KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT interval

Alicia Lundby, MSc,*[1] Lasse Steen Ravn, MD,†[1] Jesper Hastrup Svendsen, MD,† Søren-Peter Olesen, MD, PhD,* Nicole Schmitt, PhD*

*From the *Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Biomedical Sciences, The Panum Institute, University of Copenhagen, Copenhagen, Denmark, and †Danish National Research Foundation Centre for Cardiac Arrhythmia, The Heart Centre, Copenhagen University Hospital, Copenhagen, Denmark.*

BACKGROUND Atrial fibrillation (AF) and long QT syndrome (LQTS) are cardiac arrhythmia disorders that have been related to dysfunction of the voltage-gated potassium channel subunit Kv7.1 encoded by the *KCNQ1* gene.

OBJECTIVE The purpose of this study was functional assessment of a mutation in Kv7.1 identified in a proband with permanent AF and prolonged QT interval. We investigated whether this *KCNQ1* missense mutation could form the genetic basis for AF and LQTS simultaneously in this patient.

METHODS We investigated the functional consequences of the novel mutation *KCNQ1* Q147R by heterologous expression of the channel and accessory subunits in *Xenopus laevis* oocytes and mammalian cells.

RESULTS The Q147R mutation does not affect the biophysical properties of Kv7.1 in the absence of accessory subunits. Upon coexpression with the β-subunit KCNE1, the Q147R mutation induced a loss of function, observed as a decrease in current amplitude at depolarized potentials. Additionally, Q147R abolished the frequency dependence of charge carried by Kv7.1/KCNE1 channels. Coexpression with the β-subunit KCNE2 revealed a gain of function for the mutant, seen as an increase in the current amplitude at depolarized potentials. The properties of channels formed by Kv7.1 and the subunits KCNE3 and KCNE4 were unaffected by the Q147R mutation.

CONCLUSION Our data indicate that the Q147R mutation can form the molecular substrate simultaneously for different arrhythmogenic conditions. The mechanism may be heterogeneous distribution of Kv7.1 accessory subunits in the heart leading to Kv7.1 gain of function in the atria (for AF) and Kv7.1 loss of function in the ventricles (for QT prolongation).

KEYWORDS Arrhythmia; Atrial fibrillation; QT interval; Potassium channel; Kv7.1; KCNQ1; KCNE1; KCNE2; Two-electrode voltage clamp

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Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia occurring in the general population. AF is characterized by rapid irregular atrial activation. In rare cases, AF occurs in clusters in families[1] and shows some degree of heritability.[2] Its prevalence increases with age; currently AF affects more than 5% of the western population older than 65 years.[3] AF is a potentially serious disease, primarily because of thromboembolic complications and heart failure.[4] Genetic linkage analyses have identified several loci on various chromosomes associated with AF.[5–12] The genetic defect has been identified in five cases as missense mutations in *KCNQ1*, *KCNE2, KCNJ2, KCNH2,* and *KCNA5*. Interestingly, the genes identified encode cardiac potassium channel subunits. In addition, variations in the *KCNE5* and *SCN5A* genes have been associated with AF, the latter encoding the cardiac sodium channel $Na_v1.5$.[13–15] Other cases of familial AF demonstrate no linkage between the disease and these loci,[16] indicating that other genetic heterogeneity is to be expected.

Long QT syndrome (LQTS) is a genetically heterogenous cardiac disease characterized by prolonged ventricular repolarization. Affected individuals are at high risk for the ventricular tachyarrhythmia *Torsade de Pointes*, loss of consciousness, and, in the worst cases, sudden cardiac death (for review see Schwartz[17]). The surface ECG of an affected patient shows a prolonged corrected QT interval (QTc) and abnormal T-wave morphology. However, various clinical signs of life-threatening cardiac arrhythmias are required to confirm the diagnosis.[18] A QTc interval >440 ms is considered prolonged. In the absence of other clinical signs of LQTS, a patient with QTc interval ranging from 450 to 470

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Address reprint requests and correspondence: Dr. Nicole Schmitt, The Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Biomedical Sciences, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. E-mail address: nschmitt@mfi.ku.dk. (Received January 2, 2007; accepted July 21, 2007.)

