De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero

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Abstract

Objective: We describe a genetic basis for atrial fibrillation and short QT syndrome in utero. Heterologous expression of the mutant channel was used to define the physiological consequences of the mutation.

Methods: A baby girl was born at 38 weeks after induction of delivery that was prompted by bradycardia and irregular rhythm. ECG revealed atrial fibrillation with slow ventricular response and short QT interval. Genetic analysis identified a de novo missense mutation in the potassium channel KCNQ1 (V141M). To characterize the physiological consequences of the V141M mutation, Xenopus laevis oocytes were injected with cRNA encoding wild-type (wt) KCNQ1 or mutant V141M KCNQ1 subunits, with or without KCNE1.

Results: Ionic currents were recorded using standard two-microelectrode voltage clamp techniques. In the absence of KCNE1, wt KCNQ1 and V141M KCNQ1 currents had similar biophysical properties. Coexpression of wt KCNQ1 + KCNE1 subunits induced the typical slowly activating and voltage-dependent delayed rectifier K+ current, I_Ks. In contrast, oocytes injected with cRNA encoding V141M KCNQ1 + KCNE1 subunits exhibited an instantaneous and voltage-independent K+-selective current. Coexpression of V141M and wt KCNQ1 with KCNE1 induced a current with intermediate biophysical properties. Computer modeling showed that the mutation would shorten action potential duration of human ventricular myocytes and abolish pacemaker activity of the sinoatrial node.

Conclusions: The description of a novel, de novo gain of function mutation in KCNQ1, responsible for atrial fibrillation and short QT syndrome in utero indicates that some of these cases may have a genetic basis and confirms a previous hypothesis that gain of function mutations in KCNQ1 channels can shorten the duration of ventricular and atrial action potentials.

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1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice, affecting approximately 2% of the adult population. In general, the incidence of acquired...
AF increases with age and is associated with electrical remodeling [1,2]. Recent data have indicated a genetic susceptibility in 5% of the patients with AF and up to 15% of the individuals with lone AF [3]. The genetic basis for AF has been slowly unraveled over the last decade. Linkage and candidate gene analysis studies have identified five genetic loci. The first locus was isolated to chromosome 10q22–q24 in 1997, but a gene has yet to be identified [4]. Four additional loci and two genes have since been mapped to q24 in 1997, but a gene has yet to be identified [4]. Four loci. The first locus was isolated to chromosome 10q22–q24 in 1997, but a gene has yet to be identified [4]. Four additional loci and two genes have since been mapped to q24 in 1997, but a gene has yet to be identified [4].

KCNE1 (minK) subunits to form channels that conduct the additional loci and two genes have since been mapped to chromosomes 6q14–16 [5], 11p15.5 [6], 5p13 [7] and 21q22 [8]. KCNQ1 has been identified as the causative gene for the 11p15.5 [6] locus and KCNE2 for the 21q22 locus [8].

KCNQ1 (KvLQT1) channel subunits co-assemble with KCNE1 (minK) subunits to form channels that conduct the slow delayed rectifier K+ current, IKS [9,10] in the heart which is important for normal termination of the plateau phase and repolarization of atrial and ventricular action potentials. A point mutation in KCNQ1 (S140G) was reported to cause a gain of IKS function that was associated with persistent AF in a large Chinese family [6]. When expressed in COS-7 cells, the S140G mutation caused dramatic changes in IKS channel gating [6]. Normally, IKS activates extremely slowly, requiring many seconds to reach a steady-state level of activation. IKS channels containing S140G KCNQ1 subunits were constitutively open, exhibiting instantaneous activation in response to membrane depolarization. This defect in IKS gating is predicted to shorten action potential duration and is the likely molecular mechanism of AF in this family. Unexpectedly, many affected individuals in this kindred also presented with long QT syndrome. It is paradoxical that a gain of IKS function would cause long QT syndrome since loss of function mutations are clearly associated with this disorder [11]. Moreover, last year it was discovered that a gain of function mutation in KCNQ1 was the cause of an isolated case of short QT syndrome and idiopathic ventricular fibrillation [12]. In this case a point mutation (V307L) caused IKS to activate faster and at more negative potentials. Computer modeling confirmed the expectation that such a mutation would shorten the ventricular action potential. Last year, we reported that a point mutation (N588K) in KCNH2 (HERG) was the cause of short QT syndrome and ventricular arrhythmias in two families. This mutation enhances HERG current magnitude by eliminating channel inactivation. Finally, AF in two families was associated with a point mutation (R27C) in KCNE2, the gene encoding the MiRP1 K+ channel accessory subunit. The mutant MiRP1 subunits caused a gain of function when co-expressed with KCNQ1 subunits but had no effect when co-expressed with HERG or HCN channel subunits [8]. Thus, both short QT syndrome and AF can be caused by mutations in K+ channel subunits that result in a gain of function of delayed rectifier K+ currents.

