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Common Genetic Polymorphism at 4q25 Locus Predicts Atrial Fibrillation Recurrence after Successful Cardioversion

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Abstract

Background—Genome-wide association (GWA) studies have identified numerous common polymorphisms associated with atrial fibrillation (AF). The 3 loci most strongly associated with AF occur at chromosome 4q25 (near *PITX2*), 16q22 (in *ZFHX3*), and 1q21 (in *KCNN3*).

Objective—To evaluate if timing of AF recurrence after direct current cardioversion (DCCV) is modulated by common AF susceptibility alleles.

Methods—208 patients (age 65±11 years, 77% men) with persistent AF underwent successful DCCV and were prospectively evaluated at 3, 6 and 12 months for AF recurrence. Four single nucleotide polymorphisms (SNPs): rs2200733 and rs10033464 at 4q25; rs7193343 in *ZFHX3* and rs13376333 in *KCNN3* were genotyped.

Results—The final study cohort consisted of 184 patients. In 162 (88%) patients sinus rhythm was restored with DCCV, of which 108 (67%) had AF recurrence at a median of 60 (29 – 176) days. In multivariable analysis the presence of any common SNP (rs2200733, rs10033464) at the 4q25 locus was an independent predictor of AF recurrence, (hazard ratio [HR]: 2.1, 95% confidence interval [CI]:1.21–3.30, P=0.008). Furthermore, rs2200733 exhibited a graded allelic dose response for early AF recurrence (homozygous variants: 7 [4–56] days, heterozygous: 54 [28–135] days and wild type: 64 [29–180] days, P=0.03).

Conclusions—To our knowledge, this is the first study to evaluate whether genomic markers can predict timing of AF recurrence in patients undergoing elective DCCV. Our findings show that a common polymorphism on chromosome 4q25 (rs2200733) is an independent predictor of AF recurrence after DCCV and point to a potential role of stratification by genotype.

Keywords

atrial fibrillation; direct current cardioversion; genetic predictors; recurrence

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Conflicts of Interest: None

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice affecting over two million adults in the U.S and it contributes to impaired quality of life, increased morbidity and mortality^{1,2}. Symptomatic patients with AF often require direct current cardioversion (DCCV) to restore normal sinus rhythm (SR) in patients managed with a rhythm control strategy. Approximately 50% of patients who undergo successful DCCV will experience AF recurrence within the first month³. Adaptive changes including electrical and structural remodeling with involvement of neurohormones, inflammation, fibrosis and oxidative stress⁴⁻⁷, have been identified that contribute to the challenge of long-term maintenance of SR in patients with AF; however, no study to date has evaluated the ability of genomic markers to predict timing of recurrence after DCCV in patients with AF.

Recent genome wide association studies (GWAS) have identified numerous common polymorphisms associated with both lone and typical AF⁸. The 3 loci most strongly associated with AF occur on chromosomes 4q25 (near *PITX2*)⁹, 16q22 (in *ZFHX3*)¹⁰, and 1q21 (in *KCNN3*)¹¹. These findings indicate that variable mechanisms contribute to AF susceptibility, and suggest that response to therapy may also be genotype-dependent. The non-coding single nucleotide polymorphism (SNP) most strongly associated with AF, rs2200733 (4q25), conferred a 1.71-fold increased risk of AF while the other SNP, rs10033464 (4q25), conferred a 1.42-fold increased risk¹². This association has also been reported for post-cardiac surgery AF; a setting thought to be related to inflammation¹³ and has recently been reported to predict recurrence of AF after catheter ablation¹⁴ and response to AAD therapy¹⁵. The aim of this study was to prospectively evaluate if timing of AF recurrence after successful DCCV is modulated by the common AF susceptibility alleles (rs2200733 and rs10033464 at 4q25; rs7193343 in *ZFHX3* and rs13376333 in *KCNN3*).

Method

Study population

Subjects with persistent AF referred for elective DCCV were prospectively enrolled in the study, approved by the Vanderbilt Institutional Review Board. Patients > 21 years of age and scheduled to undergo an elective DCCV for AF were included in the study. Patients undergoing emergency DCCV for AF due to hemodynamic instability or post-cardiac surgery AF were excluded from the study.

Study protocol

After informed consent was obtained, baseline clinical history, blood and urine samples, and a 12-lead electrocardiogram (ECG) were obtained. Cardiac rhythm was continuously monitored for least 30 minutes prior to and following DCCV.

