Genetic Loci Associated With Atrial Fibrillation: Relation to Left Atrial Structure in the Framingham Heart Study

ORIGINAL RESEARCH

Genetic Loci Associated With Atrial Fibrillation: Relation to Left Atrial Structure in the Framingham Heart Study

Jared W. Magnani, MD, MSc; Xiaoyan Yin, PhD; David D. McManus, MD; Michael L. Chuang, MD; Susan Cheng, MD; Steven A. Lubitz, MD, MPH; Garima Arora, MD; Warren J. Manning, MD; Patrick T. Ellinor, MD, PhD;* Emelia J. Benjamin, MD, ScM*

***Background*—**Atrial fibrillation (AF) results in significant morbidity and mortality. Genome-wide association studies (GWAS) have identified genetic variants associated with AF. Whether genetic variants associated with AF are also associated with atrial structure, an intermediate phenotype for AF, has had limited investigation. We sought to investigate associations between single nucleotide polymorphisms (SNPs) and atrial structure obtained by cardiovascular imaging in the Framingham Heart Study.

***Methods and Results*—**We selected 11 SNPs that have been associated with AF in GWAS. We examined the SNPs' relations to cross-sectional left atrial (LA) dimensions (determined by transthoracic echocardiography) and LA volume (determined by cardiovascular magnetic resonance [CMR]) employing linear regression. The total sample included 1555 participants with CMR LA volume (age 60±9 years, 53% women) and 6861 participants with echocardiographic LA diameter (age 48±13 years, 52% women) measured. We employed a significance threshold of $P<0.0023$ to account for multiple testing of the 11 SNPs and 2 LA measures. In a primary analysis, no SNPs were significantly related to the LA measures. Likewise, in secondary analyses excluding individuals with prevalent AF (n=77, CMR sample; n=105, echocardiography sample) no SNPs were related to LA volume or diameter.

***Conclusion*—**In a community-based cohort, we did not identify a statistically significant association between selected SNPs associated with AF and measures of LA anatomy. Further investigations with larger longitudinally assessed samples and a broader array of SNPs may be necessary to determine the relation between genetic loci associated with AF and atrial structure. **(*J Am Heart Assoc*. 2014;3:e000616 doi: 10.1161/JAHA.113.000616)**

Key Words: atrial fibrillation • cohort studies • genetics • left atrium • single nucleotide polymorphism

Atrial fibrillation (AF) is increasing in prevalence and is associated with significant morbidity and mortality.[1] Large genome-wide association studies (GWAS) have identified novel genetic loci related to AF and PR interval. To date, 11 AF-associated, single nucleotide polymorphisms (SNPs) have been identified and replicated.[2–7] Furthermore, left atrial size has been related to the occurrence of incident AF and recurrence following radiofrequency catheter ablation.[8,9] Whether SNPs associated with AF are related to LA size or dimensions has had limited investigation. We therefore hypothesized that SNPs related to AF would be associated with measures of atrial structure, an AF intermediate phenotype. We specifically examined the relations between each SNP and left atrial (LA) dimension, as determined by

From the National Heart, Lung and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA (J.W.M., X.Y., D.D.M., M.L.C., S.C., E.J.B.); Section of Cardiovascular Medicine, Department of Medicine, Boston University, Boston, MA (J.W.M., E.J.B.); Departments of Biostatistics (X.Y.) and Epidemiology (E.J.B.), Boston University School of Public Health, Boston, MA; Section of Cardiology, Department of Medicine, University of Massachusetts, Worcester, MA (D.D.M.); Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (S.C.); Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA (S.A.L., P.T.E.); Cardiac Arrhythmia Service, Massachusetts General Hospital, Boston, MA (S.A.L., P.T.E.); Departments of Medicine and Radiology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA (G.A., W.J.M.); Section of Preventive Medicine and Epidemiology, Boston University School of Medicine, Boston, MA (E.J.B.).

This article was handled independently by Julie Johnsonn, PharmD, as a Guest Editor. The editors had no role in the evaluation of the manuscript or in the decision about its acceptance.

*Dr. Ellinor and Dr. Benjamin contributed equallty to this work.

Correspondence to: Jared W. Magnani, MD, MSc, Section of Cardiovascular Medicine, Boston University School of Medicine, 88 E. Newton Street, Boston, MA 02118. E-mail: jmagnani@bu.edu

Received October 23, 2013; accepted February 26, 2014.

transthoracic echocardiography, and LA volume, as determined by cardiovascular magnetic resonance (CMR). Demonstrating such an association would suggest that genetic associations related to AF may also play a role in atrial structure.

Methods

Study Sample

The Framingham Heart Study is a prospective, community-based investigation of the epidemiology of cardiovascular disease. In brief, the Study began in 1948 by enrolling the Original Cohort, followed by the children of the Original Cohort and their spouses in 1971, known as the Offspring Cohort.[10] In turn, children of the Offspring Cohort were enrolled in 2002 as the Third Generation Cohort.[11] Participants have undergone routine examinations every 2 to 8 years since enrollment including extensive phenotyping with periodic noninvasive cardiovascular imaging studies and comprehensive genotyping. The present analysis included participants who attended Offspring Cohort examination 6 (n=3532), examination 7 (n=3539), and the Third Generation's first examination (n=4095). The study was approved by the Boston University Medical Center Institutional Review Board, and all participants provided written informed consent.

