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

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$_2$PO$_4$, 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$_2$PO$_4$, 1 Mg$_2$SO$_4$, 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
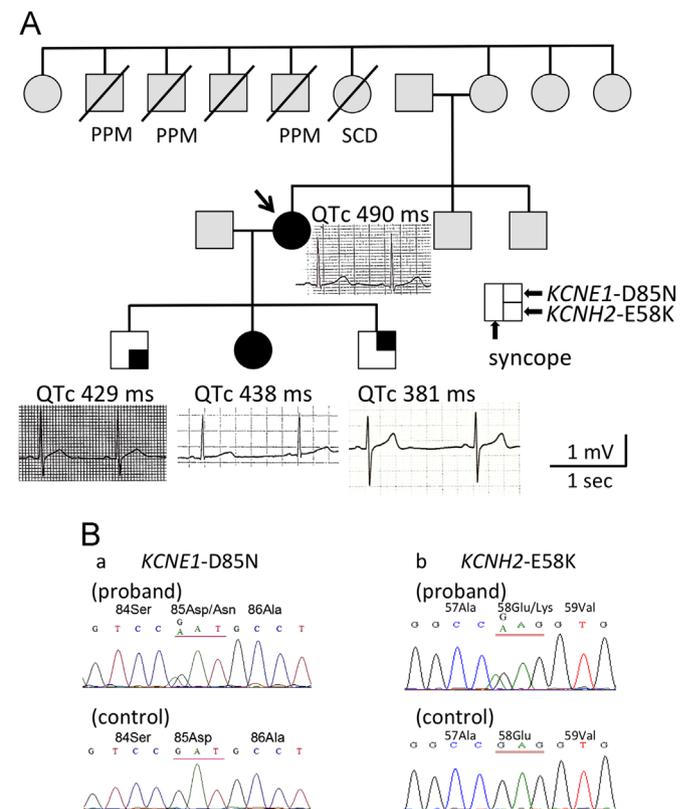
Fig. 1. Clinical Characteristics. A: Pedigree structures as well as phenotypic and genotypic information and electrocardiogram (V5) data for family members of the proband. Males and females are represented as squares and circles, respectively. Genotypes are shown on the right side of the symbols, and 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.

Boltzmann's function:

$$I_{tail} = 1/(1 + \exp[(V_{0.5} - Vt)/k]),$$

where $V_{0.5}$ is the voltage at which the current is half-activated and k is the slope factor. Time constants for deactivation (τ_{fast} and τ_{slow}) were obtained by fitting a two-exponential function to the time course of the deactivating tail currents. All data were expressed as the mean ± standard error. Statistical comparisons were made using analysis of variance, followed by a t test, and the differences were considered significant at a value of $P < 0.05$.

3. Results

3.1. Clinical features

Among our 355 probands with mutations in KCNQ1, KCNH2, or SCN5A, 206 probands (58.0%) suffered cardiac events such as ventricular arrhythmia or syncope. The average QTc interval of 355 probands was 492.4 ± 55.7 ms. Fourteen probands (3.9%) carried a heterologous KCNE1-D85N polymorphism. Coexisting mutations were identified in either KCNQ1 or KCNH2 carriers. Ten mutations were missense, and the remaining 4 were complex deletion/insertion mutations in KCNH2. The average age of these subjects was 28 ± 18 years and females were the dominant gender ($n = 10$, 71.4%). Eleven compound probands carrying KCNE1-D85N were symptomatic (78.5%), while 195 probands without KCNE1-D85N were symptomatic (57.1%, $P = 0.17$). The average QTc interval of the probands with KCNE1-D85N was a little longer (503.6 ± 92.7 ms) than that of probands without KCNE1-D85N (491.8 ± 53.2 ms, $P = 0.64$). In 7 of 14 probands, we failed to conduct genetic tests in their family members. In the remaining 7 families, we found a family in which both genetic variants were found in multiple family members (Fig. 1A). The proband (indicated by arrow in Fig. 1A) was a 51-year-old woman who was admitted to the hospital because of palpitations and repeated syncope. She experienced her first syncope at the age of 45. The standard 12-lead electrocardiogram (ECG) showed a prolonged QT interval (QT/QTc, 478/490 ms; HR, 63 bpm), notched T waves in leads II, III, and aV$_F$, and premature ventricular contraction. Blood and serological tests showed normal results. The echocardiogram, myocardial perfusion scintigraphy, and coronary angiography with/without acetylcholine test were all normal. Although ventricular fibrillation was not inducible on electrophysiological study, TdP with syncope was detected on the ECG monitor while she was talking to her doctor. Because she showed a marked sinus bradycardia (~ 40 bpm) during the day, a pacemaker was implanted and β-blocker therapy was started. Three uncles on her maternal side underwent pacemaker implantation and an aunt died suddenly before she reached 40-years-old (Fig. 1A).