The presence of AF is extremely rare in the unborn individual, and it is usually associated with structural heart disease. Here we report a case of AF that was diagnosed in utero, with concomitant bradycardia, a short QT interval and a structurally normal heart. Genetic analysis revealed a point mutation (V141M) in KCNQ1. Val141 is adjacent to Ser140, the residue mutated in the large family with AF and long QT syndrome [6] discussed above. The biophysical effects of the V141M mutation on IKS function were determined by heterologous expression studies in Xenopus oocytes. We found the electrophysiological phenotype of the V141M mutation to be the same as that previously described for the S140G mutation: a gain of function caused by a loss of voltage dependent channel gating. Our physiological studies and action potential simulations are consistent with V141M KCNQ1 causing AF, pacemaker dysfunction and short QT syndrome.

2. Methods

Studies were approved by the regional Institutional Review Board. Participants received and signed a consent form assenting to their participation. The Protocol of study conforms to the Declaration of Helsinki.

2.1. Mutational analysis

Genomic DNA was isolated from peripheral blood leukocytes using a commercial kit (Gentra System, Puregene). The coding region of KCNQ1 was amplified and analyzed by direct sequencing. Polymerase chain reaction products were purified with a commercial reagent (ExoSAP-IT, USB) and were directly sequenced in both directions with an ABI PRISM 3100 Automated DNA Sequencer. The mutation was absent in 200 unrelated control individuals from the same ethnic background. Biological relationship between the parents and the child was confirmed by segregation of highly polymorphic markers from several chromosomes.

2.2. Molecular biology

Site-directed mutagenesis of KCNQ1 in the pSP64 vector was performed using megaprimer protocols. Primers were V141MF GTCTACAACCTTCTCGAGCGTCCCTTACCACCTC-ACGGCTGGAAATGCTTCGTTTACCACTCATCTTCACGAGTCAGTCCACCAT and V141MR CATCTTCTTCTCC AGGAGTCA. The PCR product was cloned into TOPO vector (TOPO TA Cloning, Invitrogen) and the clone was digested with XhoI and BglII. The final digested product of 840 bases was inserted into the vector carrying wild type KCNQ1, replacing the equivalent wt sequence. The presence of the mutation and absence of additional changes in the clone was verified by automated DNA sequencing. DNA constructs were linearized with EcoRI and cRNA was transcribed in vitro using CapScribe and SP6 polymerase (Roche Applied Science, Indianapolis,
of experiments). Student’s t-test was used to test for statistical significance between groups. A value of $p < 0.05$ was considered statistically significant. Mean current amplitudes were measured at 0.5, 1 and 5 s following membrane depolarization.

### 2.5. Data analysis

Analysis of voltage clamp data was performed with pCLAMP 8 (Axon Instruments), Excel (Microsoft) and Origin 7 software (Microcal Software, Northampton, MA). Numerical values are reported as mean $\pm$ SEM ($n=$number of experiments). Student’s $t$-test was used to test for statistical significance between groups. A value of $p < 0.05$ was considered statistically significant. Mean current amplitudes were measured at 0.5, 1 and 5 s following membrane depolarization.

### 2.6. Cardiac myocyte modeling

A model of a human left ventricular myocyte [14] was used to simulate the effects of the heterozygous $KCNQ1$ mutation on $I_{Ks}$ and action potentials. Properties of mutant channels were modeled by assuming they were constitutively open. The channel description was combined with a model of wt $I_{Ks}$ channels [14] to reconstruct a heterozygous condition in which the $V141M$ $KCNQ1$ cRNA with or without 0.5 ng $KCNE1$ cRNA.

