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A *KCNQ1* Mutation Causes a High Penetrance for Familial Atrial Fibrillation

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Abstract

Background—Atrial fibrillation (AF) is the most common cardiac arrhythmia, and its incidence is expected to grow. A genetic predisposition for AF has long been recognized, but its manifestation in these patients likely involves a combination of rare and common genetic variants. Identifying genetic variants that associate with a high penetrance for AF would represent a significant breakthrough for understanding the mechanisms that associate with disease.

Method and Results—Candidate gene sequencing in five unrelated families with familial AF identified the *KCNQ1* missense mutation p.Arg231His (R231H). In addition to AF, several of the family members have abnormal QTc intervals, syncope, or experienced sudden cardiac arrest or death. *KCNQ1* encodes the voltage-gated K⁺ channel that conducts the slowly activating delayed rectifier K⁺ current in the heart. Functional and computational analyses suggested that R231H increases *KCNQ1* current (*I*_{KCNQ1}) to shorten the atrial action potential (AP) duration. R231H is predicted to minimally affect ventricular excitability, but it prevented the increase in *I*_{KCNQ1} following PKA activation. The unique properties of R231H appeared to be caused by a loss in voltage-dependent gating.

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Conclusions—The R231H variant causes a high penetrance for interfamilial early-onset AF. Our study indicates R231H likely shortens atrial refractoriness to promote a substrate for reentry. Additionally, R231H might cause abnormal ventricular repolarization by disrupting PKA activation of I_{KCNQ1} . We conclude genetic variants, which increase I_{Ks} during the atrial AP, decrease the atrial AP duration, and/or shorten atrial refractoriness, present a high risk for interfamilial AF.

Keywords

arrhythmia; atrial fibrillation; potassium; ion channels; KCNQ1; long-QT syndrome

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia syndrome, and it is linked to cardiovascular complications, including palpitations, syncope, stroke, and congestive heart failure.(1–4) Genetic predispositions to AF have been recognized for over 70 years. However, understanding how genetic traits influence the manifestation of AF represents a significant challenge for clinician scientists. The identification of a single genetic variant that associates with the autosomal dominant AF subtype, in more than one unrelated family, would represent a significant breakthrough in understanding the genetics and molecular mechanisms for the manifestation of AF.

Genetic linkage analysis has identified mutations that cause autosomal dominant forms of AF. Chen and colleagues (2003) identified a 'gain-of-function' missense mutation in *KCNQ1* (p.Ser140Gly or S140G), the gene encoding the voltage-gated K^+ channel (KCNQ1 or $Kv7.1$) that underlies the slowly activating delayed rectifier K^+ current (I_{Ks}) in the heart.(5–7) Several other *KCNQ1* mutations are also linked to autosomal dominant AF, but each case is limited to one family.(8–12) Surprisingly, several unrelated families that harbor the same mutation have been found to be asymptomatic for AF.(12, 13) This suggests that even the autosomal dominant AF subtype might have a missing heritability component.

In this study, we have identified five families with familial early-onset AF (< 40 years of age) who all carry the same *KCNQ1* mutation, p.Arg231His (R231H). Additionally, a few of the R231H patients are also symptomatic for syncope, prolonged QTc intervals, or sudden cardiac arrest. The purpose of this study was to use a combination of functional and computational analysis to understand how R231H might contribute to a high interfamilial incidence of AF and abnormal ventricular excitability.