The proband had 3 children (Fig. 1A), and her 2 sons were free of symptoms with normal QTc intervals (QTc, 429 ms and 381 ms, respectively). In contrast, her daughter experienced syncope several times since she was 13 years old. When the daughter was 22 years old, head-up tilt, exercise stress, and isoproterenol challenge tests were performed to examine the cause of syncope and she was suspected to have neurally mediated syncope. However, she repeated syncope while micturition at the age of 27 and she consequently underwent Holter monitoring. Because the ECG monitor demonstrated QT prolongation, she underwent an epinephrine challenge test. Intravenous administration of epinephrine (0.1 μg kg^{-1} plus 0.1 μg kg^{-1} min^{-1}) prolonged the QT interval (QTc, 438 to 658 ms) and she was diagnosed with LQTS.

3.2. Genetic analysis

DNA sequencing of the proband confirmed a G to A transition leading to amino acid substitution of aspartic acid for asparagine at position 85 (D85N) located within the C-terminal region of KCNE1 (Fig. 1B-a) and a G to A transition leading to amino acid substitution of glutamic acid for lysine at position 58 (E58K) in the N-terminus of KCNH2 (Fig. 1B-b). We identified 2 heterozygous variants, KCNH2-E58K and KCNE1-D85N, in the proband and her daughter. The proband's elder son had KCNH2-E58K and the younger son had KCNE1-D85N, respectively. In this family, therefore, a genetic double hit appeared to largely modify the clinical phenotypes (Fig. 1A).

3.3. Biophysical assays

To examine the phenotype–genotype correlation, we first examined how the KCNH2-E58K mutation affected I_{Kr} currents when reconstituted in CHO cells. Fig. 2 depicts 3 sets of typical current traces recorded from cells transfected with KCNH2-WT (A, 1 μg), KCNH2-WT/E58K (B, 0.5 μg each), and KCNH2-E58K (C, 1 μg). Cells transfected with KCNH2-WT displayed inwardly
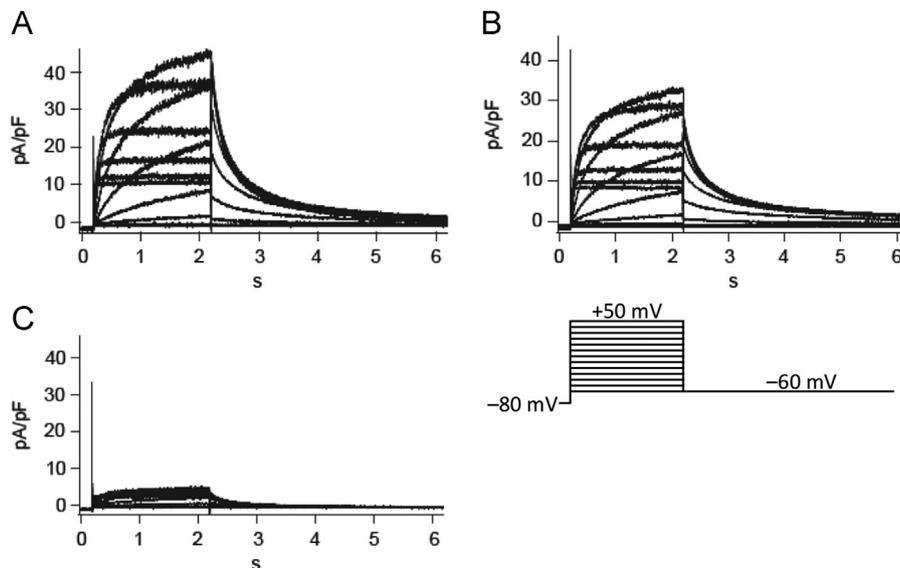
Fig. 2. Functional expression analysis of KCNH2 in Chinese hamster ovary cells. Representative current traces of KCNH2 co-expression with the WT and/or E58K. (A) KCNH2-WT (1 μg). (B) KCNH2-WT/E58K (0.5 μg of each). (C) KCNH2-E58K (1 μg).

