HEREDITARY ARRHYTHMIA CORNER

Single Nucleotide Polymorphisms in Proximity to K-Channel Genes Are Associated with Decreased Longitudinal QTc Variance

Yuliya Mints, M.S.,* Vadim Zipunnikov, Ph.D.,† Irfan Khurram, M.D.,* Hugh Calkins, M.D.,* and Saman Nazarian, M.D., Ph.D.*

*From the *The Johns Hopkins University School of Medicine, Baltimore, Maryland, and †Department of Biostatistics, Johns Hopkins University, Baltimore, Maryland*

Background: Atrial fibrillation (AF) is the most common form of cardiac arrhythmia. Despite significant progress in identification of predisposing factors, the pathophysiology of AF remains to be elucidated. Previous studies have reported that single nucleotide polymorphisms (SNPs) in potassium-channel genes associate with AF and the instantaneous corrected QT interval (QTc). The purpose of this study was to examine the association between SNPs in proximity to *KCNQ1, KCNH2, KCNE2,* and *KCNJ2* and longitudinal QTc variations in patients with AF.

Methods and Results: We conducted a retrospective cohort study of 800 electrocardiograms from 93 patients with AF. All patients were Caucasian, with an average age of 61.2 years, and 72% were male. Of all patients, 37% had persistent AF, and 63% had paroxysmal AF. Following DNA extraction from blood, SNPs at the AF-associated loci *KCNQ1, KCNH2, KCNE2,* and *KCNJ2* were genotyped using the Sequenom MassARRAY. Using a linear regression model and adapting a resampling inference, a decrease in longitudinal QTc variance was found to associate with SNPs near *KCNH2* (rs10240738) and *KCNJ2* (rs8079702) when adjusted for patient age, gender, AF type, and average QTc. On average, patients with these SNPs had a shorter QTc interval. In addition, we fitted a multilevel mixed effects regression model accounting for subject level heterogeneity and found no longitudinal association between presence of SNPs near K-channel genes and changes in QTc.

Conclusion: Polymorphisms near specific potassium-channel genes in AF patients are associated with decreased longitudinal QTc variance and a shorter average QTc. These results support the hypothesis that effects on myocardial repolarization may mediate the association of these SNPs and AF.

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Atrial fibrillation (AF) is the most common form of cardiac arrhythmia, and represents a growing health concern. The lifetime risk of developing AF is 25% above the age of 40.[1] AF is characterized by rapid and irregular activation of the atrium, and causes considerable morbidity and mortality. Although progress has been made in elucidating the pathophysiologic basis of AF, many questions remain.[2–4] Although cardiac disorders, such as coronary artery disease, valvular disease, and hypertension predispose to AF, some patients develop AF in the absence of any known risk factor.

Address for correspondence: Yuliya Mints, MS, Johns Hopkins University School of Medicine, Carnegie 592 C, 600 North Wolfe Street, Baltimore, MD 21287; E-mail: ymints1@jhmi.edu

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Therefore, genetic risk factors likely participate in the pathophysiology of AF.[5] In addition, individuals with at least one parent with a history of AF have an 85% increased relative risk of AF.[5]

Linkage analysis for monogenetic AF has mapped several loci. Of these loci, some encode subunits of potassium channels. Mutations in these cardiac potassium channel genes have been shown to cause familial AF, specifically: *KCNE2*,[6] *KCNH2*,[7] *KCNJ2*,[8] and *KCNQ1*.[9] All of the known mutations are associated with a gain of function of repolarization potassium currents. This results in a shortening of action potential duration, which facilitates the development of AF.

Prior studies have demonstrated that potassium channel gene polymorphisms are associated with a higher risk of AF.[10,11] We hypothesized that AF susceptibility in AF patients with these polymorphisms is mediated through abnormal repolarization. Because global atrial repolarization is difficult to measure we used ventricular repolarization as a surrogate. *In vitro* studies have demonstrated the presence of these four potassium channels in both the atria and ventricles.[12–14]

Rather than examining cross-sectional repolarization for patients, we examined longitudinal changes in ventricular repolarizations over months to years, which we term longitudinal variance. Therefore, we sought to uncover evidence for abnormal ventricular repolarization (QTc) in AF patients with single nucleotide polymorphisms (SNPs) near *KCNE2*, *KCNH2*, *KCNJ2*, and *KCNQ1* genes.

MATERIALS AND METHODS

Study Design

Participants in the study gave written informed consent to participate in the study, which was approved by the Johns Hopkins University School of Medicine Institutional Review Board.

The study sample was chosen from a cohort of 100 patients with AF. Patients with only one ECG in the electronic record and those with evidence of ventricular pacing or QRS duration $\geq$120 ms were excluded. Therefore, the final study cohort consisted of 93 patients (72% male and 28% female, mean age 61.5 $\pm$ 10 years, all Caucasian) who were diagnosed with persistent (37%) or paroxysmal AF (63%). Routine clinic visit and symptom prompted, 12-lead resting ECGs were retrospectively obtained from electronic patient records. To maintain consistency, the QT intervals were automatically measured by the ECG software, and corrected for heart rate by use of the Bazett's Formula (QTc).[15]

Genotyping

DNA was extracted from blood samples using Qiagen's Puregene DNA extraction kit (Qiagen, Valencia, CA). SNPs were chosen by their proximity to potassium channel genes: *KCNE2* (rs754467, rs8134775, rs9984281), *KCNH2* (rs10240738, rs11972506, rs1549760, rs1805123), *KCNJ2* (rs8079702), *KCNQ1* (rs179435, rs2301696, rs234886, rs2412115, rs3782068, rs760419). Genotyping of SNPs was performed with the MassARRAY system of the Sequenom genotyping platform as part of a single multiplex SNP analysis (Sequenom, San Diego, CA). Each sample was tested at least three times and at least two concordant results were required.

Statistical Analysis

Continuous variables are presented as mean $\pm$ SD. Categorical variables are reported as frequencies and percentages. The association of longitudinal QTc variance with SNPs near K channel genes was examined using a linear regression model and adapting a resampling inference, adjusting for age, gender, AF type, and mean QTc. To make longitudinal QTc variances comparable with mean QTc levels, we analyzed longitudinal QTc standard deviations calculated as square roots of corresponding variances. To estimate uncertainty associated with the sample size, we utilized a bootstrap study that confirmed the significance of the associations. Results are reported as significant if $P \leq 0.05$, although with Bonferroni correction for multiple comparisons, results would be considered significant at $P \leq 0.0035$. A codominant model was assumed for polymorphisms, where an intermediate effect was assigned to heterozygous variant carriers in relation to the homozygotes. Statistical analyses were performed using STATA version 12 (College Station, TX) and lm package in R statistical software (http://www.r-project.org).

Table 1. Demographic Data and Clinical Features of the Study Patients (n = 93)

Variables	All Patients (n = 93)	*KCNH2* (rs10240738)			*KCNJ2* (rs8079702)		
		Zero alleles (n = 15)	One allele (n = 51)	Two alleles (n = 27)	Zero alleles (n = 31)	One allele (n = 43)	Two alleles (n = 18)
Age (mean ± SD), year	61.2 ± 10.2	61.8 ± 13.08	61.2 ± 10.2	60.7 ± 9.05	62.8 ± 9.1	62.4 ± 9.4	55.4 ± 12.3
Male, n (%)	67 (72%)	11 (73%)	37 (73%)	8 (30%)	10 (32%)	31 (69%)	15 (88%)
Height (cm)	174 ± 21.1	178 ± 13.6	176 ± 9.5	175 ± 11	175 ± 12.3	171 ± 28.0	180 ± 10.0
BMI (kg/m^2)	28 ± 5.8	27 ± 4.5	28 ± 4.9	29 ± 5.6	29 ± 5.2	28 ± 6.8	28 ± 3.7
AF duration until first ECG	7.8 ± 6.05	11.1 ± 7.1	6.4 ± 5.5	6.4 ± 2.9	8.1 ± 6.4	7.3 ± 5.7	8.5 ± 6.6
AF type							
Paroxysmal, n (%)	59 (63%)	9 (60%)	32 (63%)	18 (67%)	20 (65%)	24 (53%)	14 (82%)
Persistent and longstanding, n (%)	34 (37%)	6 (40%)	19 (37%)	9 (33%)	11 (35%)	21 (47%)	3 (18%)
Hypertension, n (%)	50 (54%)	9 (60%)	25 (49%)	16 (59%)	17 (23%)	25 (56%)	7 (41%)
Obstructive sleep apnea, n (%)	15 (16%)	4 (27%)	6 (12%)	5 (19%)	5 (16%)	7 (16%)	3 (18%)
LVEF (mean ± SD)	56 ± 9	55 ± 10.9	55 ± 9.7	57 ± 5.4	57 ± 6.7	55 ± 10.1	58 ± 6.9
History of antiarrhythmic drug use, n (%)							
Class I	18 (19%)	4 (27%)	10 (19%)	4 (15%)	5 (16%)	8 (19%)	5 (28%)
Class III	40 (43%)	7 (47%)	23 (45%)	11 (41%)	13 (42%)	18 (42%)	9 (50%)
Class I and III	26 (28%)	3 (20%)	15 (29%)	8 (30%)	8 (26%)	14 (33%)	4 (22%)
CHADs Score, n (%)							
0	37 (40%)	5 (33%)	22 (43%)	10 (37%)	11 (61%)	15 (33%)	11 (61%)
1	35 (38%)	7 (47%)	15 (29%)	13 (48%)	14 (45%)	15 (33%)	6 (33%)
2	14 (15%)	3 (20%)	8 (16%)	3 (11%)	4 (13%)	9 (20%)	1 (6%)
≥ 3	7 (7%)	0 (0%)	6 (12%)	1 (4%)	2 (6%)	6 (14%)	0 (0%)
Mean QTc	447.4 ± 29.9	464.2 ± 28.6	445.3 ± 28.1	441.9 ± 31.5	446.8 ± 33.1	452.2 ± 30.6	436.7 ± 18.8

Table 2. Polymorphism Frequency Near Potassium Channel Genes (n = 93)

Closest Potassium Channel Gene	SNP Database	Patients with Number of Alleles		
		0	1	2
KCNE2	rs754467	73 (78.5%)	20 (21.5%)	0 (0%)
	rs8134775	67 (72.0%)	25 (26.9%)	1 (1.1%)
	rs9984281	69 (74.2%)	20 (21.5%)	4 (4.3%)
KCNH2	rs10240738	15 (16.2%)	51 (54.8%)	27 (29.0%)
	rs11972506	21 (22.6%)	57 (61.3%)	15 (16.1%)
	rs1549760	57 (61.3%)	29 (31.4%)	7 (7.4%)
	rs1805123	56 (60.2%)	37 (39.8%)	0 (0%)
KCNJ2	rs8079702	31 (33.3%)	45 (48.4%)	17 (18.3%)
KCNQ1	rs179435	55 (59.2%)	31 (33.3%)	7 (7.5%)
	rs2301696	15 (16.1%)	56 (60.2%)	22 (23.7%)
	rs234886	77 (82.8%)	15 (16.1%)	1 (1.1%)
	rs2412115	38 (40.9%)	44 (47.3%)	11 (11.8%)
	rs3782068	18 (19.3%)	38 (40.9%)	37 (39.8%)
	rs760419	23 (24.7%)	41 (44.1%)	29 (31.2%)

RESULTS

Patient Characteristics

The characteristics of patients are shown in Table 1. No significant differences were seen between the patients containing a specific SNP and the rest of the study population with regard to age, height, body mass index, and common cardiovascular factors, such as hypertension, obstructive sleep apnea, and CHADs score. History or current use of antiarrhythmic drug use (class I, class III, or both) was also similar between the groups. With regard to cardiac function, the groups did not differ significantly in left ventricular ejection fraction or type of AF (paroxysmal or persistent). The average QRS duration of the study population was 93 ms. Of the ECGs obtained, 78% were in sinus rhythm at the time of measurement, 13% in AF, and 9% in other rhythms. Polymorphism frequency near potassium channel genes has been summarized in Table 2.

Longitudinal Variance of QTc within Patients

Of the SNPs genotyped in proximity to the potassium channel genes, *KCNH2* (rs10240738) and *KCNJ2* (rs8079702) were associated with a decreased longitudinal variance in QTc for patients

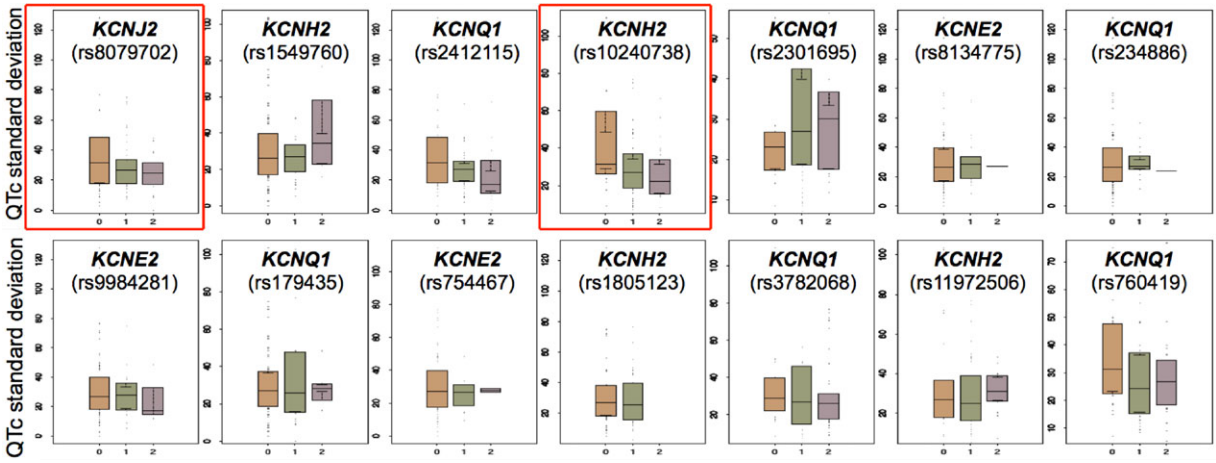

Figure 1. Longitudinal standard deviation of QTc in patients with various numbers of SNP-containing alleles (0, 1, and 2) near all 14 SNPs tested. The presence of two SNPs, *KCNJ2* (rs8079702) and *KCNH2* (rs10240738), shows a decrease in standard deviation as compared to absence of that SNP.

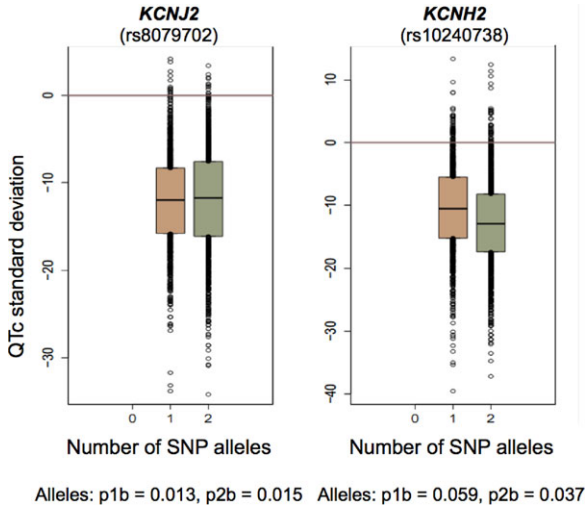

Figure 2. Simulation study: Adjusted longitudinal QTc standard deviations have been resampled with replacement 1000 times and allele coefficients have been re-estimated. The box plots show the distributions of the coefficients with corresponding P values calculated as a ratio of the number of times the coefficients were estimated to be positive over the total number of bootstrapped experiments.

Table 3a. Predictors of Longitudinal QTc Standard Deviation in a Linear Model with *KCNH2* rs10240738 Single Nucleotide Polymorphism (SNP)

Parameter	Model Coefficients	P Values
Intercept	−88.04	0.024
Mean QTc	0.34	<0.0001
One SNP allele	−10.19	0.120
Two SNP alleles	−12.55	0.082
Age	−0.42	0.058
Female gender	15.66	0.002
Paroxysmal AF type	−3.74	0.427

Table 3b. Predictors of Longitudinal QTc Standard Deviation in a Linear Model with *KCNJ2* rs8079702 Single Nucleotide Polymorphism (SNP)

Parameter	Model Coefficients	P Values
Intercept	−104.09	0.005
Mean QTc	0.38	<0.0001
1 SNP Allele	−12.36	0.015
2 SNP Alleles	−12.29	0.065
Age	−0.49	0.031
Female gender	14.41	0.005
Paroxysmal AF type	−4.04	0.388

with AF (Fig. 1; Tables 3a and 3b). The results are summarized in Tables 3a and 3b, where the longitudinal QTc standard deviation is the dependent variable. We further examined this finding by a bootstrap method[16] (Fig. 2). This is a resampling method that is useful in situations with small sample size or when the theoretical distribution of the statistic is unknown. Using this method, we created 1000 new samples of 93 patients. These new samples were taken from the original data set using sampling with replacement, so it is not identical to the original sample. For each of the 1000 samples, a linear regression has been refitted and adjusted for age, gender, AF type, and mean QTc. Figure 2 shows the distributions of the 1000 estimated regression coefficients. The bootstrap P values, defined as the proportion of positive coefficients, more accurately reflect uncertainty in small sample studies. Note that *KCNH2* rs10240738 is not reported as significant based on the linear regression fit. However, the bootstrap reports the P value for two alleles of *KCNH2* (rs10240738), p2b, equal to 0.037. Hence, we believe there is an evidence to consider this gene as significant. The model coefficient for each parameter indicates how corresponding variable changes the QTc standard deviation. Thus, the presence of one allele of *KCNH2* (rs10240738) causes a 10.19 ms decrease in QTc standard deviation as compared to absence of that SNP. In addition, the decreased longitudinal variance was the same regardless of whether the patient had one or two alleles of that SNP suggesting a dominant genetic mechanism. The other 12 SNPs were unassociated with longitudinal QTc variance.

DISCUSSION

The main finding of this study is that AF patients with SNPs in proximity to potassium channel genes *KCNH2* and *KCNJ2*, exhibit decreased longitudinal QTc variance.

Consistency with Prior Studies

Linkage analysis studies have found that polymorphisms in potassium channel genes are associated with a higher risk of AF.[10,11] In addition, characterization of mutations in *KCNE2*,[6] *KCNH2*,[7] *KCNJ2*,[8] and *KCNQ1*[9] demonstrated that AF-causing mutations are associated with a gain of function, and a shortening of QTc interval. However, few of these results have been replicated

in independent AF populations. Our results are consistent with these findings, as patients with polymorphisms near these genes exhibit a shorter average QTc. In addition, the decreased variance in longitudinal QTc implies that patients consistently have a shorter QTc interval.

We tested SNPs near *KCNE2*, *KCNH2, KCNJ2,* and *KCNQ1* for association with QTc in our population. In this study, all patients were Caucasian, excluding the possibility of linkage disequilibrium in different ethnic groups. All patients had either paroxysmal (63%) or persistent (37%) AF. Because gender and age are factors that can affect the QTc internal, they were adjusted for as potential confounders. The design excluded patients with prolonged QRS, thereby eliminating bundle branch block as a confounder.

In contrast to previous studies of the effect of SNPs on AF, we obtained all available ECGs retrospectively for our population. This allows us to observe the association of SNPs near potassium channel genes with longitudinal repolarization changes. Because AF has a high rate of recurrence,[17] we hypothesized that polymorphisms may have a long-term impact on the repolarization properties. Because we cannot easily measure atrial repolarization, we used ventricular repolarization as a proxy, because these potassium channels are also expressed in the ventricles.

AF is believed to occur as a result of functional reentry within the atria. The balance of ionic current determines the repolarization period, and therefore the length of the action potential. It is believed that reentry is at least partially propagated by a shorter action potential. Therefore, a decrease in QTc, and subsequently action potential duration, would maintain AF.[18] In addition, repolarization abnormalities may also trigger AF. In normal subjects, beat-to-beat repolarization variability is physiological, such as when changing posture.[19] It is possible that in patients with AF who have SNPs near repolarizing potassium channel genes, there is a defect in adaptation of repolarization. Abnormalities in ionic current in response to physiological changes may lead to triggered arrhythmias including AF.

Limitations

The study sample size is relatively small; however, statistical analyses were driven by a specific hypothesis. The majority of our patients were Caucasian; therefore, our results may not be generalizable to AF patients with other ethnicities. This study did not contain a validation set, however we implemented a resampling simulation study that confirmed the significance of the association. Finally, we did not investigate the mechanism for the association of polymorphisms with decreased longitudinal QTc variance.

CONCLUSIONS

We found that the alleles *KCNH2* (rs10240738) and *KCNJ2* (rs8079702) were associated with a decreased longitudinal variance in QTc. This effect does not appear to depend on the number of alleles containing the SNP. Abnormal myocardial repolarization may mediate the effect of K channel gene polymorphisms on AF susceptibility. Because a significant subset of our patients with AF have these polymorphisms, these findings may have future clinical applications to tailor treatment for individual patients.

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