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Whole exome analyses to examine the impact of rare variants on left ventricular traits in African American participants from the HyperGEN and GENOA studies

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Abstract

Left ventricular (LV) hypertrophy, highest in prevalence among African Americans, is an established risk factor heart failure. Several genome wide association studies have identified common variants associated with LV-related quantitative-traits in African Americans. To date, however, the effect of rare variants on these traits has not been extensively studied, especially in minority groups. We therefore investigated the association between rare variants and LV traits among 1,934 African Americans using exome chip data from the Hypertension Genetic Epidemiology Network (HyperGEN) study, with replication in 1,090 African American from the Genetic Epidemiology Network of Arteriopathy (GENOA) study. We used single-variant analyses and gene-based tests to investigate the association between 86,927 variants and six structural and functional LV traits including LV mass, LV internal dimension-diastole, relative wall thickness, left atrial dimension (LAD), fractional shortening (FS), and the ratio of LV early-to-late transmitral velocity (E/A ratio). Only rare variants (MAF <1% and <5%) were considered in gene-based analyses. 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

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GENOA, but the direction of effect of single variants in common was comparable. G protein-coupled receptor 55 (*GPR55)* was marginally associated with LAD 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. 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.

Keywords

genetics; epidemiology; echocardiography; left ventricular traits; rare variants; African Americans

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].

There is evidence that racial differences in LV mass and related traits may have genetic underpinnings [8–11]. 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) [12]. Recent genome wide association studies (GWAS) have identified common variants associated with LV traits in African Americans [9, 10]. However, all common variants identified to date only explain a small proportion of inter-individual variation of LV traits [9]. 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.

Material and 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 [13]. 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 [14]. 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 [15]. 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 [16]. After annotation, 86,927 exonic SNPs (including nonsynonymous, stop-gain, and stop-loss variants) were included in the analysis. Additionally, SIFT and PolyPhen values from Combined Annotation Dependent Depletion (CADD) software were used to predict the level of protein damage due to the amino acid change of significant SNPs from gene-based analysis [17].

Study outcome

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 [18]. 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 [19].

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 ×1.04× $[(IVS + LVIDD + PWT)^3 - LVIDD^3] + 0.6$ 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 [20]. RWT was calculated as twice the PWT divided by the LVIDD [21]. LAD was measured from the parasternal long-axis view and normalized for the linear measure of height [22]. 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) [18]. 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 [23]. E and A velocity were measured with pulse-wave Doppler at the mitral leaflet tips in apical 4-chamber view during diastole [18]. 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 [11, 24].

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 [25]. For single-variant analyses, we considered an additive genetic model and included common (minor allele frequency (MAF) ≥ 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. Extremely rare (MAF <0.5%) variants, including singletons and doubletons, were part of all gene-based analyses. 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 [26]. The simple burden test evaluates the cumulative effects of multiple variants in a genomic region by collapsing the rare variants within a region into a score which is tested for association with the phenotype of interest [27]. The weighted burden test uses the same approach adding a weight as a function of the MAF upweighting rarer variants [28]. Both burden and weighted burden tests assume all rare variants influence the phenotype in the same direction and with the same magnitude of effect (after incorporating known weights) [27]. The VT test

performs multiple burden tests for MAF thresholds below the upper cutoff (e.g. <5%) and reports the most significant P-value across all tests adjusting for the number of tests performed [29]. The SKAT test evaluates the distribution of the aggregated score test statistics of individual variants allowing for variants to have different directions of effect on the trait unlike burden tests [27]. Depending on the underlying genetic model different gene-based tests may perform better at different loci. Therefore, we applied this battery of tests implemented by RAREMETAL and reported significant results from any of the four tests. 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 [30]. 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). We also report results after FDR correction in supplemental material for single variant and gene-based tests. 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 [13], 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) [31]. A second study visit was completed from 2001 to 2005 (Phase II)[32]. 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 [9].

The Illumina Human Exome Beadchip v1.1 was used to genotype 1,429 individuals [33]. 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 [33]. 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 [33]. 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 ≥ 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. Seven SNPs with FDR <0.05 were also presented in Supplemental Table 2 but were not replicated in GENOA ($P>0.10$).

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 ≤ 5% and ≤ 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. These same three genes (but not others in Table 3) passed the FDR correction for multiple testing (Supplemental Table 3). Other marginally significant results from Table 3 include

phosphorylase kinase regulatory subunit beta (*PHKB*), and G protein-coupled receptor 55 (*GPR55*) 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 4). The results for *KCNH4* and *PHKB* were consistent when the analysis was restricted to participants with directly measured E/A ratio (Supplemental Table 5).

Supplemental Table 6 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 ($\alpha=0.05/5$, where 5 is the number of genes for which we attempted 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 *ENDOG* and LAD and *RAMP1* and LVIDD in AAs.

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 [34]. Though potassium transport is important to cardiovascular function [35], *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 [36]. In that study, a loss-of-function mutation was associated with increased LVM and impaired cardiac function [36]. Additionally, inhibition of *ENDOG* in cultured cardiomyocytes leads to increased cardiomyocyte size and upregulation of biomarkers indicative of hypertrophy [36]. 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 and modulate vascular physiology activity in animals [37, 38]. Overexpression of *RAMP1* is associated with lower mean arterial pressure change among hypertensive mice caused by angiotensin II [37]. Moreover, a loss-of-function mutation in *RAMP1* was found to be associated with higher blood pressure in mice [38]. Gene expression of *RAMP1* in both ventricles and atria was reported to be 3 times higher in rats with congestive heart failure compared to control rats [39]. Interestingly, an increase of *RAMP1* expression was reported to be associated with increased heart size and heart weight over body weight ratio in mice with pressure overload-induced cardiac hypertrophy [40]. Unfortunately, sequencing information for this gene was not available in GENOA and this biologically plausible finding could not be replicated in GENOA.

PHKB encodes the regulatory subunit beta of phosphorylase kinase. The gene plays an important role in glycogen breakdown and cell growth [41]. A mutation in *PHKB* is associated with autosomal phosphorylase kinase deficiency in muscle and liver [42]. The transcript level of *PHKB* was higher in the right ventricular cells among hypoplastic left heart syndrome patients in one study [43]. 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 [44]. 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 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 since treatment is associated with reduction in LVM [45, 46]. 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 has some limitations including (1) the exome array was designed based on known variant positions so it may miss novel or super-rare variants, (2) the exome array may ignore large genomic variants including insertions, deletions, duplication, inversions and other rearrangements because of their sizes, (3) the exome array is biased toward rare variants specific to Caucasian populations. 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 (including participants of other ethnicities) 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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

		Gene-based association p-values							
		MAF < 1%				MAF < 5%			
Left ventricular traits	**Gene name**	**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.