Genetic Variants Associated With Cardiac Structure and Function
A Meta-analysis and Replication of Genome-wide Association Data

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Context Echocardiographic measures of left ventricular (LV) structure and function are heritable phenotypes of cardiovascular disease.

Objective To identify common genetic variants associated with cardiac structure and function by conducting a meta-analysis of genome-wide association data in 5 population-based cohort studies (stage 1) with replication (stage 2) in 2 other community-based samples.

Design, Setting, and Participants Within each of 5 community-based cohorts comprising the EchoGen consortium (stage 1; n=12,612 individuals of European ancestry; 55% women, aged 26-95 years; examinations between 1978-2008), we estimated the association between approximately 2.5 million single-nucleotide polymorphisms (SNPs; imputed to the HapMap CEU panel) and echocardiographic traits. In stage 2, SNPs significantly associated with traits in stage 1 were tested for association in 2 other cohorts (n=4,094 people of European ancestry). Using a prespecified P value threshold of 5 × 10⁻⁷ to indicate genome-wide significance, we performed an inverse variance-weighted fixed-effects meta-analysis of genome-wide association data from each cohort.

Main Outcome Measures Echocardiographic traits: LV mass, internal dimensions, wall thickness, systolic dysfunction, aortic root, and left atrial size.

Results In stage 1, 16 genetic loci were associated with 5 echocardiographic traits: 1 each with LV internal dimensions and systolic dysfunction, 3 each with LV mass and wall thickness, and 8 with aortic root size. In stage 2, 5 loci replicated (6q22 locus associated with LV diastolic dimensions, explaining <1% of trait variance; 5q23, 12p12, 12q14, and 17p13 associated with aortic root size, explaining 1%-3% of trait variance).

Conclusions We identified 5 genetic loci harboring common variants that were associated with variation in LV diastolic dimensions and aortic root size, but such findings explained a very small proportion of variance. Further studies are required to replicate these findings, identify the causal variants at or near these loci, characterize their functional significance, and determine whether they are related to overt cardiovascular disease.

A lterations in cardiac structure and function adversely affect the prognosis of individuals in the general population. In community-based cohorts, the presence of left ventricular (LV) hypertrophy and increased LV mass predict the development of coronary heart disease, congestive heart failure (CHF), stroke, cardiovascular disease (CVD), and all-cause mortality. Likewise, increased LV wall thickness predicts CVD events, LV diastolic dysfunction predicts CHF, and asympotomatic LV systolic dysfunction predicts CHF and death. Left atrial size is related to incidence of atrial fibrillation, stroke, and death. Aortic root size

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is associated with risk of CHF, stroke, and mortality.9 Thus, traits obtained from echocardiography serve not only as measures of cardiac structure and function but also as intermediate phenotypes for clinical CVD outcomes.

These echocardiographic phenotypes are heritable10-18 and have been linked to genetic loci.19-21 Candidate gene studies have identified several single-nucleotide polymorphisms (SNPs) in genes such as ACE (GenBank J04144),22-24 PPARA (GenBank L02932),25 GNB3 (RefSeq NM_002075),26 and CYP11B2 (GenBank X54741)27 that may contribute to variability in LV mass. However, many of the studies suggesting specific genetic associations were small, based on selected samples, failed to adjust for key confounders and were not replicated.28-32

Genome-wide association analyses have led to the discovery of previously unsuspected common variants underlying risk for complex diseases unconstrained by prior knowledge.33 The present investigation uses a 2-stage approach and leverages the availability of whole genome scans in 5 community-based samples to perform a prospective combined meta-analysis of findings from these studies to identify genomic variation associated with echocardiographic traits (stage 1), followed by replication in 2 other population-based samples (stage 2).

METHODS

EchoGen Consortium Organization

The EchoGen consortium includes 7 cohort studies that enrolled participants of European ancestry and had both genome-wide variation data and echocardiographic measurements (see below for details of the cohorts); 3 of these cohorts (Cardiovascular Health Study, Rotterdam Study, and Framingham Heart Study) are part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium.34 All participating studies approved guidelines for collaboration and arrived at a consensus not only on phenotype definitions including harmonization, covariate selection, and analytic plans for within-study analyses but also on a prospective meta-analysis of results. The institutional review boards at the parent institutions for each cohort study approved the informed consent procedures, examination and surveillance components (including DNA collection), the data access and security processes, genotyping protocols, and the genome-wide association design. All participants provided written informed consent and gave permission to have their DNA used for research purposes.