Table 1
$V_{0.5}$, slope factor k and τ deactivation at $+20$ mV.

	N	$V_{0.5}$	k	τ_{fast}	τ_{slow}
KCNH2-WT	32	-18.515 ± 0.961	9.370 ± 0.437	0.184 ± 0.008	1.098 ± 0.047
KCNH2-WT/E58K	34	-18.590 ± 1.200	$7.993 \pm 0.309^*$	0.174 ± 0.010	1.050 ± 0.064
KCNH2-E58K	12	$-35.847 \pm 2.060^*$	$6.766 \pm 0.506^*$	$0.118 \pm 0.024^*$	$0.834 \pm 0.141^*$
KCNH2-WT$+$*KCNE1*-WT	23	-18.326 ± 0.775	7.373 ± 0.289	0.183 ± 0.016	1.077 ± 0.102
KCNH2-WT$+$*KCNE1*-D85N	20	$-22.069 \pm 1.560^{**}$	7.037 ± 0.389	0.193 ± 0.013	1.258 ± 0.090
KCNH2-WT/E58K$+$*KCNE1*-D85N	15	$-24.467 \pm 2.122^{***}$	7.525 ± 0.947	0.212 ± 0.033	1.092 ± 0.149

* $P < 0.05$ vs. *KCNH2*-WT.
** $P < 0.05$ vs. *KCNH2*-WT$+$*KCNE1*-WT.
*** $P < 0.005$ vs. *KCNH2*-WT$+$*KCNE1*-WT.

rectifying outward currents in response to depolarization pulses and slowly deactivating tail currents (Fig. 2A). Cells transfected with *KCNH2*-E58K alone produced very small outward currents (Fig. 2C). Compared to *KCNH2*-WT, *KCNH2*-WT/E58K had reduced peak tail currents by 25–28% at test potentials between 0 and $+50$ mV, although there was no statistical significance. Moreover, fitting of normalized data to Boltzmann's equation yielded a $V_{0.5}$ of -18.5 ± 1.0 mV for *KCNH2*-WT and of -18.6 ± 1.2 mV for *KCNH2*-WT/E58K that were not significantly different. Deactivation of tail currents could be fitted by 2 exponentials, yielding fast and slow time constants (τ_{fast} and τ_{slow}). The deactivation time constants were not significantly different between the 2 current–voltage relationships (Table 1). Taken together, the KCNH2 mutation showed no apparent dominant negative suppression effects.

We then examined how *KCNE1* and its D85N variant influence the I_{Kr} currents. Fig. 3A-a and b depict 2 sets of current traces recorded from CHO cells transfected with *KCNH2*-WT plus *KCNE1*-WT or *KCNE1*-D85N (1 μg each). Lower panel c shows current traces recorded from a cell transfected with *KCNH2*-WT and *KCNH2*-E58K (0.5 μg each, total 1 μg) and *KCNE1*-D85N (1 μg), which mimics the pathological condition of the index patients.

