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

Kanae Hasegawa, MD¹, Seiko Ohno, MD, PhD², Hideki Itoh, MD, PhD³, Takeru Makiyama, MD, PhD³, Takeshi Aiba, MD, PhD³, Yasutaka Nakano, MD, PhD³, Wataru Shimizu, MD, PhD⁴, Hiroshi Matsuura, MD, PhD⁵, Naomasa Makita, MD, PhD⁵, Minoru Horie, MD, PhD⁶,⁎

¹ Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Seta Tiukinowa-cho, Otsu 520-2192, Japan
² Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
³ Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan
⁴ Department of Cardiovascular Medicine, Nippon Medical School, Tokyo, Japan
⁵ Department of Physiology, Shiga University of Medical Science, Otsu, Japan
⁶ Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

A R T I C L E   I N F O

Article history:
Received 14 April 2013
Received in revised form 19 July 2013
Accepted 26 August 2013
Available online 26 October 2013

Keywords:
Long QT syndrome
Single nucleotide polymorphism
Modifier

A B S T R A C T

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 Ikr currents compared to co-expression with wild-type KCNE1.

Conclusions: The KCNE1-D85N polymorphism modified the clinical features of LQTS patients.

© 2013 Japanese Heart Rhythm Society. Published by Elsevier B.V. All rights reserved.

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 in Japanese individuals is 0.8% among apparently healthy individuals, but that it is significantly higher among clinically diagnosed LQTS patients (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/KCNEL channels (Iκ) and KCNH2/KCNE1 channels (Ikr) 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
Abnormal conformers were amplified by denaturing high-performance liquid chromatography using a single strand conformation polymorphism or addition to the 3 genes listed above, genetic screening for a protocol approved by our institutional ethics committees. In obtaining written informed consent in accordance with the study, their family members. Genetic analysis was performed after mutation (E58K) was constructed by overlap-extension PCR. Nucleotide sequence of the KCNH2 vector (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 Quick 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.

2.2. Mutagenesis

Complementary deoxyribonucleic acid (cDNA) for human KCNNE1 (GenBank M26685) was kindly provided by Dr. J. Barhanin (Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, Valbonne, France) and was subcloned into a pRc/CMV plasmid. 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 Quick 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 NaH2PO4, 5.4 KCl, 1.8 CaCl2, 0.5 MgCl2, 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 KH2PO4, 1 MgSO4, 3 Na2-ATP, 0.1 Li2-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 normalized) vs. test potential (Vt) to...
Boltzmann’s function:

\[ I_{\text{tail}} = \frac{1}{1 + \exp((V_{0.5} - V_t)/k)}. \]

where \( V_{0.5} \) is the voltage at which the current is half-activated and \( k \) is the slope factor. Time constants for deactivation (\( \tau_{\text{fast}} \) and \( \tau_{\text{slow}} \)) 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 \( \text{KCNQ1}, \text{KCNH2}, \) or \( \text{SCN5A} \), 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 \( \text{KCNQ1}-\text{D85N} \) polymorphism. Coexisting mutations were identified in either \( \text{KCNQ1} \) or \( \text{KCNH2} \) carriers. Ten mutations were missense, and the remaining 4 were complex deletion/insertion mutations in \( \text{KCNH2} \). The average age of these subjects was 28 ± 18 years and females were the dominant gender (\( n = 10 \), 71.4%). Eleven compound probands carrying \( \text{KCNQ1}-\text{D85N} \) were symptomatic (78.5%), while 195 probands without \( \text{KCNQ1}-\text{D85N} \) were symptomatic (57.1%, \( P = 0.17 \)). The average QTc interval of the probands with \( \text{KCNQ1}-\text{D85N} \) was a little longer (503.6 ± 92.7 ms) than that of probands without \( \text{KCNQ1}-\text{D85N} \) (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_{1}, 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 \( \beta \)-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 \( \text{KCNQ1} \) (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 \( \text{KCNH2} \) (Fig. 1B-b). We identified 2 heterozygous variants, \( \text{KCNH2-E58K} \) and \( \text{KCNQ1-D85N} \), in the proband and her daughter. The proband’s elder son had \( \text{KCNH2-E58K} \) and the younger son had \( \text{KCNQ1-D85N} \), 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 \( \text{KCNH2-E58K} \) mutation affected \( I_{\text{Kr}} \) currents when reconstituted in CHO cells. Fig. 2 depicts 3 sets of typical current traces recorded from cells transfected with \( \text{KCNH2-WT} \) (A, 1 μg), \( \text{KCNH2-WT/E58K} \) (B, 0.5 μg each), and \( \text{KCNH2-E58K} \) (C, 1 μg). Cells transfected with \( \text{KCNH2-WT} \) displayed inwardly
rectifying outward currents in response to depolarization pulses and slowly deactivating tail currents (Fig. 2A). Cells transfected with KCNH2-E85K alone produced very small outward currents (Fig. 2C). Compared to KCNH2-WT, KCNH2-WT/E85K 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 V0.5 of −18.5 ± 1.0 mV for KCNH2-WT and of −18.6 ± 1.2 mV for KCNH2-WT/E85K that were not significantly different. Deactivation of tail currents could be fitted by 2 exponentials, yielding fast and slow time constants (τfast and τslow). 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 suppressive effects.