Methods

Clinical

We identified five unrelated families who were genotype positive for R231H (Figure 1). The index patient in Figure 1B was reported previously.(14) The four additional families were referred for genetic testing because of sudden cardiac arrest while sleeping (family 1C), fetal bradycardia (family 1D), and/or familial AF (families E and F). The study was conducted according to the principles of the Helsinki Declaration. The Institutional Ethics Committees approved the respective protocols for research-based genetic analysis for patients and the patients provided informed consent before either research or genetic testing was performed. Genomic DNA was isolated from blood leukocytes and genetic screenings were performed using standard methods. Surveys for mutations in genes encoding ion channels that are linked to autosomal dominant forms of AF (*KCNH2*, *SCN5A*, *KCNJ2*, *KCNE1*, *KCNE2*) in the index patients for families B, D, and F were all negative, and index patients for families C and E were negative for mutations in *SCN5A*.(15–19)

Mutagenesis

The R231H mutation was engineered into wild type- (WT) KCNQ1 cDNA as previously described.(12) The integrity of the construct was verified by DNA sequencing (Advanced Genetic Technologies Center, University of Kentucky; Lexington, KY).

Tissue Culture

Human Embryonic Kidney (HEK293) cells were transiently transfected with WT (3μg), R231H (3μg), or WT (1.5μg) and R231H (1.5μg) plasmid DNA using the Superfect reagent (QIAGEN; Valencia, CA) as previously described.(12) KCNE1 or KCNE3 (3μg) and GFP (0.3μg) plasmid DNA were co-transfected for indicated experiments. KCNE1 is required to generate I_{Ks} -like current in heterologous expression systems.(6, 7) For perfusion studies, WT or R231H (1μg), KCNE1 (1μg), AKAP9 (Yotiao) (6μg) and GFP (0.3μg) plasmid DNA were co-transfected. Expression of AKAP9 and KCNE1 are required for the functional response of WT to PKA stimulation.(20) All cells were cultured in MEM supplemented with 10% Fetal Bovine Serum at 37°C and analyzed 24–30 hours after transfection.

Electrophysiology

The whole-cell patch clamp procedure was performed on GFP positive HEK293 cells as previously described.(12) The external solution contained (in mM) 137 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES (pH 7.4 with NaOH), and an internal pipette solution contained (in mM) 130 KCl, 1 MgCl₂, 5 EDTA, 5 MgATP, 10 HEPES (pH 7.2 with KOH). An Axopatch-200B patch clamp amplifier (Axon Instruments, Union City, CA) was used to measure membrane currents and cell capacitance. Uncompensated pipette resistances were 1–2 MΩ and series resistances were compensated up to 95%. Only cells with stable membrane resistances > 1 GΩ were studied. pCLAMP 10.0 (Axon Instruments; Union City, CA) was used to generate the voltage clamp protocols, acquire current signals, and for data analyses. Origin 7.0 (Microcal; Northhampton, MA) was used for performing Boltzmann fitting, generating current-voltage (I-V) relations, and plotting graphs. The Boltzmann equation used to describe the I-V relations was:

$$I = (I_{\text{MIN}} - I_{\text{MAX}} / 1 + e^{[V - V_{1/2}] / k}) + I_{\text{MAX}}$$

I_{MIN} is the minimally activated current, I_{MAX} is the maximally activated current, $V_{1/2}$ is the mid-point potential for half maximal activation, and k is the slope factor (mV/ e -fold change). For all experiments the holding potential was –80 mV, and the dotted line in figures corresponds to the zero current baseline. Voltage clamp experiments were performed at 22–23°C within 1–2 hours of removing the cells from their culture conditions.

For voltage clamp recordings using an atrial AP waveform, we applied a waveform generated from a computational simulation of an atrial AP at 1 Hz and recorded currents at 37°C using the TC2BIP Temperature Controller (Cell MicroControls; Norfolk, VA).(11)

For perfusion experiments, cells were plated 2 hours prior to recording on glass coverslips coated with 0.01% rat-tail collagen in 0.25% acetic acid. GFP positive cells were recorded with normal extracellular saline as previously described. The voltage protocol described was run consecutively until maximal current amplitude reached steady-state for each cell. Extracellular bath was replaced via gravity perfusion with fresh extracellular fluid containing 10 μM forskolin to activate adenylate cyclase + 0.2 mM 3-isobutyl-1-methylxanthine (IBMX) to inhibit phosphodiesterase collectively increasing intracellular levels of cAMP. The voltage protocol was run until the maximal current amplitude reached its new steady-state amplitude.

