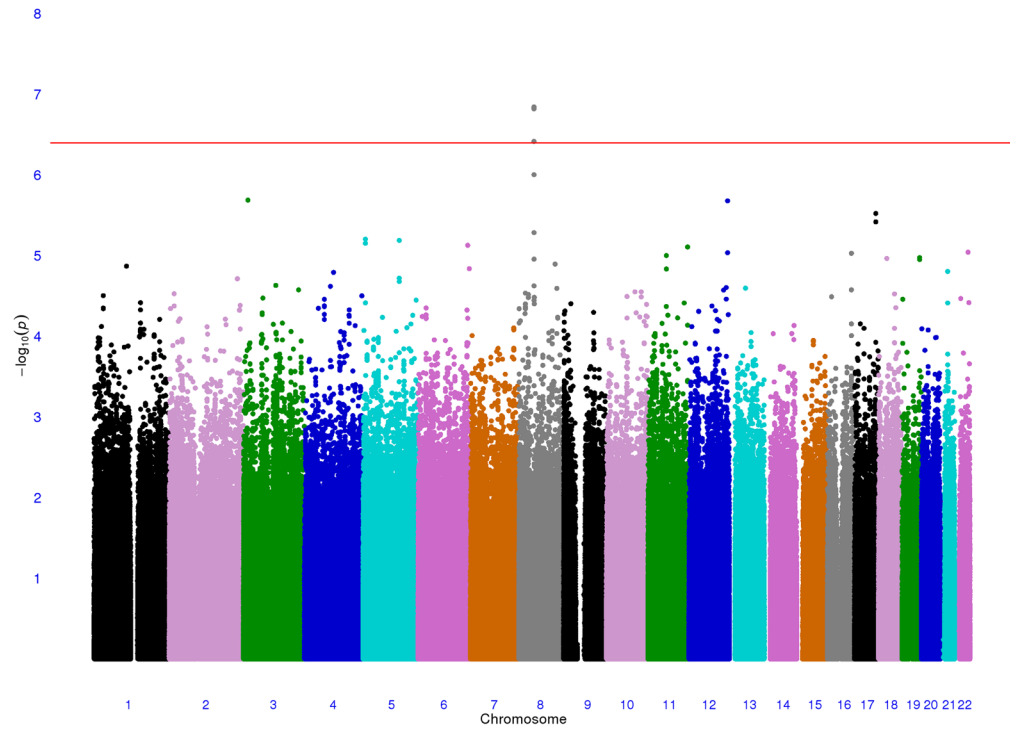
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