Association of Common Variations on Chromosome 4q25 and Left Atrial Volume in Patients with Atrial Fibrillation

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ABSTRACT

AIMS: Recent studies have shown that several genetic variants near the *PITX2* locus on chromosome 4q25 are associated with atrial fibrillation (AF). However, the mechanism that mediates this association remains unclear. Basic murine studies suggest that reduced *PITX2* expression is associated with left atrial dilatation. We sought to examine the association between single nucleotide polymorphisms (SNPs) near *PITX2* and left atrial size in patients with AF.

METHODS: We prospectively enrolled 96 consecutive patients (mean age 60 ± 10 years, 72% male) with drug-resistant AF (57% paroxysmal, 38% persistent, and 5% long-standing persistent) who underwent catheter ablation. Following DNA extraction from blood obtained pre-operatively, SNPs rs10033464 and rs2200733 were genotyped using the Sequenom MassARRAY. Left atrial volume (LAV) was determined using three-dimensional imaging (CT or MRI prior to first ablation) and by investigators blinded to genotype results.

RESULTS: The minor allele frequencies at SNPs rs10033464 and rs2200733 were 0.14 and 0.25, respectively. Using multivariable linear regression, homozygosity for the minor allele at rs10033464 (recessive model) was independently associated with larger LAV ($P = 0.002$) after adjustment for age, gender, BMI, height, type, and duration of AF, left ventricular ejection fraction, history of hypertension, valve disease, and antiarrhythmic drug use. The strength of the association was reconfirmed in a bootstrap study with 1000 resamplings. In contrast, no association was found between rs2200733 variant alleles and LAV.

CONCLUSION: SNP rs10033464 near the *PITX2* locus on 4q25 is associated with LAV. Left atrial dilatation may mediate the association of common variants at 4q25 with AF.

KEYWORDS: left atrium volume, *PITX2*, atrial fibrillation, catheter ablation, single nuclear polymorphisms

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in the US. Recent genome-wide association analyses have identified multiple loci that are associated with AF.[1,2] One of the strongest associations with AF, confirmed by validation studies, involves two non-coding single nucleotide polymorphisms (SNPs) on chromosome 4q25 (rs2200733 and rs10033464).[2] Polymorphisms at rs2200733 and rs10033464 are also associated with increased risk of AF recurrence after catheter ablation, elevated risk of cardioembolic stroke, and development of AF after coronary artery bypass grafting.[3–5]

Both SNPs are adjacent to the paired-like homeodomain transcription factor-2 (*PITX2*) gene, which has a major role in cardiac development.[6] The *PITX2* transcription factor has been linked to left atrial and pulmonary vein inflow morphogenesis.[7] In addition, Chinchilla and colleagues have demonstrated that reduced *PITX2* expression resulted in progressive enlargement of the murine left atrium (LA).[8] However, no human studies have examined left atrial structural associations with SNPs near chromosome 4q25. In the present study, we tested the hypothesis that the 4q25 AF risk alleles are associated with left atrial volume (LAV).

Methods

Patients. The Institutional Review Board approved our protocol, and all patients provided written informed consent. The study complies with the principles of the Declaration of Helsinki. During the period from September 2009 to February 2011, we enrolled 100 consecutive patients with drug-resistant AF referred for catheter ablation at our institution. We excluded four patients from analysis because a three-dimensional image of the LA prior to their first ablation was unavailable, leaving 96 patients in the cohort.

Genotyping. DNA was extracted from blood samples using Qiagen's Puregene DNA extraction kit. Genotyping of SNPs rs10033464 (HGVS name: g.111720761T>G) and rs2200733 (HGVS name: g.111710169C>T) was performed with the MassARRAY system of the Sequenom genotyping platform as part of a single multiplex SNP analysis (Sequenom). Each sample was tested at least three times, and at least two concordant results were required. Investigators were blinded to genotyping results.