Five studies contributed genome-wide association data to the discovery (stage 1) phase, and 2 studies contributed data to the replication (stage 2) phase. A description of these samples follows.

Stage 1 Cohorts

Cardiovascular Health Study. The Cardiovascular Health Study is a population-based cohort study of risk factors for coronary heart disease and stroke in adults aged 65 years or older conducted at 4 field centers.35 The original cohort of 5201 persons of primarily European ancestry was recruited in 1989-1990 from random samples of the Medicare eligibility lists and an additional 687 individuals of African ancestry were enrolled subsequently for a total sample of 5888. Those with prevalent coronary heart disease (n=1195), CHF (n=86), peripheral vascular disease (n=93), valvular heart disease (n=20), stroke (n=166), or transient ischemic attack (n=56) at baseline were excluded from the genome-wide associations. Because the other cohorts were predominantly of European descent, the African American participants were excluded from this analysis. Participants were eligible for the present investigation if their genotyping was complete and they had available echocardiographic phenotype information at their first (1989-1990) or second (1994-1995) examinations (n=3279).

Rotterdam Study. The community-based Rotterdam Study was founded in 1990 to examine the determinants of disease and health in the elderly with a focus on neurogeriatric, cardiovascular, bone, and eye diseases.36 Inhabitants of a suburb of Rotterdam, the Netherlands (n=7983), aged 55 years or older were included. Participants were visited at home for a standardized questionnaire and were subsequently examined at the research center in 1990-1993 and every 3 to 4 years thereafter. For the present investigation, data from the fourth round of examination (2002-2004) were used. Of 3550 eligible participants, 2199 were free of myocardial infarction (MI) and CHF and had both echocardiographic and genome-wide association data available.

Multinational Monitoring of Trends and Determinants in Cardiovascular Disease Study. In 1984, the World Health Organization instituted a Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study, which was continued since 1996 in the Southern German region of the Augsburg (KORA).37 The MONICA-KORA study investigated the CVD risk factor profile of randomly selected individuals of the Augsburg population (Bavaria, Germany) in cross-sectional surveys. The study design, sampling frame, and data collection have been described elsewhere.37 A total of 4856 men and women participated in the study, of which only 2376 participants residing within or close to the city of Augsburg were offered an echocardiographic examination for logistical reasons. Participants (n=3006) had a follow-up examination (KORA F3) in 2004-2005, of whom 1644 participants between 35 and 79 years of age had genome-wide associations conducted.38 Of these, 589 had available echocardiograms and were free of prevalent MI and CHF.

Framingham Heart Study. The Framingham Heart Study is a longitudinal observational, community-based cohort initiated in 1948 in Framingham, Massachusetts, to prospectively investigate CVD and its risk factors. The children (and spouses of
the present investigation. At each clinic examination, participants receive routine questionnaires, a physical examination, anthropometry, electocardiograms, and blood tests. At the second (1978-1982), fourth (1987-1990), fifth (1991-1995), and sixth (1996-1998) examination cycles participants underwent transthoracic echocardiography (see supplementary material at http://www.jama.com). The offspring cohort participants with available echocardiographic information at any of these 4 examinations and who were free of MI and CHF at these examinations (n=3245) were eligible for the present investigation.

**Gutenberg Heart Study.** The Gutenberg Heart Study was initiated in 2006 to achieve a contemporary German sex-specific cardiovascular risk score. It is a community-based, prospective cohort study including approximately 17,000 participants, aged 35 to 74 years from the city of Mainz and the district Mainz-Bingen. The sample is stratified according to sex (50% women) and decade of age. A large variety of noninvasive cardiovascular phenotypes have been assessed including 2-dimensional echocardiography. By September 2008, 5000 individuals have been enrolled; 3300 study participants with genome-wide association data and echocardiographic measurements and who were free of prevalent MI and CHF were eligible for the present investigation.