Computational Modeling

The term for the open probability was calculated from Silva and Rudy Markovian model for human I_{Ks} .⁽²¹⁾ We inserted this modification into the atrial AP model published by Abraham and colleagues or the ventricular AP model published by O'Hare and colleagues (See supplemental Figure 1C).^(11, 22) The modification for the I_{Ks} channel open probability in the atrial AP model was described previously.⁽¹²⁾ The minimal open probability was set to 0.0625 to mimic the minimally activated I_{KCNQ1} in cells expressing WT and R231H. For comparison purposes, additional simulations were performed where the I_{Ks} component was set to zero.

Statistics

Data are reported as the mean $\pm$ standard error (SE). An unpaired t-test was performed to determine if values were different from cells expressing WT. Significance was determined when the p-value was < 0.05 .

Results

R231H causes a high risk for familial early-onset atrial fibrillation

R231H is a missense mutation that disrupts a conserved charge in the KCNQ1 voltage-sensor (Figure 1A). An R231H patient was recently linked to early-onset AF (< 40 years of age) in a previous report on the prevalence of AF in a cohort of congenital long-QT syndrome patients (Figure 1B).⁽¹⁴⁾ Unfortunately, the current status of this patient and the rest of her family are not available. We now report four additional multi-generational families with familial early-onset AF that are genotype-positive for R231H (Figure 1C–F). The R231H patient data is summarized in Table 1 and additional family data are presented in detail in the Supplement (Supplemental Results, Supplemental Figure 1). Assuming the patient phenotypes do not change with age, the absolute risk for early-onset AF in the genotype positive R231H carriers is $\sim 80\%$ (11 out of 14 R231H patients). Additionally, a few R231H patients have borderline resting QTc intervals or an abnormal QTc prolongation following an epinephrine challenge. Moreover, several R231H patients have histories of syncope and one patient experienced sudden cardiac arrest while sleeping. Importantly, none of the genotype-negative subjects are symptomatic for AF or any known abnormal ventricular events thus far.

R231H increases I_{KCNQ1} at negative membrane potentials

These clinical findings suggest that R231H might generate a unique functional phenotype that causes a high risk for early-onset AF. We studied the functional properties of R231H by voltage-clamping HEK293 cells expressing WT, R231H, or co-expressing WT and R231H (to mimic the patients' heterozygous genotypes). All of these experiments were performed with the K^+ channel β -subunit KCNE1, which is obligatory for KCNQ1 to generate native-like I_{Ks} currents.^(6, 7) Macroscopic KCNQ1 current (I_{KCNQ1}) was recorded by applying step-like pulses from -80 mV to 70 mV in 10 mV increments for 5 s, immediately followed by a 'tail' pulse for 5 s to -50 mV (Figure 2A). The peak I_{KCNQ1} amplitude recorded during the step pulse, or at the start of the tail pulse, was plotted as a function of the step pulse potential (Figure 2B, 2C). The tail I–V relations for cells expressing WT or WT and R231H were described with a Boltzmann equation (Figure 2C–G). The data showed: 1) cells expressing WT conducted I_{Ks} -like currents; 2) cells expressing R231H conducted I_{KCNQ1} that was maximally activated at most potentials tested; and 3) cells expressing WT and R231H generated I_{KCNQ1} with an intermediate phenotype, which included a minimally and maximally activated component (Figure 2D–2G).

R231H increases I_{KCNQ1} that is measured with an atrial action potential waveform and predicts a shortening in the atrial AP duration

We tested whether the minimally activated I_{KCNQ1} in cells expressing WT and R231H caused an increase in I_{KCNQ1} measured during a human atrial AP waveform. To do this, we voltage-clamped cells expressing WT or WT and R231H with KCNE1 at 37°C (to mimic physiological temperature) and pulsed the cells at 1 Hz (Figure 3A). Pulsing cells expressing WT activated very little I_{KCNQ1} , whereas cells expressing WT and R231H conducted large I_{KCNQ1} (Figure 3B).