### 2.4. Two electrode voltage clamp recordings

Ionic currents were recorded 2–3 days after injection with cRNA using a Geneclamp 500 amplifier (Axon Instruments, Union City, CA) and standard two-micro-electrode voltage clamp techniques [13]. The recording chamber was perfused at $\sim 1$ ml/min with a modified ND96 solution containing (in mM): 96 NaCl, 2 KCl, 1 MgCl$_2$, 5 HEPES, pH 7.6. Data acquisition was performed using a Dell computer, a Digidata 1322A A/D interface and pClamp 8 software (Axon Instruments). Currents were elicited every 25 s by stepping the membrane to a test potential between +40 and $-120$ mV in $10$ mV increments for a duration of 1.25 or 5 s. A holding potential of $-90$ mV was used for all voltage clamp protocols.

### 3. Results

#### 3.1. Clinical analysis

Pregnancy was interrupted at 38 weeks due to severe fetal bradycardia and irregular rhythm, suggesting AF, detected since the 6th month of gestation. At birth the baby girl was hemodynamically stable and had a normal physical exam other than bradycardia at 60 bpm. This heart rate is similar to the one observed in cohorts of patients with congenital heart block [17]. Chest X-rays and blood tests were within normal limits. Echocardiogram showed a structurally normal heart and a single E wave. The basal ECG revealed an irregular rhythm and no visualization of P or f waves (Fig. 1). Suspecting AF, the patient underwent electrophysiological study which revealed irregular atrial electrograms with a normal HV interval, confirming the diagnosis of AF with slow ventricular response. Transesophageal echocardiogram excluded the presence of thrombi and the patient underwent one attempt at electrical cardioversion, which did not terminate AF. At follow-up, the patient remains asymptomatic, she is developing well and the heart is growing normally though she has conduction block with ventricular escape rhythm. Clinical analysis of both parents, grandparents and 7 of the 8 great-grandparents has revealed that only one of the latter suffers from AF. Because AF developed at an advanced age in this individual it is probably not familial.

#### 3.2. Genetics

Because of the recent association of AF [6] and short QT syndrome [12] in two different families with $KCNQ1$ mutations, we analyzed the DNA sequence of $KCNQ1$ in the proband. We identified a missense mutation, a single base G to A substitution at nucleotide 421 (GTG-ATG)
This mutation results in substitution of a valine by methionine at position 141 within transmembrane domain S1 (Fig. 2), adjacent to the previously described S140G mutation responsible for familial AF [6]. Sequence analysis of the biological parents’ DNA failed to identify the mutation indicating that V141M represents a de novo mutation in the infant.

3.3. Characterization of V141M mutation on channel function

Injection of wtKCNQ1 or V141M KCNQ1 cRNA produced small, but similar rapidly activating voltage-dependent, K+-selective currents (Fig. 3A). The shape of the current–voltage (I–V) relationships for wt and mutant channels was similar (Fig. 3B) and the voltage dependence of activation, determined by tail current analysis, was indistinguishable (Fig. 3C). Thus, the V141M mutation did not noticeably alter the gating of KCNQ1 channels expressed alone in oocytes.

As expected [9,10], co-injection of wtKCNQ1 and KCNE1 cRNA produced a slowly activating and deactivating, voltage-dependent and K+-selective current, IKs (Fig. 4B). The wtKCNQ1/KCNE1 channels exhibited a voltage-dependent threshold of activation near −50 mV and activated very slowly, increasing about 4-fold after 5 s compared to 1 s of depolarization. In sharp contrast, the V141M KCNQ1/KCNE1 channel current developed instantly at all voltages tested (Fig. 4C), consistent with the interpretation that these channels were constitutively open. Based on the reversal potential (−85 mV with 4 mM [K+]o) of the I–V relationship, the mutant channels retained
normal K\(^+\)-selectivity. Mean currents measured 1 or 5 s after step changes in the membrane voltage are compared in Fig. 4D for both wt\(KCNQ1/\)\(KCNE1\) and \(V141M\ KCNQ1/\)\(KCNE1\) channels.