**Stage 2 Cohorts**

**Study of Health in Pomerania.** The Study of Health in Pomerania (SHIP) is a longitudinal population-based cohort study conducted in West Pomerania, the northeast area of Germany. For the baseline examinations, a sample of 6267 eligible persons aged 20 to 79 years was drawn from population registries. Only individuals with German citizenship and main residency in the study area were included. Selected persons received a maximum of 3 written invitations. In case of nonresponse, letters were followed by a telephone call or by home visits if contact by telephone was not possible. The SHIP population finally comprised 4310 participants (response, 68.8%). Baseline examinations were conducted between 1997 and 2001. Between 2002 and 2006 all participants were re-invited for an examination follow-up, in which 3300 participants (83.5% of eligible persons) took part. Echocardiography at baseline was conducted only in those 45 years or older but had no age restriction at follow-up. A total of 3212 individuals who were free of prevalent MI and CHF were eligible for the present study.

**Austrian Stroke Prevention Study.** The Austrian Stroke Prevention Study is a community-based prospective cohort study on the cerebral effects of vascular risk factors in the normal elderly population of the city of Graz, Austria. From 1991-1994, 509 persons without neuropsychiatric disease were randomly selected from the official community register (stratified by sex and 5-year age groups) to undergo neuromaging, cognitive testing, and echocardiography. In 1999-2003, an additional 567 individuals were randomly selected to undergo the same imaging procedures, thereby increasing the size of the baseline cohort to 1076 individuals aged 45 to 85 years. Blood was drawn from all study participants for DNA extraction and all consented to genetic testing. Of the 996 study participants from whom DNA was extracted, 908 underwent transthoracic echocardiography. We excluded 26 individuals because of prevalent MI or CHF; leaving 882 eligible for the present analysis.

**Echocardiographic Methods**

In each cohort, participants underwent routine transthoracic echocardiography at selected examinations (1 each for the Rotterdam and Gutenberg studies; 2 for the Cardiovascular Health Study, MONICA-KORA, SHIP, and Austrian Stroke Prevention Study, and 4 for the Framingham Heart Study; data from all available echocardiographic examinations of each cohort [including the most recent ones] were included). Measurements of LV internal dimension, the thicknesses of the posterior wall and interventricular septum, and the diameter of the aortic root (all measured at end-diastole) and the left atrium at end-systole were obtained by using a leading edge technique and averaging measurements in 3 cardiac cycles according to the American Society of Echocardiography guidelines. Left ventricular wall thickness was calculated as the sum of posterior wall and interventricular septum measurements. The LV mass was calculated by using the formula 0.8 [1.04{(LV diastolic internal dimension + interventricular septum + posterior wall)² - (LV diastolic internal dimension)²}] + 0.6. The LV systolic dysfunction was defined as the presence of reduced fractional shortening (<0.29, which corresponds to an ejection fraction of 50%) on M-mode or a diminished ejection fraction (<50%) on 2-dimensional echocardiography. Details of ultrasonographic instrumentation are provided in the “Echocardiographic Methods” section and in eTable 1 of the supplementary material (available at http://www.jama.com). The present investigation focused on 6 echocardiographic traits: LV mass, LV diastolic internal dimension, LV wall thickness, aortic root, and left atrial size (continuous traits), and LV systolic dysfunction (a binary trait). For cohorts with multiple echocardiographic examinations, we used the average of all available measurements obtained at the eligible examinations for our analyses.