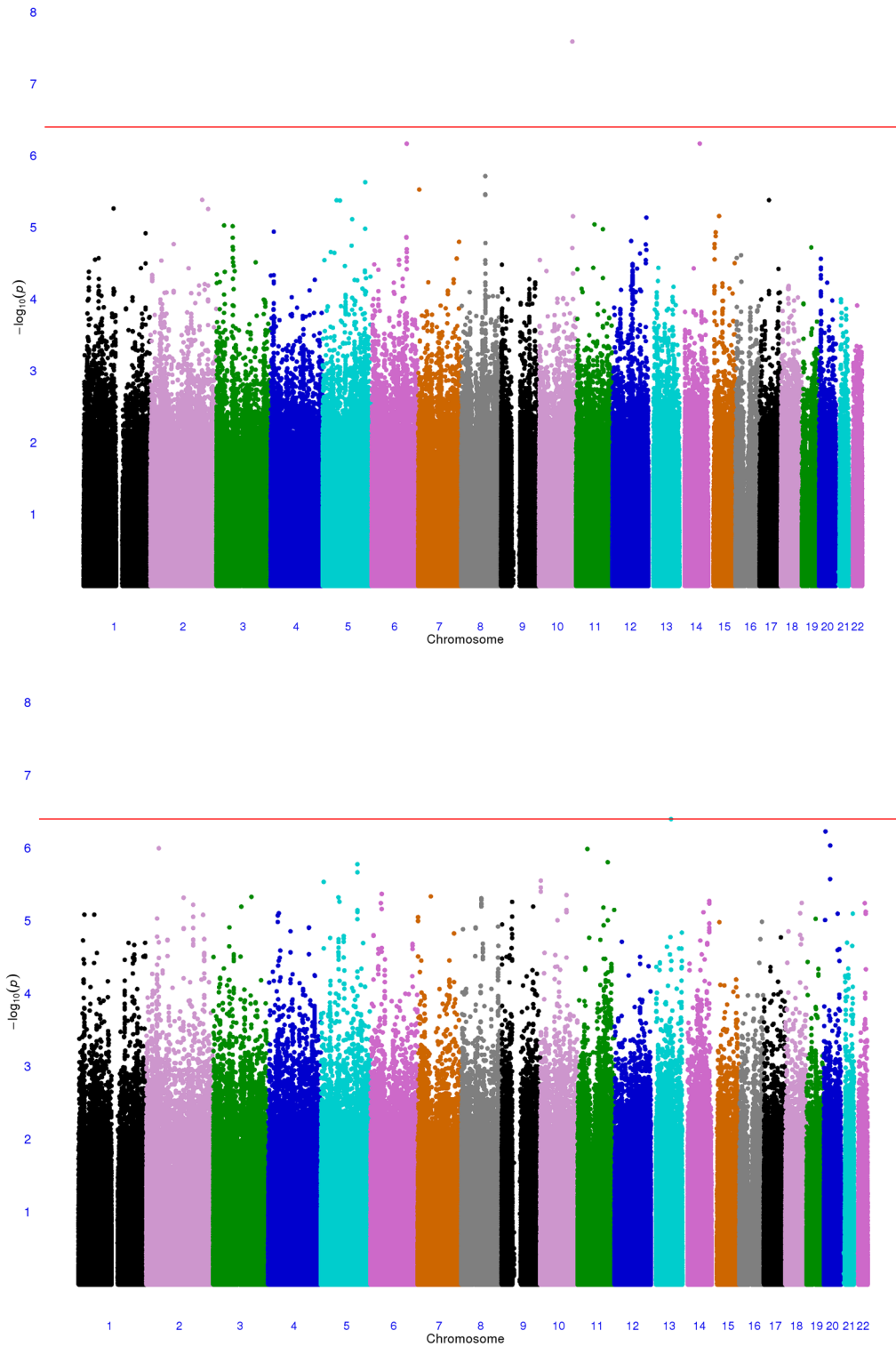
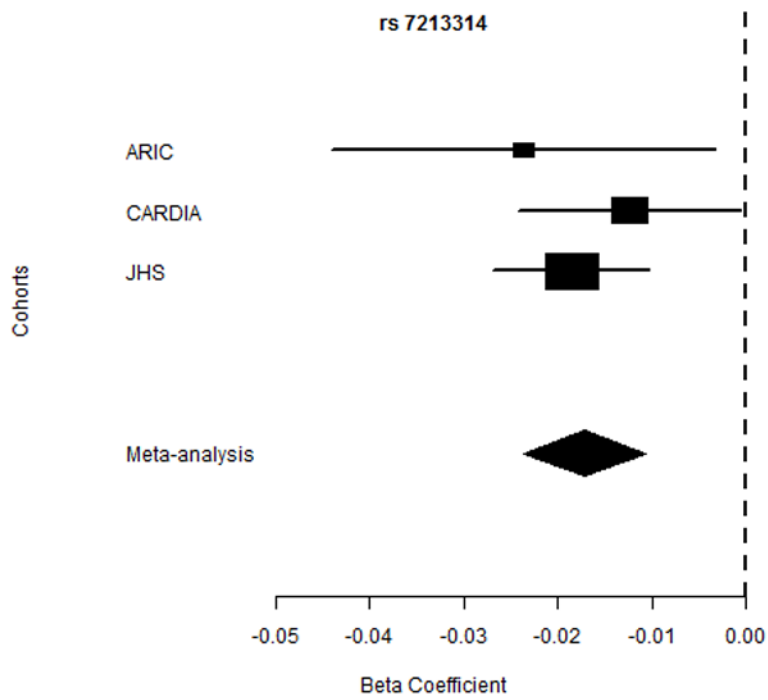