3.4. Coexpression of wt and mutant channel subunits

To simulate a heterozygous condition, we co-injected individual oocytes with equal amounts of wt\(KCNQ1\) and \(V141M\ KCNQ1\) cRNA (2.5 ng each) plus \(KCNE1\) (0.5 ng). The voltage clamp protocol used to elicit currents is illustrated in Fig. 5A. Raw currents are displayed for the wt\(KCNQ1/\)\(KCNE1\) channels (Fig. 5B), “heterozygous” wt\(KCNQ1/V141M\ KCNQ1/\)\(KCNE1\) channels (Fig. 5C) and “homozygous” \(V141M\ KCNQ1/\)\(KCNE1\) channels (Fig. 5D). Mean current was measured 0.5 s after the initial step change in membrane potential to more accurately reflect the duration of a cardiac action potential. The \(I–V\) relationships for currents measured at 0.5 s are plotted in Fig. 5E. The finding that the instantaneous current produced by co-injection of wt\(KCNQ1\) and \(V141M\ KCNQ1\) with \(KCNE1\) was not equal to half of the current that developed after 5 s of depolarization (data not shown) suggests that the resulting channel population is a mixture of heteromultimers of these two subunits, not two separate homomeric populations wt\(KCNQ1/\)\(KCNE1\) and \(V141M\ KCNQ1/\)\(KCNE1\) channels.

3.5. \(V141M\) mutation is predicted to shorten action potential duration of human cardiomyocytes

The effect of the AF-associated S140G mutation described by Chen et al. [6] on atrial myocyte electrophysiology was previously simulated. It was shown that this mutation increased \(I_{Ks}\) magnitude and greatly shortened action potential duration [18]. Because the S140G and \(V141M\) mutations have the same effect on \(I_{Ks}\) gating, the atrial myocyte simulations apply to both mutations. Here we use a newer model to simulate the effects of the mutation on...
I_{ks} and action potentials of a human ventricular myocyte [14]. The output of the model is presented in Fig. 6 which shows the currents for wt (panel A), homozygous mutant (panel B) and heterozygous mutant (panel C) conditions. We modeled the effect of the V141M mutation in KCNQ1 by assuming that in the heterozygous condition, an equal number of wt and mutant subunit-containing channels existed. This is an obvious simplification because it is most likely that wt and mutant KCNQ1 subunits would randomly coassemble (see above). Nonetheless, the modeled currents share the major features of the experimentally measured currents induced in oocytes by coexpression of KCNE1 plus equal amounts of wt and mutant subunit-containing channels—an instantaneous component followed by a slowly activating component. The I–V relationships for the three currents are shown in Fig. 6D. Numerical simulations with the myocyte model showed unaltered resting potential, marginal changes of the peak voltage, but significant differences between wt and the mutation for action potential duration (Fig. 6E). The peak voltage decreased from 23 mV in wt to 21 mV in wt/V141M. Action potential duration at 90% repolarization (APD_{90}) decreased from 314 ms in wt myocytes to 283 ms (−9.9%) in wt/V141M myocytes. Differences were also found concerning the calcium handling (Fig. 6F). Resting intracellular calcium decreased from 70 nM in wt to 66 nM (−5.8%) in wt/V141M and peak intracellular calcium decreased from 796 to 752 nM (−5.5%). Thus, the results are consistent with the observed shortening in QT interval, a body surface measure of ventricular repolarization time.

We also modeled the effects of the V141M gain of function mutation in a model of rabbit sinoatrial node cells (Fig. 7). The results indicate that the enhanced outward I_{ks} causes cessation of spontaneous activity and a stabilization of the resting membrane potential at a level positive to the normal maximum diastolic potential of these cells. Thus, modeling predicts that the V141M mutation in KCNQ1...
would shorten the duration of ventricular action potentials and halt the spontaneous firing of nodal cells.

