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Symptomatic Response to Antiarrhythmic Drug Therapy Is Modulated by a Common Single Nucleotide Polymorphism in Atrial Fibrillation

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Objectives This study tested the hypothesis that response to antiarrhythmic drugs (AADs) is modulated by 3 common loci associated with atrial fibrillation (AF).

Background Recent genome-wide association studies have identified 3 loci, on chromosomes 4q25 (near *PITX2*), 16q22 (in *ZFHX3*), and 1q21 (in *KCNN3*), that associate with either typical or lone AF. These findings indicate that variable mechanisms contribute to AF susceptibility, and suggest that response to therapy may be genotype dependent.

Methods We studied 478 and 198 Caucasian patients in the discovery cohort and validation cohort, respectively, who were prospectively enrolled in the Vanderbilt AF registry. Response was defined prospectively as successful rhythm control if the patient remained on the same AAD therapy for a minimum of 6 months with ≥75% reduction in symptomatic AF burden. We also evaluated AF recurrence by 12-lead electrocardiogram (ECG) at 3, 6, and 12 months. Symptomatic patients were also given a 24- to 48-h Holter monitor or 30-day event recorder when AF recurrence was not captured by 12-lead ECG.

Results In the discovery cohort, 399 (83%) patients were successfully rhythm controlled. Multiple clinical variables (including age, hypertension, lone AF) failed to significantly predict response to AADs; however, single nucleotide polymorphism (SNP) rs10033464 at 4q25 was an independent predictor of successful rhythm control in patients with typical AF carrying the ancestral allele (wild type) versus carriers of variant allele (odds ratio [OR]: 4.7, 95% confidence interval [CI]: 1.83 to 12, $p = 0.0013$. In the validation cohort, 143 (72%) patients met the criteria for successful rhythm control, and rs10033464 was again an independent predictor of successful rhythm control, OR: 1.5, 95% CI: 1.02 to 3.06, $p = 0.04$. This SNP (rs10033464) was an independent predictor of AF recurrence in the discovery (39% AF recurrence) and validation (38% AF recurrence) cohorts; OR: 3.27, 95% CI: 1.7 to 6, $p < 0.001$ and OR: 4.3, 95% CI: 1.98 to 9.4, $p < 0.001$, respectively.

Conclusions These results suggest that a common SNP on chromosome 4q25 associated with AF modulates response to AAD therapy and points to a potential role for stratification of therapeutic approaches by genotype. (J Am Coll Cardiol 2012;60:539–45) © 2012 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice, representing a growing epidemic with significant health consequences and affecting more than 2 million adults in the United States (1). The mean age of individuals with AF is 75 years; disease prevalence increases with age (2.3% age >40 years; 5.9% age >65 years) and with the presence of structural heart disease such as ischemic, hypertensive, myocardial, or valvular disease. Lone AF typically occurs in young and middle-age adults (mean age at diagnosis ~44 years) (2). Although novel risk factors for this arrhythmia have recently been described (3–5), the etiology of an individual's condition often remains unknown (6).

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A Mendelian pattern of inheritance has most often been reported in AF kindreds, especially in probands with lone

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Abbreviations and Acronyms

AAD = antiarrhythmic drug
AF = atrial fibrillation
CHF = congestive heart failure
CI = confidence interval
ECG = electrocardiogram
OR = odds ratio
SNP = single nucleotide polymorphism

AF. Mutations in genes encoding cardiac ion channels, gap junction proteins, atrial natriuretic peptide (ANP), and nucleoporins (NUP155) have been reported in isolated cases and small kindreds (4,7–12). Recent genome-wide association studies have identified 3 loci on chromosomes 4q25 (near *PITX2*) (13), 16q22 (in *ZFHX3*) (14,15), and 1q21 (in *KCNN3*) (16), which associate with either typical or lone AF. These findings indicate that variable mechanisms contribute to AF susceptibility, and suggest therefore that response to therapy may also be genotype dependent. Within locus 4q25, 2 noncoding single nucleotide polymorphisms (SNPs) (rs2200733 and rs10033464) were independently associated with AF, and these findings were replicated in 2 populations of European descent and 1 of Asian descent (13). Recently, this association was replicated in a study of 4 large populations with ambulatory AF (17). This association has also been reported for post-cardiac surgery AF, a setting thought to be related to inflammation (18) and has recently been reported to predict the likelihood of successful AF ablation (19).