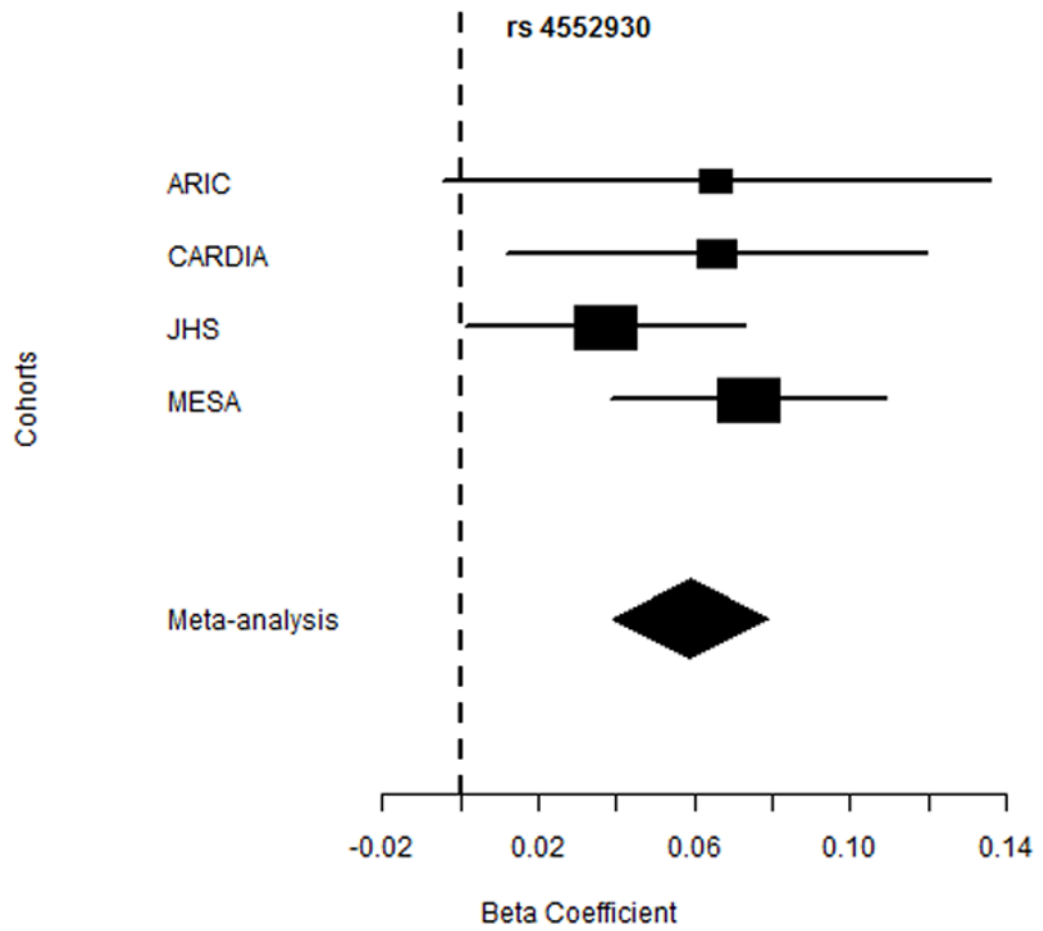
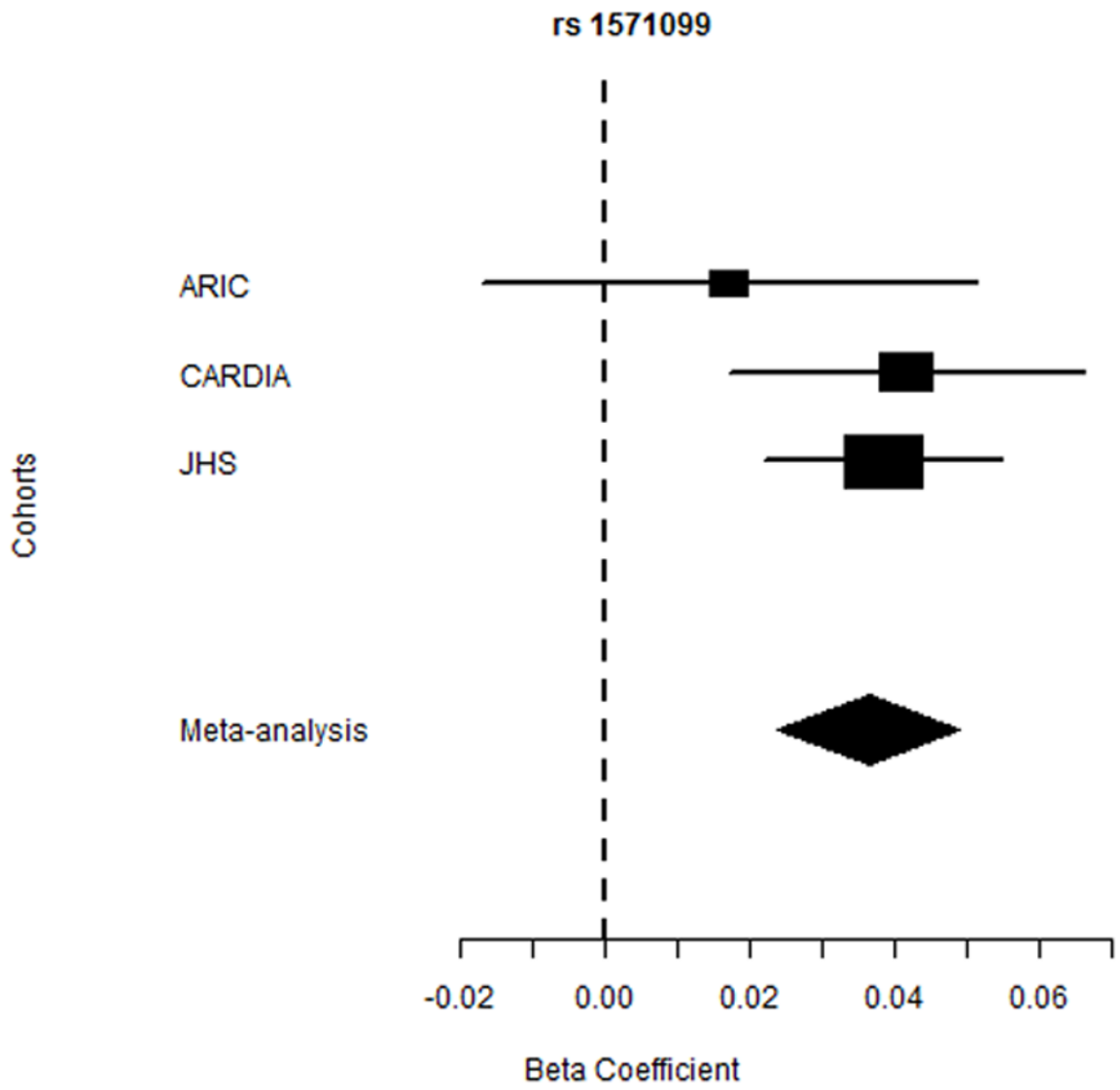


