NIH Public Access
Author Manuscript
Heart Rhythm. Author manuscript; available in PMC 2009 March 1.

Published in final edited form as:
Heart Rhythm. 2008 March ; 5(3): 427–435.

Gain of function in I_{Ks} secondary to a mutation in *KCNE5* associated with atrial fibrillation

Lasse S. Ravn, MD[*,†,‡,¶], Yoshiyasu Aizawa, MD[§], Guido D. Pollevick, PhD[§], Jacob Hofman-Bang, PhD[*,†], Jonathan M. Cordeiro, PhD[§], Ulrik Dixen, PhD[¶], Gorm Jensen, DMSc[¶], Yuesheng Wu, MS[§], Elena Burashnikov, BS[§], Stig Haunso, DMSc[†,‡], Alejandra Guerchicoff, PhD[§], Dan Hu, MD, PhD[§], Jesper H. Svendsen, DMSc[†,‡], Michael Christiansen, MD[*,†], and Charles Antzelevitch, PhD, FHRS[§]

[*]*Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark*

†*The Danish National Research Foundation Centre for Cardiac Arrhythmia, Copenhagen, Denmark*

‡*Department of Medicine, The Heart Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark*

¶*Department of Cardiology, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark*

§*Masonic and Medical Research Laboratory, Utica, New York*

Abstract

BACKGROUND—Atrial fibrillation (AF) is the most common clinical arrhythmia and a major cause of cardiovascular morbidity and mortality. Among the gene defects previously associated with AF is a gain of function of the slowly activating delayed rectifier potassium current I_{Ks}, secondary to mutations in *KCNQ1*. Coexpression of *KCNE5*, the gene encoding the *MiRP4 β*-subunit, has been shown to reduce I_{Ks}.

OBJECTIVE—The purpose of this study was to test the hypothesis that mutations in *KCNE5* are associated with AF in a large cohort of patients with AF.

METHODS—One-hundred fifty-eight patients with AF were screened for mutations in the coding region of *KCNE5*.

RESULTS—A missense mutation involving substitution of a phenylalanine for leucine at position 65 (L65F) was identified in one patient. This patient did not have a history of familial AF, and neither *KCNQ1* nor *KCNE2* mutations were found. Transient transfection of Chinese hamster ovary (CHO) cells expressing I_{Ks} *(KCNQ1+KCNE1)* with KCNE5 suppressed the developing and tail currents of I_{Ks} in a concentration-dependent manner. Transient transfection with *KCNE5*-L65F failed to suppress I_{Ks}, yielding a current indistinguishable from that recorded in the absence of *KCNE5*. Developing currents recorded during a test pulse to +60 mV and tail currents recorded upon repolarization to -40 mV both showed a significant concentration-dependent gain of function in I_{Ks} with expression of *KCNE5*-L65F vs *KCNE5*-WT.

CONCLUSION—The results of this study suggest that a missense mutation in *KCNE5* may be associated with nonfamilial or acquired forms of AF. The arrhythmogenic mechanism most likely is a gain of function of I_{Ks}.

Address reprint requests and correspondence: Dr. Charles Antzelevitch, Masonic Medical Research Laboratory, 2150 Bleecker Street, Utica, New York 13501. E-mail address: ca@mmrl.edu; Dr. Michael Christiansen, Department of Clinical Biochemistry, Statens Serum Institut, 5 Artillerivej DK2300S, Denmark..

The first two authors contributed equally to this study.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

Keywords

Electrophysiology; Arrhythmias; Genetics; Delayed rectifier potassium current

Introduction

Atrial fibrillation (AF) is the most common form of clinical arrhythmia, affecting more than 5% of the population older than 65 years. The incidence of AF has increased progressively over the past 20 years.[1,2] In approximately 12% of patients with AF, no concomitant disease is observed ("lone AF"). AF is a potentially serious disease due to thromboembolic complications[3] and heart failure.[4]

AF has long been known to cluster in families,[5] and a nationwide Icelandic study has shown strong heritability of the disease.[6] The Framingham Heart Study revealed that parental AF is a risk factor for AF in offspring.[7] Interestingly, maternal AF confers a higher risk to though AF is more prevalent among the male participants in the Framingham study.[7]

Genetic linkage analyses have identified AF loci on chromosome 10q22-24,[8] chromosome 6q14-16,[9] 5p13,[10] and 11p15.5.[11] In the case of 11p15.5, the genetic defect was identified and found to involve heterozygous missense mutations in *KCNQ1*, resulting in gain of function of the *KCNQ1-KCNE1* and *KCNQ1-KCNE2* ion channels conducting the slowly activating delayed rectifier current I_{Ks}.[11] Using a candidate gene approach, gain-of-function mutations in *KCNE2*[12] and *KCNJ2*,[13] coding for the ion channel conducting the inward rectifier potassium current I_{K1}, have been found associated with familial AF in two Chinese kindreds. However, despite identification of other *KCNQ1* mutations associated with AF,[14] the prevalence of *KCNQ1* mutations probably is low,[15] in accordance with the finding that AF is genetically heterogeneous.[5] Studies have also associated *GJA5* and *KCNA5* with human AF.[16,17] *KCNA5* encodes the α-subunit of the $K_v1.5$ channel responsible for I_{Kur}, whereas GJA5 encodes the gap junctional protein connexin40. Olson et al[16] identified loss of function of $K_v1.5$ channels due to a mutation in *KCNA5* as a cause of AF. Interestingly, Gollob et al[17] reported that somatic mutations in *GJA5* predispose patients to idiopathic AF by impairing gap junction assembly.

The *KCNE* proteins *KCNE1-KCNE5* constitute a homologous group of regulators of voltage-gated (Kv) potassium channels, and several are involved in cardiac arrhythmia.[18] Thus, mutations in *KCNE1* may cause Jervell and Lange-Nielsen disease,[19] and mutations in *KCNE2*, coding for minK-related peptide 1 (*MiRP1*), have been associated with drug-induced arrhythmia,[20,21] long QT syndrome (loss-of-function phenotype),[22] and AF (gain-of-function phenotype).[12] A controversial relationship exists between hypokalemic paralysis and a mutation/polymorphism in *KCNE3*.[23,24] The *KCNE* proteins are single polypeptide chains of 100 to 200 amino acids. They contain an extracellular N-terminus, a single putative transmembrane segment, and an intracellular C-terminus.[18] They modify the function of the different repolarizing K^+ channels.[18,25,26] Thus, the *KCNE1* protein *minK* confers the characteristic slow activation of I_{Ks}.[25] The interaction between individual *KCNE* proteins and K^+ ion channel α-subunits is not specific.[10] Thus, *minK* interacts with both *KCNQ1* and *HERG*, and *KCNE1-3* proteins interact with *Kv3.1* and *Kv3.2* causing diversification of channel gating.[26] Other channels also interact with *KCNE* proteins, but the functional significance of such interactions in vivo has not been clarified.[18] All *KCNE* genes are expressed in the heart but at varying levels[20,23,27] and, for *KCNE1* and *KCNE3*, with several splice transcripts.[27] The balance between different *KCNE* proteins with varying effects on Kv channels may enable variable modulation of I_{Ks} and other K^+ currents.[28]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

KCNE5 is expressed in both right and left atria and ventricles.[27,28] The relative amount of *KCNE5* mRNA is thought to be greater than that of *KCNE2*.[28] The *KCNE5* gene product[29] *MiRP4* suppresses the I_{Ks} current in heterologous systems and is suggested to function as a down-regulating *β*-subunit to the *KCNQ1 α*-subunit in competition with the stimulating *KCNE1 β*-subunit.[30] This action of *KCNE5* suggests that it may be a candidate gene for AF. We examined this hypothesis by screening a cohort of AF patients for mutations in the coding region of *KCNE5*.