Image acquisition. All individuals had either cardiac CT angiography (CTA) or MR angiography (MRA) to delineate end-diastolic left atrial dimensions. In patients who were undergoing their second or third ablation at the time of enrollment, we used CTA or MRA prior to the first ablation for LAV measurements. Of all patients, 81 (84%) underwent CTA and 15 (16%) underwent MRA prior to their ablation. CTA was performed at the end of the expiratory phase after intravenous administration of 70–80 mL of contrast (Isovue 370) using a multi-detector CT scanner (Toshiba's Aquilion). MRA was performed after administration of 0.2 mmol/kg of gadopentetate dimeglumine (Magnevist; Bayer HealthCare) with a 1.5-T magnetic resonance scanner (Avanto; Siemens) and a body phased-array coil. All scans were ECG-gated and were acquired at atrial end-diastole, just before opening of the mitral valve. Of all scans, 24 (25%) were obtained during AF and the remaining scans were acquired during sinus rhythm. Our detailed image acquisition protocols for CTA and MRI have previously been reported.[9]

Measurement of LAV. A single experienced reader, who was blinded to patient genetic profile and demographic characteristics, performed image analysis. LAV was calculated using the prolate ellipsoid formula as previously described and validated.[10] Using the endocardial border, we measured three orthogonal dimensions of the LA, including transverse (T), anterior–posterior (AP), and longitudinal (LO) diameters. Transverse diameter of the LA was defined as the distance between the midpoint of the right- and left-sided pulmonary veins in oblique axial images. The AP and LO diameters were measured at the midpoint of the transverse diameter in oblique axial and sagittal images (Fig. 1). These dimensions were then entered into the prolate ellipsoid formula: LAV = [(T × AP × LO) × (0.523)].

Statistical analysis. Continuous variables are presented as mean ± SD. Categorical variables are reported as frequencies and percentages. The association of LAV with 4q25 genetic variants was examined using multivariable linear regression models, adjusting for age, ethnicity, gender, BMI, height type and duration of AF, left ventricular ejection fraction, history of hypertension, antiarrhythmic drug use, and valve disease. Based upon univariate analyses of the association of LAV with 4q25 genetic variants, a recessive model was assumed for polymorphisms. To estimate uncertainty associated with the sample size, we utilized a nonparametric bootstrap study to confirm the significance of the associations. This method involves resampling observations with replacement from the data 1000 times, therefore, estimating the sampling distribution, standard error, and confidence intervals without assumptions about the distribution of the underlying population. Thus, the method is useful in situations with small sample size or when the theoretical distribution of the statistic is complicated. Using this method, we created new samples of 95 patients. We then executed the multivariable analysis, including those variables from model 3 that reached or approached statistical significance, and repeated the process for a total of 1000 times. These new samples were taken from the original data set using sampling with replacement, so it is not identical to the original sample.[11,12] Results are reported as significant if $P \leq 0.05$, and with Bonferroni correction for multiple comparisons, results should be considered significant at $P \leq 0.025$. Statistical analyses were performed using STATA version 12.

Results

Of 96 patients in the cohort (mean age 60 ± 9.9 years, 72% male), 89 (93%) were Caucasians, 3 (3%) were African American and 4 (4%) were self-identified as other ethnicities. AF types were defined according to rhythm status prior to each patient's first ablation procedure. Overall, 55 (57%) patients had paroxysmal AF, 36 (38%) had persistent AF, and 5 (5%) had long-standing persistent AF. The $CHADS_2$ risk score was low (0 or 1) in 73 (76%) participants. Valve disease was present in six (6%) patients, including four with mild-to-moderate mitral regurgitation, one with severe mitral regurgitation, and one with mild aortic stenosis. There was a trend toward higher LA volume in patients with valve disease (143.7 ± 33.2 cm^3 vs. 103.8 ± 5.4 cm^3, $P = 0.08$).