Understanding the molecular mechanisms of AF supports the idea that variability in drug therapy response is modulated by various underlying disease mechanisms and that drug response is as heterogeneous as the arrhythmia itself. Antiarrhythmic agents remain the cornerstone of treatment for patients with symptomatic AF despite the high recurrence rate: 30% to 50% over 1 year. We tested the hypothesis that response to antiarrhythmic drug (AAD) therapy may also be genotype dependent by determining the relationship between response to conventional AADs and common SNPs that predict AF: rs2200733 and rs10033464 at 4q25, rs7193343 in *ZFHX3*, and rs13376333 in *KCNN3*.

Methods

Study population. The study was performed on prospectively enrolled patients in the Vanderbilt AF registry. Inclusion criteria included age >18 years and documented history of AF with concurrent use of at least 1 conventional AAD. At the time of enrollment, a detailed medical and drug history was obtained along with a symptom questionnaire (20). The symptom questionnaire is a modification of the validated University of Toronto AF severity scale (21) and is repeated at 3, 6, and 12 months follow-up along with 12-lead electrocardiograms (ECGs) after starting an AAD. In addition, any patient who experienced symptoms consistent with AF not captured by 12-lead ECG was provided with either a 24- to 48-h Holter monitor or 30-day event recorder. Written informed consent was obtained from all patients under a protocol approved by the Vanderbilt University Institutional Review Board.

Definitions. Arterial hypertension was defined by a history of hypertension and/or the presence of antihypertensive therapy. Criteria for coronary artery disease included a history of myocardial infarction or typical angina, previous bypass surgery or angioplasty, and drug treatment. Congestive heart failure (CHF) was defined by a history of CHF and/or drug treatment for heart failure. Left atrial and left ventricular measurements from the M-mode echocardiograms were made at the time enrollment if a recent echo (<3 months) was not available in the medical records. Echocardiograms were read by an experienced physician blinded to the genotype status of the patient. The echocardiograms were evaluated according to the recommendations of the American Society of Echocardiography. Lone AF was defined as AF occurring in patients ≤65 years of age without hypertension or overt structural heart disease by clinical examination, ECG, and echocardiography. AF recurrence was defined as presence of documented AF on 12-lead ECG, Holter monitoring, or pacemaker interrogations.

Response to AAD therapy. This was defined prospectively as successful rhythm control if the patient remained on the same AAD therapy for a minimum of 6 months with ≥75% reduction in symptomatic AF burden (the composite score for frequency, duration, and severity of symptoms) on AAD therapy (20). Nonresponse was defined as <75% reduction in symptomatic AF burden score necessitating a change to another AAD or to nonpharmacological therapy such as atrioventricular node ablation and pacemaker implantation. We also evaluated AF recurrence by 12-lead ECG at 3-, 6-, and 12-month follow-up, Holter monitoring, 30-day event recorder, and, in some patients (2%), from pacemaker interrogations (pacemakers inserted for sick sinus syndrome).

Genotyping. Genomic DNA was isolated from whole blood by a commercial kit (Purgene, Gentra Systems, Minneapolis, Minnesota). Genotyping of the 2 4q25 SNPs (rs2200733, rs10033464) was performed using real-time polymerase chain reaction (PCR), iPlex single-base primer extension, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in a 384-well format (Sequenom, San Diego, California) as previously described (17). Genotyping at the 16q22 (rs7193343 in *ZFHX3*) and the 1q21 SNPs (rs13376333 in *KCNN3*) was performed using TaqMan assays (Applied Biosystems, Foster City, California), as previously described (14,16), by laboratory personnel who had no knowledge of the response to rhythm control therapy.

