Biochimica et Biophysica Acta 1812 (2011) 488–494

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Biophysical properties of mutant KCNQ1 S277L channels linked to hereditary long QT syndrome with phenotypic variability

Parwez Aidery [1], Jana Kisselbach [1], Patrick A. Schweizer, Rüdiger Becker, Hugo A. Katus, Dierk Thomas *

Department of Cardiology, Medical University Hospital Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 6 January 2011
Accepted 10 January 2011
Available online 15 January 2011

Keywords:
Cardiac electrophysiology
I_{Ks} current
KCNQ1 (KVLQT1)
Long QT syndrome
Mutation
Sudden cardiac death

ABSTRACT

Hereditary long QT syndrome (LQTS) is associated with ventricular torsade de pointes tachyarrhythmias and sudden cardiac death. Mutations in a cardiac voltage-gated potassium channel, KCNQ1, induce the most frequent variant of LQTS. We identified a KCNQ1 missense mutation, KCNQ1 S277L, in a patient presenting with recurrent syncope triggered by emotional stress (QTc = 528 ms). This mutation is located in the conserved S5 transmembrane region of the KCNQ1 channel. Using *in vitro* electrophysiological testing in the *Xenopus* oocyte expression system, the S277L mutation was found to be non-functional and to suppress wild type currents in dominant-negative fashion in the presence and in the absence of the regulatory ß-subunit, KCNE1. In addition, expression of S277L and wild type KCNQ1 with KCNE1 resulted in a shift of the voltage-dependence of activation by −8.7 mV compared to wild type I_{Ks}, indicating co-assembly of mutant and wild type subunits. The electrophysiological phenotype corresponds well with the severe clinical phenotype of the index patient. However, investigation of family members revealed three patients that exhibit asymptomatic QT interval prolongation (QTc = 493–518 ms). In conclusion, this study emphasizes the value of biophysical testing to provide mechanistic evidence for pathogenicity of ion channel mutations identified in LQTS patients. The inconsistent association of the KCNQ1 S277L mutation with the clinical presentation suggests that additional genetic, epigenetic, or environmental factors play a role in defining the individual clinical LQTS phenotype.

1. Introduction

Hereditary long QT syndromes (LQTS) are cardiac repolarization abnormalities characterized by a prolonged QT interval on the surface ECG. LQTS are associated with syncope and a high risk of sudden cardiac death due to ventricular tachyarrhythmias [1]. More than 700 mutations in twelve different genes have been described to date [2]. LQTS1, the most frequent form of LQTS, is associated with KCNQ1 (KvLQT1) gene mutations [3]. Co-expression of the pore-forming α-subunit KCNQ1 and its regulatory β-subunit KCNE1 (minK) elicits slowly activating potassium currents, resembling the slow component of the cardiac delayed rectifier potassium current I_{Ks} [4,5]. I_{Ks} contributes to cardiac repolarization and is a target for class III antiarrhythmic drugs such as dronedarone [6]. Loss-of-function mutations in KCNQ1 lead to prolonged cardiac repolarization and cause congenital long QT syndrome 1 [7–12]. In contrast, KCNQ1 gain-of-function mutations may induce short QT syndrome [13] or hereditary atrial fibrillation [14].

Previous work on genotype–phenotype correlations has indicated that the individual LQTS genotype influences the clinical course [15,16]. Approximately one third of KCNQ1 mutations identified in LQTS patients are located in the pore region or in adjacent transmembrane regions, S5 and S6 [17]. Carriers of these mutations exhibit a higher risk for cardiac events and tend to be affected at younger ages compared to patients with mutations in the C-terminal region [18,19]. Furthermore, Moss et al. [20] revealed that location of KCNQ1 mutations in the pore and transmembrane regions and dominant-negative current suppression *in vitro* may serve as predictors of severe clinical courses independent of traditional clinical risk factors, suggesting potential significance for therapy planning in LQTS patients.