The minor allele frequencies at SNPs rs10033464 and rs2200733 were 0.14 and 0.25, respectively. The genotype frequencies for rs10033464 and rs2200733 were in Hardy–Weinberg equilibrium ($\chi^2 = 1.959$, $P = 0.162$; $\chi^2 = 0.474$, $P = 0.491$, respectively). The r^2 between rs1003464 and rs2200733 in our cohort was 0.003. In 44 (46%) patients, no risk variants at either rs10033464 or rs2200733 were observed. At least one risk allele at either rs10033464 or rs2200733 was detected in 12 (13%) or 31 (32%) patients, respectively. The remaining nine (9%) patients had at least one risk allele at both rs10033464 and rs2200733. The baseline characteristics of all participants and of participant groups by genotype have been summarized in Table 1.
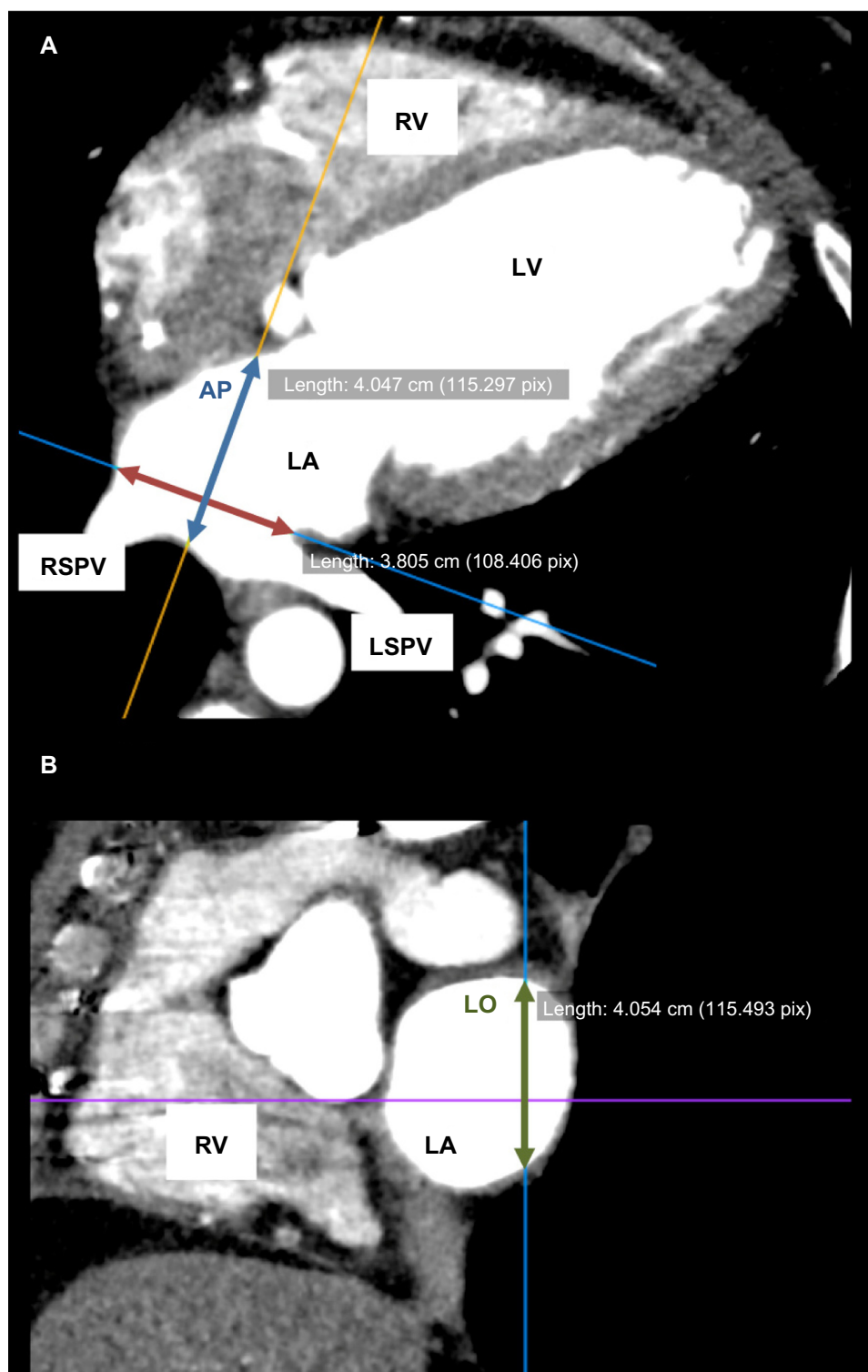
Figure 1. The figure illustrates the methodology for measurement of LAV on a CT angiogram image. The transverse diameter of the LA (T) was defined as the distance between the midpoint of the right- and left-sided pulmonary veins in oblique axial images (**A**). The AP and LO left atrial diameters were measured at the midpoint of the transverse diameter in oblique axial (**A**) and sagittal images (**B**). These dimensions were then entered into the prolate ellipsoid formula: LAV = [(T × AP × LO) × (0.523)].

The TT haplotype at rs10033464 was strongly associated with larger LAV (Table 2). In contrast, an association was not identified in those with the AF risk allele at rs2200733. In the unadjusted model, homozygosity at the T allele at rs10033464 was associated with 104.6 ± 29.7 cm^3 increase in LAV (*P* = 0.001). To evaluate the effects of potential confounders, multivariable regression models with age, ethnicity, gender, AF duration, type of AF, BMI, height, left ventricular ejection

Table 1. Patients' demographics ($n = 96$).