The familial form of AF is relatively uncommon, accounting for 5% of all cases of AF and up to 15% of cases of lone AF.[5] The majority of cases of AF are acquired and related to structural abnormalities. However, not all individuals with the same cardiac pathology develop AF, suggesting that genetic factors predispose to the development of acquired AF.[31] Little is known about the genetic predisposition to acquired AF. This report presents evidence in support of this hypothesis.

Materials and methods

Patients

The study consisted of 158 patients from the Copenhagen SAFIR (Signal Averaged ECG as a prognostic factor in atrial FIbRillation) investigation.[32] Patients were recruited at the 3-year follow-up of an initial group of 244 patients (20 had died and 66 did not want to participate) with ECG-documented AF who, at the time of inclusion, were in sinus rhythm by spontaneous, pharmacologic, or electrical cardioversion. Demographic and clinical data of the patients are given in Table 1.

Bioinformatics

The mRNA and protein sequences of the intronless human *KCNE5* (accession number NM012282), *KCNE1* (NM000219), *KCNE2* (NM172201), *KCNE3* (NM005472), and *KCNE4* (NM080671) genes, the mouse *KCNE5* gene (NM021487), and the *Xenopus laevis KCNE5.1* gene (AF545502) were obtained from the NCBI Entrez Nucleotide database *(http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)*.

KCNE5 protein sequences were retrieved by basic local alignment search tool (BLAST) searching the genomes via *http://www.ncbi.nlm.nih.gov/Genomes/* against the human KCNE5 mRNA sequence. The open reading frame was located, translated to protein, and aligned with the clustalW algorithm using DNASTAR software (DNASTAR, Inc, Madison, WI) and parameters—gap penalty: 10, gap length penalty: 0.2, delay divergent sequences: 30, protein weight matrix: Gonnet series. Visualization of the alignment was performed using BIOEDIT software *(http://www.mbio.ncsu.edu/BioEdit/bioedit.html)*. KCNE1-4 protein sequences were retrieved via *http://genome.ucsc.edu/cgi-bin/hgGateway*.

Ethics

Collection of clinical data and analysis of samples was approved by the ethics committees of Copenhagen and Frederiksberg counties (KF01-147/02).

Mutation screening

Mutation screening was performed as described previously.[33] Briefly, genomic DNA was extracted using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) was used to amplify the DNA fragment corresponding to the single exon of the gene. Reactions containing 50 ng genomic DNA and 10 pmol of the appropriate primers were prepared and underwent PCR through 33 cycles at an annealing temperature of 60°C. The PCR product was cleaved by incubating overnight with BstEII in order to obtain two

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

fragments of an appropriate size. Single-strand conformation polymorphism (SSCP) electrophoresis of the fragments was performed using GeneGel Excel 12.5/24 kits (Amersham Biosciences AB, Uppsala, Sweden). Aberrant conformers were directly sequenced on a 3100-Avant Genetic analyzer (Applied Biosystems, Foster City, CA, USA) using big dye chemistry. Mutation screening of KCNQ1 and KCNE2 was performed as described previously.[34,35]

Wild-type and mutant *KCNE5* constructs

The complete coding region of *KCNE5* was amplified from both patient and control human genomic DNA using PCR. The following primers were designed from *KCNE5* GenBank sequence (accession number: BC035330): forward: 5′-GGAAGATCT GCTAGCGCCG-CCATGAACTGCAGCGAGAGCCAGC-3′ and reverse 5′-CCGCTCGAGGGATCCTTAGACCCGCT CAGCGCCCTGG-3 having additional 5′ sequences specifying NheI/BglII or XhoI/BamHI restriction sites used for cloning. Each gene was amplified beginning at the respective ATG start codon and included a modified translation initiation sequence (GCCGCCATG). *KCNE5* PCR products from wild-type (WT) and mutant (L65F) were subcloned into the NheI (5′) and BamHI (3′) sites of the plasmid pIRES2-AcGFP1 or pIRES2-DsRed-Express (BD Biosciences-Clontech, Mountain View, CA, USA), sequenced in their entirety, and the mutation confirmed. High-quality plasmid DNA for transfections was prepared using a QIAGEN Plasmid Maxi Kit (QIAGEN, Valencia, CA, USA).

Cell culture and transient transfection

A Chinese hamster ovary (CHO) cell line stably expressing I_{Ks} (KCNQ1+KCNE1 cDNAs in a bicistronic vector; kind gift of Dr. Alfred L. George, Vanderbilt University) was cultured in F-12 nutrient mixture (Ham) medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and penicillin (100 units/mL)-streptomycin (100 μg/mL), and 600 μg/mL hygromycin in a humidified 5% CO_2 incubator at 37°C. Cultured cells were seeded in 35-mm dishes 1 day before transfection and transiently transfected with various plasmids by FuGENE6 lipid-based transfection reagent (Roche, Basel, Switzerland). In the electrophysiologic experiments, 1.5, 0.75, 0.3, or 0.15 μg of WT *KCNE5* and 1.5, 0.75, or 0.3 μg of mutant *KCNE5* was transfected into I_{Ks}/CHO cells. Cells displaying green fluorescence 24 to 48 hours after transfection were studied electrophysiologically.

To visualize CHO cells expressing green fluorescent protein, cells were excited at 480 nm using a 100-W mercury bulb, and fluorescence emission was detected using a 520-nm band-pass filter. To visualize cells expressing red fluorescent protein, cells were excited at 540 nm, and the fluorescence emission was detected using a 605-nm band-pass filter.