Figure 1. Manhattan plots showing the genome-wide $-\log_{10} p$ values for interrogated single nucleotide polymorphisms across the 22 autosomal chromosomes for (A) left ventricular

mass, (B) left ventricular internal diastolic diameter, (C) interventricular septal wall thickness, (D) and left ventricular ejection fraction. Chr; Chromosome





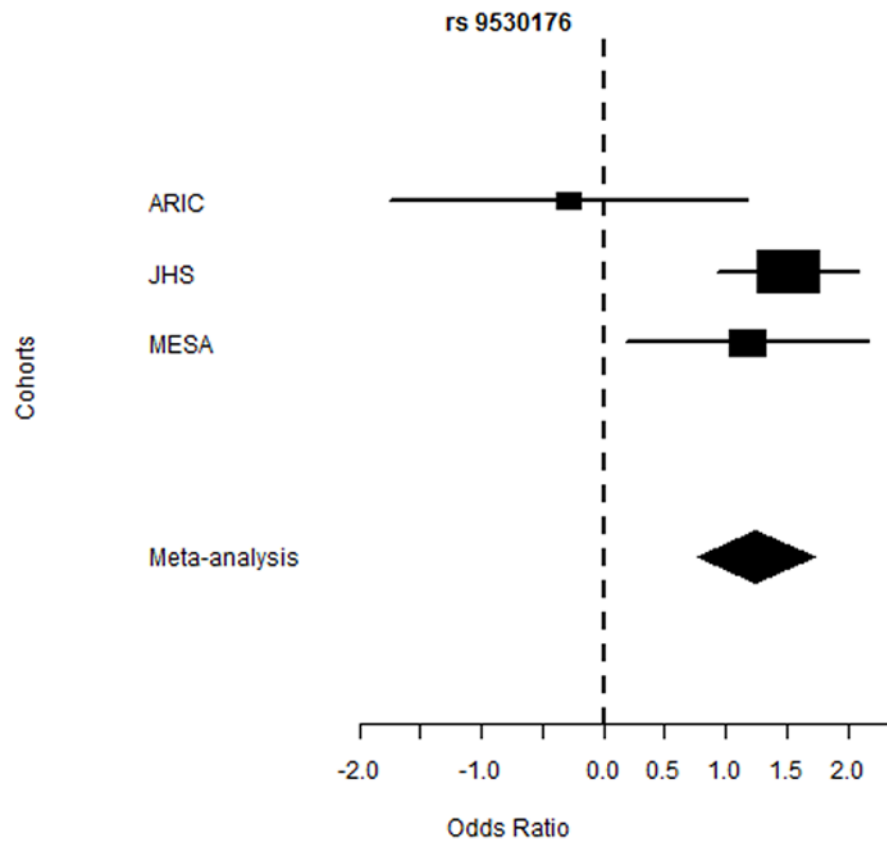
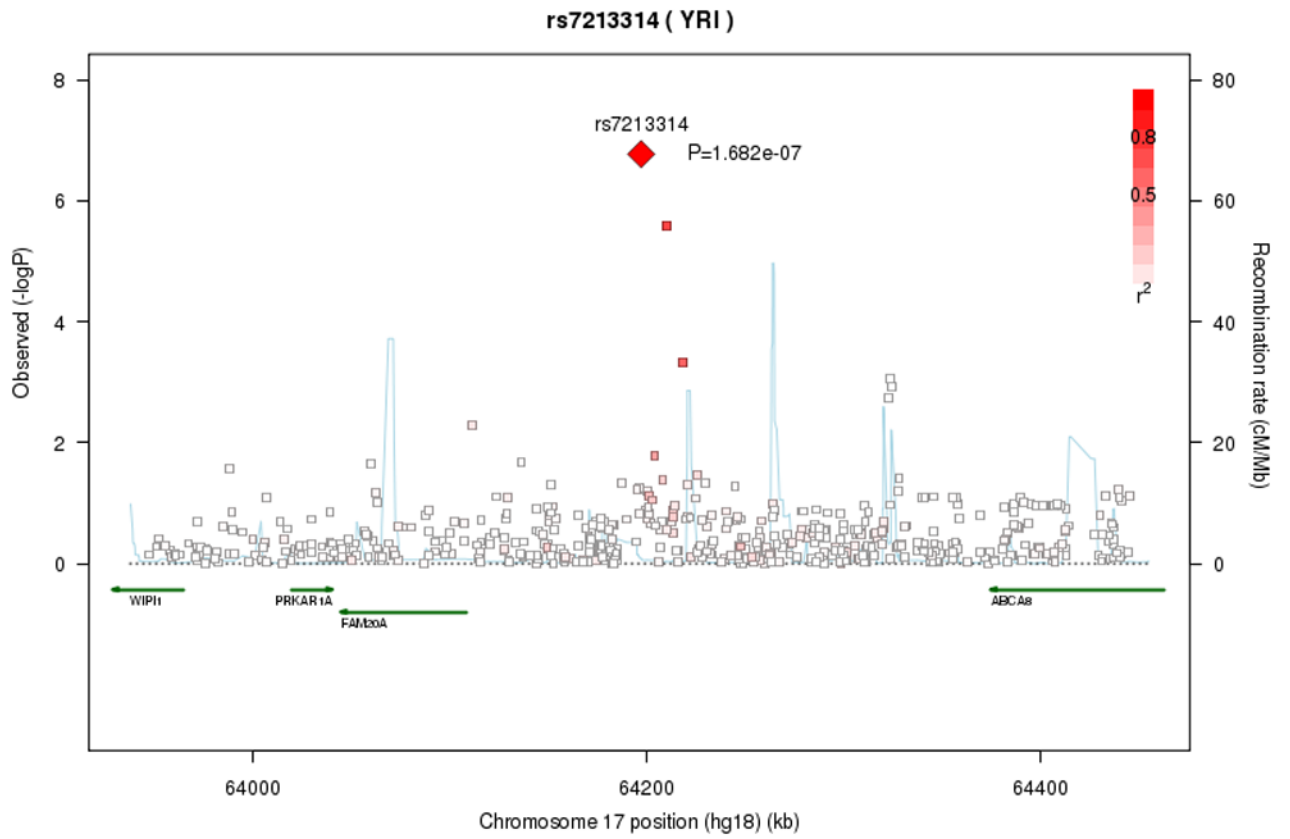
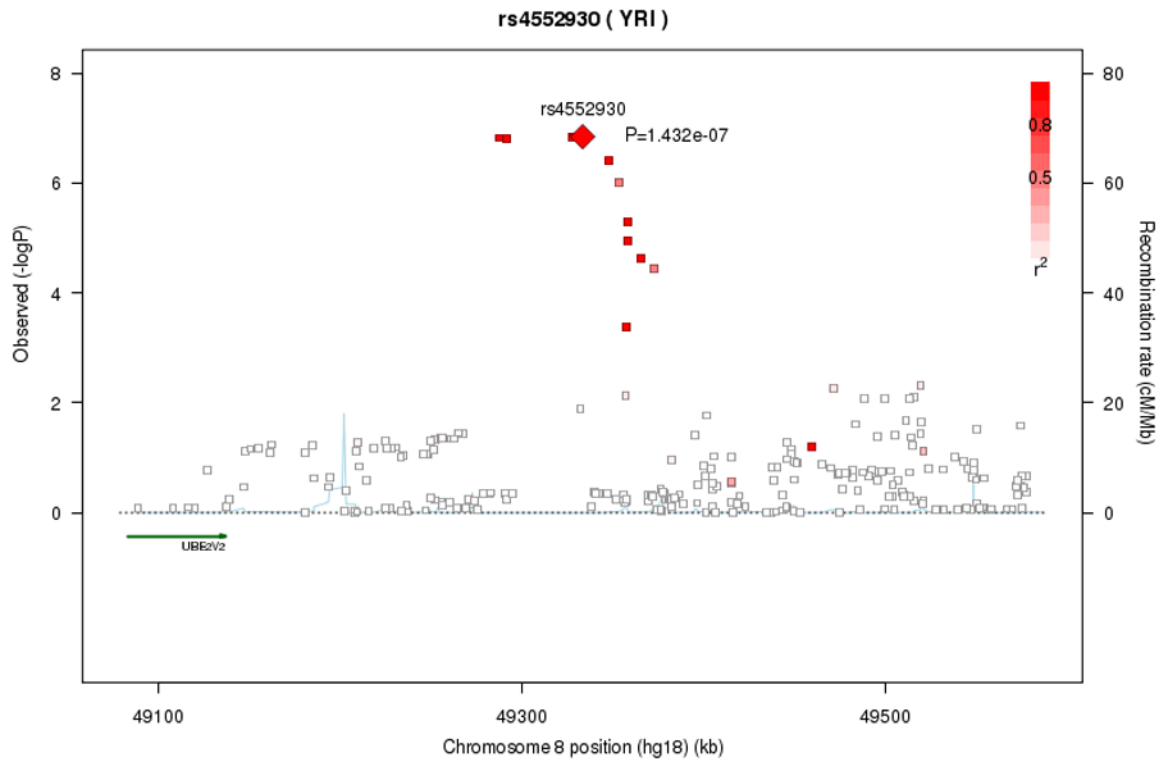
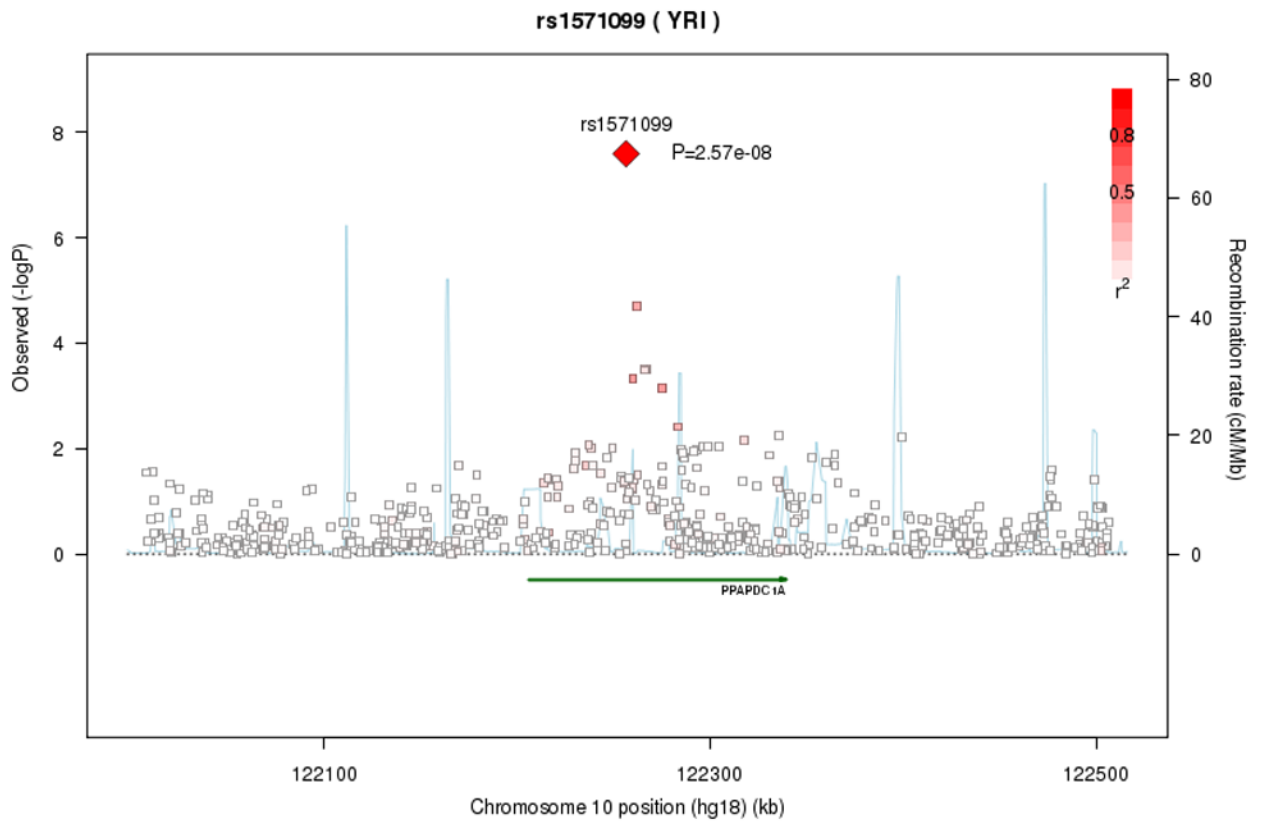