Statistical analysis. Data are expressed as mean ± SD. The Mann-Whitney *U* test was performed on continuous variables and chi-square test on discrete variables. Logistic regression analysis was done to determine odds ratio (OR) and adjusted for type of AF (paroxysmal or persistent) and sex using dominant and additive models. Due to expected low numbers of homozygous variants, it was defined a priori to include homozygous variants of all SNPs with their

respective heterozygous variants. We used a similar analysis to test the association between AF recurrence within 12 months, as well as genetic polymorphism. Final dominant model for AF recurrence was adjusted for age, sex, AF type, history of hypertension, coronary artery disease, heart failure, diabetes, use of AADs, left ventricular ejection fraction, and left atrial size in the discovery cohort and age, sex, AF type, hypertension, and use of AADs in the validation cohort.

In the dominant model, an identical effect is expected in heterozygous and homozygous variant carriers, whereas in the additive model, heterozygous variant carriers have an intermediate effect in relation to the homozygotes. We used Bonferroni correction to correct for multiple testing with even distribution of significance (alpha) among the 4 tested SNPs in the discovery cohort, with a 2-sided p value <0.0125 considered statistically significant. For validation cohort (only 1 SNP tested), a 2-sided p value <0.05 was considered statistically significant. All genetic analyses were performed with PLINK.

Results

Our study population consisted of 478 and 198 Caucasian patients enrolled in the discovery and validation cohorts, respectively.

Clinical characteristics. The clinical characteristics of the study cohorts are summarized in Table 1. The discovery cohort consisted of 325 (68%) men, 152 (32%) women, age 63 ± 13 years, 148 (31%) with lone AF, and hypertension was the most common underlying comorbidity in 207 (43%) patients. The validation cohort followed a similar trend: 129 (65%) men, 69 (35%) women, age 63 ± 14 years, 53 (27%) with lone AF, and hypertension was found in 100 (50%) patients. Both study cohorts were similar with respect to baseline demographics except for larger left atrial size (46 ± 8.6 mm) in the discovery cohort versus the validation cohort (44 ± 9 mm), p = 0.005, and a marginal difference in left ventricular ejection fraction; 52 ± 14% in the discovery cohort versus 53 ± 11% in the validation cohort, p = 0.05 in patients with typical AF.

Genotyping. The frequencies of the common AF variants between responders and nonresponders did not deviate significantly from Hardy-Weinberg equilibrium. Table 2 shows the genotype frequencies of the tested SNPs in both study cohorts.

Response to AAD therapy. In the discovery cohort, 399 (83.5%) patients met the criteria of successful rhythm control. Response to therapy (successful rhythm control) was significantly associated with rs10033464 in patients with typical AF; allelic chi-square = 0.0026, genotypic chi-square = 0.0008. There was marginal genotypic association of the same SNP in the cumulative discovery cohort (typical and lone AF); chi-square = 0.03.

Multiple clinical variables (including age, hypertension, lone AF) failed to significantly predict response to AADs. By contrast, rs10033464 at 4q25 in regression analysis under both the dominant and additive models was significantly associated with successful rhythm control in patients with typical AF carrying the ancestral allele (wild type) versus carriers of variant allele (odds ratio [OR]: 4.59, 95% confidence interval [CI]: 1.82 to 11.5, p = 0.0012 and OR: 3.03, 95% CI: 1.41 to 6.50, p = 0.004, respectively). This association persisted after correction for multiple clinical factors (OR: 4.70, 95% CI: 1.83 to 12.0, p = 0.0013 and OR: 3.44, 95% CI: 1.52 to 7.76, p = 0.003, respectively) (Table 3).

There was a differential response to the class of AADs in variants of rs10033464. Wild types responded better to class III AADs, p = 0.02, whereas carriers of variant allele responded better to class I AADs, p = 0.02 (Table 4).