Echocardiography

Participants in Offspring examination 6 (1995–1998) and Third Generation examination 1 (2002–2005) underwent transthoracic echocardiography employing a standardized protocol. Echocardiography in the Framingham Heart Study has been well described and was performed using a Hewlett-Packard Sonos 1000 in the Offspring Cohort and Hewlett-Packard Sonos 5500 in the Third Generation Cohort (Hewlett-Packard Medical Products) by certified sonographers and over-read by technicians and cardiologists.[12,13] LA diameter measurement was conducted according to guidelines from the American Society of Echocardiography[14] and entailed M-mode measurement recorded at end-systole in the parasternal long- or short-axis views. The maximum distance from the wall of the posterior aortic root to the posterior LA was measured in up to 4 cycles for each participant. Prior analyses have described excellent inter- and intra-rater reproducibility for Framingham Heart Study LA diameter measurement using this approach.[15] In total, 6861 Framingham Heart Study participants underwent echocardiography at the examinations relevant to this analysis. Left ventricular mass and fractional shortening also were calculated as described previously.[12] All echocardiography reading was done offline by readers who were blinded to participant clinical characteristics.

Cardiovascular Magnetic Resonance

A subset of participants in the Offspring examination 7 (1998–2001) was included in a study using noncontrast CMR. Exclusions for CMR included any identified contraindication to CMR (eg, defibrillator or pacemaker implantation) or severe claustrophobia. Participants underwent imaging in the supine position in a 1.5 Tesla MRI scanner (Gyroscan NT; Philips Healthcare). After scout images to determine the position and orientation of the heart within the thorax an electrocardiogram-gated, steady-state free precession cine sequence was acquired in the anatomic 2-chamber and 4-chamber orientations. Imaging measures included TR=3.2 ms, TE=1.6 ms, flip angle=60°, slice thickness=10 mm with 1.9×1.6-mm^2 in-plane spatial resolution. Images were processed off-line using commercially available software (MASS v.6.1; QT-Medis) by a single observer (GA). In each view, the systolic frame immediately prior to mitral valve opening was identified and the cross-sectional area of the LA was manually planimetered. Particular care was taken to exclude left atrial appendage and pulmonary vein ostia from left atrial area. Left atrial volume was then calculated using the biplane formula: $=8\times A_{2c}\times A_{4c}/(3\pi L_{min})$ for which A_{2c} and A_{4c} are the planimetered areas and L_{min} is the minimum of the 2-chamber and 4-chamber LA lengths. In total, 1810 participants from the Offspring Cohort underwent CMR using a standardized protocol.[16] All readers were blinded to participant identity and clinical characteristics.

Genotyping and SNP Selection

Genotyping of Framingham Heart Study participants has been described elsewhere.[17] In the present study, SNPs were selected from their established association with AF in large GWAS including Framingham Heart Study Offspring and Original Cohort participants.[2–7] Only SNPs that had reached statistical significance in GWAS meta-analyses after application of Bonferroni correction ($P<5.0\times10^{-8}$) were included in the present analysis.

Covariates

Covariates were selected for their established associations with AF in the Framingham Heart Study and other cohorts, and for their potential relation to longitudinal modification of atrial structure.[13,18,19] Covariates selected for inclusion were age, sex, body mass index, systolic blood pressure, treatment for hypertension, valvular heart disease, and prevalent

cardiovascular disease and heart failure. Participants with prevalent AF, ie, a history of documented AF according to Framingham Heart Study criteria that were present at or prior to the imaging assessment, were included in our primary analysis. The Framingham Heart Study protocol for adjudicating AF has been described elsewhere.[19]

Statistical Analyses

We examined the distributions of continuous variables (means, standard deviations, summaries). We examined correlations between clinical characteristics and cardiac imaging measures using the Pearson product-moment correlation coefficient. For CMR analysis we employed linear mixed models to determine the associations between selected SNPs and measures of LA volume with a random effect to account for sibling correlation. For echo analysis considering that the sample was composed of 2 generations, we used generalized estimating equations to obtain robust standard errors in evaluating the association between SNPs and LA dimension. All correlation and regression models were adjusted for age and sex. We conducted a primary analysis including participants with prevalent AF, and a secondary analysis that excluded participants with prevalent AF. We conducted a post hoc power calculation using the method described by Cohen.[20] All analyses were performed using SAS version 9.2 (SAS Institute). A P value <0.0023 (0.05 with 11 SNPs and 2 LA phenotypes) was deemed statistically significant to account for multiple testing.

Results

The study sample for the examination of selected SNPs and LA measures consisted of 6861 participants with echocardiographic LA dimension and 1555 participants with CMR LA volume. The LA imaging measures were considered as separate assessments in relation to the selected AF SNPs. In total 1465 participants had both CMR and echocardiography, 90 CMR only, and 5396 echocardiography only. We analyzed them separately because they were done at different time points and with different technology.

The characteristics of the study samples and the distribution of LA measures are described in Table 1. The sample with echocardiographic LA dimension was larger and younger than the CMR LA volume sample, reflecting the contribution of the younger Third Generation Cohort and earlier examination of the Offspring Cohort. Mean left ventricular mass differed between echocardiographic and CMR assessments. The mean LA volume by CMR was 73.5 ± 23.8 mL. Echocardiographic LA volume was not determined. The number of participants with prevalent AF was limited (5.0% of the CMR cohort and 1.5% of the echocardiography cohort).

Table 2 describes correlations between the clinical characteristics and the imaging measures. The correlation between LA volume and LA diameter was moderate ($r=0.41$, $P<0.001$). We observed a significant correlation ($r=0.33$, $P<0.001$) between LA volume and left ventricular mass as measured by CMR. We also found significant, modest associations between both LA volume and LA dimension with prevalent AF.