**Genotyping Methods and Imputation**

The 7 studies included in this meta-analysis used different genotyping platforms: the Illumina HumanCNV370-Duo for the Cardiovascular Health Study, the Illumina Infinium HumanHap550-chip v3.0 for the Rotterdam Study, Illumina Human610-Quad BeadChip for the Austrian Stroke Prevention Study, Affymetrix Human Mapping 500K Array Set for MONICA-KORA, Affymetrix Human Mapping 500K Array Set and 50K Human GeneFocused Panel for the
Framingham Heart Study, and the Affymetrix Human SNP Array 6.0 for the Gutenberg Study and SHIP. Therefore, to facilitate meta-analyses, all studies used their genotype data to impute to the 2.5 million nonmonomorphic autosomal, SNPs described in HapMap (CEU population, release 22, build 36; http://hapmap.org).45, 46 Imputation of unmeasured genotypes in order to combine results data across genotyping platforms is an essential and accepted tool in the conduct of genome-wide association studies.39 Stated simply, the application of imputation techniques on each specific genotyping platform allowed us to estimate the association of all 2.5 million polymorphic HapMap SNPs in each study. The Cardiovascular Health Study used the BIMBAM algorithm software for imputation (available at http://stephenslab.uchicago.edu/software.html),47 whereas the Rotterdam, Framingham, Gutenberg, Austrian Stroke and Prevention, and MONICA-KORA studies used the MACH algorithm software (http://www.sph.umich.edu/csg/abecasis/MaCH). SHIP used the IMPUTE algorithm software (http://www.stats.ox.ac.uk/~marchini/software/gwas/impute.html). All studies imputed the genotype dosage, from 0 to 2, which is the expected number of minor alleles. Extensive quality control analyses were performed in each cohort. Imputation methods and quality control filters are described in the “Genotyping Methods” section of the supplementary material (available at http://www.jama.com).

**Statistical Methods**

We chose a 2-stage design with a larger stage 1 (followed by joint analysis) to combine statistical efficiency with power for detecting variants with modest effects.48 For stage 1, separate within-cohort analyses (n = 5 cohorts) were performed for each echocardiographic trait using an additive genetic model relating the trait to genotype dosage (0-2 copies of the minor allele) for each SNP, adjusting for age, sex, height, and weight. The Cardiovascular Health Study additionally adjusted for study site. For continuous phenotypes, linear regression was used. For LV systolic dysfunction, we used a logistic model in unconditional logistic regression to compare those with and without the condition. In the Framingham Heart Study alone, we used mixed-effects models (linear or logistic depending on trait) to account for familial correlations. The association of each echocardiographic trait to each genotype was quantified by the regression slope (β), its standard error [SE(β)], and P value. Genomic control correction was applied in each study prior to the meta-analysis.49

After verifying strand alignment across studies, we conducted a prospective meta-analysis of results from within-cohort analysis (n = 5 cohorts) for each echocardiographic trait. We combined the results from individual studies with inverse-variance weighting for each SNP using the R software (http://www.r-project.org). The approach did not pool raw participant-specific data, which could induce problems associated with phenotypic heterogeneity or population structure admixture, hence, the approach is robust. We selected an a priori genome-wide statistical significance threshold of 5 × 10^−7, the threshold used by the Wellcome Trust Case-control Consortium.50 For 2.5 million tests, this threshold provides an expectation of less than 1.25 false-positive results across the genome. Post-meta-analytic filters were an average weighted minor allele frequency of more than 0.005 for continuous traits and more than 0.03 for the binary trait of LV systolic dysfunction.

For stage 2, we selected the top SNP at each genetic locus that was associated with an echocardiographic trait and achieved genome-wide significance in stage 1 (as defined above); a locus was defined as a set of HapMap SNPs associated with the most significantly associated SNP with an R^2 of 0.5 or greater. We related the top SNPs to corresponding echocardiographic traits in the 2 replication samples. To be considered replication, we required that the direction of the β (for a SNP) must be in the same direction in the replication study as in the discovery analysis. Using a 1-sided P value is therefore necessary in order for the P value distribution to be correct under the null hypothesis. Accordingly, we only calculated replication P values for SNPs with βs in the appropriate direction and defined statistical significance based on a 1-sided P value less than .05 (uncorrected). We queried HapMap for evaluating if any of the replicated SNPs at a locus was correlated with a nonautosomal SNP (R^2 > 0.5). We estimated that our stage 2 sample size of 4094 individuals yielded more than 80% power to detect associations of a magnitude similar to that observed in stage 1 for each trait at a 1-sided α of .05 (eTable 2 available at http://www.jama.com).