In the validation cohort, 143 (72%) patients met the criteria for rhythm control. Response to therapy was again significantly associated with rs10033464; chi-square = 0.042. In similar genetic regression models as the discovery

Table 1 Clinical Characteristics of the Study Cohort

	Cohort					
	Discovery (n = 478)		Validation (n = 198)		p Value*	p Value†
AF type %	Typical 69	Lone 31	Typical 73	Lone 27	0.28	0.28
Age, yrs	67 ± 10	53 ± 13	65 ± 11	51 ± 14	0.07	0.3
Age at onset, yrs	54 ± 13	39 ± 14	55 ± 13	40 ± 14	0.18	0.6
Male	217 (66)	108 (73)	94 (65)	35 (66)	0.9	0.3
Comorbidities						
Hypertension	207 (43)	0 (0)	100 (50)	0 (0)	0.11	—
Coronary artery disease	102 (21)	0 (0)	36 (18)	0 (0)	0.25	—
Congestive heart failure	66 (14)	0 (0)	23 (12)	0 (0)	0.39	—
Diabetes mellitus	65 (14)	0 (0)	30 (15)	0 (0)	0.85	—
Echocardiographic variables						
Left atrial size, mm	46 ± 8.6	42 ± 10.5	43 ± 8	41 ± 6.0	0.005	0.56
LVEF, %	52 ± 14	56 ± 9.0	53 ± 11	56 ± 12	0.05	0.6

Values are n (%) and mean ± SD. *Comparison between typical AF; †comparison between lone AF.
AF = atrial fibrillation; LVEF = left ventricular ejection fraction.

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Table 2 Genotype Frequencies in the Study Population

SNP	Response	MAF	Wild Type (%)	Variant Allele Carriers (%)
Discovery cohort				
rs2200733 (4q25) n = 477	Responders (398)	16	70	30
	Nonresponders (79)	15	71	29
rs10033464 (4q25) n = 477	Responders (398)	11	79	21
	Nonresponders (79)	16	68	32
rs7193343 (16q22) n = 464	Responders (386)	20	63	37
	Nonresponders (78)	21	63	37
rs13376333 (1q21) n = 466	Responders (387)	38	39	61
	Nonresponders (79)	35	41	59
Validation cohort				
rs10033464 (4q25) n = 198	Responders (143)	16.7	67	33
	Nonresponders (55)	12	71	29

MAF = minor allele frequency; SNP = single nucleotide polymorphism.

cohort, polymorphism at rs10033464 was a significant predictor of successful rhythm control in wild type: dominant model OR: 1.496, 95% CI: 1.02 to 3.06, p = 0.04. In the additive model, we observed a similar trend as in the discovery cohort; however, it did not reach statistical significance: OR: 1.318, 95% CI: 0.62 to 2.79, p = 0.46 (Table 3).

Response to AADs in the validation cohort followed the same trend as the discovery cohort, with wild types responding favorably to class III AADs (61% vs. 34%, p = 0.02) versus carriers of variant allele at rs10033464 responding favorably to class I AADs (39% vs. 67%, p = 0.01) (Table 4).

AF recurrence and genetic polymorphism. In 12-month follow-up, 184 (39%) patients in the discovery cohort had AF recurrence (n = 478), of which 57 (12% of 478) patients had AF captured by Holter or event monitor, and 10 (2% of 478) patients had AF captured by pacemaker interrogations. In the validation cohort (n = 198), 73 (38%) patients had AF recurrence, of which 20 (10% of 198) patients had AF captured by Holter or event monitor. AF recurrence among carriers of the variant allele and wild type for each SNP in each cohort is shown in Table 5.

In both cohorts under univariate analysis, only rs10033464 showed significance associated with AF recurrence. Carriers of the variant allele at rs10033464 had greater AF recurrence compared with wild types; 46% versus 36%, p = 0.06, in the discovery cohort and 51% versus 32%, p = 0.013, in the validation cohort. In the multivariable dominant adjusted model, carriers of the variant allele at rs10033464 had greater OR of AF recurrence: OR: 3.27, 95% CI: 1.77 to 6.04, p < 0.001 in the discovery cohort and OR: 4.31, 95% CI: 1.98 to 9.4, p < 0.001 in the validation cohort (Table 6).

