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Original Article

A rare *KCNE1* polymorphism, D85N, as a genetic modifier of long QT syndrome

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ABSTRACT

Background: The gene *KCNE1* encodes the β-subunit of cardiac voltage-gated K^+ channels and causes long QT syndrome (LQTS). LQTS is characterized by the prolongation of QT interval and lethal arrhythmias such as torsade de pointes (TdP). A *KCNE1* polymorphism, D85N, has been shown to modify the phenotype of LQTS through a loss-of-function effect on both KCNQ1 and KCNH2 channels when co-expressed and reconstituted in a heterologous expression system.
Methods: A screening for the D85N polymorphism was performed in 355 LQTS families with mutations in *KCNQ1*, *KCNH2*, or *SCN5A*. Among the probands who had a heterozygous status with the polymorphism, we focused on a family with a *KCNH2* mutation (E58K), a N-terminal missense mutation, and examined the clinical significance of this polymorphism. We also conducted biophysical assays to analyze the effect of the polymorphism in mammalian cells.
Results: In 355 probands, we found 14 probands (3.9%) who had a heterozygous compound status with the D85N polymorphism. In the family with a *KCNE1*-D85N polymorphism and a *KCNH2*-E58K mutation, the proband and her daughter carried both the *KCNH2* mutation and the *KCNE1*-D85N polymorphism. They experienced repetitive syncope and TdP. Two sons of the proband had either *KCNH2*-E58K mutation or *KCNE1*-D85N, but were asymptomatic. Biophysical assays of *KCNE1*-D85N with *KCNH2*-E58K variants produced a larger reduction in the reconstituted I_{Kr} currents compared to co-expression with wild-type *KCNE1*.
Conclusions: The *KCNE1*-D85N polymorphism modified the clinical features of LQTS patients.

1. Introduction

Long QT syndrome (LQTS) is characterized by cardiac repolarization abnormalities that lead to TdP, syncope, and sudden cardiac death [1]. The disease is genetically heterogeneous and caused by mutations in > 10 genes, including *KCNH2* and *KCNE1* [2–4]. In LQTS probands with heterozygous genetic variants, compound mutations usually exacerbate the disease severity compared to other family members who carry a single mutation [5–7]. Previously, the coexistence of the single nucleotide polymorphism (SNP) *KCNH2*-K897T with the latent *KCNH2* mutation A1116V was shown to modify the clinical symptoms [8].

A *KCNE1* C-terminal polymorphism, D85N, has been found in the normal population. The sequence, a nucleotide replacement from G to A at 253, causes an amino acid change from aspartic acid to asparagine at position 85 [9]. The allele frequency of the polymorphism is reported to be 0.7% in apparently healthy Asians [10]. Paulussen et al. demonstrated that the allele frequency of the same variant among Europeans is 5% in drug-induced LQTS patients who experienced TdP, but 0% in the control population [11]. More recently, we demonstrated that the D85N allele frequency is 0.8% among apparently healthy Japanese individuals and that it is significantly higher among clinically diagnosed LQTS probands (3.9%) [9]. In a patch-clamp experiment using a heterologous expression system in a mammalian cell line, *KCNE1*-D85N was found to reduce the current densities in *KCNQ1*/*KCNE1* channels (I_{Ks}) and *KCNH2*/*KCNE1* channels (I_{Kr}) by 28% and 31%, respectively [9].

In the present study, we screened for the D85N polymorphism in 355 LQTS probands in which we could identify a mutation in

Abbreviations: CHO cell, Chinese hamster ovary cell; LQTS, long QT syndrome; PPM, post pacemaker implantation; SCD, sudden cardiac death; SNP, single nucleotide polymorphism; TdP, torsade de pointes

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KCNQ1, *KCNH2*, or *SCN5A*, and found 14 patients that carried the polymorphism in addition to a single pathologic LQTS-related gene mutation. Among them, in a family with *KCNH2*-E58K, D85N appeared to modulate the phenotype of family members. In order to clarify the phenotype–genotype correlation, we then conducted functional assays of the variants by using a heterologous expression system in Chinese hamster ovary (CHO) cells.

2. Material and methods

2.1. Genetic analysis

The cohort of this study was 355 LQTS probands who were identified as having mutations in *KCNQ1*, *KCNH2*, or *SCN5A* and their family members. Genetic analysis was performed after obtaining written informed consent in accordance with the study protocol approved by our institutional ethics committees. In addition to the 3 genes listed above, genetic screening for *KCNE1* was performed by single strand conformation polymorphism or denaturing high-performance liquid chromatography using a WAVE System Model 3500 (Transgenomic, Omaha, NE, USA). Abnormal conformers were amplified by polymerase chain reaction (PCR). Sequencing was performed with an ABI PRISM3130 DNA sequencer (Applied Biosystems, Wellesley, MA, USA).

2.2. Mutagenesis

Complementary deoxyribonucleic acid (cDNA) for human *KCNE1* (GenBank M26685) was kindly provided by Dr. J. Barhanin (Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, Valbonne, France) and was subcloned into a pIRES-CD8 vector. cDNA for human *KCNH2* (GenBank AF363636) was kindly donated by Dr. M. Sanguinetti (University of Utah, Salt Lake City, UT, USA) and was subcloned into a pRc-CMV vector. A *KCNE1*-D85N variant was constructed using a Quik Change II XL Site-Directed Mutagenesis Kit, according to the manufacturer's instructions (Stratagene, La Jolla, California, USA). A *KCNH2* mutation (E58K) was constructed by overlap-extension PCR. Nucleotide sequence analysis was performed on each variant construct before the expression study to confirm their sequences.

