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LQT5 masquerading as LQT2: a dominant negative effect of *KCNE1*-D85N rare polymorphism on *KCNH2* current

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Aims *KCNE1* encodes an auxiliary subunit of cardiac potassium channels. Loss-of-function variations in this gene have been associated with the LQT5 form of the long QT syndrome (LQTS), secondary to reduction of I_{Ks} current. We present a case in which a D85N rare polymorphism in *KCNE1* is associated with an LQT2 phenotype.

Methods and results An 11-year old competitive athlete presented with mild bradycardia and a QTc interval of 470 ms. An LQT2 phenotype, consisting of low-voltage bifid T waves, was evident in the right precordial electrocardiogram leads. During the tachycardia phase following adenosine, QTc increased to 620 ms. Genetic analysis revealed a rare heterozygous polymorphism in *KCNE1* predicting the substitution of asparagine for aspartic acid at position 85 of minK (D85N). Patch clamp experiments showed that *KCNE1*-D85N, when co-expressed with *KCNH2* in TSA201 cells, significantly reduced I_{Kr}. Homozygous co-expression of the mutant with *KCNH2* reduced I_{Kr} tail current by 85%, whereas heterozygous co-expression reduced the current by 52%, demonstrating for the first time a dominant-negative effect of D85N to reduce I_{Kr}. Co-expression of the mutant with *KCNQ1*, either homozygously or heterozygously, produced no change in I_{Ks}.

Conclusions Our results suggest that a rare polymorphism *KCNE1*-D85N underlies the development of an LQT2 phenotype in this young athlete by interacting with *KCNH2* to cause a dominant-negative effect to reduce I_{Kr}. Our data provide further evidence in support of the promiscuity of potassium channel β subunits in modulating the function of multiple potassium channels leading to a diversity of clinical phenotypes.

Keywords Long QT syndrome • Electrophysiology • Arrhythmia • Athlete

Introduction

Long QT syndrome (LQTS) is a congenital ion channelopathy that predisposes affected individuals to sudden cardiac death (SCD).[1,2] Although some affected LQT patients may have symptoms ranging from syncope to severe arrhythmias such as torsade de pointes (TdP), in most cases patients are asymptomatic.[3–5] In some the QT interval is even within normal range.[6] To date, mutations in 13 genes have been identified. These genes encode proteins that function as cardiac channel α subunits[7–9] (*KCNQ1, KCNH2, SCN5A, KCNJ2, CACNA1C, KCNJ5*), cardiac channel auxiliary or β subunits,[10–12] (*KCNE1, KCNE2, SCN4B, SNTA1*), and structural membrane proteins[13,14] (*ANK2, Caveolin-3*).

Acquired LQT presents similarly as congenital LQT;[15] however, these patients typically present with the signs and symptoms of LQTS secondary to pathophysiologic conditions (e.g. bradycardia,

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hypertrophic, or dilated cardiomyopathy) or exposure to drugs.[5,16] The most common cause is drug administration.[17] Many cases of drug-induced LQT have been shown to be associated with a genetic variation that creates a subclinical form of the syndrome, which becomes manifest following exposure drugs with QT prolonging actions.[5,16] D85N, a *KCNE1* rare single-nucleotide polymorphism (SNP), has been shown in several studies to be associated with acquired LQT.[17–19] However, a recent study[20] demonstrated that D85N can also be considered as an LQT disease-causing gene. We present the case of a young female competitive athlete with a *KCNE1*-D85N SNP presenting with mild QTc prolongation and LQT2 T wave morphology. Functional expression studies demonstrate that this genetic variation exerts a dominant-negative effect to reduce I_{Kr}, thus accounting for the clinical phenotype.

Methods

Clinical evaluation

An 11-year-old female world-class swimmer was referred for evaluation of weakness. During January/February 2007 she developed a 'viral' infection with extreme fatigue from which she recovered uneventfully and resumed swimming (about 3 miles daily). On first evaluation, her electrocardiogram (ECG) showed: sinus rhythm of 58 beats per minute, normal axis, normal PR interval, and QTc of 470 ms with bifid T waves in leads V2 and V3 (*Figure 1*). At the time the ECG was recorded, she was not taking any medication and had a normal body temperature. The QT interval was measured and adjusted to heart rate (QTc), according to Bazett's formula.[21] The end of the T wave was defined as the intersection with the isoelectric line of a tangent drawn to the descending portion of the T wave. The QT interval was measured in lead V3 whenever possible, because it often had the largest T wave amplitude.[22] Her evaluation included: two-dimensional echocardiography, and adenosine and epinephrine tests. Using the methods of Viskin *et al.*,[23] adenosine was injected intravenously as a single bolus until a high-degree atrioventricular (AV) block was achieved. Epinephrine was continuously infused intravenously in escalating doses, from 0.05, 0.1, to 0.2 μg/kg/min for 20, 5, and 5 min, respectively.