4. Discussion

Repolarization of cardiac action potentials is dependent on activation of $I_{Ks}$ ($\text{KCNQ1}/\text{KCNE1}$ channels) and $I_{Kr}$ (HERG channels). We previously reported that a gain of function mutation in HERG also causes AF and short QT syndrome [19]. Here we have identified a missense mutation ($V141M$) in $\text{KCNQ1}$ which causes AF and shortens the QT interval by altering the gating of $I_{Ks}$ channels. The mutation causes $\text{KCNQ1}/\text{KCNE1}$ channels to remain constitutively open. Our computer modeling predicts that this defect in gating would shorten ventricular action potential duration, consistent with the clinical observation of short QT interval. The role of $I_{Ks}$ in human atrial repolarization is uncertain. Wang et al. (1994) reported the presence of $I_{Ks}$ in isolated human atrial myocytes [20] whereas others [21] were unable to detect the current in these cells. However, similar variability in recording $I_{Ks}$ in isolated human ventricular myocytes has been noted [22] despite clear evidence that mutations in the genes encoding the channel greatly affect ventricular repolarization [11,23]. Given the likely importance of $I_{Ks}$ in repolarization of the human atria, [20] it is reasonable to expect that the $V141M$ mutation would also shorten atrial action potentials and cause AF in the affected child.

It is unclear how the $V141M$ mutation abolishes the normal voltage-dependent gating of $\text{KCNQ1}/\text{KCNE1}$ channels, especially considering that the mutation was without significant effect on the gating of homomeric $\text{KCNQ1}$ channels. It is intriguing that the $V141M$ mutation induced abnormal $\text{KCNQ1}$ channel gating that resembles the normal effect of channels formed by coassembly of $\text{KCNQ1}$ with other $\text{KCNE1}$-like proteins, $\text{KCNE2}$ and $\text{KCNE3}$ [24]. Unlike $\text{KCNE1}$, channels formed by coassembly of $\text{KCNQ1}$ and $\text{KCNE2}$ [25] or $\text{KCNE3}$ [26] subunits are constitutively open, similar to the behavior caused by the $V141M$ or S140G mutations in $\text{KCNQ1}$. The underlying mechanism of the abnormal gating of $V141M$ $\text{KCNQ1}/\text{KCNE1}$, $\text{KCNQ1}/\text{KCNE2}$ or $\text{KCNQ1}/\text{KCNE3}$ channels remains to be determined. The finding that two adjacent mutations in $\text{KCNQ1}$ can convert slowly activating $I_{Ks}$ channels into constitutively open channels suggests that a previously unrecognized interaction between $\text{KCNE1}$ subunits and the C-terminal end of the $\text{KCNQ1}$ S1 domain mediates the unusual gating of these heteromultimeric channels.

The gain in $I_{Ks}$ function caused an intriguing effect on the atrio-ventricular (AV) conduction system of the individual with the $V141M$ mutation. The child presented with severe bradycardia while in utero. When born, her heart rate was in the 60’s, much lower than the expected average heart rate of 140 bpm [27]. Computer modeling suggests a possible explanation for this bradycardia and the absence of P waves. The model rabbit SAN cell ceased spontaneous activity when the gating and magnitude of $I_{Ks}$ was altered in a manner consistent with changes in the current induced by heterologous expression of wild-type and $V141M$ $\text{KCNQ1}$ proteins in oocytes. In the absence of or slowed SAN pacemaker activity, atrial electrical activity would initiate in the AVN, explaining the bradycardia and lack of apparent P waves. P waves resulting from retrograde activation of the atria might be hidden in the ventricular activation.

Electrophysiological analysis showed a normal HV interval, suggesting that this was not an infra-nodal block. The role of $\text{KCNQ1}$ in AV conduction has been investigated in transgenic mice over-expressing a dominant-negative isoform of human $\text{KCNQ1}$. These mice had a prolonged QT and AV nodal block [28], suggesting that in addition to the well understood long QT syndrome phenotype, loss of $I_{Ks}$ can also cause AV nodal dysfunction in mice. Pacemaker activity can evidently also be disrupted by a gain of $I_{Ks}$ function. Modeling of the $V141M$ mutation predicts a cessation of spontaneous activity of sinoatrial node cells. The effect of the mutation on pacemaker activity in the human heart likely depends on many factors not included in the rabbit model, but it is clear that gain of $I_{Ks}$ function should affect nodal automaticity.
In summary, we have described a novel de novo mutation in KCNQ1 which presented with a severe cardiac phenotype in utero. The V141M mutation alters channel gating and causes a gain of function in \( I_{Ks} \) that is responsible for AF and short QT syndrome.

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References