VARIABLES	ALL PATIENTS	rs10033464				rs2200733			
	(n = 96)	GG (n = 75)	TG (n = 18)	TT (n = 3)	P	CC (n = 56)	CT (n = 33)	TT (n = 7)	P
Age (mean ± SD), y	60 ± 9.9	61 ± 9.4	58 ± 12.6	61 ± 6.5	0.8	62 ± 8.7	58 ± 12	60 ± 7.3	0.7
Male, n (%)	69 (72%)	52 (69%)	14 (78%)	3 (100%)	0.4	41 (73%)	21 (63%)	7 (100%)	0.14
Height, cm	176 ± 10.5	176 ± 10.9	175 ± 10.1	178 ± 4.4	0.94	175 ± 10.9	176 ± 9.3	183 ± 10.7	0.16
BMI, kg/m^2	28.7 ± 5.4	29 ± 5.5	27 ± 4.6	31 ± 7.2	0.25	29 ± 5	29 ± 5.8	26 ± 6	0.45
Atrial fibrillation duration until imaging									
<3 years, n (%)	33 (34%)	27 (36%)	5 (28%)	1 (33%)	0.81	18 (32%)	12 (36%)	3 (43%)	0.82
≥3 years, n (%)	63 (66%)	48 (64%)	13 (72%)	2 (67%)		38 (68%)	21 (64%)	4 (57%)	
Atrial fibrillation type									
Paroxysmal, n (%)	55 (57%)	41 (55%)	14 (78%)	0	0.83	31 (55%)	20 (61%)	4 (57%)	0.9
Persistent & long-standing persistent, n (%)	41 (43%)	34 (45%)	4 (22%)	3 (100%)		25 (45%)	13 (39%)	3 (43%)	
Congestive heart failure, n (%)	12 (12.5%)	9 (12%)	2 (11%)	1 (33%)	0.54	8 (14%)	3 (9%)	1 (14%)	0.76
Valve Disease, n (%)	6 (6%)	5 (7%)	0 (0%)	1 (33%)	0.08	4 (7%)	2 (6%)	0 (05)	0.77
LA Volume (cm^3)	106.3 ± 53.6	103.4 ± 53.0	101.7 ± 39.5	207.6 ± 59.2	0.003	107.4 ± 53.0	100.2 ± 57.3	126.6 ± 39.5	0.489
Hypertension, n (%)	53 (55%)	42 (56%)	10 (56%)	1 (33%)	0.74	26 (46%)	24 (72%)	3 (43%)	0.043
Diabetes mellitus, n (%)	10 (10%)	9 (12%)	1 (5%)	0	0.6	5 (9%)	5 (15%)	0	0.4
Stroke/TIA, n (%)	6 (6%)	5 (6%)	1 (5%)	0	0.78	2 (4%)	4 (12%)	0	0.4
Coronary artery disease, n (%)	16 (17%)	11 (14%)	5 (28%)	0	0.3	8 (14%)	6 (18%)	2 (28%)	0.6
Obstructive sleep apnea, n (%)	18 (19%)	14 (19%)	3 (17%)	1 (33%)	0.79	11 (20%)	6 (18%)	1 (14%)	0.94
LVEF, (mean ± SD)	55 ± 9	55 ± 9	60 ± 5	50 ± 15	0.58	55 ± 10	57 ± 6	52 ± 12	0.23
Prior cardioversion, n (%)	41 (43%)	34 (45%)	4 (22%)	3 (100%)	0.03	25 (45%)	13 (39%)	3 (43%)	0.89
Antiarrhythmic drug use prior to imaging, n (%)	89 (93%)	70 (93%)	16 (89%)	3 (100%)	0.72	51 (91%)	31 (94%)	7 (100%)	0.66
CHADS$_2$ Score, n (%)									
0	37 (39%)	27 (36%)	8 (44%)	2 (67%)	0.89	25 (45%)	8 (24%)	4 (57%)	0.55
1	36 (37%)	29 (39%)	7 (39%)	0		22 (36%)	14 (43%)	2 (29%)	
2	16 (17%)	14 (19%)	1 (6%)	1 (33%)		6 (11%)	9 (27%)	1 (14%)	
≥3	7 (7%)	5 (7%)	2 (11%)	0		5 (8%)	2 (6%)	0	