Peak tail current densities measured at -60 mV were calculated in the respective cells and are plotted as a function of test potential in Fig. 3B. Compared to *KCNE1*-WT, *KCNE1*-D85N reduced the peak tail currents by 31–36% at test potentials between 0 and $+50$ mV ($P < 0.005$ vs. *KCNH2*-WT plus *KCNE1*-WT). *KCNH2*-WT/E58K plus *KCNE1*-D85N reduced peak tail currents by 60–65% at test potentials between 0 and $+50$ mV ($P < 0.0001$ vs. *KCNH2*-WT plus *KCNE1*-WT). Fitting of normalized data to Boltzmann's equation yielded a $V_{0.5}$ of -18.3 ± 0.8 mV for *KCNH2*-WT/*KCNE1*-WT, of -22.1 ± 1.6 mV for *KCNH2*-WT/*KCNE1*-D85N ($P < 0.05$), and of -24.5 ± 2.1 mV for *KCNH2*-WT/E58K plus *KCNE1*-D85N ($P < 0.005$). These data suggest that the presence of both *KCNH2*-E58K and *KCNE1*-D85N caused a significantly negative shift of I_{Kr} activation kinetics (Fig. 3C and Table 1). Fast and slow deactivation time constants (τ_{fast} and τ_{slow}) were not significantly different between the 2 types of I_{Kr} (Table 1).

4. Discussion

The present study provides clinical, molecular, and in vitro electro-physiological evidence that a rare SNP (*KCNE1*-D85N) can act as a genetic modifier in LQTS. In general, SNPs are thought to be non-pathological, but some have been reported to modify the clinical features of the disease. For example, the *KCNH2*-K897T polymorphism [8] has been shown to aggravate LQTS phenotypes directly by reducing cardiac K^+ channel function in association with the *KCNH2* mutation, A1116V. The prevalence of the *KCNH2*-K897T polymorphism is estimated to be up to 33% in Caucasians [10,12,13].

A rare genetic variant of LQTS, *KCNE1*-D85N, was originally reported by Tesson et al. [14], and we have previously demonstrated that the prevalence of the SNP is 0.8% in healthy Japanese individuals

and 3.9% in clinically diagnosed LQTS probands [9]. In a heterologous expression system with *Xenopus* oocytes, *KCNE1*-D85N has been shown to reduce I_{Ks} by approximately 50% [5]. In our previous experiments [9] using CHO cells, D85N also significantly reduced I_{Ks} by 28% ($P < 0.05$ vs. WT), although this was a smaller reduction than that shown in *Xenopus* oocytes [5]. In contrast, when *KCNH2*-WT was co-expressed with the variant, it was found to decrease I_{Kr} significantly by 31–36% ($P < 0.005$ vs. WT) [9]. Regarding the current reduction, the interaction between KCNE1 and KCNH2 was, therefore, stronger than that between KCNE1 and KCNQ1. Since in the range of a normal heart rate I_{Kr} plays a more essential role for ventricular repolarization than I_{Ks}, carriers of loss-of-function *KCNH2* mutations generally display longer QT prolongation and bradycardia than those of *KCNQ1* mutations.