Electrophysiologic recordings

To investigate the effects of the L65F mutation on channel function, we performed whole-cell patch clamp experiments on I_{Ks}/CHO cells transfected with WT or mutant *KCNE5* channels. Briefly, cells were placed in a perfusion chamber (PDMI-2, Medical Systems Corp., Greenvale, NY, USA), mounted on the stage of an inverted microscope with epi-fluorescence capabilities (TE2000, Nikon, EL Segundo, CA, USA). Cells were superfused with normal external solution of the following composition (in mmol/L): 132 NaCl, 4.8 KCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 5 glucose, and 10 HEPES-Na (pH 7.4 adjusted with NaOH). Patch pipettes were fabricated from borosilicate glass capillaries (1.5-mm outer diameter; Fisher Scientific, Pittsburgh, PA, USA). The pipettes were pulled using a gravity puller (Narishige Corp., Greenvale, NY, USA) and filled with pipette solution of the following composition (in mmol/L): 110 aspartic acid, 5 ATP-K_2, 11 EGTA, 5 HEPES, and 1 $MgCl_2$ (pH 7.35 adjusted with KOH). Pipette resistance ranged from 1 to 4 MΩ when filled with the internal solution.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

After a gigaseal was formed, the cell membrane was ruptured by applying negative pressure. Current signals were recorded using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA, USA), and series resistance errors were reduced by approximately 60% to 70% with electronic compensation. All signals were acquired at 0.5 to 5 kHz (Digidata 1322, Axon Instruments) with a microcomputer running Clampex 9 software (Axon Instruments) and filtered at 5 kHz with a four-pole Bessel low-pass filter. Membrane currents were analyzed with Clampfit 9 software (Axon Instruments). Peak developing current (measured at the end of the depolarizing test pulse) and peak tail current (measured immediately following repolarization to -40 mV) were quantitated.

Data analysis

Results are expressed as mean ± SEM. Difference between groups were tested by one-way analysis of variance, followed by Scheffe modified F-test for multiple comparisons. $P < .05$ was considered significant.

Results

Mutation screening

Aberrant conformers were found on SSCP analysis for mutations in KCNE5 in one patient, a 66-year-old woman with persistent AF but no family history. DNA sequencing revealed a heterozygous substitution of T for C in nucleotide 193 predicting substitution of phenylalanine (TTC) for leucine (CTC) in codon 65 (L65F; Figure 1A and B). The mutation is located in a highly conserved transmembrane domain of the protein. Table 2 shows an alignment of the three known KCNE5 homologues in humans, monkey, dog, cattle, mouse, chicken, and frog as well as the other human KCNE proteins. The L-to-F substitution changes the residue from the one conserved in the different KCNE5 proteins to the F residue specific for *KCNE3*. The mutation was not found in 200 normal random controls, and it was not registered as a Caucasian variant in PharmGKB *(www.pharmgkb.com)*. No mutations were found in *KCNQ1* or *KCNE2*.

The family of the proband was small, and both parents were dead. None of the first-degree relatives had AF. The family had no history of sudden death or sudden infant death syndrome. The patient, who was 66 years old at the time of evaluation, had a history of AF, first documented at age 59 years. The ECG of the proband recorded during sinus rhythm showed normal PR and QT intervals and no signs of hypertrophy (Figure 1C). The patient had ischemic heart disease and mild hypertension, with a blood pressure of 155/85 mmHg at the time of presentation. Echocardiography revealed a normal left ventricular ejection fraction and a slightly dilated left atrium, with a left atrial diameter of 44 mm. The patient was treated with a statin, aspirin, diuretics, sotalol, and an angiotensin-converting enzyme inhibitor.

Electrophysiologic impact of WT and mutant *KCNE5* on I_{Ks} currents

Patch clamp experiments were conducted on cells transiently transfected with WT or mutant *KCNE5*. I_{Ks}/CHO cells exhibited slowly activating outward current compatible with I_{Ks} recorded in native cardiac myocytes (Figure 2A). Transient transfection with increasing amounts of *KCNE5* resulted in marked suppression of I_{Ks} (Figure 2B-D).

Figure 3 shows the summarized current-voltage relation for I_{Ks} in the absence and presence of various amounts of *KCNE5*. Addition of *KCNE5* suppressed both developing (Figure 3A) and tail (Figure 3B) currents. At a voltage of +60 mV, the amplitude of the peak developing (Figure 3C) and tail currents (Figure 3D) in the cells transiently transfected with 0.15, 0.3 and 1.5 μg *KCNE5-WT* were significantly reduced in a concentration-dependent manner.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Transient transfection with the *KCNE5-L65F* mutant gene failed to suppress the current, yielding an I_{Ks} current indistinguishable from that recorded in the absence of KCNE5 (Figure 4A and B). Figure 4C and D shows the average current-voltage relation for the effect of *KCNE5-L65F* mutant on I_{Ks}. Cells transfected with *KCNE5-L65F* showed significantly larger developing (Figure 4C) and tail (Figure 4D) currents relative to cells transfected with *KCNE5-WT*.

The effects of 0.3 and 1.5 μg *KCNE5-WT* or *KCNE5-L65F* on I_{Ks} recorded at +60 mV are summarized in Figure 5. Suppression of I_{Ks} developing and tail currents by 1.5 μg *KCNE5-WT* was not observed with the mutant (1.5 μg *KCNE5-L65F*). At the lower concentration of KCNE5, suppression of developing and tail currents tended to be smaller. A statistically significant gain of function was observed at both concentrations of the β-subunit when current recorded after transfection with the *KCNE5* mutant was compared to WT.

The effects of equal amounts of *KCNE5-WT* and *KCNE5-L65F* on I_{Ks} were determined next. I_{Ks}/CHO cells were cotransfected with either 0.75 μg *KCNE5-WT* and *KCNE5-L65F* (Figure 6A) or 1.5 μg *KCNE5-WT* and *KCNE5-L65F* (Figure 6B). No suppression of I_{Ks} developing or tail currents by *KCNE5-WT* in the presence of the *KCNE5-L65F* mutation was observed, indicating a dominant positive effect of the mutant. Similar results were obtained when either 0.75 μg or 1.5 μg KCNE5-WT and KCNE5-L65F were cotransfected (Figure 6E and F).

Discussion

This is the first demonstration of a mutation in *KCNE5* associated with AF. Thus, *KCNE5* is the 10th AF gene identified following *KCNE1* in 2002,[36] *KCNQ1* and *ANK2* in 2003,[11,37] *KCNH2* and *KNCE2* in 2004,[12,38,39] *KCNJ2* in 2005,[13] and *KCNA5*[16] and *GJA5* in 2006,[17] and *ABCC9* in 2007.[40] In contrast to some of the genes for which mutations were found in three large kindreds,[11-13] our mutation in *KCNE5* was found in one of 158 patients attending a cardiology clinic. There was no selection for familial occurrence of AF and no family history in the index family. Our findings suggest that *KCNE5* mutations are a relatively rare cause (<1%) of sporadic AF. Of note, a polymorphism (97C) in *KCNE5*[33] has been associated with increased propensity for AF.[36,41]

Functional analysis of the L65F mutant demonstrates concentration-dependent suppression of I_{Ks} by *KCNE5-WT*, consistent with previously published results,[28,30,42] and reversal of this suppression by the L65F mutation in *KCNE5.* Thus, the L65F missense mutation in *KCNE5* produces an important gain of function in I_{Ks} (or *KCNQ1+KCNE1+KCNE5* current).