Note: Values are presented in mean ± SD or n (%). Parentheses contain column percentages.
Abbreviation: LVEF, left ventricular ejection fraction.

fraction, history of valve disease, history of antiarrhythmic drug use, and history of hypertension were utilized. Model 1 controlled for demographic features such as age, ethnicity, and type of AF. As illustrated in Table 3, the magnitude of association for homozygosity at the T allele at rs10033464 with LAV was unchanged at $104.4 ± 31.6$ cm^3, and statistical significance was maintained ($P = 0.001$). In the fully adjusted model 2, the magnitude of association of homozygosity at the T allele at rs10033464 with LAV and statistical significance were maintained ($102.7 ± 32.1$, $P = 0.0002$, Table 3).

We further examined this finding by a nonparametric bootstrap method.[11,12] This method confirmed that the magnitude of association of homozygosity for the T allele at rs10033464 with LAV withstands resampling ($91.9 ± 38.2$ cm^3, $P = 0.016$,

Table 4) and that the finding is unlikely to be incidental. Additionally, left ventricular ejection fraction ($−1.3 ± 0.6$ cm^3 per 1% increase in ejection fraction, $P = 0.026$) and AF duration ≥3 years ($22.0 ± 9.9$ cm^3, $P = 0.038$) were associated with LAV.

Discussion

The main finding of this study is that homozygosity for the T allele at SNP rs10033464 is associated with larger LAV in models adjusting for potential confounders of left atrial size. Importantly, the association of homozygosity for the T allele at SNP rs10033464 with LA volume was independent of the association of left ventricular ejection fraction and AF duration with LA volume. To the best of our knowledge, this is the

Table 2. Association of LAV, CHADS$_2$ score, and left ventricular ejection fraction with common variations on chromosome 4q25.

SNPs VARIATIONS	rs10033464		P	rs2200733		P
	(TT)	(TG OR GG)		(TT)	(CT OR CC)	
LAV (cm^3)	**207.6± 34.2**	103.1 ± 5.2	**<0.001**	126.6 ± 14.9	104.7 ± 5.8	0.30
CHADS$_2$						
0	2	35	0.638	4	33	0.842
1	0	36		2	34	
2	1	15		1	15	
3	0	6		0	6	
4	0	1		0	1	
LVEF (%)	50.0 ± 8.7	55.9 ± 0.95	0.271	52.2 ± 4.8	55.9 ± 0.96	0.327

Note: Values are in cm^3.
Abbreviations: LAV, left atrial volume; LVEF, left ventricular ejection fraction; SNPs, single nucleotide polymorphisms.

first report of an association between a SNP in close proximity to the *PITX2* gene with left atrial structure in patients with AF. We also observed that variation at both rs10033464 and rs2200733 was common in our cohort of patients with drug-refractory AF referred for catheter ablation. In our cohort, 54% of patients had at least one of the risk variants. This prevalence is higher than the prevalence of 35% reported in a population sample of European descent.[2]

Left atrial dilatation can be the cause or the consequence of AF. It has previously been shown that left atrial size is independently associated with AF incidence.[13] Increased LAV is also associated with decreased efficacy of catheter ablation for AF. Each 10 mL increase in volume is associated with a 14% increased risk of AF recurrence after ablation.[14] However, the mechanisms that explain these associations are not yet well understood. Atrial dilatation and stretch may induce the activation of extracellular signal-related kinase Erk$_1$/ERk$_2$, leading to atrial fibrosis and thereby atrial arrhythmia.[15] It appears, however, that atrial dilatation can increase AF susceptibility and perpetuation independent of variations in atrial fibrosis or tissue refractoriness.[16] Conversely, it has also been shown that atrial dilatation may occur as a consequence of AF in patients without prior left atrial dilatation.[17]

The non-coding SNPs at rs10033464 and rs2200733 have been associated with AF, and are close to the *PITX2* gene.[2] The *PITX2* gene product is a member of the pituitary homeobox family of transcription factors and appears to play an important role in embryologic morphogenesis.[18] Chinchilla and

colleagues have demonstrated that the expression of *PITX2C* (the predominant cardiac isoform of *PITX2*) is decreased in left atrial biopsies of patients with AF.[19] Additionally, using a murine model, the same group demonstrated that reduced *PITX2* expression in atrial myocardium resulted in enlarged atrial chambers.[19] This effect of reduced *PITX2* expression on chamber size is likely mediated in part by selective upregulation of *Bmp10*, a regulator of physiologic hypertrophy in the LA.[19] Our findings support the findings of Chinchilla and colleagues in the murine model. After adjusting for potential confounders, variation at rs10033464 was associated with increased LAV in patients with drug-refractory AF referred for catheter ablation.