The associations between the relevant SNPs and the LA measures are shown in Table 3. Two SNPs were associated with CMR LA volume as determined by CMR. Both SNPs were in proximity to the *PITX2* gene, which regulates cardiac development and atrial electrical function.[21] When we adjusted for body surface area, the associations between these 2 SNPs and LA volume were no longer significant.

In secondary analyses we excluded participants with prevalent AF (n=77 in the CMR sample and n=105 in the echocardiography sample). Results of the secondary analysis are reported in Table 4. The statistical association between 2 of the *PITX2* SNPs and LA volume as measured by CMR were nominally significant, but none of the findings met our threshold for statistical significance. A single SNP (rs2200733) in proximity to *PITX2* was associated ($\beta=-1.22\pm0.60$, $P=0.04$) with LA volume following adjustment for body surface area. A second SNP (rs2106261) in proximity to the *ZFHX3* gene was related ($\beta=-0.22\pm0.11$, $P=0.04$) to LA diameter. We were further concerned about potential misclassification of atrial structure by including individuals with valvular heart disease. Therefore, we conducted an analysis excluding the $\approx$2% of individuals with valvular heart disease; the results were not substantively affected (Table 5).

In view of our negative findings, we conducted a post-hoc power calculation. Among the 11 SNPs, rs2200733 had the smallest P value in association with LA volume ($P=0.02$), explaining 0.23% of residual variability in LA volume. Given the limited-sized sample (n=1555), we had only 14% statistical power to detect such an effect size at alpha=0.0023 level. With this sample size, we would have 40% and 60% power to detect a SNP explaining 0.50% and 0.69% variability in LA volume, respectively. Rs2106261 had the smallest P value in association with LA diameter as determined by echocardiography ($P=0.06$), explaining 0.10% of the residual variability in LA diameter. Given the sample size (n=6861), we had 38% power to detect such an effect size at alpha=0.0023 level. We would have 50% and 70% power to detect a SNP explaining 0.14% and 0.19% variability in LA dimension, respectively.

Discussion

We hypothesized that selected SNPs, identified from large GWAS for AF, are associated with measures of atrial structure

Table 1. Clinical Characteristics of the Framingham Heart Study Samples According to Cardiac Imaging Modality

	CMR (n=1555)	Echocardiography (n=6861)
Clinical characteristics		
Age, y	60±9	48±13
Women, %	827 (53)	3626 (52)
Body mass index, kg/m^2	27.8±4.8	27.3±5.3
Body surface area, m^2	1.90±0.23	1.91±0.25
Systolic blood pressure, mm Hg	125±18	122±17
Hypertension treatment, %	450 (29)	1144 (17)
Prevalent cardiovascular disease, %	93 (6.0)	186 (2.7)
Prevalent atrial fibrillation, %	77 (5.0)	104 (1.5)
Valvular heart disease, %	31 (2.0)	124 (1.8)
Imaging		
LV ejection fraction, %	67±7	—
LV mass, g	106±30	159±43
LA volume, mL	73.5±23.8	—
LA dimension, mm	—	38.2±5.3
LV fractional shortening	—	0.36±0.05

Continuous measures provided as means±SD and categorical as N (%). CMR indicates cardiovascular magnetic resonance; LA, left atrium; LV, left ventricular.

determined by noninvasive cardiac imaging in a community-based cohort. Our hypothesis was informed by animal and human electrophysiologic studies and further consideration of genetic contributions to atrial electrical and structural remodeling. As atrial structure serves as an intermediate phenotype for AF and is directly related to risk,[8,22] we postulate that AF genetic variants mediated atrial structural remodeling. However, our analysis did not identify a significant association between the genetic loci and atrial structures.

The AF SNPs examined in our analysis were selected from GWAS. Specifically, 3 of the SNPs studied here (rs2200733, rs17570669, and rs3853445) are on chromosome 4q25 and are located upstream of the *PITX2* gene, which is relevant for atrial tissue development. *PITX2c*-deficient mice do not develop pulmonary myocardial cells or pulmonary myocardial sleeves.[23] The *PITX2c* isoform impedes LA sinus node development.[24] *PITX2c* −/− increases vulnerability to atrial arrhythmias.[25] In studies of human atrial tissue, *PITX2* expression levels are ≈2 orders of magnitude higher in the LA compared to the right atrium or the ventricles.[26] Finally, *PITX2c* heterozygote mice were susceptible to AF induced by pacing and had shorter atrial action potential durations

Table 2. Age- and Sex-Adjusted Correlations (r) Between Selected Clinical and Imaging Variables in the Framingham Heart Study

	Cardiovascular Magnetic Resonance LA Volume (mL)	Echocardiography LA Dimension (mm)
Body mass index, kg/m^2	0.17*	0.45*
Systolic blood pressure, mm Hg	0.08†	0.14*
Hypertension treatment	0.13*	0.18*
Prevalent cardiovascular disease	0.13*	0.10*
Prevalent atrial fibrillation	0.21*	0.14*
LV ejection fraction, %‡	−0.03	−0.03
LV mass, g	0.33*	0.60*
LA diameter, mm	0.41*	—

LA indicates left atrium; LV, left ventricular.
*P<0.001. †P<0.05. ‡Refers to fractional shortening for echocardiography assessment.