**RESULTS**

Table 1 displays the clinical and echocardiographic characteristics of the 7 samples contributing to the 2 stages of the present investigation. The genomic inflation factor (λ) was small in each of the 5 studies contributing to stage 1 (<1.09 for all traits in all cohorts). The quantile-quantile (Q-Q) plots of observed against expected P value distributions are shown in eFigure 1 (available at http://www.jama.com) and the meta-analytic λ for all traits was 1.02 or less. The Q-Q plots show a marked excess of statistically significant associations over what expected by chance alone for LV diastolic dimensions and aortic root size (eFigure 1, panels B and E, respectively).

**SNPs Related to Echocardiographic Traits Meeting Threshold for Genome-wide Significance in Stage 1**

Figure 1 illustrates the primary findings from the stage 1 meta-analysis and displays the genome-wide P values for interrogated SNPs across the 22 autosomal chromosomes separately for each of the 6 echocardiographic traits. Table 2 lists the 16 genetic loci (and the SNP with the lowest P value at each locus) associated with echocardiographic...
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graphic traits that were marked by 1 or more SNPs with \( P < 5 \times 10^{-5} \), the pre-specified genome-wide significance threshold: 3 loci each for LV mass and LV wall thickness, 1 locus each for LV diastolic internal dimension and LV systolic dysfunction, and 8 loci for aortic root diameter. There are 18 SNPs representing the 16 loci because 2 LV diastolic internal dimensions SNPs are correlated, as are 2 SNPs on chromosome 17 that are related to aortic root size \( (R^2 \geq 0.5) \). No SNP was associated with left atrial size at the genome-wide significance threshold. The section “Loci Associated With Echocardiographic Traits in Stage 1” of the supplementary material provides a description of these genetic loci and eTable 3 amplifies the details of the SNPs listed in Table 2 with regard to their imputation status and the quality of imputation. We provide in eTables 4 through 9 a list of all SNPs associated with each of the echocardiographic traits at a meta-analytic \( P < 1 \times 10^{-3} \) level. (All supplemental material is available at http://www.jama.com.)

SNPs Related to Echocardiographic Traits in Stage 2 (Replication)

Table 2 shows the association and 1-sided \( P \) value for each stage 1 locus in the stage 2 replication samples. Seven of the 17 SNPs (representing 15 loci; 1 LV mass SNP was not subjected to replication, given very low minor allele frequency) tested in Table 2 replicated, including 2 for LV diastolic dimensions, and 5 for aortic root dimensions. Five of these 7 replicated SNPs were genotyped in at least 1 of the replication cohorts (eTable 3 available at http://www.jama.com). The replicated SNPs explained only a modest proportion in the variance of LV diastolic dimensions (increments in \( R^2 \) attributable to SNPs were 0.0 in the Rotterdam Study, 0.002 in the Cardiovascular Health Study, 0.004 in the Gutenberg Heart Study, and 0.005 in KORA and the Framingham Heart Study) and aortic root size (increments in \( R^2 \) attributable to SNPs were 0.01 in the Cardiovascular Health Study and KORA, 0.02 in the Rotterdam Study, and 0.03 in the Framingham Heart Study; eTable 10 available at http://www.jama.com). Figure 2 displays the stage 1 forest plots for each of these 7 SNPs. eFigure 2 (Panels A-B, available at http://www.jama.com) shows the regional plots for the associations centered on these 7 SNPs.

Table 2 also displays the \( P \) values for combined meta-analysis of the 17 SNPs in stages 1 and 2.

COMMENT

We identified novel findings for 5 genetic loci that are associated with LV structure (1 locus) and aortic root diameter (4 loci). The effect sizes for the observed associations were generally very modest, and the proportion of variance explained was 1% to 3% for aortic root size, and 0.2% to 0.5% for LV diastolic dimensions. However, since the causal variants have not

Table 1. Study Sample Characteristics

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<th>Clinical Characteristics</th>
<th>Cardiovascular Health Study</th>
<th>Rotterdam Study</th>
<th>KORA</th>
<th>Framingham Heart Study</th>
<th>Gutenberg Heart Study</th>
<th>Study of Health in Pomerania</th>
<th>Austrian Stroke Prevention Study</th>
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<td>594 (18)</td>
<td>867 (27)</td>
<td>97 (11)</td>
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</table>