To predict how R231H might affect the atrial AP duration over a wide range of cycle-lengths, we performed simulations using a computational model of a human atrial AP. To mimic the minimally activated I_{KCNQ1} at negative membrane potentials, we modeled 6.25% of the I_{Ks} as always being open. This is the fraction of I_{Ks} that would be always activated assuming random co-assembly between WT and R231H subunits, and it is similar to what was seen experimentally (Figure 2). Additionally, we performed atrial AP simulations that lacked the I_{Ks} component to determine how much I_{Ks} contributed to atrial AP duration. Compared to the control simulation, the simulation that mimicked R231H predicted a dramatic shortening in atrial APD90 at all the cycle lengths, but the simulation that mimicked a complete loss of I_{Ks} showed only a modest prolongation (Figure 3C).

R231H prevents PKA activation of I_{KCNQ1}

Several of the R231H patients showed borderline resting QTc intervals and one patient presented with epinephrine induced QTc prolongation. Simulations using a computational model of a human ventricular AP predicted that R231H would minimally affect the ventricular AP duration at different cycle lengths (Supplemental Figure 1C). An important functional role of I_{Ks} in the ventricular is to increase in response to β -adrenergic stimulation to prevent a prolongation in the ventricular AP.(20, 23, 24) This functional response is achieved by PKA stimulation of I_{Ks} . We tested whether R231H prevented PKA activation of I_{KCNQ1} from cells expressing WT or R231H, KCNE1, and AKAP9, which is an A-kinase anchoring protein that is required for PKA activation of I_{KCNQ1} . PKA activation was achieved by perfusing cells in forskolin + IBMX.(20) I_{KCNQ1} was measured by applying a depolarizing step pulse to 50 mV for 5 s followed by a tail pulse to -50 mV for 5 s before and after PKA activation (Figure 4A). Similar to what has been previously shown, PKA activation increased the mean peak tail I_{KCNQ1} in cells expressing WT by ~100% (Figure 4B).(20) Cells expressing R231H showed only a small increase in the mean peak tail I_{KCNQ1} that was not significantly different (Figure 4B). These data suggest that R231H suppresses PKA regulation of I_{KCNQ1} .

R231H decreases voltage-dependent gating of I_{KCNQ1} in cells expressing KCNE3

Although KCNE1 is necessary to generate native-like cardiac I_{Ks} , evidence suggests that other KCNE subunits also regulate WT.(25–28) WT is uniquely regulated by KCNE3, because KCNE3 stabilizes the KCNQ1 voltage-sensor in a 'partially-open' configuration to generate a minimally activated I_{KCNQ1} at negative potentials.(26, 29, 30) We expressed WT or R231H and KCNE3 to determine if KCNE3 regulated R231H differently. I_{KCNQ1} was measured and analyzed similar to Figure 2. Cells expressing WT or R231H and KCNE3 generated a minimally and maximally activated I_{KCNQ1} (Figure 5A–5C), and the tail I_{KCNQ1} plotted as a function of the step pulse was described using a Boltzmann equation to calculate I_{MIN} , I_{MAX} , $V_{1/2}$, and k (Figure 5D–G). Cells expressing R231H and KCNE3 conducted a minimally activated I_{KCNQ1} comparable to cells expressing WT, but the maximally activated I_{KCNQ1} was much smaller (Figure 5D–E). This result is essentially opposite to what was observed in cells expressing R231H and KCNE1 (Figure 2D–E), where cells expressing R231H and KCNE1 expressed primarily maximally activated I_{KCNQ1} . Although

KCNE1 and KCNE3 show bipartite regulation of R231H, both data sets demonstrate that R231H disrupted voltage-dependent gating.