A gain of function in I_{Ks} secondary to a *KCNE1* deletion in mice[43] or *KCNQ1* mutation in humans[11,14,15] has been associated with the development of AF. Our results suggest that a gain of function in I_{Ks} secondary to a missense mutation in KCNE5 likely contributes to the manifestation of AF in our patient. Of interest is that the expected gain of function in I_{Ks} was not accompanied by an abbreviation of QTc in our patient. This apparent discrepancy may be explained by much higher expression of KCNE5 in atria than in ventricles demonstrated by Lundquist et al.[28] Accordingly, a loss-of-function mutation in KCNE5 would be expected to cause a much greater change in I_{Ks} in atria than in ventricles and thus produce little, if any change, in QTc.

The alignment in Table 2 shows that the L65F mutation is located in the transmembrane region known to be decisive for proper function.[27,28] When a phylogenetic tree was constructed based on the sequences shown in Table 2 (data not shown), *KCNE3* and *KCNE5* were seen to more closely related than the other genes, so we used available knowledge on the *KCNE3* structure-function relationship to model the tentative functional consequences of the L65F mutation. Using a series of chimeras of *KCNE1* and *KCNE3*,[44,45] it has been possible to define

the particular gating characteristics of *KCNE3*. Based on this structural modeling, a mutation in which a small aliphatic amino acid (Leu) is substituted by a large hydrophobic amino acid (Phe) is expected to interfere with function. The suggested role of the *KCNE5* subunit under physiologically relevant conditions is suppression of I_{Ks} via a positive shift of *KCNQ1* activation by >140 mV so that only strong and sustained voltage depolarizations activate the channel.[30] Therefore, a mutation in *KCNE5* can result in a gain of function of *KCNQ1*. The abbreviated refractoriness that develops as a consequence can render the myocardium more vulnerable to the development of reentrant tachyarrhythmias. Interestingly, coexpression of WT and mutant KCNE5 demonstrated a dominant-positive effect as suppression of I_{Ks} current by WT KCNE5 was eliminated in the presence of the mutant *KCNE5*.

Study limitations

The results of this study suggest that a gain of function of I_{Ks} produced by the L65F mutation in *KCNE5* is associated with AF. However, translation of these findings to the clinic must be approached with caution. Although we evaluated the modulatory effect of *KCNE5* over a wide range of concentrations, the amount of *KNCE5* used in our expression studies may not accurately reflect the amount present in the human heart. The precise stoichiometry of *KCNQ1/KCNE1* and *KCNE5* subunits present in the human heart is not clearly defined. Bendahhou et al[42] reported that *KCNE5* mRNA was virtually absent in human left atrial tissue. In contrast, Lundquist et al[28] found that *KCNE5* expression levels were comparable to both *KCNE1a* and *KCNE1b* expression levels in human left atrium. Although our results suggest that the L65F mutation contributes to AF in this setting, a definitive mechanistic link cannot be made because only a single patient with this mutation was identified.

It can be argued that at age 66 years, our patient might be better categorized as a case of acquired AF, as blood pressure was mildly elevated and this trait has been shown to be associated with development of AF. Of note, all patients with high blood pressure develop AF; the majority remain in sinus rhythm. Although it is possible that individuals who do not develop the arrhythmia are genetically protected, it is equally probable that those who develop AF are genetically predisposed. Thus, genetics may play a role in older individuals with one or more acquired risk factors. The degree to which “environmental” or acquired risk factors vs genetic factors contributes to the development of arrhythmia ultimately will distinguish between what we term acquired vs familial or congenital AF. Our results suggest that, in the case of our patient, who was identified in a study population of nonselected patients consecutively recruited based on presentation with AF, a genetic predisposition likely contributes to the clinical phenotype.

KCNE5 has been reported to modulate the function of *Kv4.3*.[46] It is possible that the L65F mutant contributes other changes to the balance of current responsible for the characteristics of repolarization of the atrial action potential via its interaction with Kv4.3 or other channels. Additional studies are required to address this issue.

Conclusion

The data from this study suggest that AF in this patient is associated with L65F mutation. Whether the mutation merely functions as a predisposing factor or as an initiator can only be addressed with larger studies involving selected patients with hereditary AF as well as a larger population of unselected AF patients. Our data provide support for the hypothesis that a genetic defect in an auxiliary subunit composing the I_{Ks} channel is associated with AF, which potentially could lead to new therapeutic strategies.

Acknowledgments

We thank Ryan Pfeiffer and Jette Rasmussen for excellent technical assistance.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Supported by Grant HL47678 from the National Heart, Lung, and Blood Institute to Dr. Antzelevitch; the American Health Assistance Foundation to Dr. Cordeiro; the NYS, Florida Grand Lodges F.&A.M, The John and Birthe Meyer Foundation to Drs. Haunso and Christiansen; and The Danish National Research Foundation Centre for Cardiac Arrhythmia and The Danish Cardiovascular Research Academy to Dr. Ravn.