Echocardiographic Characteristics

| LV mass, g               | 172 (48)                    | 133 (33)       | 162 (36)| 186 (40)               | 145 (37)             | 185 (33)                    | 212 (57)                      |
| LV diastolic dimensions, cm | 5.1 (0.6)               | 4.7 (0.5)       | 5.3 (0.5)| 5.0 (0.4)              | 4.6 (0.3)            | 5.1 (0.3)                   | 4.7 (0.3)                     |
| LV wall thickness, cm    | 1.8 (0.3)                   | 1.7 (0.2)       | 1.6 (0.3)| 2.0 (0.3)              | 2.0 (0.2)            | 1.8 (0.2)                   | 2.1 (0.3)                     |
| Left atrial size, cm     | 4.1 (0.8)                   | 3.8 (0.6)       | 4.2 (0.6)| 4.0 (0.4)              | 3.7 (0.5)            | 4.0 (0.4)                   | NA NA                         |
| Aortic root, cm          | 3.5 (0.6)                   | 3.0 (0.3)       | 3.6 (0.4)| 3.1 (0.4)              | 3.4 (0.4)            | 2.9 (0.3)                   | NA NA                         |
| LV systolic dysfunction, No. (%) | 237 (7)                  | 266 (12)       | 48 (8) | 159 (5)                | 165 (5)             | 405 (13)                    | 111 (13)                      |

Abbreviations: BP, blood pressure; KORA, Cooperative Health Research in the Region of Augsburg study; LV, left ventricular; NA, not available in Gutenberg Heart Study.

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been identified, our investigation may underestimate the proportion of variance explained by these loci. Four of the replicated SNPs (2 each that were associated with LV diastolic dimensions and aortic root size) were within genes.

**Novel Loci Associated With LV Structure and Function**

Left ventricular diastolic dimension was associated with 2 SNPs presumably marking the same 6q22 locus and that included the SLC35F1 (GenBank BC028615) and C6orf204 (GenBank AF308284) genes. SLC35F1 codes a membrane protein that belongs to the solute transporter family. Its role in cardiac physiology is unknown, although the protein is expressed in cardiac tissue. Some of the associated SNPs at the 6q22 locus are in C6orf204, which is expressed in cardiac tissue and encodes a protein (coiled-coil domain containing protein C6orf204) whose function is unclear. One of the top SNPs in this gene (rs11968176), is about 100 kb from PLN (RefSeq NM_002667, which encodes phospholamban, a protein that inhibits cardiac muscle sarcoplasmic reticulum Ca2+/ATPase and regulates diastolic function. Mutations in PLN have been implicated in the pathogenesis of dilated cardiomyopathy.

**Figure 1. Genome-wide Signal Intensity Plots**

The plots show the single-nucleotide polymorphism–wise log $P$ values (based on the fixed-effects meta-analysis) against their genomic position for left ventricular mass, internal dimensions, wall thickness, and systolic dysfunction and for the aortic root diameter and left atrial size. Within each chromosome, shown on the x-axis, the results are plotted from the p-terminal end. The horizontal dotted lines indicate the significance threshold of $P=5 \times 10^{-7}$.
Novel Loci Associated With Aortic Root Diameter

Aortic root size was associated with 5 SNPs presumably representing 4 genetic loci. The SNP at 17p13, rs10852932, is in the gene SMG6 ([GenBank:AB018275] Smg-6 homologue, nonsense mediated mRNA decay factor). SMG6 is expressed in aortic tissue and encodes a component of the telomerase ribonucleoprotein (RNP) complex that is essential for the replication of chromosome termini. This protein may have a general role in telomere regulation, including promoting the ability of telomerase reverse transcriptase to elongate telomeres. Of note, telomerase activity is up-regulated in the aorta of spontaneous hypertensive rats, and PDE3A ([Ref-Seq:NM_000921] phosphodiesterase 3A, cGMP-inhibited; 291 kb), all 3 genes are expressed in aortic tissue. CCDC100 encodes a centrosomal protein that has a role in development of the neocortex; its function in cardiac or vascular tissue remains unclear. HMGA2 encodes a protein with structural DNA-binding domains that acts as a transcriptional regulator. It is expressed largely during embryogenesis and has been linked to vascular tumors including angiomyxomas and pulmonary hamartomas.