DCCV protocol

DCCV was performed under deep conscious sedation with either propofol or etomidate. A biphasic R-wave synchronized shock was delivered at 125J. If the initial shock failed to terminate AF, energy was gradually increased to 200, 300, and then 360J serially. If DCCV terminated AF successfully, the patient's cardiac rhythm was continuously monitored for at least 30 min to detect early AF recurrence.

Follow-up

All patients were evaluated in the outpatient clinic for 12 months after the DCCV. During this follow-up period, continuous cardiac rhythm monitoring was done for at least 30

minutes immediately after the restoration of sinus rhythm followed by performance of a 12-lead ECG at 3, 6 and 12 months. Report of symptoms suggestive of AF were evaluated with additional ambulatory (Holter, event recorder) monitoring. At each follow up visit, detailed history and medical records were obtained about outpatient visits and hospital admissions to outside facilities. An AF recurrence was defined as a documented AF episode lasting longer than 30 seconds.

Definitions

Documentation of AF on an ECG, rhythm strip, event recorder, or Holter monitor recording was necessary. Lone AF was defined as AF occurring in patients <66 years of age without hypertension or overt structural heart disease by clinical examination, ECG and echocardiography. Paroxysmal AF was defined as AF lasting more than 30 seconds with spontaneous termination. Persistent AF was defined as AF lasting more than seven days and requiring either pharmacologic or electrical cardioversion for termination. Time since AF diagnosis was defined as the time in years from the date of initial AF diagnosis to the date of study enrollment.

Genotyping

Genomic DNA was isolated from whole blood by a commercial kit (Purgene; Gentra Systems, Minneapolis, MN). Genotyping was performed for rs2200733 C>T, rs10033464 G>T, rs13376333 C>T and rs7193343 C>T, using previously described methods¹⁵.

Statistical analysis

Data is expressed as median with interquartile range (IQR) or frequency (percentage). Mann-Whitney U test was performed on continuous variables and Pearson Chi-square test or Fisher's exact test on discrete variables. Primary outcome was defined as time to AF recurrence. Kruskal-Wallis test was performed to determine the difference in time to AF recurrence by genotype. Association between genotype and AF recurrence was assessed in an additive genetic model where a graded allelic dose response is expected (heterozygous variant carriers have an intermediate effect in relation to the homozygotes) and dominant genetic model (an identical effect is expected in heterozygous and homozygous variant carriers).

Cox proportional hazard analysis was used to determine the hazard ratios (HRs) and their 95% confidence intervals (CIs). Covariates adjusted for in the final multivariable model included the SNP, age, time since AF diagnosis, gender, left atrial size and a propensity score for AF recurrence. The propensity score variable included age, gender, time since AF diagnosis, history of diabetes, coronary artery disease, hypertension, prior DCCV, use of antiarrhythmic drugs including amiodarone, β -blockers, angiotensin converting enzyme inhibitors, left atrial size and left ventricular ejection fraction determined by echocardiogram. We used Bonferroni correction to correct for multiple testing with even distribution of significance (α) between the four tested SNPs, with a two-sided P value < 0.0125 considered statistically significant. Haplotypes were estimated using the two SNPs (rs2200733 and rs10033464 at 4q25) by applying standard E-M algorithms and measured for degree of linkage disequilibrium (LD) represented by r^2 .

A sub-group analysis was done dichotomized by prior history of DCCV to evaluate the effects of genetic variants on time to AF recurrence in patients with and without prior DCCV. This model was adjusted for age and gender. All genetic and statistical analysis was performed using PLINK version 1.07¹⁶ and R version 2.14.0 (2011-10-31), platform: x86_64-redhat-linux-gnu (64-bit). Kaplan-Meier curves were generated using SPSS version 19.0.0.

Results

A total of 208 consecutive patients with persistent AF undergoing elective DCCV were enrolled in the study between 2008–2012, of which 13 did not undergo DCCV and 11 were excluded from the final analysis due to unsuccessful DNA extraction (Figure 1).

Patient demographics and clinical characteristics

The clinical characteristics of the study participants are listed in Table 1. Our study cohort consisted of 184 subjects; 142 men, 42 women; 179 Caucasian and five African American. The median age of the cohort was 66 (58–72) years and the median time since AF diagnosis was 2.8 (1.7–4.0) years. Eighty subjects had prior history of DCCV, 109 were on β -blockers (BB), 39 on amiodarone, 52 on other AADs and 56 on angiotensin converting enzyme inhibitors.