Figure 2. Forest plots for (A) left ventricular mass, (B) left ventricular internal diastolic diameter, (C) interventricular septal wall thickness, and (D) left ventricular ejection fraction are shown. For each cardiac trait the odds ratios are shown for cohort specific analyses and for the meta-analysis. ARIC, Atherosclerosis Risks in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; Chr; Chromosome





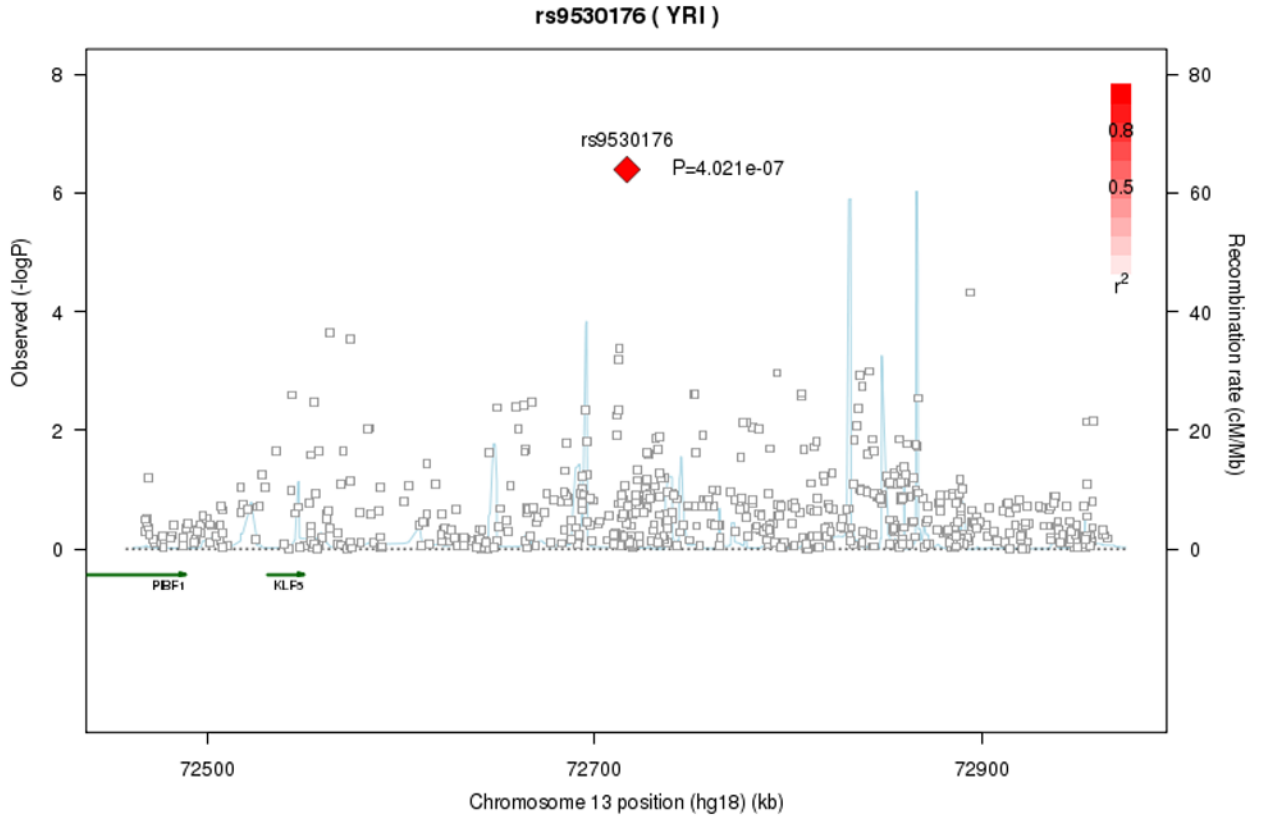


Figure 3. “Results ($-\log_{10} P$) are shown for SNPs in the region flanking 250kb on either side of the marker SNPs. The marker SNPs and r^2 values of other SNPs are shown in red color. The genes within the region of interest are annotated and are shown in green arrow We investigated the association between genome-wide markers with cardiac structure and systolic function using data from four community-based cohorts of African Americans in the Candidate-gene Association Resource study. Findings from this analysis may help us better understand the role genes play in 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 to phenotypic findings on cardiac imaging.

Table 1

Study Sample Characteristics

	Atherosclerosis Risk in Communities		Coronary Artery Risk Development in Young Adults Study		Jackson Heart Study		Multi-Ethnic Study of Atherosclerosis	
	Women	Men	Women	Men	Women	Men	Women	Men
Age (mean±SD), y	59±6	59±6	30±4	29±4	55±13	54±13	62±10	62±10
Height, cm	163±6	176±7	164±7	177±7	164±7	177±7	162±7	176±7
Weight, lbs	186±41	190±36	167±47	183±40	196±47	209±48	182±39	196±36
Echocardiographic Traits								
N	698	415	854	589	1884	1115	647	563
LV Mass, g	242±78	289±96	136±40	177±44	140±34	167±42	137±30	184±39
LV diastolic dimension, cm	4.6±0.6	4.9±0.6	4.8±0.45	5.1±0.47	4.87±0.41	5.1±0.4		