Critical role in vascular remodeling in hypertension.

Aortic root diameter was also associated with SNPs at 3 genetic loci that were intergenic, located at variable distances from CCDC100 ([GenBank:AK095646] centrosomal protein 120kDa [also referred to as CEPT120]; 149 kb), HMGA2 ([GenBank:U28754] high mobility group AT-hook 2; 35 kb), and PDE3A ([Ref-Seq:NM_000921] phosphodiesterase 3A, cGMP-inhibited; 291 kb), all 3 genes are expressed in aortic tissue. CCDC100 encodes a centrosomal protein that has a role in development of the neocortex; its function in cardiac or vascular tissue remains unclear. HMGA2 encodes a protein with structural DNA-binding domains that acts as a transcriptional regulator. It is expressed largely during embryogenesis and has been linked to vascular tumors including angiomyxomas and pulmonary hamartomas.

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Table 2. Genetic Loci in Which Single-Nucleotide Polymorphisms Associated With Echocardiographic Traits With $P < 5 \times 10^{-7}$ (Stage 1) and Replication of These SNPs (Stage 2)

<table>
<thead>
<tr>
<th>Echocardiographic Trait</th>
<th>SNP</th>
<th>Locus</th>
<th>SNP Type</th>
<th>Nearest Gene</th>
<th>Major/Minor Allele (Minor Allele Frequency)$^b$</th>
<th>Stage 1 Effect Size (SE)$^c$</th>
<th>Meta-analysis $P$ Value$^d$</th>
<th>Stage 2, One-sided $P$ Value$^e$</th>
<th>Stages 1 + 2, Meta-analysis $P$ Value$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass, gm</td>
<td>rs17568359</td>
<td>14q12</td>
<td>Intergenic</td>
<td>NOA1</td>
<td>G/C (0.07)</td>
<td>-4.78 (0.89)</td>
<td>$8.53 \times 10^{-8}$</td>
<td>DNR</td>
<td>$1.66 \times 10^{-5}$</td>
</tr>
<tr>
<td>LV internal diastolic dimensions, cm</td>
<td>rs756161</td>
<td>2p21</td>
<td>Intergenic</td>
<td>CALM2</td>
<td>G/A (0.40)</td>
<td>-3.01 (0.59)</td>
<td>$3.19 \times 10^{-7}$</td>
<td>DNR</td>
<td>$9.64 \times 10^{-5}$</td>
</tr>
<tr>
<td>LV wall thickness, cm</td>
<td>rs8031633</td>
<td>15q14</td>
<td>Intergenic</td>
<td>MEIS2</td>
<td>T/C (0.006)</td>
<td>16.62 (2.27)</td>
<td>$3.71 \times 10^{-7}$</td>
<td>Not done</td>
<td>-</td>
</tr>
<tr>
<td>LV systolic dysfunction</td>
<td>rs89107</td>
<td>6p22</td>
<td>Intergenic</td>
<td>SLCS3F1</td>
<td>A/G (0.50)</td>
<td>-0.03 (0.005)</td>
<td>$1.14 \times 10^{-6}$</td>
<td>$0.003e$</td>
<td>$1.21 \times 10^{-9}$</td>
</tr>
<tr>
<td>Aortic root size, cm$^f$</td>
<td>rs1113376</td>
<td>6p22</td>
<td>Intergenic</td>
<td>SORT204,PLN</td>
<td>C/T (0.45)</td>
<td>0.03 (0.005)</td>
<td>$4.61 \times 10^{-7}$</td>
<td>$0.002e$</td>
<td>$1.67 \times 10^{-8}$</td>
</tr>
<tr>
<td>Aortic root size, cm$^f$</td>
<td>rs7910620</td>
<td>10q23</td>
<td>Intergenic</td>
<td>GRID1</td>
<td>C/G (0.009)</td>
<td>0.17 (0.03)</td>
<td>$5.62 \times 10^{-7}$</td>
<td>NS</td>
<td>$6.69 \times 10^{-7}$</td>
</tr>
<tr>
<td>Aortic root size, cm$^f$</td>
<td>rs2059238</td>
<td>16q23</td>
<td>Intergenic</td>
<td>I WWOX</td>
<td>C/A (0.22)</td>
<td>-0.02 (0.004)</td>
<td>$1.89 \times 10^{-7}$</td>
<td>NS</td>
<td>$2.84 \times 10^{-4}$</td>
</tr>
<tr>
<td>Aortic root size, cm$^f$</td>
<td>rs71132611</td>
<td>5q21</td>
<td>Intergenic</td>
<td>SLC25A46</td>
<td>C/T (0.015)</td>
<td>0.086 (0.05)</td>
<td>$3.38 \times 10^{-7}$</td>
<td>$0.007$</td>
<td>$3.92 \times 10^{-8}$</td>
</tr>
<tr>
<td>Aortic root size, cm$^f$</td>
<td>rs2234587</td>
<td>16p13</td>
<td>Intergenic</td>
<td>HN1L</td>
<td>A/G (0.22)</td>
<td>-0.38 (0.07)</td>
<td>$1.98 \times 10^{-7}$</td>
<td>$0.10$</td>
<td>$6.53 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