Discussion

This study demonstrates a pharmacogenomics interaction: significant modulation of symptomatic AF burden and AF recurrence between a common SNP on chromosome 4q25 (rs10033464) and response to AAD therapy in patients with AF. It further provides evidence for an important role of genetic polymorphisms, not only in the pathophysiology of AF, but also in response to pharmacological therapy.

The potential mechanism of action of variants at the chromosome 4q25 locus tagged by the 2 noncoding SNPs is unknown, and may be mediated through effects of distant genes. However, it is interesting that the closest gene, located approximately 90 kb centromeric, is the paired-like homeodomain transcription factor 2 (*PITX2*) with an isoform implicated in early cardiac development (22–24). Mouse *pitx2* knockouts have demonstrated a critical role for 1 isoform, *pitx2c*, in left–right asymmetry, specifically the development of the left atrium (24). Loss of *pitx2c* leads to right atrial isomerization and a failure to suppress a default pathway for sinus node development of the pulmonary myocardium, or the sleeve of cardiomyocytes extending from the left atrium into the initial portion of the pulmonary vein. Importantly, this area is now recognized as a common source for the ectopic atrial activity necessary for the initiation and propagation of AF (24,25). Recently, novel AF susceptibility alleles in addition to rs2200733 and rs10033464 have been identified on chromosome 4q25. This provides evidence of multiple susceptibility signals at chromosome 4q25 and may help localize regulatory elements at this locus with biological relevance in the pathogenesis of AF (26).

Microarray analysis of *pitx2* null-mutant and control mice hearts revealed up-regulation of *Kcnq1*, a potassium channel gene that has been associated with gain-of-function mutations in familial AF (8,27). A recent study by Voigt et al. (28) looked at unequal left-to-right distribution of inward rectifier K^+ currents in subjects with paroxysmal AF. They found a 2-fold greater basal current in left atria compared with right atria, and 2-fold greater protein expression of I_{K1} (Kir2.1) in paroxysmal and chronic AF. Physiologically, a gain-of-function potassium channel mutation would be predicted to respond to higher doses of

Table 3 Logistic Regression Analysis of Genetic Predictors of Response to AADs

	Unadjusted			Adjusted*		
SNP	OR	95% CI	p Value	OR	95% CI	p Value
Discovery cohort						
rs2200733 additive						
All AF	0.91	0.57–1.45	0.69	0.91	0.57–1.45	0.68
Typical	0.98	0.42–2.33	0.97	0.94	0.39–2.29	0.89
Lone	0.67	0.36–1.27	0.22	0.66	0.35–1.27	0.21
rs2200733 dominant						
All AF	0.99	0.58–1.68	0.95	0.98	0.57–1.67	0.93
Typical	0.89	0.31–2.53	0.83	0.82	0.28–2.37	0.71
Lone	0.75	0.37–1.50	0.41	0.75	0.36–1.53	0.42
rs10033464 additive						
All AF	1.49	0.91–2.4	0.11	1.48	0.91–2.42	0.11
Typical	3.03†	1.41–6.50	0.004	3.44†	1.52–7.76	0.003
Lone	1.07	0.50–2.26	0.86	1.01	0.47–2.19	0.97
rs10033464 dominant						
All AF	1.73	1.02–2.95	0.04	1.74	1.02–2.97	0.04
Typical	4.59†	1.82–11.5	0.0012	4.70†	1.83–12.0	0.0013
Lone	1.15	0.52–2.51	0.73	1.1	0.49–2.45	0.82
rs7193343 additive						
All AF	1.07	0.69–1.66	0.75	1.08	0.69–1.66	0.75
Typical	1.14	0.49–2.67	0.77	1.11	0.46–2.67	0.80
Lone	0.90	0.52–1.57	0.71	0.92	0.52–1.61	0.76
rs7193343 dominant						
All AF	0.99	0.60–1.65	0.98	0.99	0.62–1.65	0.98
Typical	0.80	0.29–2.16	0.65	0.75	0.27–2.06	0.58
Lone	0.99	0.50–1.94	0.96	1.1	0.52–2.00	0.97
rs13376333 additive						
All AF	0.89	0.63–1.27	0.51	0.9	0.63–1.28	0.55
Typical	0.59	0.29–1.20	0.14	0.6	0.29–2.23	0.16
Lone	0.87	0.53–1.42	0.57	0.9	0.55–1.49	0.68
rs13376333 dominant						
All AF	0.89	0.55–1.46	0.64	0.91	0.55–1.49	0.70
Typical	0.58	0.23–1.45	0.25	0.61	0.24–1.53	0.29
Validation cohort						
rs10033464 additive						
All AF	1.318	0.62–2.79	0.46	1.317	0.72–2.79	0.47
rs10033464 dominant						
All AF	1.496†	1.02–3.06	0.04	1.498†	1.02–3.06	0.04