Left atrial dimension, cm	3.9±0.6	3.9±0.6	3.5±0.5	3.6±0.48				
Aortic root diameter, cm	3.0±0.4	3.4±0.4	2.6±0.3	3.0±0.35	3.0±0.28	3.4±0.3		
Posterior wall thickness, cm	1.1±0.2	1.2±0.2	0.8±0.1	0.9±.14	0.8±0.1	0.9±0.1		
Fractional shortening, % < 0.29	19.6	9.2	6.4	7.6	3.8	8.0		
Ejection Fraction, % < 0.50	5.3	2.5	*0.7	*1.2	1.6	4.2	1.1	5.0
LV systolic dimension, cm					2.9±0.4	3.2±0.5		
Interventricular septal wall thickness, cm	1.2±0.2	1.2±0.3	0.9±0.2	1.0±0.16	0.9±0.1	0.9±0.15		

MRI, magnetic resonance imaging; g, grams; cm, centimeters;

* 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.

Table 2

Genome-Wide significant SNPs associated with cardiac traits ($P < 4.0 \times 10^{-7}$) and results from replication analyses

Trait	Meta-Analysis of 3 CARe Cohorts										Replication Studies					
	Locus	SNP	SNP position (bp)	Minor/Major	SNP type	Nearest gene	MAF	Effect size β (SE)	Meta-analysis P	GENOA (n=651) Beta	P	HyperGEN (n=1316) Beta	P	CHS (n=501) Beta	P	
LV Mass	8q11	rs4552930	49170958	G/A [†]	Intergenic	<i>UBE2V2</i>	0.05	0.059 (0.010)	1.42×10^{-7}	0.042 (.029)	0.14	-5.49 (3.35)	0.10	0.034 (.036)	0.34	
LV internal diastolic diameter	17q24	rs7213314	64197442	T/C [†]	Intergenic	<i>WPI1; PRKAR1A; FAM20A; ABCA8</i>	0.17	0.017 (0.003)	1.68×10^{-7}	0.009 (.011)	0.40	-0.03 (.012)	0.14	-0.008 (.01)	0.41	
Intraventricular septal wall thickness	10q26	rs1571099	122256604	C [†] /T	intronic	<i>PPAPDC1A</i>	0.11	-0.036 (0.007)	2.57×10^{-8}					-0.003 (.019)	0.88	
Ejection fraction	13q22	rs9530176	72716729	A [†] /T	Intergenic	<i>KLF5; PIBF1</i>	0.07	1.240 (0.240)	4.02×10^{-7}			-0.34 (0.56)	0.55			

SNP, single nucleotide polymorphism; bp, base pairs; MAF, minor allele frequency; LV, left ventricular;

[†] Coded allele