Table 3. Age- and Sex-Adjusted Associations Between AF SNPs and LA Measures

	SNP			Cardiovascular Magnetic Resonance				Echocardiography	
				LA Volume (mL)		LA Volume, Indexed to BSA		LA Dimension (mm)	
	Chr	Coded Allele*	Closest Gene	Estimate±SE	*P* Value	Estimate±SE	*P* Value	Estimate±SE	*P* Value
rs2200733	4	T	*PITX2*	−2.52±1.19	0.04	−0.86±0.60	0.15	0.10±0.12	0.41
rs17570669	4	T	*PITX2*	−4.49±1.88	0.02	−1.59±0.94	0.09	−0.09±0.18	0.59
rs3853445	4	C	*PITX2*	−1.12±0.90	0.18	−0.51±0.45	0.26	−0.06±0.09	0.51
rs13376333	1	T	*KCNN3*	0.95±1.02	0.35	0.45±0.51	0.38	−0.05±0.10	0.62
rs2106261	16	T	*ZFHX3*	−0.41±1.14	0.72	−0.51±0.57	0.37	−0.21±0.11	0.06
rs6666258	1	C	*KCNN3*	0.95±1.02	0.35	0.45±0.52	0.38	−0.05±0.10	0.62
rs3807989	7	A	*CAV1*	0.29±0.84	0.73	0.33±0.42	0.42	−0.08±0.08	0.35
rs10821415	9	A	*C9orf3*	−0.58±0.82	0.48	−0.22±0.41	0.56	−0.10±0.11	0.38
rs10824026	10	G	*SYNPO2L*	0.00±1.09	0.99	0.06±0.55	0.91	−0.002±0.08	0.98
rs1152591	14	A	*SYNE2*	0.68±0.82	0.41	0.49±0.42	0.24	0.05±0.09	0.57
rs7164883	15	G	*HCN4*	0.52±1.07	0.63	0.14±0.54	0.80	−0.12±0.11	0.28

All analyses age- and sex-adjusted, and echocardiography results further adjusted for cohort. AF indicates atrial fibrillation; BSA, body surface area; Chr, chromosome; LA, left atrium; SNP, single nucleotide polymorphism.

*All coded alleles are minor alleles and refer to the allele associated with AF risk as identified in genome-wide association studies referenced in text.

compared with the wild type controls.[27] Our study did not identify an association between the 4q25 SNPs and measures of atrial structure. Further studies of the AF-associated SNPs, *PITX2* and expression are essential to determine their relevance towards atrial structure and function.

Many of the other AF-risk SNPs do not have a clear relation to cardiac development or atrial structure. SNP rs13376333 is intronic to *KCNN3*, a gene that encodes a calcium-activated potassium channel.[4] SNP rs2106261 is found in a zinc finger homeobox transcription factor, *ZFHX3*, which participates in myogenic and neuronal differentiation. The association of this SNP with AF has been replicated in an Asian cohort and associated with coronary disease in an African American cohort.[27,28] SNP rs3807989 is intronic to *CAV1*, and has been associated with AF and the PR and QRS intervals.[17,29,30]

Table 4. Age- and Sex-Adjusted Associations Between AF SNPs and LA Measures, Excluding Prevalent AF

	SNP			Cardiovascular Magnetic Resonance				Echocardiography	
				LA Volume (mL)		LA Volume, Indexed to BSA		LA Dimension (mm)	
	Chr	Coded Allele*	Closest Gene	Estimate±SE	*P* Value	Estimate±SE	*P* Value	Estimate±SE	*P* Value
rs2200733	4	T	*PITX2*	−3.15±1.18	0.01	−1.22±0.60	0.05	0.03±0.11	0.80
rs17570669	4	T	*PITX2*	−4.63±1.81	0.01	−1.74±0.92	0.06	−0.05±0.18	0.78
rs3853445	4	C	*PITX2*	−1.13±0.89	0.20	−0.47±0.45	0.30	−0.03±0.09	0.75
rs13376333	1	T	*KCNN3*	0.58±1.00	0.56	0.31±0.51	0.55	−0.04±0.09	0.66
rs2106261	16	T	*ZFHX3*	−0.91±1.12	0.42	−0.77±0.57	0.18	−0.22±0.11	0.04
rs6666258	1	C	*KCNN3*	0.58±1.00	0.56	0.31±0.51	0.55	−0.04±0.09	0.66
rs3807989	7	A	*CAV1*	0.04±0.83	0.96	0.15±0.42	0.73	0.01±0.08	0.91
rs10821415	9	A	*C9orf3*	−1.03±0.81	0.21	−0.36±0.41	0.38	−0.04±0.09	0.66
rs10824026	10	G	*SYNPO2L*	1.47±1.07	0.17	0.72±0.55	0.19	−0.08±0.11	0.48
rs1152591	14	A	*SYNE2*	0.61±0.81	0.45	0.31±0.41	0.46	0.06±0.08	0.44
rs7164883	15	G	*HCN4*	1.18±1.05	0.27	0.50±0.54	0.35	−0.02±0.11	0.87

All analyses age- and sex-adjusted, and echocardiography results further adjusted for cohort. AF indicates atrial fibrillation; BSA, body surface area; Chr, chromosome; LA, left atrium; SNP, single nucleotide polymorphism.

*All coded alleles are minor alleles and refer to the allele associated with AF risk as identified in genome-wide association studies referenced in text.