*Adjusted for sex and AF subtype. †$p < 0.05$.
CI = confidence interval; OR = odds ratio; other abbreviations as in Tables 1 and 2.

potassium channel–blocking drugs, which in real-life practice has a higher probability of patients experiencing deleterious side effects of these drugs and ultimate discontinuation. In our cohorts, carriers of the rs10033464 variant allele responded better to class I AADs: 41% versus 28% in the discovery cohort and 67% versus 39% in the validation cohort, $p < 0.05$, suggesting that the 4q25 SNP likely modulates other cardiac ion channels in addition to potassium channels.

In a novel study, Kirchhof et al. (29), utilizing expression arrays in *Pitx2*$^{+/-}$ mice, identified alteration in a number of cellular and molecular pathways in addition to potassium channels, which might explain our findings. Although studies in *Pitx2*-null mice have identified increased expression of potassium channel genes (*kcna3*, *kcnk5*), the functional consequences are unknown. Chinchilla et al. (30) recently looked at the electrophysiological defects in atrial-specific *Pitx2*-deficient mice. Their data show significantly more depolarized resting membrane potential in *Pitx2*-deficient mice (−84 mV vs. −87 mV) as compared with littermate control mice ($p < 0.05$), suggesting decreased expression and/or function of the channels that generate ionic currents involved in the control of the resting membrane potential, for instance, the inward rectifier current (I_{k1}). By utilizing quantitative RT-PCR, they show severely reduced expression of a number of potassium channels genes such as *Kcnj2*, and a gain-of-function consequence for both inward and outward Kir2.1 currents. In addition, atrial deletion of Pitx2 lead to down-regulation of *Scn5a* and *Scn1b*, both of which have been associated with familial

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Table 4 Response (%) Distribution of AAD by Class Among Responders for SNP rs10033464

Drug Class	Wild Type	Variant Allele Carriers	p Value
Discovery cohort, n	309	90	
Class 1	87 (28%)	37 (41%)	0.02
Class III	222 (72%)	53 (59%)	0.02
Validation cohort, n	97	46	
Class 1	37 (39%)	30 (67%)	0.01
Class III	60 (61%)	16 (34%)	0.02

Values are n (%).
AAD = antiarrhythmic drugs; SNP = single nucleotide polymorphism.

cases of AF. These recent data strongly support our finding that response to AADs may also be modulated by the 4q25 SNPs, with the mechanism related to modulation of cardiac ion channel expression in the heart. As there is likely to be differential expression of ion channels in the human heart compared with mice, it is not surprising that there was a differential response to the class of AADs.

The *ZFHX3* (zinc finger homeobox 3) gene on chromosome 16q22 encodes a transcription factor that was originally identified as a regulator of alpha-fetoprotein expression (31). *ZFHX3* has been associated with Kawasaki disease, as well as malignancies such as prostate cancer. However, in human cardiac and pulmonary tissue, its expression pattern and mechanism are not fully known (32). Two separate genome-wide association studies established an AF susceptibility locus on chromosome 16q22 near *ZFHX3* (14,15). More recently, a novel genetic locus on chromosome 1q21 was identified for early-onset or lone AF. The SNP (rs13376333) is located in the intronic region of chromosome 1q21 between the first and second exons of the calcium-activated potassium channel gene *KCNN3* (16). The precise mechanism by which it causes AF remains to be determined.