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ms has intermediate probability of having the syndrome. To date, nine genes have been associated with LQTS, seven of which encode ion channel subunits involved in shaping the cardiac action potential. The non-ion channel proteins ankyrin-B and caveolin are required for localization or regulation of ion channels in cardiomyocytes, respectively.[19,20]

The most common subtype of LQTS, known as LQT1, is associated with mutations in the Kv7.1 potassium channel causing loss of channel function. Kv7.1 α-subunits coassemble with the accessory β-subunit KCNE1 to form channels that conduct the slow delayed rectifier K^+ current in the heart I_{Ks}, which is important for termination of the plateau phase and repolarization of atrial and ventricular action potentials.[21,22] Moreover, Kv7.1 has been postulated to form complexes with other β-subunits of the KCNE type in the heart.[23–25]

This study investigated the functional consequences of the novel missense mutation Q147R in *KCNQ1* identified in a patient with permanent AF and concomitant prolonged QT interval. The mutation leads to modification of the current conducted by Kv7.1 channels, which require the presence of either KCNE1 or KCNE2 in the channel complex to manifest. Q147R was found to cause loss of function when coexpressed with KCNE1 and gain of function when coexpressed with KCNE2. Previous studies reported that gain of function of the I_{Ks} current can cause AF, whereas loss of function can cause LQTS. We suggest that, depending on the regional distribution of KCNE1 and KCNE2 subunits in the heart, the Q147R mutation in *KCNQ1* can explain the molecular basis for AF and prolonged QT interval in the same patient.

Methods

Patient presentation

The investigation conforms to the principles outlined in the Declaration of Helsinki. Collection of clinical data and analysis of samples were approved by the ethics committees of Copenhagen and Frederiksberg counties (KF01-147/02).

Patients initially participated in the Copenhagen SAFIR investigation[26] and were included consecutively from the Department of Cardiology, Copenhagen University Hospital Hvidovre, Denmark, from April 1999 to August 2001. The basic inclusion criterion was ECG-documented AF followed by restored sinus rhythm (SR). Three years later, 158 (50 women and 108 men) of the 220 invited survivors underwent follow-up testing, which consisted of Holter recording, registration of disease history and demographic data, signal-averaged ECG, echocardiography, and evaluation of blood samples. The study design was cross-sectional, based on a follow-up study of a population characterized by earlier ECG-documented AF followed by SR at the original time of inclusion into the study.

QTc calculation

Measurement of the QT interval and calculation of QTc were performed by standard 12-lead ECG recorded at a paper speed of 25 mm/s. QT intervals were measured for 13 consecutive cycles from one of the 12 ECG leads. Mean QTc was calculated from the mean QT interval and mean RR interval of the 13 cycles. The Fridericia formula [$QTc = QT/(RR)^{1/3}$] was used for QT interval correction.

Mutation screening

Genomic DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The coding region of the *KCNQ1* gene was amplified by polymerase chain reaction (PCR) using primers designed to obtain fragments of appropriate size. Single-strand conformation polymorphism (SSCP) analysis 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.

Molecular biology

The Q147R mutation (CGG for CAG) was introduced into human Kv7.1 cDNA (GenBank Accession No. NM_000218) by site-directed PCR mutagenesis using standard overlap PCR techniques and subsequently cloned into the pGEM-HE and pcDNA3 vectors and sequenced using an ABI 377 DNA sequencer (Perkin Elmer/Applied Biosystems). cRNA was synthesized from hKv7.1-WT in pXOOM, hKv7.1-Q147R in pGEM-HE, and human KCNE1-5 in pXOOM (GenBank Accession No. NM_000219, NM_172201, NM_005472, NM_080671, and NM_012282, respectively) using the Ambion T7 m-Message Machine kit according to the manufacturer's instructions (Ambion, Austin, TX, USA). cRNA was extracted and dissolved using the MegaClear kit (Ambion). cRNA quality was examined by agarose gel electrophoresis, quantified by ultraviolet spectroscopy, and stored at −80°C until injection.

Transient expression in *Xenopus laevis* oocytes and mammalian cells

Oocyte isolation and injection

Isolation, maintenance, and cRNA injection of stage V and VI *X. laevis* oocytes were performed as previously described.[27] Currents were recorded from oocytes injected with Kv7.1-WT or Kv7.1-Q147R cRNA (2.5 ng per oocyte) as well as from oocytes coinjected with Kv7.1-WT or Kv7.1-Q147R subunits (2.5 ng per oocyte) and accessory subunits KCNE1 (0.5 ng per oocyte), KCNE2 (0.5 ng per oocyte), KCNE3 (0.4 ng per oocyte), KCNE4 (0.7 ng per oocyte), or KCNE5 (0.5 ng per oocyte), corresponding to a 1:1 molar ratio between channel subunits and accessory subunits. For paired comparisons, only results from oocytes isolated from the same frog were used, and oocytes from a minimum of three different batches were tested.

Transient transfections

CHO-K1 (Chinese hamster ovary) cells were cultured in Dulbecco modified Eagle medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (Life Technologies) and 40 mg/L L-proline and grown in T25 flasks (Nunc) at 37°C in 5% CO_2. On the day