Discussion

This is the first study to identify a single *KCNQ1* variant (R231H) in unrelated families with familial AF. Voltage-clamp and computational analyses suggest that R231H increased I_{KCNQ1} during the atrial AP to shorten its duration. This is expected to decrease the distance an electrical impulse travels during the refractory period (the cardiac wavelength). If the cardiac wavelength becomes shorter than the path length, then multiple reentry circuits can develop to cause fibrillation.(31–33)

Additionally, several R231H families are symptomatic for abnormal ventricular excitability. In fact, R231H was originally classified as a type 1 long-QT syndrome (LQT1) mutation, which is typically caused by loss-of-function mutations in *KCNQ1*.(6, 7, 14, 34) Our data show that R231H did not cause a loss-of-function and computational modeling suggested R231H does not predict a prolongation in the ventricular AP duration. An important functional role for I_{Ks} in the ventricle is to prevent excessive ventricular AP prolongation following β -adrenergic stimulation.(24) Indeed, LQT1 mutations that are resistant to PKA activation confer a high risk for life-threatening events.(35, 36) We found that R231H was also resistant to PKA stimulation. We suspect that a loss of I_{KCNQ1} regulation by PKA might account for the borderline or prolonged QTc intervals seen in some of the R231H patients at rest or following epinephrine challenge. One R231H patient even experienced sudden cardiac arrest while sleeping; however, since sleep is not a common trigger for LQT1-related cardiac events, the mechanism(s) by which R231H might have contributed to this event warrants further investigation.(37)

R231H directly disrupts one of the conserved charged residues in the KCNQ1 voltage-sensor. The S4 of the KCNQ1 voltage-sensor moves in response to the membrane depolarization to favor the maximally activated state. R231H stabilized the maximally activated state in cells expressing KCNE1. In contrast to KCNE1, KCNE3 modulates KCNQ1 to favor a partially conducting closed state rather than a non-conducting closed state.(26, 30) Interestingly in cells expressing KCNE3, R231H stabilized the partially conducting closed state rather than the maximally activated state (inset, Figure 5A). In other words, KCNE1 and KCNE3 appear to stabilize different configurations of the R231H voltage-sensor.

Although speculative, the loss of I_{KCNQ1} in cells expressing R231H and KCNE3 might contribute to the borderline resting QTc interval in some patients. KCNE3 is expressed in the heart and might contribute to I_{Ks} .(38, 39) Mutations in KCNE3 that decrease I_{KCNQ1} are linked to long-QT syndrome in some patients.(29) We performed additional experiments in cells expressing WT or R231H and other KCNE subunits, but these cells only conducted small I_{KCNQ1} that did not show any obvious differences (data not shown).

There are several limitations to our approach. The functional data were obtained in a widely used heterologous expression system and may not completely recapitulate the native condition. The prevalence of the R231H mutation as a cause of lone atrial fibrillation (AF) in large cohorts remains unknown (and likely represents a small number). Additionally, some AF-susceptibility genes were not screened in these patients. However, the presence of this rare *KCNQ1* variant in multiple kindreds with appropriate co-segregation and AF, the biophysical findings observed, and the known association of *KCNQ1* with familial AF, strongly supports a contribution of R231H to AF vulnerability.

Conclusions

In summary, R231H provides a molecular link to the manifestation of AF in unrelated families. Our studies indicate that R231H likely increases the amount of I_{KCNQ1} during the atrial AP to dramatically shorten its duration. R231H also disrupts PKA regulation of I_{KCNQ1} and is associated with borderline and adrenergic-induced QT prolongation in patients. We conclude genetic variants that shorten atrial refractoriness will present a high risk for interfamilial early-onset AF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. R231H confers a high risk for early-onset AF

A. Topology of KCNQ1 embedded in the plasma membrane. Shown are pedigrees of five non-related families (**B**, **C**, **D**, **E**, and **F**) identified with the R231H mutation. Individual males or females are represented as squares or circles, respectively; each generation is denoted by a Roman numeral; and the patients' clinical phenotypes/genotypes are defined in the key. Prolonged QTc is defined as > 450 ms for males or > 460 ms for females.