We then examined how KCNE1 and its D85N variant influence the Ik1 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-E85K (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/E85K 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 V0.5 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/E85K plus KCNE1-D85N (P < 0.005). These data suggest that the presence of both KCNH2-E85K and KCNE1-D85N caused a significantly negative shift of Ik1 activation kinetics (Fig. 3C and Table 1). Fast and slow deactivation time constants (τfast and τslow) were not significantly different between the 2 types of Ik1 (Table 1).

4. Discussion

The present study provides clinical, molecular, and in vitro electrophysiological evidence that a rare SNP (KCN1E-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 Ik by approximately 50% [5]. In our previous experiments [9] using CHO cells, D85N also significantly reduced Ik 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 Ik 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 Ik plays a more essential role for ventricular repolarization than Ik, 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 homo-heterologous background, and the same mutation, KCNH2-E85K, to show that the KCNE1-D85N polymorphism could act as a modifier. In fact, the proband and her daughter carried both KCNH2-E85K and KCNE1-D85N and had a longer QT interval than the proband’s son, who carried KCNH2-E85K only. In a biophysical assay, KCNH2-WT/KCNE1-D85N 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 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. KCNE1-W5 and KCNE1-W7; 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 Ik. 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

| Table 1 | V0.5, slope factor k and τ deactivation at +20 mV. |
|------------------------|------------------------|------------------------|------------------------|
| N | V0.5 | k | τfast | τslow |
| KCNH2-WT | 32 | −18.515 ± 0.961 | 9.370 ± 0.437 | 0.184 ± 0.008 | 1.098 ± 0.047 |
| KCNH2-WT/E85K | 34 | −18.590 ± 1.200 | 7.993 ± 0.309 | 0.174 ± 0.010 | 1.050 ± 0.064 |
| KCNH2-E85K | 12 | −35.847 ± 2.060 | 6.766 ± 0.506 | 0.118 ± 0.024 | 0.834 ± 0.141 |
| KCNH2-WT + KCNE1-WT | 23 | −18.326 ± 0.775 | 7.373 ± 0.289 | 0.183 ± 0.016 | 1.077 ± 0.012 |
| KCNH2-WT + KCNE1-D85N | 20 | −22.069 ± 1.560 | 7.037 ± 0.389 | 0.193 ± 0.013 | 1.258 ± 0.090 |
| KCNH2-WT/E85K + KCNE1-D85N | 15 | −24.467 ± 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.
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

This work was supported by Grants-in-Aid in Scientific Research from the Ministry of Education, Culture, Science, and Technology of Japan, a Health Sciences Research Grant from the Ministry of Health, Labor, and Welfare of Japan, and Translational Research Funds from the Japan Circulation Society.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

The authors are grateful to the Japanese LQT families for their willingness to participate in this study and to Dr. Y. Yoshikane and Dr. M. Yoshinaga for keen discussion. We thank Arisa Ikeda, Kazu Toyooka, and Aya Umehara for excellent technical assistance.
References