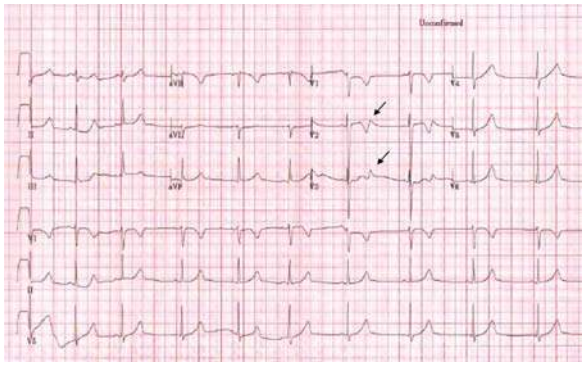

Figure 1 Baseline electrocardiogram demonstrating a QTc of 470. Arrows depict bifid T waves in leads V2 and V3.

Genetic analysis

After informed consent was obtained, blood was collected from the proband. Genomic DNA was extracted from peripheral blood leukocytes with a commercial kit (Puregene, Gentra Systems Inc., Minneapolis, MN, USA). The genomic DNA was amplified on by polymerase chain reaction (PCR) on GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). All exons and intron borders of the following LQT-susceptibility genes were amplified and analysed by direct sequencing: *KCNQ1, KCNH2, KCNE1, KCNE2,* and *SCN5A*. Polymerase chain reaction products were purified with a commercial reagent (ExoSAP- IT, USB, Cleveland, OH, USA) and directly sequenced from both directions using ABI PRISM 3100 Automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). Electropherograms were visually examined for heterozygous peaks and compared with reference sequences for homozygous variations (GenBank accession number NM_000219) using the CodonCode Aligner Ver. 2.0.4 (CodonCode Corporation, Dedham, MA, USA).

Site-directed mutagenesis and transfection of the TSA201 cell line

Site-directed mutagenesis was performed with QuikChange (Stratagene, La Jolla, CA, USA) on full-length human wild type (WT) and D85N in *KCNE1* cDNA was cloned in the pcDNA3.1 vector. The *KCNE1*-D85N plasmid was sequenced to ensure the presence of the mutation without spurious substitutions. *KCNH2*-WT and *KCNQ1*-WT were also cloned in the pcDNA3.1 vector.

I_{Kr} channels were expressed in a modified human embryonic kidney cell line, TSA201, as previously described.[24] Briefly, transient transfection using fugene6 (Roche Diagnostics, Indianapolis, IN, USA) was carried out with *KCNH2*-WT and *KCNQ1*-WT and *KCNE1* (WT or D85N) with a molar ratio of 1:1. CD_8 cDNA was co-transfected as a reporter gene to visually identify transfected cells using Dynabeads (M-450 CD_8 Dynal, Invitrogen Corp., Carlsbad, CA, USA). The cells were grown in GIBCO Dulbecco's Modified Eagle Medium (DMEM) medium (No. 10566, Gibco, Invitrogen Corp., Carlsbad, CA, USA) with FBS (No. 16000) and antibiotics (No. 15140) on polylysine-coated 35 mm culture dishes (Cell+, Sarstedt, Newton, NC, USA). Cells were placed in a 5% CO_2 incubator at 37°C for 24 to 48 h prior to patch clamp study.

Electrophysiology studies

Membrane currents were measured using whole-cell patch-clamp techniques in transfected TSA201 cells. All recordings were obtained at room temperature (22°C) using an Axopatch 200B amplifier equipped with a CV-201A head stage (Axon Instruments, Union City, CA, USA). Macroscopic whole-cell K^+ current was recorded with cells bathed in solution containing (in mmol/L): 130 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 2.8 Na acetate, 10 HEPES, and 10 glucose (pH 7.3 with NaOH). Patch pipettes were pulled

from borosilicate glass (Model PP-89, Narashige, Toyko, Japan) to have resistances between 1 and 2.5 MΩ when filled with a solution containing(in mmol/L): 10 KCL, 125 K-Aspartate, 1.0 $MgCl_2$, 11 EGTA, 5 MgATP, and 10 HEPES (pH 7.3 with KOH). Currents were filtered with a four-pole Bessel filter at 1 kHz and were digitized at 5 kHz.

Data and statistical analysis

All data acquisition and analysis were performed using the suite of pCLAMP programmes V9.2 (Axon Instruments, Union City, CA, USA), EXCEL (Microsoft Corp., Redmond, WA, USA), and ORIGIN 6.1 (Microcal Software, Northampton, MA, USA). Data are expressed as mean ± SEM. Two-tailed Student's *t*-test was performed using SigmaPlot 2000 statistical software (Systat Software Inc., Chicago, IL, USA). Differences were considered to be statistically significant at a value of $P < 0.05$.

Results

Clinical findings

The proband is the only child of an unreachable father (apparently healthy) and a healthy mother, with no known family history of SCD or syncope. Physical examination was within normal limits. Echocardiography demonstrated an ejection fraction of 60%, normal left ventricle dimensions (end systolic: 2.3 cm, end diastolic: 4.1 cm) without hypertrophy and without segmental wall abnormalities.