Abbreviations: DNR, did not replicate; LV, left ventricular; SNP, single-nucleotide polymorphisms.

$^a$See eTables 4 through 9 (available at www.jama.com) for exact location. No replication attempted for rs8031633 because of low minor allele frequency. Note that the 18 SNPs likely represent 16 genetic loci: rs89107 and rs11133768 are correlated ($r^2 = 0.5$), as are rs10852932 and rs45239575 ($r^2 = 0.84$) and so may represent the same loci, but are shown separately in the table because they are in different genes.

$^b$Alleles for the SNP on the forward strand of human genome reference sequence (National Center for Biotechnology Information Build 36) were modeled.

$^c$Inverse variance-weighted meta-analysis performed as detailed in the "Methods" section.

$^d$SNPs that replicated (based on 1-sided $P < .05$). DNR indicates that the $P$ was in the opposite direction (no $P$ values provided as tests were 1-sided).

$^e$Only 4 studies in stage 1 (Cardiovascular Heart Study, Rotterdam Study, Cooperative Health Research in the Region of Augsburg study, Framingham Heart Study) contributed to genome-wide association of aortic root size.

Strengths and Limitations

The large community-based studies, the common method of M-mode echocardiography; and the implementation of quality control procedures in individual
Several limitations of our investigation merit comment. First, phenotypic and study design heterogeneity diminished statistical power to detect modest genetic effects in genome-wide association. Measurement errors would bias the estimates toward the null hypothesis of no association of SNPs. In this context, it should be noted that M-mode measurements of the aortic root may be less accurate and can result in underestimation of aortic diameter (compared with 2-dimensional images). Furthermore, our approach has limited statistical power to evaluate associations of traits with rare SNPs or with poorly imputed SNPs. We evaluated additive models using pooled sex analy-
GENETIC VARIANTS AND CARDIAC STRUCTURE AND FUNCTION

CONCLUSIONS

Our prospective meta-analysis of echocardiographic data from more than 12,000 participants in 5 community-based cohorts with replication in more than 4000 people from 2 other cohorts identified 5 genetic loci that are associated with interindividual variation in cardiac dimensions and aortic root size. These findings are novel, but the loci explained a very small proportion of the variance of the traits. Additional investigations are required to replicate our findings, to identify the underlying causal variants and characterize their functional importance, to understand the biological mechanisms underlying the observed associations, and to determine whether they are related to overt cardiovascular disease.

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Author Contributions: Dr Vasan had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The following authors contributed equally to the manuscript: Drs Ramachandran S. Vasan, Nicole J. Glazer, and Stephen F. Wild. Drs S. Felix, J. Felix, and Wild designed the study; Drs S. Felix, J. Felix, and Wild performed the analysis; Drs S. Felix, J. Felix, and Wild interpreted the results; and Drs S. Felix, J. Felix, and Wild drafted the manuscript.


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REFERENCES


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It is nonsense for you to talk of old age as long as you outrun young men in the race for service and in the midst of anxious times fill rooms with your laughter and inspire youth with hope when they are on the brink of despair.

—Mohandas K. Ghandhi (1869-1948)