References

1. Frost L, Engholm G, Moller H, et al. Decrease in mortality in patients with a hospital diagnosis of atrial fibrillation in Denmark during the period 1980-1993. Eur Heart J 1999;20:1592–1599. [PubMed: 10529328]
2. Friberg J, Scharling H, Gadsboll N, et al. Sex-specific increase in the prevalence of atrial fibrillation (The Copenhagen City Heart Study). Am J Cardiol 2003;92:1419–1423. [PubMed: 14675577]
3. Frost L, Engholm G, Johnsen S, et al. Incident stroke after discharge from the hospital with a diagnosis of atrial fibrillation. Am J Med 2000;108:36–40. [PubMed: 11059439]
4. Grogan M, Smith HC, Gersh BJ, et al. Left ventricular dysfunction due to atrial fibrillation in patients initially believed to have idiopathic dilated cardiomyopathy. Am J Cardiol 1992;69:1570–1573. [PubMed: 1598871]
5. Darbar D, Herron KJ, Ballew JD, et al. Familial atrial fibrillation is a genetically heterogeneous disorder. J Am Coll Cardiol 2003;41:2185–2192. [PubMed: 12821245]
6. Arnar DO, Thorvaldsson S, Manolio TA, et al. Familial aggregation of atrial fibrillation in Iceland. Eur Heart J 2006;27:708–712. [PubMed: 16428254]
7. Fox CS, Parise H, D'Agostino RB Sr, et al. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. JAMA 2004;291:2851–2855. [PubMed: 15199036]
8. Brugada R, Tapscott T, Czernuszewicz GZ, et al. Identification of a genetic locus for familial atrial fibrillation. N Engl J Med 1997;336:905–911. [PubMed: 9070470]
9. Ellinor PT, Shin JT, Moore RK, et al. Locus for atrial fibrillation maps to chromosome 6q14-16. Circulation 2003;107:2880–2883. [PubMed: 12782570]
10. Oberti C, Wang L, Li L, et al. Genome-wide linkage scan identifies a novel genetic locus on chromosome 5p13 for neonatal atrial fibrillation associated with sudden death and variable cardiomyopathy. Circulation 2004;110:3753–3759. [PubMed: 15596564]
11. Chen YH, Xu SJ, Bendahhou S, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 2003;299:251–254. [PubMed: 12522251]
12. Yang Y, Xia M, Jin Q, et al. Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. Am J Hum Genet 2004;75:899–905. [PubMed: 15368194]
13. Xia M, Jin Q, Bendahhou S, et al. A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation. Biochem Biophys Res Commun 2005;332:1012–1019. [PubMed: 15922306]
14. Hong K, Piper DR, Diaz-Valdecantos A, et al. De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. Cardiovasc Res 2005;68:433–440. [PubMed: 16109388]
15. Ellinor PT, Moore RK, Patton KK, et al. Mutations in KCNQ1 are an uncommon cause of atrial fibrillation. Circulation 2003;108:IV-182–IV-183.
16. Olson TM, Alekseev AE, Liu XK, et al. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. Hum Mol Genet 2006;15:2185–2191. [PubMed: 16772329]
17. Gollob MH, Jones DL, Krahn AD, et al. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. N Engl J Med 2006;354:2677–2688. [PubMed: 16790700]
18. Abbott GW, Goldstein SA. Potassium channel subunits encoded by the KCNE gene family: physiology and pathophysiology of the MinK-related peptides (MiRPs). Mol Interv 2001;1:95–107. [PubMed: 14993329]
19. Schulze-Bahr E, Wang Q, Wedekind H, et al. KCNE1 mutations cause Jervell and Lange-Nielsen syndrome. Nat Genet 1997;17:267–268. [PubMed: 9354783]
20. Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 1999;97:175–187. [PubMed: 10219239]
21. Lu Y, Mahaut-Smith MP, Huang CL, et al. Mutant MiRP1 subunits modulate HERG K^+ channel gating: a mechanism for pro-arrhythmia in long QT syndrome type 6. J Physiol 2003;551:253–262. [PubMed: 12923204]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

22. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation 2000;102:1178–1185. [PubMed: 10973849]
23. Abbott GW, Butler MH, Bendahhou S, et al. MiRP2 forms potassium channels in skeletal muscle with Kv3.4 and is associated with periodic paralysis. Cell 2001;104:217–231. [PubMed: 11207363]
24. Sternberg D, Tabti N, Fournier E, et al. Lack of association of the potassium channel-associated peptide MiRP2-R83H variant with periodic paralysis. Neurology 2003;61:857–859. [PubMed: 14504341]
25. Barhanin J, Lesage F, Guillemare E, et al. KvLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. Nature 1996;384:78–80. [PubMed: 8900282]
26. Lewis A, McCrossan ZA, Abbott GW. MinK, MiRP1, and MiRP2 diversify Kv3.1 and Kv3.2 potassium channel gating. J Biol Chem 2004;279:7884–7892. [PubMed: 14679187]
27. Lundquist AL, Turner CL, Ballester LY, et al. Expression and transcriptional control of human KCNE genes. Genomics 2006;87:119–128. [PubMed: 16303284]
28. Lundquist AL, Manderfield LJ, Vanoye CG, et al. Expression of multiple KCNE genes in human heart may enable variable modulation of I(Ks). J Mol Cell Cardiol 2005;38:277–287. [PubMed: 15698834]
29. Piccini M, Vitelli F, Seri M, et al. KCNE1-like gene is deleted in AMME contiguous gene syndrome: identification and characterization of the human and mouse homologs. Genomics 1999;60:251–257. [PubMed: 10493825]
30. Angelo K, Jespersen T, Grunnet M, et al. KCNE5 induces time- and voltage-dependent modulation of the KCNQ1 current. Biophys J 2002;83:1997–2006. [PubMed: 12324418]
31. Brugada R. Is atrial fibrillation a genetic disease? J Cardiovasc Electrophysiol 2005;16:553–556. [PubMed: 15877629]
32. Dixen U, Joens C, Parner J, et al. Prolonged signal-averaged P wave duration after elective cardioversion increases the risk of recurrent atrial fibrillation. Scand Cardiovasc J 2004;38:147–151. [PubMed: 15223712]
33. Hofman-Bang J, Jespersen T, Grunnet M, et al. Does KCNE5 play a role in long QT syndrome? Clin Chim Acta 2004;345:49–53. [PubMed: 15193977]
34. Larsen LA, Andersen PS, Kanters JK, et al. A single strand conformation polymorphism/heteroduplex (SSCP/HD) method for detection of mutations in 15 exons of the KVLQT1 gene, associated with long QT syndrome. Clin Chim Acta 1999;280:113–125. [PubMed: 10090529]
35. Larsen LA, Andersen PS, Kanters J, et al. Screening for mutations and polymorphisms in the genes KCNH2 and KCNE2 encoding the cardiac HERG/MiRP1 ion channel: implications for acquired and congenital long Q-T syndrome. Clin Chem 2001;47:1390–1395. [PubMed: 11468227]
36. Lai LP, Su MJ, Yeh HM, et al. Association of the human minK gene 38G allele with atrial fibrillation: evidence of possible genetic control on the pathogenesis of atrial fibrillation. Am Heart J 2002;144:485–490. [PubMed: 12228786]
37. Mohler PJ, Schott JJ, Gramolini AO, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature 2003;421:634–639. [PubMed: 12571597]
38. Brugada R, Hong K, Dumaine R, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation 2004;109:30–35. [PubMed: 14676148]
39. Hong K, Bjerregaard P, Gussak I, et al. Short QT syndrome and atrial fibrillation caused by mutation in KCNH2. J Cardiovasc Electrophysiol 2005;16:394–396. [PubMed: 15828882]
40. Olson TM, Alekseev AE, Moreau C, et al. KATP channel mutation confers risk for vein of Marshall adrenergic atrial fibrillation. Nat Clin Pract Cardiovasc Med 2007;4:110–116. [PubMed: 17245405]
41. Ravn LS, Hofman-Bang J, Dixen U, et al. Relation of 97T polymorphism in KCNE5 to risk of atrial fibrillation. Am J Cardiol 2005;96:405–407. [PubMed: 16054468]
42. Bendahhou S, Marionneau C, Haurogne K, et al. In vitro molecular interactions and distribution of KCNE family with KCNQ1 in the human heart. Cardiovasc Res 2005;67:529–538. [PubMed: 16039274]
43. Temple J, Frias P, Rottman J, et al. Atrial fibrillation in KCNE1-null mice. Circ Res 2005;97:62–69. [PubMed: 15947250]