In the present study, we analyse biophysical properties of mutant KCNQ1 S277L potassium channels identified in a German LQTS family. This mutation is located in the S5 transmembrane region of the KCNQ1 channel and causes loss of channel function. However, closely related family members display phenotypic variability ranging from asymptomatic QTc prolongation to recurrent ventricular tachycardia despite harbouring the same mutation associated with a severe cellular phenotype. We conclude that modifying factors contribute to the individual clinical phenotype to a larger extent than previously appreciated.

* Corresponding author. Tel.: +49 6221 568855; fax: +49 6221 565514.
E-mail address: dierk.thomas@med.uni-heidelberg.de (D. Thomas).
[1] These authors contributed equally to this work.

0925-4439/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2011.01.008

2. Materials and methods

2.1. Pedigree and mutation analysis

Clinical evaluation and blood samples are based on an LQTS family of German origin (Fig. 1A). The index patient (II.4, female) was brought to our attention after recurrent syncope and aborted sudden death had occurred during emotional stress. The investigation conforms to the principles outlined in the Declaration of Helsinki. Informed consent was obtained for genetic analyses. DNA sequence analyses were performed by the Center for Human Genetics and Laboratory Medicine (Martinsried, Germany).

2.2. Molecular biology

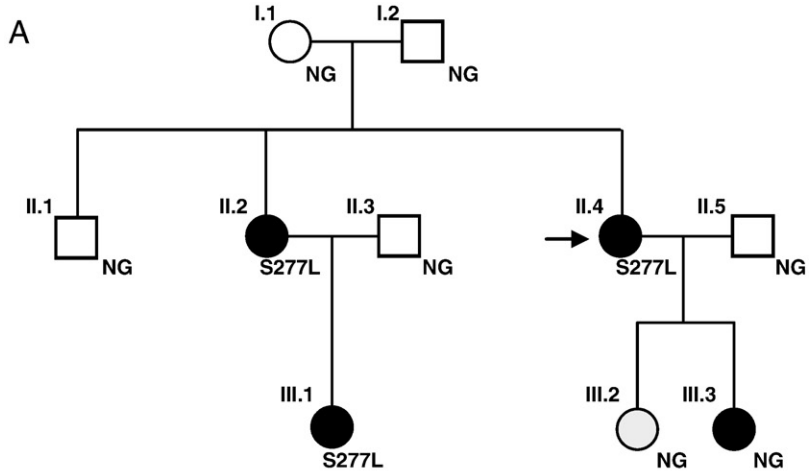
Complementary DNA encoding human KCNQ1 and human KCNE1 was kindly provided by Dr. Steve Goldstein (Chicago, USA). To introduce the S277L mutation into KCNQ1, site-directed PCR-mutagenesis was performed using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, USA) as described [21], and resulting cDNA was analysed by DNA sequence analysis.

2.3. Heterologous gene expression in Xenopus laevis oocytes

Procedures for *in vitro* transcription and oocyte injection have been published previously [9]. Briefly, WT KCNQ1, KCNQ1 S277L, and KCNE1 cRNAs were prepared with the mMESSAGE mMACHINE kit (Ambion, Austin, USA) using T7 RNA polymerase. Stage V–VI defolliculated *Xenopus* oocytes were injected with 46 nl of cRNA per oocyte, and electrophysiological measurements were performed 2 days after injection. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996). The European Community guidelines for the use of experimental animals have been adhered to.