The minor allele frequency (MAF) of each SNP in our cohort was 19.7% for rs2200733, 10% for rs10033464 (no homozygous variant carriers), 32.6% for rs13376333, and 23.8% for rs7193343. The genotype frequencies for all SNPs did not deviate significantly from Hardy-Weinberg equilibrium (P for all >0.2).

DCCV outcomes

Of the 184 subjects who underwent elective DCCV, sinus rhythm was successfully restored in 162 (88%). There were no significant differences in baseline characteristics of subjects who were successfully cardioverted to sinus rhythm and those who remained in AF (Table 1).

Genotype call rates for subjects who underwent successful DCCV ($n=162$) were 96.3% for rs2200733 and 95.1% for rs10033464 at 4q25 locus, 93.8% for rs13376333 (*KCNJ3*) and 93.8% for rs7193343 (*ZFHX3*) (Table 2). We did not observe any significant difference in frequency of carriers of the variant allele between subjects who were successfully cardioverted vs. those who remained in AF (data not shown).

Study follow up and AF recurrence

For the 162 subjects who were successfully cardioverted to sinus rhythm, our median follow-up time was 118 (36–333) days. One hundred and eight subjects (67%) had documented AF recurrence in 60 (29–176) days while 54 subjects (33%) had no AF recurrence in 321 (129–365) days. Sixty-one (38%) subjects had symptoms suggestive of AF not captured by 12-lead ECG and hence were given either a 48-hour Holter monitor (42 subjects) or an event recorder (15 subjects). Holter monitor detected AF recurrence in 19 subjects and event recorder detected AF in 4 subjects.

Subjects who had AF recurrence had higher prevalence of coronary artery disease (42% vs. 26%, $P=0.05$) and prior use of AADs including amiodarone (57% vs. 35%, $P=0.008$); however, there was no significant difference in current use of amiodarone between the two groups; 24% (AF recurrence) vs. 13% (no AF recurrence), $P=0.09$ (Table 2). Frequency of carriers of variant allele of each SNP in subjects with AF recurrence vs. no AF recurrence was 37.5% vs. 23.1% for rs2200733 ($P=0.07$), 21.2% vs. 20% for rs10033464 ($P=0.9$), 58.8% vs. 48% for rs13376333 ($P=0.2$) and 4.1% vs. 38% for rs7193343 ($P=0.47$).

Time to AF recurrence by genotype

One hundred and eight subjects (67%) had documented AF recurrence. Only SNP rs2200733 (4q25) exhibited a graded allelic dose response for time to AF recurrence; homozygous carriers: 7 (4–56) days, heterozygous carriers: 54 (28–135) days and wild type:

64 (29–180) days, $P=0.03$ (Table 3), Kaplan-Meier (KM) log rank 0.02 (dominant model) and log rank 0.001 (additive model) (Figure 2). Polymorphisms at other loci did not show significant difference in time to AF recurrence, $P>0.1$ for all and KM log rank $P>0.1$ for all.

In univariate analysis, carriers of variant allele (rs2200733) had significantly higher rate of AF recurrence than wild type; Hazard ratio (HR) 1.68, 95% CI 1.18–2.40, $P=0.004$ (Table 4). None of the other clinical variables or SNPs showed significant effect on rate of AF recurrence. In multivariate analysis, under both additive and dominant genetic model, only SNP rs2200733 (4q25) remained an independent predictor of time to AF recurrence with carriers of the variant allele having nearly two and half folds higher rate of AF recurrence in 12 months; HR 2.41, 95% CI 1.45–4.00, $P=0.0006$ and HR 2.01, 95% CI 1.20–3.36, $P=0.007$ respectively. Furthermore, presence of any common SNP (rs2200733 or rs10033464; $r^2=0.03$) at the 4q25 locus was an independent predictor of the rate of AF recurrence; HR: 2.1, 95% CI 1.21–3.30, $P=0.008$.

Sub-group analysis dichotomized by prior DCCV

Seventy-four (46%) of 162 subjects that were successfully converted to SR had undergone prior DCCV and 88 (54%) subjects had no prior DCCV. Three subjects from each group failed genotyping; call rate 71/74 (96%) in prior DCCV group and 85/88 (96.5%) in no prior DCCV group.