44. Melman YF, Krumerman A, McDonald TV. A single transmembrane site in the KCNE-encoded proteins controls the specificity of KvLQT1 channel gating. J Biol Chem 2002;277:25187–25194. [PubMed: 11994278]

45. Melman YF, Domenech A, de la Luna S, et al. Structural determinants of KvLQT1 control by the KCNE family of proteins. J Biol Chem 2001;276:6439–6444. [PubMed: 11104781]

46. Radicke S, Cotella D, Graf EM, et al. Functional modulation of the transient outward current Ito by KCNE beta-subunits and regional distribution in human non-failing and failing hearts. Cardiovasc Res 2006;71:695–703. [PubMed: 16876774]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

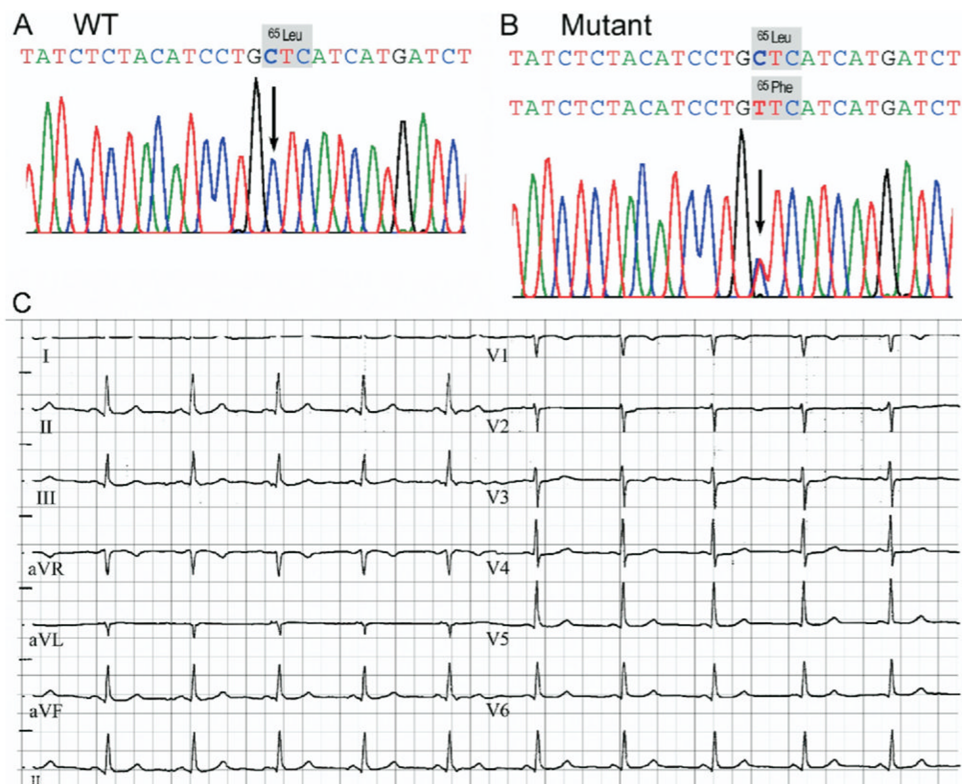

Figure 1.
Mutation screening of *KCNE5*. **A:** DNA sequence chromatogram showing the wild type (WT) and the corresponding amino acid sequence. **B:** DNA sequence chromatogram showing the heterozygous mutation (in *bold)* and the corresponding amino acid sequence. A heterozygous substitution of T for C in nucleotide 193 predicts substitution of phenylalanine (TTC) for leucine (CTC) in codon 65 (L65F). **C:** ECG of the proband recorded during normal sinus rhythm between episodes of atrial fibrillation.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

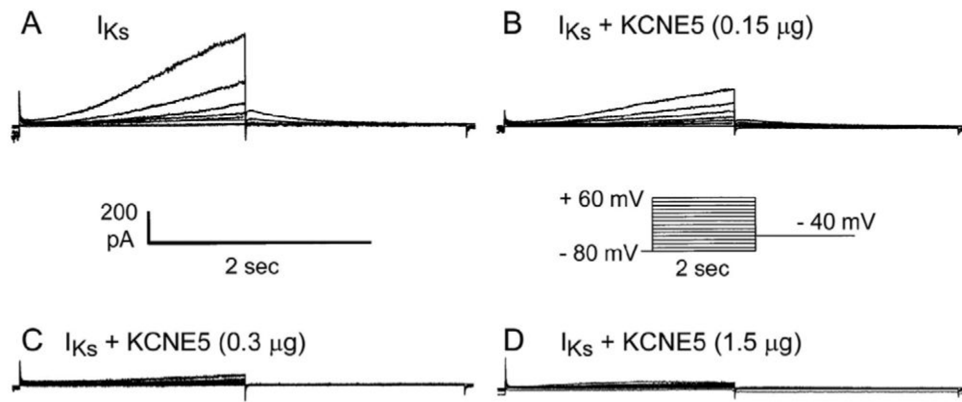

Figure 2.

Representative current trace recorded from I_{Ks}/CHO cells transiently transfected with *KCNE5-WT*. **A:** Current from stable cell line of I_{Ks}/CHO. **B:** Current from I_{Ks}/CHO transfected with 0.15 μg *KCNE5-WT*. **C:** Current from I_{Ks}/CHO transfected with 0.3 μg *KCNE5-WT*. **D:** Current from I_{Ks}/CHO transfected with 1.5 μg *KCNE5-WT*. Pulse protocol is shown in the *inset* in the center right.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

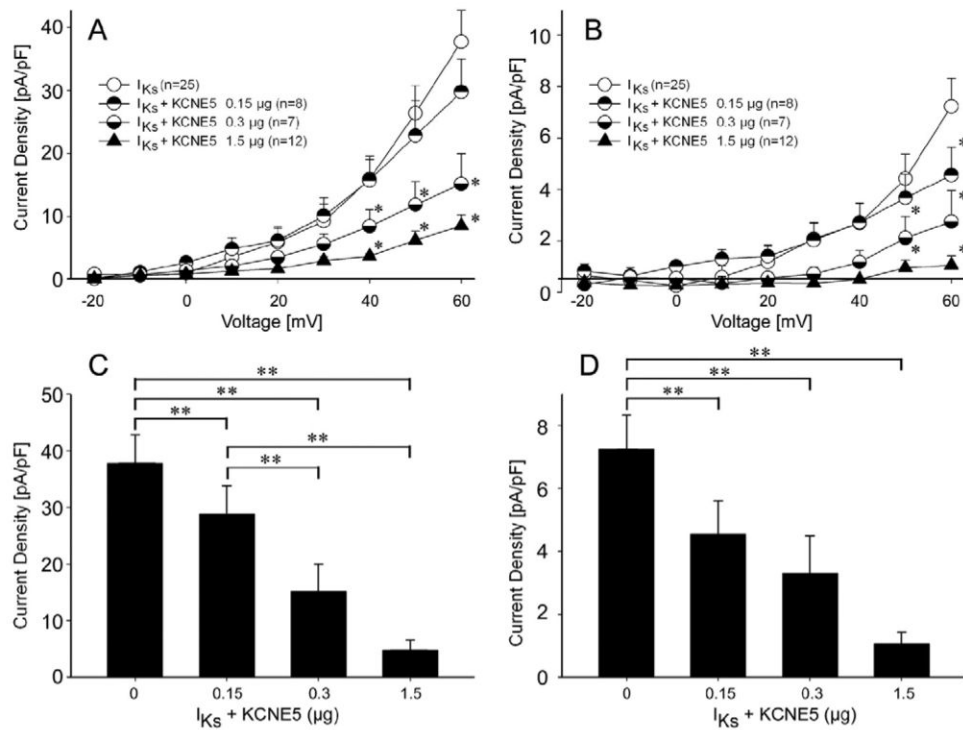

Figure 3.