Study limitations. The study sample size is small and underpowered, and the findings may potentially be driven by false-positive, random findings. However, statistical analyses were driven by a specific hypothesis and limited to associations of polymorphisms at two specific sites with LAV. The majority of our patients were Caucasian; therefore, our results may not be generalizable to AF patients with other ethnicities. Conversely, the inhomogeneity of ethnicity may also bias the results; however, the reported associations persisted after adjustment for ethnicity in our multivariable model. Our cohort included patients with paroxysmal, persistent, and long-standing persistent AF; however, the associations persisted after adjustment for the type of AF. The LAV was measured using two modalities (CTA or MRA), which may introduce differential measurement error and/or variance.

Table 3. Magnitude of association between LAV and common variations on chromosome 4q25 in multivariable models adjusting for potential confounders.

VARIABLE	UNIVARIATE		MODEL 1*		MODEL 2†	
	COEFFICIENT	P VALUE	COEFFICIENT	P VALUE	COEFFICIENT	P VALUE
rs10033464	104.6 ± 29.7 cm^3	**0.001**	104.4 ± 31.6 cm^3	**0.001**	102.7 ± 32.1 cm^3	**0.002**
rs2200733	21.8 ± 21.0 cm^3	0.303	21.6 ± 21.2 cm^3	0.310	9.26 ± 23.0 cm^3	0.689

Notes: *Model 1, adjusted for age, ethnicity, and type of AF. †Model 2, adjusted for age, ethnicity, AF duration, type of AF, BMI, height, gender, left ventricular ejection fraction, history of valve disease, history of antiarrhythmic drug use, and history of hypertension.

Table 4. Magnitude of association between LAV and the rs10033464 SNP in a 1000-fold bootstrap simulation study.

	COEFFICIENT ± BOOTSTRAP STANDARD ERROR (CM³)	P VALUE
rs10033464	**91.9 ± 38.2**	**0.016**
Age (years)	0.3 ± 0.5	0.607
BMI (kg/m²)	1.0 ± 0.9	0.266
Left ventricular ejection fraction (%)	**−1.3 ± 0.6**	**0.026**
Hypertension	−11.7 ± 11.4	0.306
History of anti-arrhythmic drug use	−4.0 ± 16.8	0.810
AF duration ≥3 years	**22.0 ± 9.9**	**0.027**

However, Wen and colleagues reported that measurements of LAV using these two modalities are very similar.[20] In all patients with long-standing-persistent AF and 13 (36%) patients with persistent AF, images were obtained during AF. In comparison, patients with paroxysmal AF underwent imaging during sinus rhythm. In all cases, however, images were acquired just before the opening of the mitral valve, thus measuring maximum atrial volume. In addition, we controlled for type of AF in our multivariable models. Finally, no association was observed between polymorphism at rs2200733 and LAV. Previous reports have observed discrepant associations between the two SNPs and risk of AF in different populations[21] and validation samples.[2] These discrepant associations likely reflect variations in linkage disequilibrium of the two SNPs across different population samples. We implemented a resampling simulation study that confirmed the significance of the association; however, our results lack external validation/control and will need to be confirmed in future studies. We did not investigate the mechanism for the association of polymorphism at rs10033464 with atrial volume. However, the results are consistent with functional studies previously reported by Chinchilla and colleagues.[19]

Conclusion

Polymorphism at rs10033464, but not rs2200733, near the *PITX2* locus on chromosome 4q25 is associated with increased LAV. Left atrial dilatation may mediate the association of common variants at 4q25 with AF. Additional studies with larger sample size are warranted to confirm these findings.

Author Contributions

Conceived and designed the experiments: SN. Analyzed the data: YM, HY, SN, VZ. Wrote the first draft of the manuscript: HY. Contributed to the writing of the manuscript: all authors. Agree with manuscript results and conclusions: all authors. Jointly developed the structure and arguments for the paper: all authors. Made critical revisions and approved final version: all authors. All authors reviewed and approved of the final manuscript.

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