Subjects with prior DCCV compared to those with no prior DCCV were similar in their baseline clinical characteristics except for greater time since AF diagnosis: 3.3 (2–4.6) years vs. 2.5 (1.4–3.8) years ($P=0.02$), larger left atrial size: 48 (42–53) millimeter (mm) vs. 45 (40–48) mm, ($P=0.007$) and higher current use of AADs; 50 of 74 (68%) vs. 31 of 88 (35%), ($P<0.001$). Table 6 shows the univariate and multivariate model and only SNP rs2200733 (4q25) remained a significant predictor of the rate of AF recurrence with the carriers of the variant allele having higher rate of AF recurrence in both groups; HR 1.72, 95% CI 1.0–2.9, $P=0.05$ (prior DCCV) and HR 1.8, 95% CI 1.1–2.9, $P=0.02$ (no prior DCCV).

Discussion

This is the first study to test the hypothesis that common genetic polymorphisms associated with AF modulate response to DCCV. We show that rs2200733 at the 4q25 locus is an independent predictor of AF recurrence after successful DCCV. A graded allelic effect was observed such that median time to AF recurrence in patients who were homozygous carriers of the variant allele (rs2200733) was 7 (4–56) days compared to 54 (28–135) days for heterozygous carriers and 65 (29–180) days for wild-type (Figure 2).

Recently several GWAS have confirmed an association between AF and intergenic variation (rs2200733) on chromosome 4q25^{9,17}. While the exact mechanism by which this variant increases susceptibility to AF is not completely clear, modulation of the paired-like homeodomain transcription factor 2 (*PITX2*) gene has been postulated. *PITX2* is a homeobox transcription factor that plays a regulatory role in early left/right determination during embryonic development, downstream of the *nodal/lefty*-signaling pathway¹⁸. *PITX2* has also been demonstrated to play a role in development of the pulmonary vein myocardium, the predominant source of AF triggers¹⁹. Mice models with heterozygous deletion of *Pitx2c*, the cardiac isoform of *Pitx2*, have shown to be at increased risk of AF associated with a shortening of the left atrial action potential duration²⁰. *PITX2c* (the cardiac isoform of *PITX2*) plays a pivotal role in a number of cardiac developmental functions, particularly as a mediator in the left-right patterning pathway of mammalian embryos²¹ and has marked chamber specificity in the adult heart: mRNA transcripts are

expressed almost 100-fold higher in the left as compared to the right adult human and murine atrium ²⁰.

Recently Chinchilla et al confirmed that subjects carrying the genetic variant (rs2200733) have significantly decreased *PITX2c* expression, thus providing a molecular link between loss of function of *PITX2* and AF ²². They further showed altered morphological, molecular, and electrophysiological characterization of chamber-specific *Pitx2* conditional mouse mutants along with altered sodium and potassium channel expression ²². *Pitx2* loss of function leads to down regulation of *Scn5a* and *Scn1b*, point mutations in which have been associated with familial cases of AF ²³.

We and others have shown that variants on 4q25 are important genetic predictors of response to AF therapy ^{14, 15}. Husser et al showed that these variants also modulate the risk for AF recurrence after successful catheter ablation in a cohort of 195 subjects. The presence of these variants was associated with two to three-fold higher risk of early (within the first week after ablation) and late AF recurrence (between 3 to 6 month after ablation) ¹⁴. We recently tested the hypothesis that response to AAD therapy might also be modulated by common SNPs associated with AF. Our data, in two independent cohorts of 478 and 198 subjects, showed that presence of variant rs10033464 at 4q25 locus was significantly associated with response to AAD therapy (symptom reduction and AF recurrence). Carriers of the variant allele at this position were at three to four-fold higher risk of AF recurrence within 6 month of initiation of AAD therapy. We also observed that carriers of the variant allele responded more favorably to Class I AAD, thus providing an important link between clinical and animal model evidence of altered sodium/potassium channel expression ¹⁵.

The exact mechanism by which 4q25 SNPs modulate response to AF therapies is unknown, but the relationship between *PITX2* and development of the PV myocardium suggests the hypothesis that variant carriers possess a greater degree of AF triggers from the PVs. In turn, increased PV triggers may secondarily increase adverse atrial structural remodeling over time and the combination of enhanced triggers and adverse structural remodeling could modulate the clinical response to a variety of AF therapies, including AF ablation and AADs.