It is also possible that common genetic variants identify AF subtypes with differing drug responses. For example, recognizing that activation of the renin-angiotensin system is implicated in AF, 1 study examined the impact of the common angiotensin-converting enzyme insertion/deletion (ID) polymorphism on response to AAD therapy. An analysis of 213 subjects treated with rhythm control showed that lone AF and double-deletion (DD)/ID genotypes were highly significant predictors of failure of drug therapy (20). By contrast, no relationship between common SNPs in the beta1-adrenergic receptor (resulting in G389R and S49G) and response to ventricular rate-control therapy in a group of 279 patients managed with rate control was identified (33). These findings indicate that variable mechanisms contribute to AF susceptibility, and suggest therefore that response to therapy may also be genotype dependent.

Table 5 Distribution of AF Recurrence by Genotype

SNP	Carriers of Variant Allele	Wild Type	p Value*
Discovery cohort			
rs2200733	60/139 (43%)	123/334 (37%)	0.19
rs10033464	50/108 (46%)	133/365 (36%)	0.06
rs7193343	67/173 (37%)	114/287 (40%)	0.83
rs13376333	113/279 (40%)	68/183 (37%)	0.47
Validation cohort			
rs10033464	30/59 (51%)	43/134 (32%)	0.013

Numerator indicates total number of patients with AF recurrence, and denominator indicates total number of patients by genotype. *Univariate analysis (chi-square).
Abbreviations as in Tables 1 and 2.

Table 6 Multivariable Logistic Regression Analysis for Genetic Predictors of AF Recurrence

SNP	OR	95% CI	p Value
Discovery cohort			
rs2200733	1.26	0.70–2.14	0.40
rs10033464	3.27	1.77–6.04	<0.001
rs7193343	0.80	0.40–1.33	0.40
rs13376333	1.10	0.60–1.80	0.70
Validation cohort			
Rs10033464	4.31	1.98–9.40	<0.001

Abbreviations as in Tables 3 and 5.

Study limitations. There are certain limitations to this study that should be acknowledged. Although this was a retrospective analysis, patients were enrolled prospectively, and more important, response to AAD therapy was defined a priori without knowledge of the genotypes. The recessive logistic regression model was not performed due to the limited number of homozygous carriers. The sample size for the validation cohort was underpowered to detect allelic effect in the additive genetic model. With a follow-up period of 12 months and our criteria to document AF recurrence by 12-lead ECG at 3, 6, and 12 months, or by Holter monitoring or 30-day event recorder, and in some patients, from pacemaker interrogations, we might not have been able to capture asymptomatic AF in all patients. Both study cohorts were similar with respect to baseline demographics, but patients with typical AF in the validation cohort had well-preserved left ventricular ejection fraction and small left atrial size. Finally, our study looked at both reduction in symptomatic AF burden score as a reflection of successful rhythm control and AF recurrence, with both outcomes showing similar association with the same polymorphism at 4q25, but considering the retrospective nature of our study, a larger prospective randomized study is needed to fully appreciate this difference.

Conclusions

Our data support the hypothesis that a common SNP on chromosome 4q25 associated with AF modulates response to AAD therapy and points to a potential role for stratification of therapeutic approaches by genotype. Our findings may potentially impact the management of a large number of patients with AF because 19% to 22% of different AF populations carry this variant (17). However, this hypothesis

can only be truly evaluated in the context of a clinical trial, and these findings should be confirmed in randomized studies before tailoring AAD therapy for AF based on 4q25 genotype. The high recurrence of AF (30% to 50%) on AADs, added to the risk, albeit small, of life-threatening proarrhythmia, strongly supports the need for randomized pharmacogenetic trials and suggests that genetic assessment of therapeutic efficacy should be incorporated into all future trials of therapy for AF. If the current findings are confirmed, tailoring therapy on the basis of the 4q25 genotype is not only feasible, but may reduce the cost burden associated with this arrhythmia.

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