2.3. Cell transfection

CHO cells were maintained at 37 °C in Dulbecco's modified Eagle medium and Ham's F12 nutritional mixture (Gibco-BRL, Rockville, Maryland, USA) containing 10% fetal bovine serum supplemented with 1% penicillin and 1% streptomycin. Wild-type (WT) and/or variant *KCNH2*, and WT and/or variant *KCNE1* clones were transiently expressed in CHO cells by using the Lipofectamine method according to the manufacturer's instructions (Invitrogen, Carlsbad, California, USA).

To identify the cells that were positive for *KCNH2* expression, CHO cells were co-transfected with 0.5–1 μg of the pRc-CMV/*KCNH2* vector and 0.5 μg of a pEGFP-N1/CMV vector. About 48–72 h after transfection, green fluorescent protein (GFP) positive cells and anti-CD8 antibody-coated bead (Dynabeads CD8; Dynal Biotech, Oslo, Norway) decorated cells were used for the patch-clamp study.

2.4. Electrophysiological assays

Whole-cell configuration of the patch-clamp technique was employed to record membrane currents at 37 °C with an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany). Pipette resistance ranged from 2.5 to 4 MΩ when filled with pipette solutions, as described in the following text. The series resistance was electronically compensated for at 70–85%. The extracellular solution contained (mmol/l): 140 NaCl, 0.33 NaH_2PO_4, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.5 glucose, and 5 HEPES and the pH was adjusted to 7.4 with NaOH. The internal (pipette) solution contained (mmol/l): 70 potassium aspartate, 70 KOH, 40 KCl, 10 KH_2PO_4, 1 Mg_2SO_4, 3 Na_2-ATP, 0.1 Li_2-GTP, 5 EGTA, and 5 HEPES and the pH was adjusted to 7.2 with KOH.

KCNH2/KCNE1-encoded currents were elicited by depolarizing pulses from a holding potential of −80 mV to test potentials between −60 and +50 mV (with a 10-mV step increment), and then repolarized to −60 mV to measure tail currents. Current densities (pA/pF) were calculated for each cell studied by normalizing peak tail current amplitude to cell capacitance (Cm). The Cm was calculated by fitting a single exponential function to the decay phase of the transient capacitive current in response to ±5 mV voltage steps (20 ms) from a holding potential of −50 mV. The liquid junction potential between the test solution and the pipette solution was measured as approximately −10 mV and was corrected. Data were collected and analyzed using Patch master and Igor Pro (WaveMetrics, Lake Oswego, Oregon, USA).

2.5. Data analyses

The voltage-dependence of current activation was determined by fitting the normalized tail current (I_{tail}) vs. test potential (Vt) to

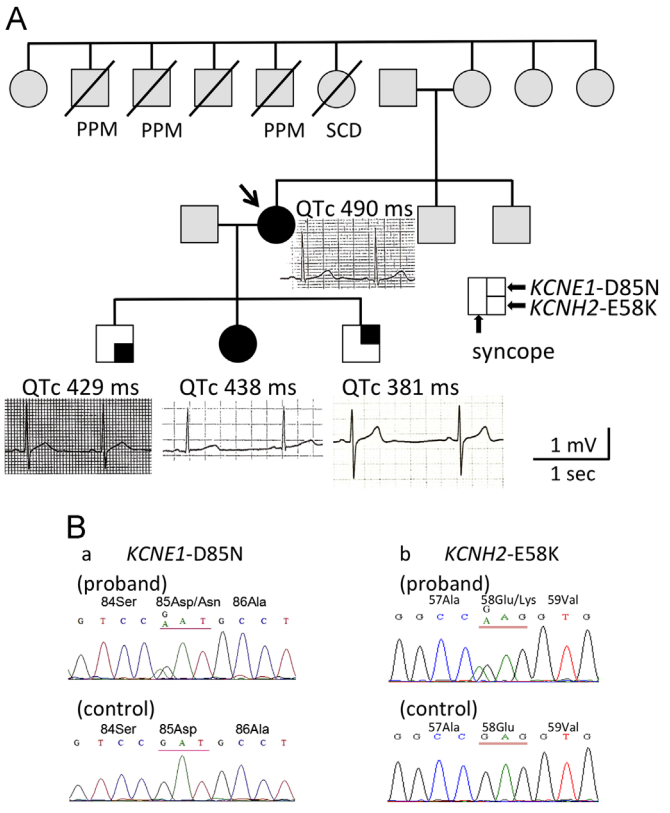

Fig. 1. Clinical Characteristics. A: Pedigree structures as well as phenotypic and genotypic information and electrocardiogram (V5) data for family members of the proband. Males and females are represented as squares and circles, respectively. Genotypes are shown on the right side of the symbols, and the presence of variants is indicated as shown in the inset. Phenotypes are shown in the left half of symbols. Filled symbols indicate symptomatic cases. Individuals with uncertain genotype and phenotype are indicated by a gray color. Deceased family members are indicated by symbols with slashes. PPM, post pacemaker implantation; SCD, sudden cardiac death. B: (a) The DNA sequence of D85N *KCNE1*; part of the nucleotide sequence of the *KCNE1* showing a G to A transition at codon 253 leading to an amino acid substitution of aspartic acid for asparagine at position 85. (b) The DNA sequence of E58K *KCNH2*; part of the nucleotide sequence of the *KCNH2* showing a G to A transition at codon 172 leading to an amino acid substitution of glutamine for lysine at position 58.