The present study attests that 3.9% of the LQTS subjects genotyped had the *KCNE1*-D85N variant in addition to a LQT-related mutation. The average QT interval of *KCNE1*-D85N carriers was longer than that of non-carriers. The incidence of symptoms in patients with *KCNE1*-D85N was higher than that of the patients without *KCNE1*-D85N, although the differences were not significant. Here, we clinically evaluated the family members as a group with a relatively homogeneous genetic background and/or the same mutation, *KCNH2*-E58K, to show that the *KCNE1*-D85N polymorphism could act as a modifier. In fact, the proband and her daughter carried both *KCNH2*-E58K and *KCNE1*-D85N and had a longer QT interval than the proband's son, who carried *KCNH2*-E58K only. In a biophysical assay, *KCNH2*-WT/E58K induced a small decrease in current densities, compared to *KCNH2*-WT, suggesting no dominant negative suppression, but a small mutant effect (Fig. 2). Co-expression of *KCNH2*-WT with/without *KCNH2*-E58K and *KCNE1*-D85N showed a significantly negative shift of the activation curve compared to *KCNH2*-WT with *KCNE1*-WT (-6 mV and -4 mV, respectively); in other words, a gain of function. However, there was a massive decrease in current densities by *KCNE1*-D85N, about 60–65% ($P < 0.0001$ vs. *KCNH2*-E58K and *KCNE1*-WT; Fig. 3B). Therefore, the latter change led to a loss-of-function effect by *KCNE1*-D85N. The proband and her daughter carried both these 2 genetic variants and both of them experienced TdP resulting in repeated syncope. From these clinical features, the D85N variant was suggested to aggravate the clinical phenotypes by largely reducing I_{Kr}. Thus, loss-of-function effects caused by the combination of the 2 genetic variants may explain the significant prolongation of the QTc intervals and the severe symptoms in the family.