Current-voltage relationship of developing and tail currents in the absence and presence of 0.15 to 1.5 μg *KCNE5-WT*. **A:** Current-voltage relationship of peak developing current. **B:** Current-voltage relationship of tail current measured upon repolarization to -40 mV. **C:** Bar graphs showing current densities of peak developing current measured during test pulse to +60 mV. **D:** Bar graphs showing current densities of tail current measured upon repolarization to -40 mV following a test pulse to +60 mV. **P* <.05; ***P* <.01 vs I_{Ks}.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

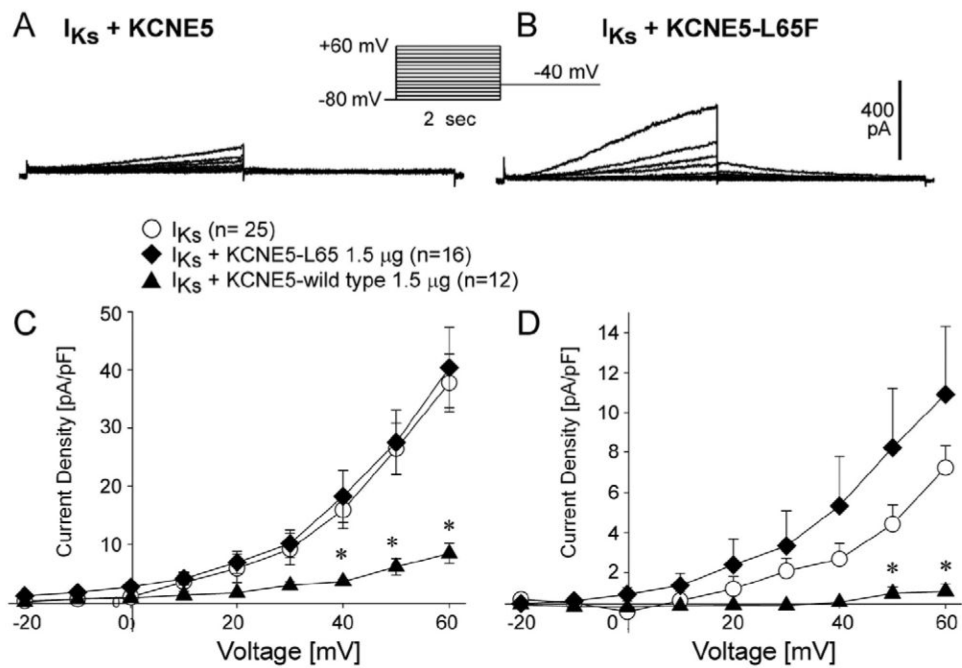

Figure 4.

Current-voltage relationship of developing and tail currents in the absence and presence of 1.5 μg *KCNE5-WT* or *KCNE5-L65F*. **A:** Representative current trace of I_{Ks}/CHO cells transfected with 1.5 μg WT *KCNE5*. **B:** Representative current trace of I_{Ks}/CHO cells transfected with 1.5 μg L65F *KCNE5*. Pulse protocol is shown in the *inset* in the center right. **C:** Current-voltage relationship of peak developing current. **D:** Current-voltage relationship of tail current measured upon repolarization to -40 mV. *P <.05 vs I_{Ks}+*KCNE5-WT*.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

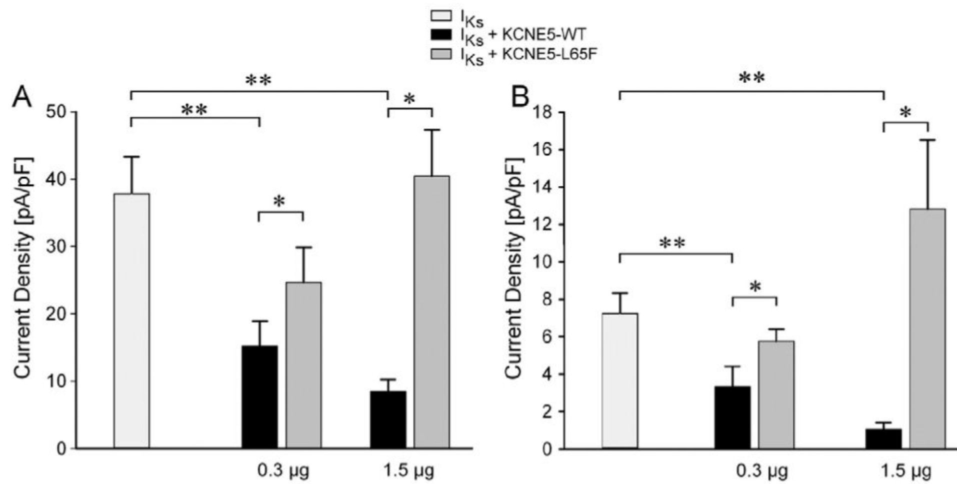

Figure 5.