Studies have shown that the use of AADs is an independent predictor of maintenance of SR following successful DCCV ^{24, 25} and for prevention of AF following cardiac surgery ^{26, 27}. In our study cohort, use of AADs including amiodarone was associated with higher rate of AF recurrence (HR 1.49, 95% CI 1.02–2.18, P=0.04) which is likely confounded by the characteristics of patients taking AADs such as longer time since AF diagnosis (4.25 years vs. 3.68 years), increased weight (104 kg vs. 99 kg), left atrial enlargement (47 mm vs. 45 mm) and lower ejection fraction (48% vs. 50%), thus predisposing them to higher risk of AF recurrence.

There are certain limitations to this study that should be acknowledged. First, ~ 12% (24 of 208) subjects enrolled in the study were excluded from the final analysis for reasons including failure to undergo DCCV and unsuccessful DNA extraction (Figure 1); however, given our sample size calculation, we had enough power to detect significant modulatory effect of genetic variants on AF recurrence following DCCV. Second, compared to other studies evaluating AF recurrence after DCCV, ^{24, 28} our study population was relatively older with longer time since AF diagnosis and with higher percentage of comorbidities such as coronary artery disease and hypertension. Furthermore, only 68% of patients with a prior history of DCCV were on an AAD compared to 35% of patients without prior DCCV. As these patients were recruited from the procedural suite on the day of DCCV and patients were on an AAD at the discretion of the attending cardiologist, there are likely many reasons

for the low rate of amiodarone/AAD use in the group with a history of prior DCCV. These include whether they were referred by an electrophysiologist or general cardiologist, the intervening time from the prior DCCV, co-morbid conditions precluding the use of many AADs, and patient/provider preferences.

Finally, AF recurrence was evaluated by serial ECGs at 3, 6 and 12 months in all patients, 48-hour ambulatory holter monitor in 42 of 162 patients and 30 days event recorder in 19 of 162 in patients who complained of symptoms suggestive of AF not captured by 12 lead ECG. This was supplemented by review of medical records from other institutions and physician notes to capture time to AF recurrence in all patients. Since the use of continuous rhythm monitoring was at the discretion of the attending physician, some asymptomatic AF was likely missed.

Conclusions

Our study shows that a common polymorphism on chromosome 4q25 (rs2200733) is an independent predictor of AF recurrence after successful restoration of sinus rhythm following DCCV. Patients homozygous for the rs2200733 risk allele experienced a clinically significantly earlier recurrence of AF (median time 7days) compared to heterozygous carriers (median time 54 days) and wild-type patients (median time 64 days). This finding has the potential to change clinical management such that homozygous carriers at rs2200733 may be considered poor candidates for DCCV due to early recurrence and suggests the need to evaluate alternative management strategies for symptomatic AF in these patients.

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Glossary of Abbreviations

AF	atrial fibrillation
CI	confidence interval
DCCV	direct current cardioversion
GWAS	genome wide association study
HR	Hazard ratio
SNP	single nucleotide polymorphism;

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Figure 1.
Consort diagram of the study.

Figure 2.

Kaplan-Meier curve of Genetic polymorphism (top panel [dominant model]; wild type [green] and carriers of variant allele [red]; bottom panel [additive model]; wild type [green], heterozygous carriers of variant allele [blue] and homozygous carriers of variant allele [red]) and time to AF recurrence after successful DC-cardioversion. Numerator represents events (AF recurrence) and denominator represents total subjects by genotype.

Table 1

Study cohort characteristics.

Characteristics	Entire cohort (n=184)	DC-Cardioversion		P value*
		Successful (n=162)	Unsuccessful (n=22)	
Age (years)	66 (58–72)	67 (59–72)	65 (58–70)	0.73
Time since AF diagnosis (years)	2.8 (1.7–4.0)	3 (1.8–4.1)	2.1 (0.7–3.1)	0.13
Gender, n (% male)	142 (77)	125 (77)	17 (77)	0.99
Ethnicity, n (% white)	179 (97)	157 (97)	22 (100)	0.40
Height (cm)	179 (173–185)	179 (170–185)	180 (173–185)	0.98
Weight (kg)	98 (87–113)	98.6 (86–113)	93 (88–114)	0.87
Diabetes, n (%)	35 (19)	32 (20)	3 (14)	0.49
Coronary artery disease, n (%)	66 (36)	59 (36)	7 (32)	0.67
Hypertension, n (%)	120 (65)	107 (66)	13 (59)	0.52
History of cardioversion, n (%)	80 (44)	74 (46)	6 (27)	0.10
Lone AF, n (%)	8 (4)	6 (4)	2 (9)	0.24
Use of AAD, n (%)	91 (50)	81 (50)	10 (46)	0.68
Amiodarone, n (%)	39 (21)	33 (20)	6 (27)	0.45
Use of BB, n (%)	109 (59)	98 (61)	11 (50)	0.34
Use of ACEi, n (%)	56 (30)	51 (32)	5 (23)	0.40
LVEF	55 (45–60)	55 (45–60)	55 (54–56)	0.10
Left atrial size (mm)	45 (42–50)	46 (42–50)	44 (43–50)	0.79
Genotype distribution [^]				
SNP	Entire cohort	DC-Cardioversion		Undetermined
		Successful	Unsuccessful	
rs2200733 (4q25)	114/50/8	105/45/6	9/5/2	12
rs10033464 (4q25)	136/34/0	123/31/0	13/3/0	14
rs13376333 (KCNN3)	74/77/16	68/71/13	6/6/3	17
rs7193343 (ZFHX3)	97/62/9	88/55/9	9/7/0	16

* Between DC-cardioversion successful and unsuccessful (Mann-Whitney U test, chi-square or Fisher's exact were appropriate), AF = atrial fibrillation, AAD = antiarrhythmic drugs, BB = β -blockers, ACEi = angiotensin converting enzyme inhibitors, LVEF = left ventricular ejection fraction, continuous variables expressed as median with IQR, SNP = single nucleotide polymorphism

[^] wild type/heterozygous carriers/homozygous carriers

Table 2

Characteristics of patients by AF recurrence (n=173).

Characteristics	AF recurrence		P value *
	Yes (n=108)	No (n=54)	
Age (years)	67 (59–72)	66 (58–72)	0.89
Time since AF diagnosis (years)	3.4 (1.9–4.4)	2.3 (1.5–3.8)	0.052
Gender, n (% male)	82 (76)	43 (80)	0.59
Ethnicity, n (% white)	106 (98)	51 (94)	0.19
Height (cm)	179 (170–185)	180 (173–185)	0.54
Weight (kg)	99 (86–113)	99 (87–114)	0.75
Diabetes, n (%)	22 (20)	10 (19)	0.78
CAD n (%)	45 (42)	14 (26)	0.05
Hypertension, n (%)	73 (68)	33 (63)	0.55
History of DCCV, n (%)	54 (50)	20 (37)	0.18
Lone AF, n (%)	4 (4)	2 (4)	1.00
Use of AAD, n (%)	62 (57)	19 (35)	0.008
Amiodarone, n (%)	26 (24)	7 (13)	0.09
Use of BB, n (%)	63 (58)	35 (65)	0.42
Use of ACEi, n (%)	34 (32)	17 (32)	1.00
LVEF	55 (45–55)	55 (45–55)	0.40
Left atrial size (mm)	46 (41–50)	44 (42–48)	0.30
Genotype distribution [^]			
SNP	AF recurrence		Undetermined
	Yes	No	
rs2200733 (4q25)	65/34/5	40/11/1	6
rs10033464 (4q25)	83/21/0	40/10/0	8
rs13376333 (KCNN3)	42/52/8	26/19/5	10
rs7193343 (ZFHX3)	57/40/5	31/15/4	10

* Mann-Whitney U test, chi-square or Fisher's exact were appropriate, AF = atrial fibrillation, AAD = antiarrhythmic drugs, CAD= coronary artery disease, BB = β -blockers, ACEi = angiotensin converting enzyme inhibitors, LVEF = left ventricular ejection fraction, continuous variables expressed as median with IQR, SNP = single nucleotide polymorphism,

[^] wild type/heterozygous carriers/homozygous carriers

Table 3

Time (days) to AF recurrence by genotype.

Genetic Polymorphisms	N by genotype [‡]	Days till AF recurrence			P value [*]
		Wild type	Heterozygous carriers	Homozygous carriers	
rs2200733 (4q25)	67/34/5	64 [29–180]	54 [28–135]	7 [4–56]	0.03
rs10033464 (4q25)	83/21/0	59 [27–177]	43 [17–118]	–	0.35
rs13376333 (KCNN3)	42/52/8	54 [26–180]	68 [35–167]	29 [9–100]	0.16
rs7193343 (ZFHX3)	57/40/5	58 [28–195]	65 [28–163]	35 [20–73]	0.58

^{*} Kruskal-Wallis H test except for rs10033464 (Mann-Whitney U test), continuous variables expressed as median with IQR

[‡] wild type/heterozygous carriers/homozygous carriers

Table 4

Univariate analysis of predictors of time to AF recurrence.

Clinical variables	Hazard Ratio	95% Confidence Interval	P value
Age (5 years)	1.00	0.92–1.09	0.99
Time since AF diagnosis (year)	1.05	0.99–1.10	0.057
Gender (male)	1.00	0.69–1.66	0.77
Ethnicity (white)	0.49	0.12–1.99	0.32
Diabetes	0.92	0.57–1.47	0.72
Coronary artery disease	1.16	0.79–1.71	0.44
Hypertension	1.04	0.69–1.55	0.86
History of cardioversion	1.16	0.79–1.70	0.44
Lone AF	0.65	0.46–3.43	0.64
Use of AAD	1.49	1.02–2.18	0.04
Amiodarone	1.19	0.76–1.85	0.44
Use of BB	0.72	0.49–1.06	0.09
Use of ACEi	0.94	0.66–1.48	0.94
LVEF (%)	1.00	0.98–1.10	0.90
Left atrial size (mm)	1.00	0.97–1.04	0.63
Genetic Polymorphisms (Additive model)			
rs2200733 (4q25)	1.68	1.18–2.40	0.004
rs10033464 (4q25)	1.03	0.64–1.67	0.89
rs13376333 (KCNN3)	1.20	0.88–1.65	0.24
rs7193343 (ZFHX3)	1.14	0.82–1.58	0.42
Genetic Polymorphisms (Dominant model)			
rs2200733 (4q25)	1.58	1.06–2.36	0.02
rs10033464 (4q25)	1.07	0.67–1.71	0.78
rs13376333 (KCNN3)	1.26	0.85–1.87	0.25
rs7193343 (ZFHX3)	1.19	0.80–1.76	0.38

Abbreviations: see Table 1.

Table 5

Multivariate Cox regression analysis of genetic variants and time to AF recurrence

Genetic Polymorphisms	Hazard Ratio	95% Confidence Interval	P value
Additive model			
rs2200733 (4q25)	2.41	1.45–4.00	0.0006
rs10033464 (4q25)	1.30	0.65–2.59	0.44
rs13376333 (KCNN3)	1.47	0.99–2.18	0.06
rs7193343 (ZFHX3)	1.45	0.96–2.17	0.07
Dominant model			
rs2200733 (4q25)	2.01	1.20–3.36	0.007
rs10033464 (4q25)	1.30	0.65–2.59	0.44
rs13376333 (KCNN3)	1.44	0.88–2.37	0.14
rs7193343 (ZFHX3)	1.57	0.96–2.57	0.06
Haplotype model			
Any 4q25 variant	2.10	1.21–3.30	0.008

Model adjusted for age, time since AF diagnosis, gender, left atrial size and a propensity score variable consisting of age, gender, time since AF diagnosis, history of diabetes, coronary artery disease, hypertension, prior DCCV, use of AADs including amiodarone, β -blockers, ACEIs, left atrial size and LVEF determined by echocardiogram, † adjusted plus false discovery rate corrected.

Table 6

Cox regression analysis of genetic variants and time to AF recurrence after successful restoration of sinus rhythm dichotomized by prior DC-cardioversion

Genetic Polymorphisms	Prior DCCV (n=71)		No Prior DCCV (n=85)	
	HR	95% CI	HR	95% CI
Univariate				
rs2200733 (4q25)	1.6	0.9–2.6	0.08	1.8
rs10033464 (4q25)	1.2	0.6–2.3	0.62	0.9
rs13376333 (KCNN3)	1.3	0.8–1.9	0.23	1.1
rs7193343 (ZFHX3)	0.9	0.6–1.6	0.99	1.3
Multivariate (additive model)*				
rs2200733 (4q25)	1.7	1.0–2.9	0.05	1.8
rs10033464 (4q25)	1.1	0.5–2.3	0.79	0.9
rs13376333 (KCNN3)	1.3	0.8–2.0	0.19	1.1
rs7193343 (ZFHX3)	1.0	0.6–1.6	0.99	1.3

* multivariate model adjusted for age and gender; CI = confidence interval; DCCV = direct current cardioversion; HR = hazard ratio.