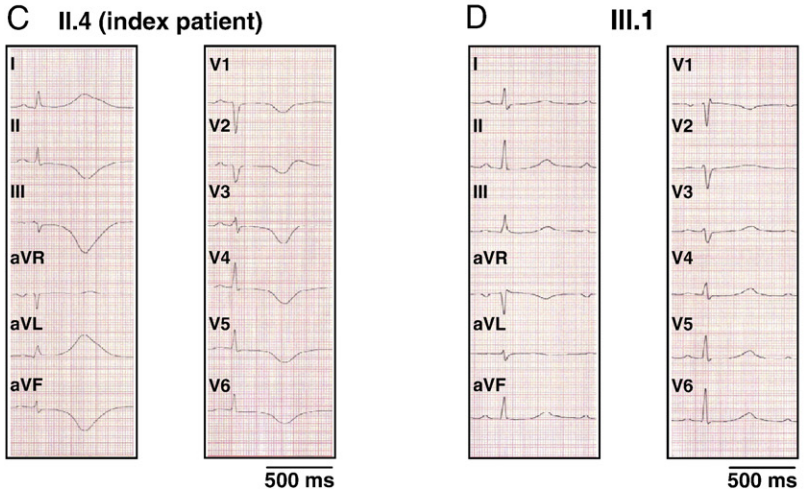
2.4. Electrophysiology and data analysis

Two-microelectrode voltage-clamp recordings from *Xenopus laevis* oocytes were carried out as published previously [9]. Voltage clamp measurements of *Xenopus* oocytes were performed in a solution containing (in mM): 5 KCl, 100 NaCl, 1.5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH 7.4, adjusted with NaOH). Current and voltage electrodes were filled with 3 M KCl solution. All experiments were carried out at room



B

Patient ID	Age (years)	Sex	QTc (ms)	LQTS score	Trigger of syncope
II.2	50	F	511	4	n/a
II.4	49	F	528	5.5	emotion
III.1	19	F	493	4	n/a
III.3	11	F	518	4	n/a



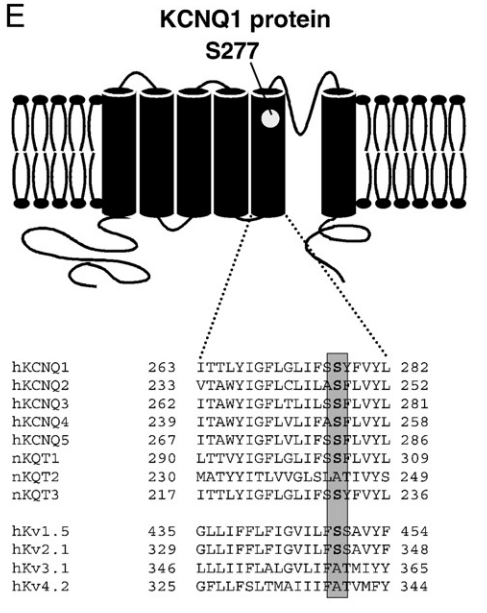


Fig. 1. Patient characteristics and identification of the KCNQ1 S277L mutation. (A) pedigree of the LQTS family analysed in this study (arrow indicates the index patient). Closed symbols denote definite long QT syndrome (i.e., Schwartz Score ≥ 4 [1,23]), open symbols indicate unaffected family members. Circles refer to women, squares indicate men. S277L, heterozygous S277L genotype; NG, not genotyped. (B) clinical evaluation of LQTS family members. (C, D) resting ECGs of the index patient (II.4; C) and patient III.1 (D) reveal significant QTc prolongation. (E) membrane folding model of the KCNQ1 protein, indicating the location of the mutated amino acid residue, S227. Residue S277 is highly conserved, as demonstrated by amino acid sequence alignments of S5 transmembrane segments of human KCNQ family potassium channels KCNQ1-5, *Caenorhabditis elegans* KCNQ homologs KQT1-3, and select human voltage gated potassium (Kv) channels. S277 and its homologs are boxed. The respective GenBank accession numbers are AF000571, NM_172107, NM_004519, NM_004700, and NM_019842 for KCNQ1-5, NM_171709, NM_076991, and NM_064474 for KQT1-3, and NM_002234 (hKv1.5), NM_004974 (hKv2.1), NM_004976 (hKv3.1), NM_012281 (hKv4.2).