Figure 2. Co-expression of R231H and WT increases I_{KCNQ1} at negative membrane potentials
A. Representative families of currents recorded from HEK 293 cells expressing WT (black squares), R231H (grey triangles), or WT and R231H (open grey triangles). The voltage protocol used to record the currents is in the inset. The mean peak step (**B.**) or tail (**C.**) current is plotted as a function of the step potential (WT, n=18; R231H, n=11; WT and R231H, n=15). The individual peak tail I-V relations were described with the Boltzmann equation (grey line, C) to calculate the mean I_{MIN} (**D.**) and I_{MAX} (**E.**), $V_{1/2}$ (**F.**), and slope factor, k (**G.**) (* $p < 0.05$).

Figure 3. Cells co-expressing R231H and WT increase current measured using a human atrial AP waveform

A. Shown are representative currents from cells expressing WT or WT and R231H (n=10 or n=11, respectively) measured using the atrial AP waveform at 37°C. **B.** The mean peak current $\pm$ SE measured during the plateau phase of the atrial AP waveform for cells expressing WT or WT and R231H (* $p < 0.05$) is plotted. **C.** Atrial AP simulations plotting the time to 90% atrial AP repolarization (APD90) as a function of cycle length for control I_{Ks} (black squares), a mutation causing a minimum I_{Ks} open probability of 6.25% (open triangles), and a mutation causing a loss-of-function of I_{Ks} (open circles) are shown.

Figure 4. Cells expressing R231H have a blunted response to PKA stimulation

A. Shown are representative currents from cells expressing WT or R231H with KCNE1 and Yotiao using the voltage protocol in the inset before (grey line) or after (black line) perfusion of Forskolin + IBMX in the extracellular bath. **B.** The bar graph represents the mean peak tail current $\pm$ SE (WT, n=5; R231H, n=5) (* $p < 0.05$ vs before perfusion).

Figure 5. Cells co-expressing R231H and KCNE3 decrease maximal I_{KCNQ1}

A. Shown are representative currents from cells expressing WT (black squares) or R231H (open triangles) with KCNE3 using the voltage protocol shown above. Tail currents are enlarged in the inset (scale bars represent 0.2 nA and 10 ms). Also shown is the mean step (**B.**) and tail (**C.**) current plotted as a function of the step potential (WT & KCNE3, $n=17$; R231H & KCNE3, $n=18$). The individual peak tail I-V relations were described with the Boltzmann equation (grey line, C) to calculate the mean I_{MIN} (**D.**) and I_{MAX} (**E.**), $V_{1/2}$ (**F.**), and slope factor, k (**G.**) (* $p < 0.05$).

Table 1

Clinical characteristics of genotype positive R231H patients. Early-onset AF is defined as age < 40 years. Prolonged QTc interval is defined as > 460 ms for females and > 450 ms for males.

Genotype positive R231H families, n	5
Genotype positive subjects, n (female, n)	14 (9)
Early-onset AF, n (female, n)	11 (8)
Mean age of onset $\pm$ SD (years)	15 $\pm$ 8
Female mean age of onset $\pm$ SD (years)	15 $\pm$ 10
Male mean age of onset $\pm$ SD (years)	16 $\pm$ 4
Mean QTc $\pm$ SD (ms)	444 $\pm$ 20
Female mean QTc $\pm$ SD (ms)	446 $\pm$ 16
Male mean QTc $\pm$ SD (ms)	441 $\pm$ 27
Prolonged QTc interval, n (female, n)	2 (1)
Sudden cardiac arrest, n (female, n)	1 (0)
Syncope, n (female, n)	2 (2)