Table 5. Age- and Sex-Adjusted Associations Between AF SNPs and LA Measures, Excluding Prevalent AF and VHD

	SNP			Cardiovascular Magnetic Resonance				Echocardiography	
				LA Volume (mL)		LA Volume, Indexed to BSA		LA Dimension (mm)	
	Chr	Coded Allele*	Closest Gene	Estimate±SE	*P* Value	Estimate±SE	*P* Value	Estimate±SE	*P* Value
rs2200733	4	T	*PITX2*	−2.81±1.16	0.02	−1.08±0.60	0.07	0.03±0.11	0.78
rs17570669	4	T	*PITX2*	−4.64±1.79	0.01	−1.86±0.92	0.04	0.01±0.18	0.96
rs3853445	4	C	*PITX2*	−1.15±0.88	0.19	−0.55±0.45	0.22	−0.01±0.09	0.92
rs13376333	1	T	*KCNN3*	0.87±0.99	0.38	0.43±0.51	0.40	−0.02±0.10	0.80
rs2106261	16	T	*ZFHX3*	−1.56±1.10	0.16	−0.1.05±0.56	0.06	−0.25±0.11	0.02
rs6666258	1	C	*KCNN3*	0.87±0.99	0.38	0.43±0.51	0.40	−0.02±0.10	0.80
rs3807989	7	A	*CAV1*	0.38±0.81	0.65	0.32±0.42	0.44	0.04±0.08	0.64
rs10821415	9	A	*C9orf3*	−1.01±0.80	0.65	−0.38±0.41	0.36	0.05±0.09	0.54
rs10824026	10	G	*SYNPO2L*	0.91±1.05	0.39	0.53±0.54	0.33	−0.08±0.11	0.45
rs1152591	14	A	*SYNE2*	0.72±0.80	0.37	0.38±0.41	0.36	0.09±0.08	0.27
rs7164883	15	G	*HCN4*	1.27±1.04	0.22	0.58±0.54	0.28	−0.05±0.11	0.64

All analyses age- and sex-adjusted, and echocardiography results further adjusted for cohort. AF indicates atrial fibrillation; BSA, body surface area; Chr, chromosome; LA, left atrium; SNP, single nucleotide polymorphism; VHD, valvular heart disease.

*All coded alleles are minor alleles and refer to the allele associated with AF risk as identified in genome-wide association studies referenced in text.

We considered that individuals with genetic polymorphisms making them susceptible to AF may experience altered LA structure.[8] However, all the 11 AF SNPs we examined were not significantly associated with LA volume and dimension in our samples. Further work with a larger sample and longitudinal measures of LA structure may discern if there is a genetic contribution towards atrial structure and remodeling.

We are not aware of other literature examining SNPs related to AF and measures of LA structure. A large (n=12 612) GWAS investigating genetic variants associated with echocardiographic traits did not identify genetic loci associated with LA size.[31] Comparison between these 2 studies is difficult as the prior echocardiographic study examined 2.5 million SNPs in relation to LA diameter in meta-analysis involving 5 cohorts. In the Framingham Heart Study, there may be further misclassification of LA size by conducting echocardiographic measurements in the anteroposterior diameter. M-mode LA measurement has a poor correlation with LA volume determined by echocardiography.[32]

Atrial structural characteristics are likely modified by multiple clinical factors, eg, hypertension, left ventricular diastolic properties, and unrecognized AF. Our analysis did not include left ventricular diastolic function or other structural abnormalities that could influence LA diameter or volume. Our study is not able to examine the association of the AF SNPs and functional assessments such as the left atrial functional index or conduction as measured by P wave indices.

Our study has several important strengths. The Framingham Heart Study echocardiographic LA dimension and the CMR LA volume measurements were reproducible and conducted using standardized techniques. The SNPs selected for inclusion were identified from a series of large GWAS. However, we note that our study has multiple limitations. First, the Framingham Heart Study is primarily of European descent, limiting our ability to comment on the generalizability of our results to more diverse cohorts. Second, we used strict criteria in the selection of the AF-related SNPs for association with LA dimensions. It is possible that other genetic loci associated with AF below stringent genome-wide significance thresholds may be related to atrial structure. Third, we performed the assessments of LA size at a single time-point. The AF associated SNPs may have a relation to longitudinal changes in LA structure that our analysis was not designed to assess. Consequently, we are not able to comment on the relation of the SNPs to longitudinal change that would distinguish atrial remodeling. Whether and how SNPs related to AF are related to trajectories or changes in LA dimensions remains an important question. Fourth, our echocardiography studies relied on M-mode for determining LA dimension, potentially introducing systematic misclassification of the echocardiography measures. Fifth, 43% of participants included in the echocardiography cohort also participated in the AF GWAS. While the overlap in participants may have biased our results, Framingham participants were included as part of large consortia for determining the GWAS related to AF. Finally, our power calculation indicates that our sample size had limited statistical power. Our study would be enhanced by a far larger sample size.

In summary, our primary analysis did not identify a relation between SNPs associated with AF and LA structure in a community-based study employing echocardiography and CMR for LA structural characterization. A secondary analysis

excluding individuals with prevalent AF identified marginal associations. Further efforts in larger cohorts are indicated; demonstrating the relation between genetic exposures and changes in atrial structure and function may identify key intermediate steps in the pathogenesis of AF. Future studies that examine additional genetic loci, genomic expression, more diverse cohorts, and the association with longitudinal changes in atrial structure and function may more completely characterize atrial remodeling and its pathophysiologic relations with AF.

Sources of Funding

This work was supported by grants from the American Heart Association (Award 09FTF219028) to Dr Magnani and (13EIA14220013) to Dr Ellinor, and from the NIH to Dr Magnani (5R21HL106092), Drs Benjamin and Ellinor (2RO1HL092577), Dr. Benjamin (RC1-HL101056, 1R01HL102214), Dr Manning (RO1 HL70279), and Dr Ellinor (1RO1HL104156, 1K24HL105780) and 6R01-NS17950, N01-HC25195. This work was further supported by a Boston University School of Medicine Department of Medicine Career Investment Award to Dr Magnani.

Disclosures

None.

References

1. Magnani JW, Rienstra M, Lin H, Sinner MF, Lubitz SA, McManus DD, Dupuis J, Ellinor PT, Benjamin EJ. Atrial fibrillation: current knowledge and future directions in epidemiology and genomics. *Circulation*. 2011;124:1982–1993.
2. Benjamin EJ, Rice KM, Arking DE, Pfeufer A, van Noord C, Smith AV, Schnabel RB, Bis JC, Boerwinkle E, Sinner MF, Dehghan A, Lubitz SA, D'Agostino RB Sr, Lumley T, Ehret GB, Heeringa J, Aspelund T, Newton-Cheh C, Larson MG, Marciante KD, Soliman EZ, Rivadeneira F, Wang TJ, Eiriksdottir G, Levy D, Psaty BM, Li M, Chamberlain AM, Hofman A, Vasan RS, Harris TB, Rotter JI, Kao WH, Agarwal SK, Stricker BH, Wang K, Launer LJ, Smith NL, Chakravarti A, Uitterlinden AG, Wolf PA, Sotoodehnia N, Kottgen A, van Duijn CM, Meitinger T, Mueller M, Perz S, Steinbeck G, Wichmann HE, Lunetta KL, Heckbert SR, Gudnason V, Alonso A, Kaab S, Ellinor PT, Witteman JC. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. *Nat Genet*. 2009;41:879–881.
3. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Müller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dörr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW, Wakili R, Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Völker U, Völzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE, Witteman JCM, Kao WHL, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjögren M, Newman AB, Liu Y, Gollob MH, Melander O, Tanaka T, Stricker BHC, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kääb S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet*. 2012;44:670–675.
4. Ellinor PT, Lunetta KL, Glazer NL, Pfeufer A, Alonso A, Chung MK, Sinner MF, de Bakker PI, Mueller M, Lubitz SA, Fox E, Darbar D, Smith NL, Smith JD, Schnabel RB, Soliman EZ, Rice KM, Van Wagoner DR, Beckmann BM, van Noord C, Wang K, Ehret GB, Rotter JI, Hazen SL, Steinbeck G, Smith AV, Launer LJ, Harris TB, Makino S, Nelis M, Milan DJ, Perz S, Esko T, Kottgen A, Moebus S, Newton-Cheh C, Li M, Mohlenkamp S, Wang TJ, Kao WH, Vasan RS, Nothen MM, MacRae CA, Stricker BH, Hofman A, Uitterlinden AG, Levy D, Boerwinkle E, Metspalu A, Topol EJ, Chakravarti A, Gudnason V, Psaty BM, Roden DM, Meitinger T, Wichmann HE, Witteman JC, Barnard J, Arking DE, Benjamin EJ, Heckbert SR, Kaab S. Common variants in KCNN3 are associated with lone atrial fibrillation. *Nat Genet*. 2010;42:240–244.
5. Gudbjartsson DF, Arnar DO, Helgadottir A, Gretarsdottir S, Holm H, Sigurdsson A, Jonasdottir A, Baker A, Thorleifsson G, Kristjansson K, Palsson A, Blondal T, Sulem P, Backman VM, Hardarson GA, Palsdottir E, Helgason A, Sigurjonsdottir R, Sverrisson JT, Kostulas K, Ng MC, Baum L, So WY, Wong KS, Chan JC, Furie KL, Greenberg SM, Sale M, Kelly P, MacRae CA, Smith EE, Rosand J, Hillert J, Ma RC, Ellinor PT, Thorgeirsson G, Gulcher JR, Kong A, Thorsteinsdottir U, Stefansson K. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature*. 2007;448:353–357.
6. Gudbjartsson DF, Holm H, Gretarsdottir S, Thorleifsson G, Walters GB, Thorgeirsson G, Gulcher J, Mathiesen EB, Njolstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Kucera G, Stubblefield T, Carter S, Roden D, Ng MC, Baum L, So WY, Wong KS, Chan JC, Gieger C, Wichmann HE, Gschwendtner A, Dichgans M, Kuhlenbaumer G, Berger K, Ringelstein EB, Bevan S, Markus HS, Kostulas K, Hillert J, Sveinbjornsdottir S, Valdimarsson EM, Lochen ML, Ma RC, Darbar D, Kong A, Arnar DO, Thorsteinsdottir U, Stefansson K. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke. *Nat Genet*. 2009;41:876–878.
7. Lubitz SA, Sinner MF, Lunetta KL, Makino S, Pfeufer A, Rahman R, Veltman CE, Barnard J, Bis JC, Danik SP, Sonni A, Shea MA, Del Monte F, Perz S, Muller M, Peters A, Greenberg SM, Furie KL, van Noord C, Boerwinkle E, Stricker BH, Witteman J, Smith JD, Chung MK, Heckbert SR, Benjamin EJ, Rosand J, Arking DE, Alonso A, Kaab S, Ellinor PT. Independent susceptibility markers for atrial fibrillation on chromosome 4q25. *Circulation*. 2010;122:976–984.
8. Vaziri SM, Larson MG, Benjamin EJ, Levy D. Echocardiographic predictors of nonrheumatic atrial fibrillation. The Framingham Heart Study. *Circulation*. 1994;89:724–730.
9. Montserrat S, Gabrielli L, Borras R, Poyatos S, Berruezo A, Bijnens B, Brugada J, Mont L, Sitges M. Left atrial size and function by three-dimensional echocardiography to predict arrhythmia recurrence after first and repeated ablation of atrial fibrillation. *Eur Heart J Cardiovasc Imaging*. 2013; Epub ahead of print. PMID 24168909.
10. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281–290.
11. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB Sr, Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D. The third generation cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *Am J Epidemiol*. 2007;165:1328–1335.
12. Lieb W, Xanthakis V, Sullivan LM, Aragam J, Pencina MJ, Larson MG, Benjamin EJ, Vasan RS. Longitudinal tracking of left ventricular mass over the adult life course: clinical correlates of short- and long-term change in the Framingham Offspring Study. *Circulation*. 2009;119:3085–3092.
13. McManus DD, Xanthakis V, Sullivan LM, Zachariah J, Aragam J, Larson MG, Benjamin EJ, Vasan RS. Longitudinal tracking of left atrial diameter over the adult life course: clinical correlates in the community. *Circulation*. 2010;121:667–674.
14. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation*. 1978;58:1072–1083.
15. Sundstrom J, Sullivan L, Selhub J, Benjamin EJ, D'Agostino RB, Jacques PF, Rosenberg IH, Levy D, Wilson PW, Vasan RS. Relations of plasma homocysteine to left ventricular structure and function: the Framingham Heart Study. *Eur Heart J*. 2004;25:523–530.
16. Scheffler K, Lehnhardt S. Principles and applications of balanced SSFP techniques. *Eur Radiol*. 2003;13:2409–2418.
17. Sotoodehnia N, Isaacs A, de Bakker PI, Dorr M, Newton-Cheh C, Nolte IM, van der Harst P, Muller M, Eijgelsheim M, Alonso A, Hicks AA, Padmanabhan S, Hayward C, Smith AV, Polasek O, Giovannone S, Fu J, Magnani JW, Marciante KD, Pfeufer A, Gharib SA, Teumer A, Li M, Bis JC, Rivadeneira F, Aspelund T, Kottgen A, Johnson T, Rice K, Sie MP, Wang YA, Klopp N, Fuchsberger C, Wild SH, Mateo Leach I, Estrada K, Volker U, Wright AF, Asselbergs FW, Qu J, Chakravarti A, Sinner MF, Kors JA, Petersmann A, Harris TB, Soliman EZ, Munroe PB, Psaty BM, Oostra BA, Cupples LA, Perz S, de Boer RA, Uitterlinden AG, Volzke H, Spector TD, Liu FY, Boerwinkle E, Dominiczak AF, Rotter JI, van Herpen G, Levy D, Wichmann HE, van Gilst WH, Witteman JC, Kroemer HK, Kao WH, Heckbert SR, Meitinger T, Hofman A, Campbell H, Folsom AR, van Veldhuisen DJ, Schwienbacher C, O'Donnell CJ, Volpato CB, Caulfield MJ, Connell JM, Launer L, Lu X, Franke L, Fehrmann RS, te Meerman G, Groen HJ, Weersma RK, van den Berg LH, Wijmenga C, Ophoff RA, Navis G, Rudan I, Snieder H, Wilson JF, Pramstaller PP, Siscovick DS, Wang TJ, Gudnason V, van Duijn CM, Felix SB, Fishman GI, Jamshidi Y, Stricker BH,

Samani NJ, Kaab S, Arking DE. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet*. 2010;42:1068–1076.

18. Chamberlain AM, Agarwal SK, Folsom AR, Soliman EZ, Chambless LE, Crow R, Ambrose M, Alonso A. A clinical risk score for atrial fibrillation in a biracial prospective cohort (from the atherosclerosis risk in communities [ARIC] study). *Am J Cardiol*. 2011;107:85–91.
19. Schnabel RB, Sullivan LM, Levy D, Pencina MJ, Massaro JM, D'Agostino RB Sr, Newton-Cheh C, Yamamoto JF, Magnani JW, Tadros TM, Kannel WB, Wang TJ, Ellinor PT, Wolf PA, Vasan RS, Benjamin EJ. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. *Lancet*. 2009;373:739–745.
20. Cohen J. *Statistical Power Analysis for Behavioral Sciences*. Hillsdale: Lawrence Erlbaum Associates, Inc; 1988.
21. Chinchilla A, Daimi H, Lozano-Velasco E, Dominguez JN, Caballero R, Delpon E, Tamargo J, Cinca J, Hove-Madsen L, Aranega AE, Franco D. PITX2 insufficiency leads to atrial electrical and structural remodeling linked to arrhythmogenesis. *Circ Cardiovasc Genet*. 2011;4:269–279.
22. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, White R, Furberg CD, Rautaharju PM. Incidence of and risk factors for atrial fibrillation in older adults. *Circulation*. 1997;96:2455–2461.
23. Mommersteeg MT, Hoogaars WM, Prall OW, de Gier-de Vries C, Wiese C, Clout DE, Papaioannou VE, Brown NA, Harvey RP, Moorman AF, Christoffels VM. Molecular pathway for the localized formation of the sinoatrial node. *Circ Res*. 2007;100:354–362.
24. Mommersteeg MT, Brown NA, Prall OW, de Gier-de Vries C, Harvey RP, Moorman AF, Christoffels VM. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium. *Circ Res*. 2007;101:902–909.
25. Wang J, Klysik E, Sood S, Johnson RL, Wehrens XH, Martin JF. Pitx2 prevents susceptibility to atrial arrhythmias by inhibiting left-sided pacemaker specification. *Proc Natl Acad Sci USA*. 2010;107:9753–9758.
26. Kirchhof P, Kahr PC, Kaese S, Piccini I, Vokshi I, Scheld HH, Rotering H, Fortmueller L, Laakmann S, Verheule S, Schotten U, Fabritz L, Brown NA. PITX2C is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression. *Circ Cardiovasc Genet*. 2011;4:123–133.
27. Li C, Wang F, Yang Y, Fu F, Xu C, Shi L, Li S, Xia Y, Wu G, Cheng X, Liu H, Wang C, Wang P, Hao J, Ke Y, Zhao Y, Liu M, Zhang R, Gao L, Yu B, Zeng Q, Liao Y, Yang B, Tu X, Wang QK. Significant association of SNP rs2106261 in the ZFHX3 gene with atrial fibrillation in a Chinese Han GeneID population. *Hum Genet*. 2011;129:239–246.
28. Lettre G, Palmer CD, Young T, Ejebe KG, Allayee H, Benjamin EJ, Bennett F, Bowden DW, Chakravarti A, Dreisbach A, Farlow DN, Folsom AR, Fornage M, Forrester T, Fox E, Haiman CA, Hartiala J, Harris TB, Hazen SL, Heckbert SR, Henderson BE, Hirschhorn JN, Keating BJ, Kritchevsky SB, Larkin E, Li M, Rudock ME, McKenzie CA, Meigs JB, Meng YA, Mosley TH, Newman AB, Newton-Cheh CH, Paltoo DN, Papanicolaou GJ, Patterson N, Post WS, Psaty BM, Qasim AN, Qu L, Rader DJ, Redline S, Reilly MP, Reiner AP, Rich SS, Rotter JI, Liu Y, Shrader P, Siscovick DS, Tang WH, Taylor HA, Tracy RP, Vasan RS, Waters KM, Wilks R, Wilson JG, Fabsitz RR, Gabriel SB, Kathiresan S, Boerwinkle E. Genome-wide association study of coronary heart disease and its risk factors in 8,090 African Americans: the NHLBI CARe Project. *PLoS Genet*. 2011;7:e1001300.
29. Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV, Tarasov KV, Muller M, Sotoodehnia N, Sinner MF, Verwoert GC, Li M, Kao WH, Kottgen A, Coresh J, Bis JC, Psaty BM, Rice K, Rotter JI, Rivadeneira F, Hofman A, Kors JA, Stricker BH, Uitterlinden AG, van Duijn CM, Beckmann BM, Sauter W, Gieger C, Lubitz SA, Newton-Cheh C, Wang TJ, Magnani JW, Schnabel RB, Chung MK, Barnard J, Smith JD, Van Wagoner DR, Vasan RS, Aspelund T, Eiriksdottir G, Harris TB, Launer LJ, Najjar SS, Lakatta E, Schlessinger D, Uda M, Abecasis GR, Muller-Myhsok B, Ehret GB, Boerwinkle E, Chakravarti A, Soliman EZ, Lunetta KL, Perz S, Wichmann HE, Meitinger T, Levy D, Gudnason V, Ellinor PT, Sanna S, Kaab S, Witteman JC, Alonso A, Benjamin EJ, Heckbert SR. Genome-wide association study of PR interval. *Nat Genet*. 2010;42:153–159.
30. Smith JG, Magnani JW, Palmer C, Meng YA, Soliman EZ, Musani SK, Kerr KF, Schnabel RB, Lubitz SA, Sotoodehnia N, Redline S, Pfeufer A, Muller M, Evans DS, Nalls MA, Liu Y, Newman AB, Zonderman AB, Evans MK, Deo R, Ellinor PT, Paltoo DN, Newton-Cheh C, Benjamin EJ, Mehra R, Alonso A, Heckbert SR, Fox ER. Genome-wide association studies of the PR interval in African Americans. *PLoS Genet*. 2011;7:e1001304.
31. Vasan RS, Glazer NL, Felix JF, Lieb W, Wild PS, Felix SB, Watzinger N, Larson MG, Smith NL, Dehghan A, Grosshennig A, Schillert A, Teumer A, Schmidt R, Kathiresan S, Lumley T, Aulchenko YS, Konig IR, Zeller T, Homuth G, Struchalin M, Aragam J, Bis JC, Rivadeneira F, Erdmann J, Schnabel RB, Dorr M, Zweiker R, Lind L, Rodeheffer RJ, Greiser KH, Levy D, Haritunians T, Deckers JW, Stritzke J, Lackner KJ, Volker U, Ingelsson E, Kullo I, Haerting J, O'Donnell CJ, Heckbert SR, Stricker BH, Ziegler A, Reffelmann T, Redfield MM, Werdan K, Mitchell GF, Rice K, Arnett DK, Hofman A, Gottdiener JS, Uitterlinden AG, Meitinger T, Blettner M, Friedrich N, Wang TJ, Psaty BM, van Duijn CM, Wichmann HE, Munzel TF, Kroemer HK, Benjamin EJ, Rotter JI, Witteman JC, Schunkert H, Schmidt H, Volzke H, Blankenberg S. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. *JAMA*. 2009;302:168–178.
32. Badano LP, Pezzutto N, Marinigh R, Cinello M, Nucifora G, Pavoni D, Gianfagna P, Fioretti PM. How many patients would be misclassified using M-mode and two-dimensional estimates of left atrial size instead of left atrial volume? A three-dimensional echocardiographic study. *J Cardiovasc Med (Hagerstown)*. 2008;9:476–484.