More recently, Yoshikane and his colleagues [15] reported a family in which 2 genetic variants were harbored in the presence or absence of the *KCNE1*-D85N polymorphism. They compared the symptoms among the family members who carried one or more of the genetic variants *KCNH2*-N45D, *SCN5A*-A1428S, or *KCNE1*-D85N. They demonstrated that only the proband carrying all 3 variants (triple hit) experienced ventricular fibrillation, and his ECG showed marked bradycardia. His brother and mother carried either *KCNH2*-N45D or *SCN5A*-A1428S, in addition to *KCNE1*-D85N (double hit). His father carried only *KCNH2*-N45D. All family members except for the proband
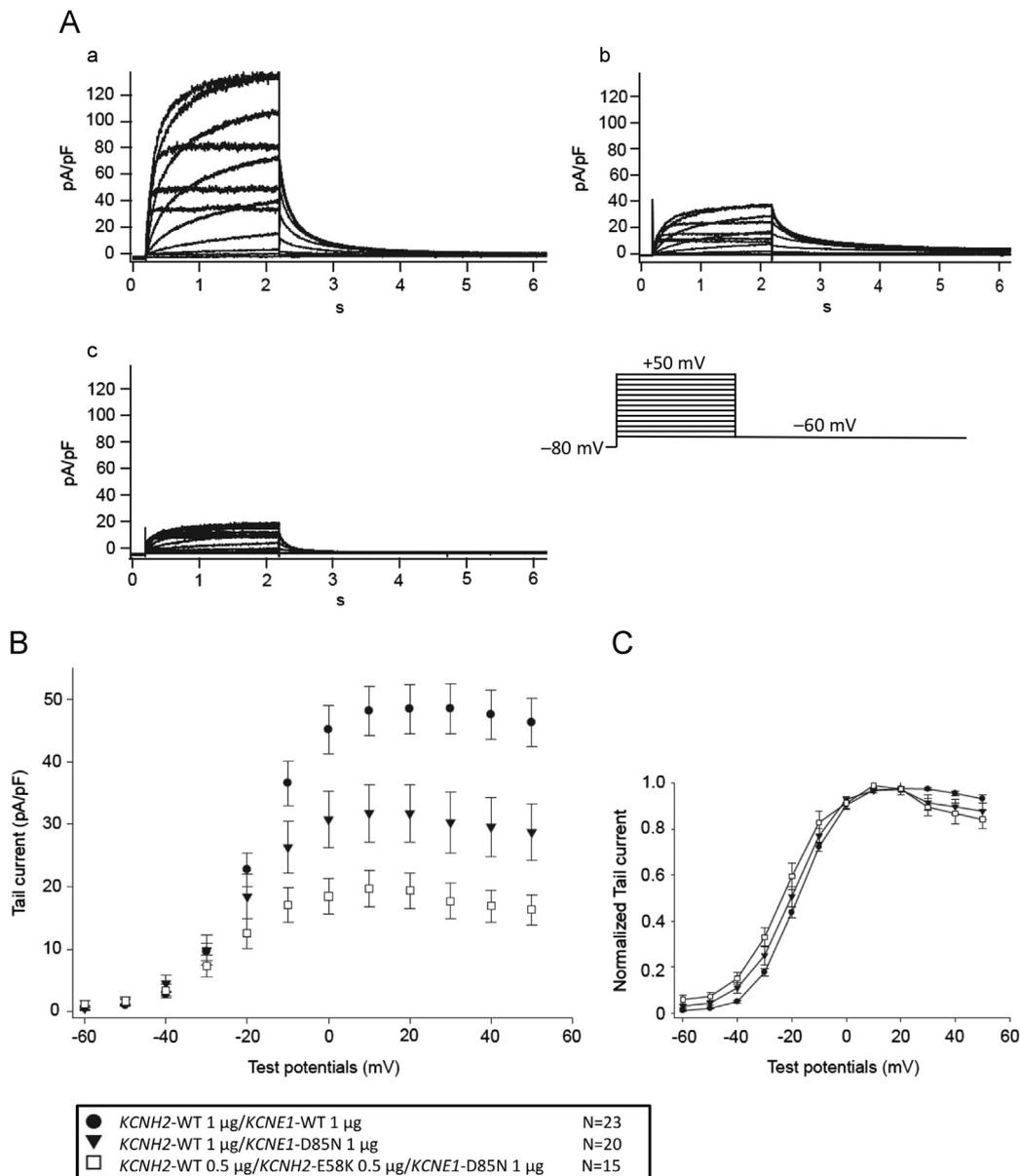
Fig. 3. Functional expression analysis of *KCNH2* with *KCNE1* in Chinese hamster ovary cells. A: Representative current traces of *KCNH2* WT and/or E58K co-expression with *KCNE1*-WT or *KCNE1*-D85N. (a) *KCNH2*-WT (1 μg) plus *KCNE1*-WT (1 μg). (b) *KCNH2*-WT (1 μg) plus *KCNE1*-D85N (1 μg) ($P < 0.005$ vs. *KCNH2*-WT (1 μg) plus *KCNE1*-WT (1 μg)). (c) *KCNH2*-WT/E58K (0.5 μg of each, total 1 μg) plus *KCNE1*-D85N (1 μg) ($P < 0.0001$ vs. *KCNH2*-WT (1 μg) plus *KCNE1*-WT (1 μg)). (B and C) Functional consequences of *KCNH2* WT and/or E58K with *KCNE1*-WT or D85N ([B] activation curve; [C] normalized activation curve). Solid circles indicate data from 23 cells that were transfected with *KCNH2*-WT (1 μg) plus *KCNE1*-WT (1 μg). Solid triangles indicate data from 20 cells that were transfected with *KCNH2*-WT (1 μg) plus *KCNE1*-D85N (1 μg). Open squares indicate data from 15 cells that were transfected with *KCNH2*-WT/E58K (0.5 μg each, total 1 μg) and *KCNE1*-D85N (1 μg).

remained asymptomatic. When compared, the phenotype of the proband's brother and father (both carry *KCNH2*-N45D, but D85N is present only in the brother), the QTc intervals were longer in the brother (500 vs. 430 ms) and a Holter ECG revealed the presence of bradycardia in the brother. Thus, *KCNE1*-D85N appeared to modify the disease phenotypes, providing another example of D85N as a genetic modifier of LQTS.

In conclusion, a rare *KCNE1*-D85N polymorphism may modify the LQTS phenotype in combination with other pathogenic LQTS-related gene mutations.

Disclosures

Conflict of interest

There are no conflicts of interest.

Acknowledgments

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