Effect of 0.3 and 1.5 *μ*g *KCNE5-WT* or *KCNE5-L65F* on $I_{Ks.}$ **A:** Bar graph showing density of peak developing current recorded during a test pulse to +60 mV. **B:** Bar graph showing density of tail currents recorded during repolarization to -40 mV following a test pulse to +60 mV. Statistical significance determined using analysis of variance. *P <.05; **P <.01.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

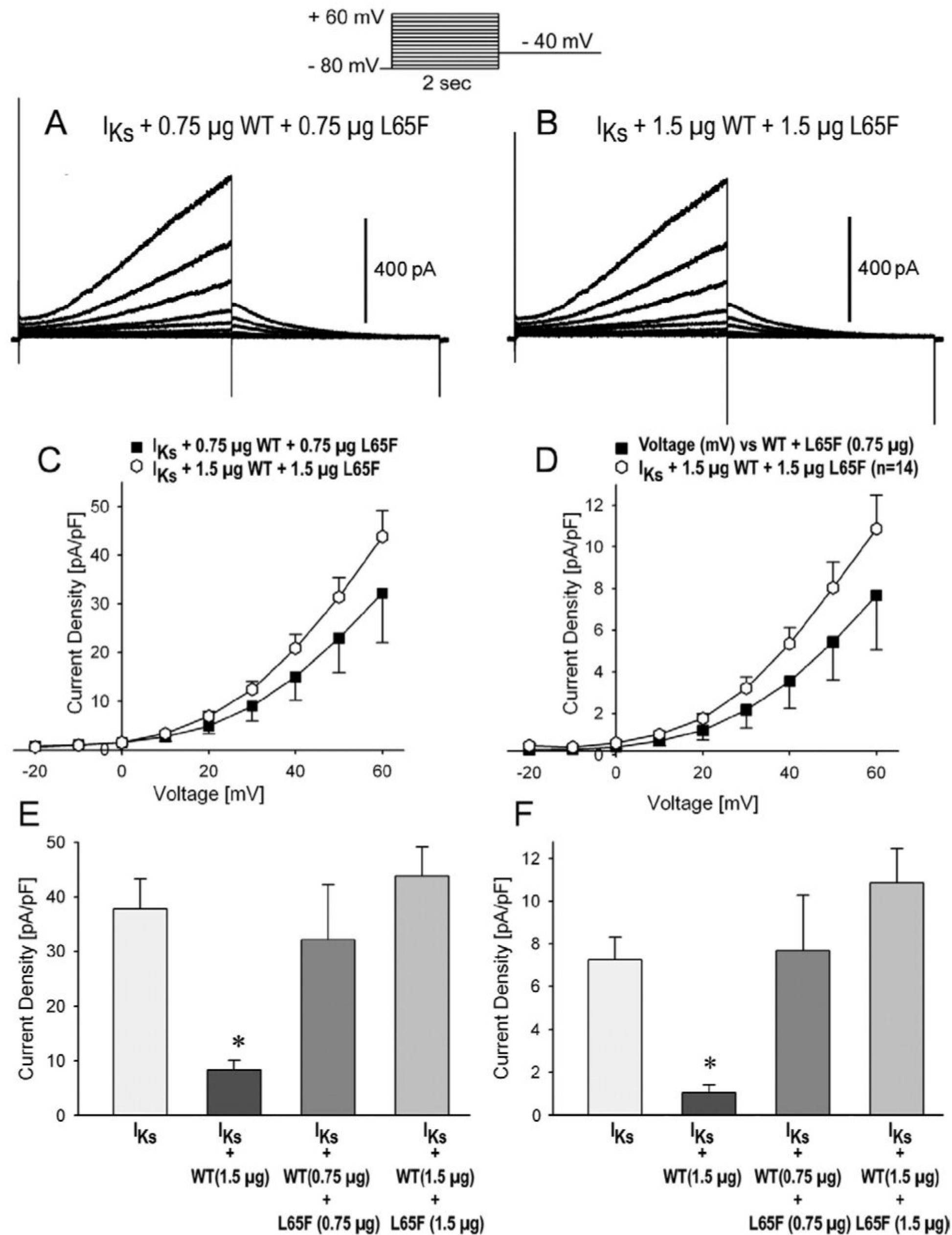

Figure 6.

Current-voltage relationship of developing and tail currents in cells cotransfected with equal amounts of *KCNE5-WT* and *KCNE5-L65F*. **A:** Representative current trace of I_{Ks}/CHO cells transfected with 0.75 μg WT *KCNE5* and 0.75 μg L65F *KCNE5.* **B:** Representative current trace of I_{Ks}/CHO cells transfected with 1.5 μg WT *KCNE5* and 1.5 μg L65F *KCNE5.* **C:** Current-voltage relationship of peak developing current. **D:** Current-voltage relationship of tail current measured upon repolarization to -40 mV. **E:** Bar graph showing density of peak developing current recorded during a test pulse to +60 mV (I_{Ks}; I_{Ks}+1.5 μg KCNE5-WT; I_{Ks}+0.75 μg KCNE5-WT+0.75 μg KCNE5-L65F; I_{Ks}+1.5 μg KCNE5-WT+1.5 μg KCNE5-L65F). **F:** Bar graph showing density of tail currents recorded during repolarization to -40 mV following a test pulse to +60 mV (I_{Ks}; I_{Ks}+1.5 μg KCNE5-WT; I_{Ks}+0.75 μg KCNE5-WT+0.75 μg KCNE5-L65F; I_{Ks}+1.5 μg KCNE5-WT+1.5 μg KCNE5-L65F).

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Demographic and clinical characteristics of the 158 patients with atrial fibrillation

Characteristic	
Male	108 (68%)
Age (years)	66.2 (32-86)
Smoker	29 (18.4%)
Body mass index	27.1 (17-43)
Hypertension	70 (44.3%)
Ischemic heart disease	29 (18.4%)
Diabetes	17 (10.8%)
Ejection fraction <45%	15 (9.6%)
Ejection fraction 46%-55%	18 (11.5%)
Ejection fraction >55%	123 (78.9%)
Paroxysmal atrial fibrillation	19 (12.0%)
Persistent atrial fibrillation	93 (58.9%)
Permanent atrial fibrillation	46 (29.1%)

Age and body mass index are given as mean values.

Proportion of all patients (in percent) or range is given parentheses.

Table 2

Alignment of amino acid sequence of *KCNE5* in various species and comparison with human *KCNE1-4*

		↓	
kcne5_human	56	G D D A Y L Y I L ~ L ~ I M I F Y A C L A G G L I L A Y T R S R K	86
kcne5_monkey	56	. . . . ~ . ~	86
kcne5_dog	56	. . . . ~ . ~ . . . . N	86
kcne5_cattle	56	. . . . ~ . ~ . . V	86
kcne5_horse	56	. . . . ~ . ~	86
kcne5_mouse	56	. N . . . . ~ . ~	86
kcne5_chicken	36	. G . . S . . . . ~ . ~ . . . . . G	66
kcne5_frog_1	35	N . . . . ~ . ~ . . . . . G	65
kcne5_frog 2	35	N . . . . ~ . ~ . . . . . G	65
kcne4_human	34	N E ~ ~ . F . . . ~ V ~ V . S . . G I F L I . I M . G . M K . K R	62
kcne3_human	54	D . N S . M . . . ~ F ~ V . F L F . V T V . S . . . G	84
kcne2_human	46	F Y Y V I . . L M ~ V ~ M I G M F S F I I V A I L V S T V K . K R	76
kcne1_human	40	. K L E A . . V . ~ M ~ V L G . F G F F T L . I M . S . I . . K .	70

Dots represent identical amino acids. *Gray shaded boxes* represent similar amino acids according to the human KCNE5 protein sequence. *Arrow* points to the highly conserved leucine at position 65 in *KCNE5*.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript