D85N, a KCNE1 Polymorphism, Is a Disease-Causing Gene Variant in Long QT Syndrome

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Objectives
This study aims to address whether D85N, a KCNE1 polymorphism, is a gene variant that causes long QT syndrome (LQTS) phenotype.

Background
KCNE1 encodes the beta-subunit of cardiac voltage-gated K+ channels and causes LQTS, which is characterized by the prolongation of the QT interval and torsades de pointes, a lethal arrhythmia. D85N, a KCNE1 polymorphism, is known to be a functional variant associated with drug-induced LQTS.

Methods
In order to elucidate the prevalence and clinical significance of this polymorphism, we performed genetic screening in 317 LQTS probands. For comparison, we examined its presence in 496 healthy control subjects. We also conducted biophysical assays for the D85N variant in mammalian cells.

Results
The allele frequency for D85N carriers was 0.81% in healthy people. In contrast, among LQTS probands, there were 1 homozygous and 23 heterozygous carriers (allele frequency 3.9%). Seven of 23 heterozygous carriers had additional mutations in LQTS-related genes, and 3 female subjects had documented factors predisposing to the symptom. After excluding these probands, the D85N prevalence was significantly higher compared with control subjects (allele frequency 2.1%, \( p < 0.05 \)). In a heterologous expression study with Chinese hamster ovarian cells, KCNE1-D85N was found to exert significant loss-of-function effects on both KCNQ1- and KCNH2-encoded channel currents.

Conclusions
The KCNE1-D85N polymorphism was significantly more frequent in our LQTS probands. The functional variant is a disease-causing gene variant of LQTS phenotype that functions by interacting with KCNH2 and KCNQ1. Since its allele frequency was ~1% among control individuals, KCNE1-D85N may be a clinically important genetic variant.

Long QT syndrome (LQTS) is a disorder that is characterized by repolarization abnormalities in the heart, leading to torsades de pointes (TdP), syncope, and sudden death. Among the LQTS-related genes identified to date, KCNQ1 and KCNE1 are known to encode the alpha and beta subunits of voltage-gated K+ channels, which carry \( I_{Ks} \), a slowly activating component of delayed rectifier K+ current (1,2). KCNE1 is also known to regulate KCNH2 (3), which encodes the Kv11.1 protein, the alpha subunit of rapidly activating delayed rectifier K+ current (\( I_{Kr} \)) (4–6).

A KCNE1 C-terminal polymorphism, D85N, has been found in the normal population and is known to cause a G-to-A transition at codon 253 (c.253G>A), which leads to the amino acid substitution of aspartic acid for asparagine (7). This has been shown to cause an approximately 50% reduction in KCNQ1-encoded currents in a heterologous expression system using \textit{Xenopus} oocytes (8), although biophysical study data are not available for mammalian cells.
The allele frequency of the polymorphism is reported to be 0.7% in apparently healthy Asians (7). Paulussen et al. (9) demonstrated in a European population that the allele frequency of D85N was 5% in acquired LQTS patients who experienced TdP as a result of drug administration, but was 0% in the control group. Iwasa et al. (10) reported that the allele frequency was 2% in 100 Japanese cases, but their cohort contained both LQTS patients and normal individuals.

In the present study, we examined the incidence rate of KCNE1-D85N polymorphisms in 317 LQTS probands from unrelated families and 496 control healthy individuals. We identified 23 heterozygous and 1 homozygous probands (allele frequency 3.9%), described the demographics of these index patients, and examined the possibility that the D85N polymorphism is an LQTS-causing genetic variant. We also conducted detailed functional assays of the variant while it was coexpressed with the 2 alpha subunits of cardiac delayed rectifier K⁺ channels, KCNQ1 and KCNH2, by using a heterologous expression system involving Chinese hamster ovarian (CHO) cells.

Methods

Study subjects. Three hundred and seventeen consecutive LQTS probands who showed a prolongation of the QT interval were referred to our laboratory for genetic evaluation and were enrolled in our analysis. The electrocardiogram diagnostic criteria of Keating and Sanguinetti (11) included a corrected QT interval (QTc) of ≥470 ms in asymptomatic individuals and a QTc of >440 ms for male subjects and of >460 ms for female subjects that had 1 or more of the following: 1) stress-related syncope; 2) documented TdP; or 3) a family history of early sudden cardiac death.

The protocol for genetic analysis was approved by our institutional ethics committee and was performed under its guidelines. Informed consent was obtained from all individuals or their guardians before the analysis. The QT intervals were measured from electrocardiographic lead II or an available rhythm strip and were corrected for heart rate according to Bazett’s formula. As for the control cohort, we screened the frequency of the D85N polymorphism in 496 randomly selected cases, consisting of healthy volunteers and mutation-negative family members such as probands’ spouses. Their QTc were 440 ms for male subjects and of 470 ms in asymptomatic individuals and a QTc of >440 ms for male subjects and of >460 ms for female subjects that had 1 or more of the following: 1) stress-related syncope; 2) documented TdP; or 3) a family history of early sudden cardiac death.

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Genotyping. Genomic deoxyribonucleic acid (DNA) was isolated from venous blood by use of the QIAamp DNA midikit (Qiagen, Hilden, Germany). Genetic screening for KCNE1-D85N was performed by direct polymerase chain reaction. Other LQTS-related genes, including KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and KCNJ2, were assayed by denaturing high-performance liquid chromatography using a WAVE System Model 3500 (Transgenomic, Omaha, Nebraska). Abnormal conformers were amplified by polymerase chain reaction. Sequencing was performed with an ABI PRISM3100 DNA sequencer (Applied Biosystems, Wellesley, Massachusetts).

Site-directed mutagenesis. Complementary deoxyribonucleic acid (cDNA) for human KCNQ1 (GenBank AF000571) and KCNE1 (GenBank M26685) were kindly provided by Dr. J. Barhanin (Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, Valbonne, France). The cDNAs were subcloned into pIRE2-EGFP (for KCNQ1) and pIRE2-CD8 (for both wild-type and mutated KCNE1) vectors. cDNA for human KCNH2 (GenBank AF363636) was kindly donated by Dr. M. Sanguinetti (University of Utah, Salt Lake City, Utah). The cDNA 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). Nucleotide sequence analysis was performed on each variant construct before the expression study to confirm their sequences.

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) containing 10% fetal bovine serum supplemented with 1% penicillin and 1% streptomycin. Wild-type KCNQ1, KCNH2, and wild-type and/or variant KCNE1 clones were expressed transiently in CHO cells using the LipofectAMINE method according to the manufacturer’s instructions (Invitrogen, Carlsbad, California).

To identify the cells that were positive for KCNH2 expression, CHO cells were cotransfected with 1 µg of pRc-CMV/KCNH2 vector and 0.5 µg of pEGFP-N1/CMV vector. Forty-eight to 72 h after transfection, green fluorescent protein-positive cells and anti-CD8 antibody-coated bead (Dynabeads CD8, Dynal Biotech, Oslo, Norway) decorated cells were used for the patch-clamp study.

Electrophysiological assays. Whole-cell configuration of patch-clamp techniques was employed to record membrane currents at 37°C in an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany). Pipette resistance ranged from 2.5 to 4 MΩ when filled with the pipette solutions described in the following text. The series resistance was electronically compensated for at 70% to 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 Na₃-ATP, 0.1 Li₂-GTP, 5 EGTA, and 5 HEPES, and the pH was adjusted to 7.2 with KOH.

KCNQ1/KCNE1-encoded currents were measured by depolarizing pulses from a holding potential of −90 mV to test potentials between −70 and +50 mV (with a 10-mV step increment), before being repolarized to −50 mV in

Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>CHO</td>
<td>Chinese hamster ovarian</td>
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<tr>
<td>LQTS</td>
<td>long QT syndrome</td>
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<tr>
<td>QTc</td>
<td>corrected QT interval</td>
</tr>
<tr>
<td>TdP</td>
<td>torsades de points</td>
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order to monitor tail current amplitude. KCNH2/KCNE1-encoded currents were elicited by depolarizing pulses from a holding potential of −80 mV to test potentials between −60 to +50 mV (with a 10-mV step increment), before being repolarized to −60 mV in order to monitor tail current amplitude. Current densities (pA/pF) were calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm). The Cm was calculated for each cell studied, by normalizing peak tail current amplitude. Current densities (pA/pF) were calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm). The Cm was calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm).

**Data analyses.** The voltage-dependence of current activation was determined by fitting the normalized tail current amplitude to cell capacitance (Cm). The Cm was calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm). The Cm was calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm).

The transient capacitive current in response to voltage steps (20 ms) from a holding potential of 80 mV to test potentials between ±5 mV was determined by fitting the normalized tail current amplitude to cell capacitance (Cm). The Cm was calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm).

**Results**

**Clinical characteristics and genotyping.** Of the 496 control volunteers, 8 (mean QTc 420.5 ± 7.5 ms) had heterozygous D85N genotypes (allele frequency 0.81%). In contrast, 23 of the 317 LQTS probands had heterozygous D85N genotypes and 1 (Table 1) (Patient #24) had a homozygous D85N genotype (allele frequency 3.9%). Table 1 and Figure 1 summarize the demographics of the 24 index patients. Their mean age was 34.8 ± 4.4 years, and their mean QTc was 507.9 ± 9.2 ms. Among the D85N-negative cases, we identified 116 probands that were positive for other LQTS-related gene mutations (Fig. 1), and their mean QTc was significantly longer (540.6 ± 6.1 ms) than those of the 24 D85N carriers (p < 0.05).

Seven of the 23 heterozygous probands (30%) had other LQTS-related gene variants (KCNQ1 or KCNH2), and 3 female patients (13%; Patients #1, #6, and #10) had documented predisposing factors, such as electrolyte disturbances, QT prolonging drug intake, or bradycardia (Table 1). The allele frequency of the remaining 13 patients (2.1%) was significantly higher than that in the control subjects (p < 0.05). Six of these 13 patients (46%) had syncope and/or TdP while 9 of 10 patients (90%) with multiple genetic variants or triggering factors were symptomatic (Fig. 1).

The mean onset age of the 6 symptomatic heterozygous D85N carriers without compromised factors to affect QT

<table>
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<th>Table 1</th>
<th>Clinical Characteristics of the LQTS Probands Who Carried the KCNE1-D85N Variant</th>
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<tr>
<td>Patient #</td>
<td>Age (F/M)</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>9 (M)</td>
</tr>
<tr>
<td>3</td>
<td>21 (F)</td>
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<tr>
<td>4</td>
<td>42 (F)</td>
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<td>5</td>
<td>51 (F)</td>
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<tr>
<td>6</td>
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<tr>
<td>7</td>
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<tr>
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<tr>
<td>23</td>
<td>51 (M)</td>
</tr>
<tr>
<td>24</td>
<td>39 (F)</td>
</tr>
</tbody>
</table>

*Novel variant.
LQTS = long QT syndrome; QTc = corrected QT interval; TdP = torsades de pointes.
interval was 35.5 ± 10.4 years. It was significantly older than the mean onset age of the other genotyped symptomatic LQTS patients (21.0 years in our cohort of 94 genotyped LQTS) (Horie M. et al., unpublished data, September 2008). Although the clinical features of KCNE1-D85N–positive probands differed with respect to the QTc and the onset age from those of other genotyped LQTS patients, this variant appeared to be a disease-causing gene variant in congenital LQTS.

**Biophysical assays of the genetic variant. KCNE1-D85N with KCNQ1.** In order to confirm that the D85N is a disease-causing variant, we conducted functional assays using a heterologous expression system with a mammalian cell line (CHO cells). In the first line of experiments, we examined how KCNE1-D85N affected the reconstituted KCNQ1/KCNE1 currents. Figure 2 depicts representative current traces recorded from cells that coexpressed KCNQ1 and wild-type (Fig. 2A-a) or D85N (Fig. 2A-b) KCNE1 (1 μg each). Peak tail current densities measured after repolarization to −50 mV from various test pulses were calculated in individual cells and are plotted as a function of test potential in Figure 2B. Solid circles indicate the mean peak current densities from 21 cells that were transfected with KCNQ1 and wild-type KCNE1; open circles indicate the mean peak current densities from 25 cells that were transfected with KCNQ1 and D85N, and solid triangles indicate the mean peak current densities from 25 cells that were transfected with KCNQ1 alone. D85N reduced the peak tail currents of wild-type KCNQ1/KCNE1-encoded currents by 28% at 0 mV test potential (p < 0.05 vs. wild type).

In Figure 2C, peak tail current densities have been normalized using the current densities recorded after a test pulse to +50 mV and are plotted as a function of test potential. Fitting of data plots to Boltzmann’s equation yielded \( V_{0.5} \) values of −4.36 ± 1.8 mV for the wild type and 3.38 ± 1.7 mV for D85N (p < 0.05), suggesting that the KCNE1 variant produced a significantly positive shift in KCNQ1-encoded current activation kinetics (Table 2). The deactivation process of tail currents could be fitted by 2 exponentials, yielding fast and slow time constants. No significant difference with respect to the fast time constants was evident between the wild-type and D85N genotypes; however, slow deactivation was significantly accelerated by coexpression of D85N (Table 2).

**KCNE1-D85N with KCNH2.** In the next line of experiments, we examined how KCNE1 and its D85N variant influence KCNH2-encoded currents. Figures 3A-a and 3A-b depict 2 sets of current traces recorded from CHO cells that had been transfected with KCNH2 plus wild-type or D85N KCNE1 (1 μg each). Peak tail current densities at −60 mV were calculated in the respective cells and are plotted as a function of test potential in Figure 3B. Solid circles and open circles indicate the mean current densities calculated from 23

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**Figure 1** Summary of LQTS Probands

LQTS = long QT syndrome; QTc = corrected QT interval; TdP = torsades de pointes.
and 20 cells, respectively, which were transfected with 1 μg of KCNH2 and 1 μg of wild-type or D85N KCNE1.

D85N reduced the peak tail currents of wild-type KCNH2/KCNE1-encoded currents by 31% to 36% at test potentials between 0 and +50 mV (p < 0.005 vs. wild type). Fitting of normalized data to Boltzmann’s equation yielded a V_0.5 of −18.33 ± 0.8 mV for the wild-type KCNH2/KCNE1 and of −22.07 ± 1.6 mV for KCNH2/KCNE1-D85N (p < 0.05), suggesting that the KCNE1 variant causes a significantly negative shift of KCNH2/KCNE1-encoded current activation kinetics (Fig. 3C, Table 2). Deactivation of tail currents could be fitted by 2 exponentials, yielding fast and slow time constants. The fast and slow kinetics were not significantly different between the 2 types of KCNH2 channel currents (Table 2).

**Discussion**

The present study demonstrates that the allele frequency of KCNE1-D85N is significantly higher in LQTS patients than in control subjects after excluding cases with compromised factors to prolong QT interval (p < 0.05). A biophysical assay of D85N showed that the variant affected both reconstituted IKs and IKr channel function, leading to a prolongation of the QTc with D85N working as a disease-causing variant. In a heterologous expression system with *Xenopus* oocytes (8),
KCNE1-D85N has been reported to cause an approximately 50% reduction in KCNQ1-encoded currents, although data for mammalian cells is not available. In our experiments using CHO cells, D85N significantly reduced KCNQ1-encoded currents by 28% (p < 0.05 vs. wild type), although this effect was smaller than that in Xenopus oocytes.

When KCNH2 was coexpressed with the wild-type or D85N variant of KCNE1, D85N significantly reduced wild-type KCNH2/KCNE1-encoded currents by 31% to 36% (p < 0.005 vs. wild type). Regarding the interaction between KCNE1 and KCNH2, McDonald et al. (3) demonstrated that KCNE1 forms a stable complex with KCNH2 and

![Figure 3](image-url)

**Figure 3** Functional Expression of KCNH2 With KCNE1-D85N in Chinese Hamster Ovarian Cells

(A) Representative current traces of KCNH2 coexpression with the wild-type (WT) or D85N KCNE1. (a) KCNH2 (1 μg) plus WT KCNE1 (1 μg). (b) KCNH2 (1 μg) plus D85N KCNE1 (1 μg). (B and C) Functional consequences of KCNH2 coexpression with the WT or D85N of KCNE1 ([B] activation curve; [C] normalized activation curve). Solid circles indicate data from 23 cells that were transfected with KCNH2 and WT KCNE1 (1 μg of each). Open circles indicate data from 20 cells that were transfected with KCNH2 and D85N KCNE1 (1 μg of each).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>V_{0.5}</th>
<th>Slope Factor k</th>
<th>τ_{fast}</th>
<th>τ_{slow}</th>
</tr>
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<tr>
<td>KCNQ1 (WT) 1 μg</td>
<td>25</td>
<td>-20.86 ± 1.034</td>
<td>8.223 ± 0.421</td>
<td>0.070 ± 0.005</td>
<td>0.136 ± 0.019</td>
</tr>
<tr>
<td>KCNQ1 (WT) 1 μg/KCNE1 (WT) 1 μg</td>
<td>21</td>
<td>-4.364 ± 1.834*</td>
<td>12.724 ± 0.407*</td>
<td>0.145 ± 0.013*</td>
<td>0.586 ± 0.070†</td>
</tr>
<tr>
<td>KCNQ1 (WT) 1 μg/KCNE1 (D85N) 1 μg</td>
<td>25</td>
<td>0.382 ± 1.177‡</td>
<td>12.566 ± 0.429*</td>
<td>0.141 ± 0.013*</td>
<td>0.409 ± 0.050‡</td>
</tr>
<tr>
<td>KCNH2 (WT) 1 μg</td>
<td>23</td>
<td>-18.326 ± 0.775</td>
<td>7.373 ± 0.289</td>
<td>0.183 ± 0.016</td>
<td>1.077 ± 0.102</td>
</tr>
<tr>
<td>KCNH2 (WT) 1 μg/KCNE1 (D85N) 1 μg</td>
<td>20</td>
<td>-22.069 ± 1.560‡</td>
<td>7.037 ± 0.389</td>
<td>0.193 ± 0.013</td>
<td>1.258 ± 0.090</td>
</tr>
</tbody>
</table>

* p < 0.0001 versus KCNQ1 (wild type [WT]) 1 μg; † p = 0.0001 versus KCNQ1 (WT) 1 μg; ‡ p < 0.05 versus KCNQ1 (WT) 1 μg/KCNE1 (WT) 1 μg; § p < 0.05 versus KCNH2 (WT) 1 μg/KCNE1 (WT) 1 μg.
up-regulates \( I_{Kr} \)-like currents by 50% in CHO cells. Bianchi et al. (12) also showed interactions between the KCNE1-D85N mutation and both KCNQ1 and KCNH2 in HEK cells. In atrial tumor myocytes that expressed \( I_{Kr} \) alone, Yang et al. (13) demonstrated that antisense oligonucleotides against minK cDNA (KCN1E) significantly reduced the \( I_{Kr} \) by \(-62\%\). More recently, Ohno et al. (14) identified a missense KCNE1 mutation, A8V, in a sporadic case of LQTS and reported that the mutation significantly reduced the magnitude of KCNH2- but not KCNQ1-encoded currents.

Collectively, it is of clinical importance that the KCNE1-D85N variant modifies not only KCNQ1- but also KCNH2-coded channel currents. Furthermore, its inhibitory action on KCNH2 was even stronger than that on KCNQ1. The KCNE1-D85N polymorphism may therefore cause phenotypes similar to those observed in type 2 LQTS such as bradycardia (15,16). The deactivation process of \( I_{Kr} \) plays a significant role in maintaining the appropriate rate of pacemakers (17) and, therefore, a decreased \( I_{Kr} \) will lead to sinus bradycardia. In the present study, 3 D85N carriers (13%) had sinus bradycardia (Table 1).

The mean onset age of 6 symptomatic heterozygous D85N carriers (Table 1) was 35.5 years, and this was significantly older than the mean age of other genotyped carriers (13%) had sinus bradycardia (Table 1).

The mean ages of first event were 11 \pm 8 years and 13 \pm 9 years. Nagaoka et al. (19) also demonstrated that in 118 Japanese LQT2 patients with pore mutations or nonpore mutations, the mean ages of first event were 16 \pm 10 years and 20 \pm 13 years. In addition, the mean QTc of 13 D85N carriers was prolonged (498.5 \pm 13.6 ms) but significantly shorter than that in 116 probands with other LQTS-related gene mutations (541 ms) (Fig. 1). These different phenotypes appear to reflect the fact that D85N causes a milder channel dysfunction than other LQTS mutations, and reveals a “forme fruste” phenotype (20).

The allele frequency of the KCNE1-D85N polymorphism was 0.81% among apparently healthy control individuals. We found only 1 report concerning D85N frequency (0.7%) (7) in control subjects, which showed equivalent results to our study. Based on 2008 healthy French individuals, Gouas et al. (21) demonstrated that the allele frequency of D85N was significantly higher in the 200 subjects with the longest QTc than in those with the shortest QTc (3.1% vs. 0.75%), suggesting that this single nucleotide polymorphism may influence the QTc length in healthy individuals.

LQTS can remain latent or subclinical because of “repolarization reserve” (22), and can become unmasked upon the intake of QT-prolonging drugs. Heterozygous D85N carriers in the control group may be at a potentially higher risk of long QT-related arrhythmias. Assuming that genetic surveys are feasible before drug therapy, D85N carriers may be recommended to avoid the secondary factors that predispose them to further QT prolongation such as QT prolonging drugs (23) and electrolyte disturbances (23–25). It is also clinically useful to search for other variants of long QT-related genes (8,26,27).

**Study limitations.** In the present study, we screened the mutations that are responsible for LQT1, 2, 3, 5, 6, and 7. Therefore, the comorbidity of other types of LQTS was not completely excluded, although their frequency was quite low. In general, single nucleotide polymorphisms are thought to be nonpathological although some may modify the clinical features of a disease. For example, the KCNH2-K897T polymorphism is a typical genetic modifier that aggravates LQTS phenotypes directly by reducing channel function in association with the KCNH2 mutation A1116V (28). Such a role for KCNE1-D85N was not addressed in this study and warrants further study.

**Conclusions**

KCNE1-D85N was a highly frequent variant in our LQTS probands and was found to cause loss-of-function effects on both \( I_{Kr} \) and \( I_{Kr} \) and work as a disease-causing variant. Since its allele frequency was 0.81% among control healthy individuals, KCNE1-D85N may be a clinically important genetic variant.

**Acknowledgment**

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**REFERENCES**


Key Words: long QT syndrome • single nucleotide polymorphism • disease-causing variant.