A bolus of 18 mg adenosine was administered intravenously to achieve a high degree AV block. The R–R interval increased from 900 to 1560 ms. The transient bradycardia was followed by a period of tachycardia. The QTc interval changed from 538 to 408 ms during the bradycardia phase of the adenosine test. The bifurcated T wave became more accentuated with an increase in the amplitude of the second component of the T wave (T2) (*Figure 2A*). During the tachycardia phase, QTc increased to 620 ms (*Figure 2B*). The ΔQT interval between baseline and under low epinephrine dose (0.1 μg/kg/min) was −20 ms (from 540 to 520 ms).

Based on these results, a definite diagnosis of LQTS was made and the patient was advised to stop swimming competitively and to avoid medications known to prolong the QT interval. Her blood was sent for genetic evaluation.

Genetic analysis revealed a heterozygous transition of G to A at position 253 of *KCNE1* predicting substitution of an asparagine for aspartic acid at position 85 of mink (D85N). D85N is a rare polymorphism with a 1.5–2% incidence in the general population.[19,25] Another polymorphism was found in *SCN5A* (H558R). This is a common finding in the general population with an incidence of up to 20%.[26] No other SNPs were found in any of the LQT genes analysed.

Biophysical characteristics of *KCNE1*-D85N co-expressed with *KCNH2* and *KCNQ1*

Figure 3 illustrates the voltage-dependent properties of macroscopic human ether-à-go-go-related gene (*HERG*) current (I_{Kr}).

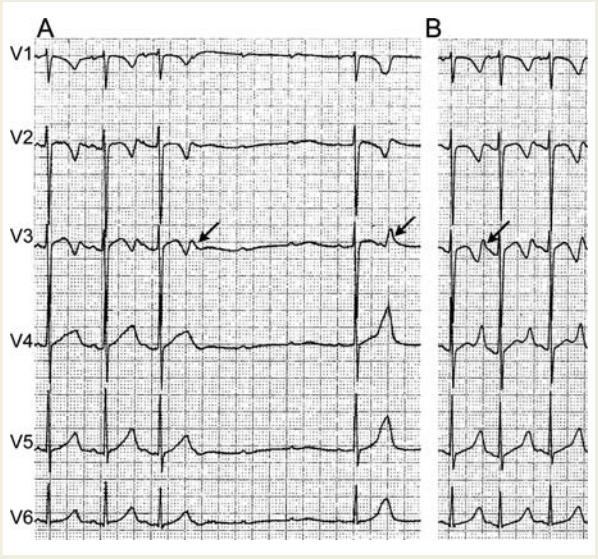

Figure 2 (*A*) High-degree atrioventricular block during an adenosine challenge. Note the accentuation of the second component of the T wave (T2; shown by arrows). (*B*) Adenosine-induced tachycardia causing a marked prolongation of QTc.

The currents shown are the result of co-expression of *KCNH2* with either *KCNE1*-WT (*Figure 3A*) or *KCNE1*-D85N expressed homozygously (*Figure 3B*) or heterozygously (*Figure 3C*). The transfected cells were clamped at a holding potential of −80 mV and depolarized to voltages between −50 and 70 mV for 800 ms to activate I_{Kr}. Tail currents were recorded on return to −40 mV. I_{Kr} developing and tail current was significantly reduced with co-expression of the D85N variant. *Figure 3D* shows the *I–V* plot of tail and developing I_{Kr} amplitude (*Figure 3D and E*, respectively), average values based on three different transfections for each group. Homozygous co-expression of D85N reduced tail current by 85%, whereas heterozygous co-expression reduced the current by 52%, pointing to a dominant-negative effect of the rare polymorphism (*Figure 3D*). Developing I_{Kr} in *KCNH2*-WT co-expressed with *KCNE1*-WT shows strong inward rectification. Homozygous co-expression of the *KCNE1*-D85N with *KCNH2* reduced I_{Kr} tail current by 85%, whereas heterozygous co-expression reduced the current by 52%, demonstrating a dominant-negative effect of D85N to reduce I_{Kr}. (*Figure 3E*).

Figure 4 shows the results of co-expression of *KCNQ1* with either *KCNE1*-WT (*Figure 4A*) or *KCNE1*-D85N expressed homozygously (*Figure 4C*) or heterozygously (*Figure 3B*). The transfected cells were clamped at a holding potential of −80 mV and depolarized to voltages between −80 and 100 mV for 5 s to activate I_{Ks}. Tail currents were recorded on return to −40 mV. Functional co-expression of D85N-*KCNE1* with *KCNQ1*, either homozygously or heterozygously, did not significantly reduce I_{Ks}. *Figure 4D* shows the *I–V* plot of tail and developing I_{Ks} amplitude (*Figure 4D and E*, respectively); average values based on three different transfections for each group.

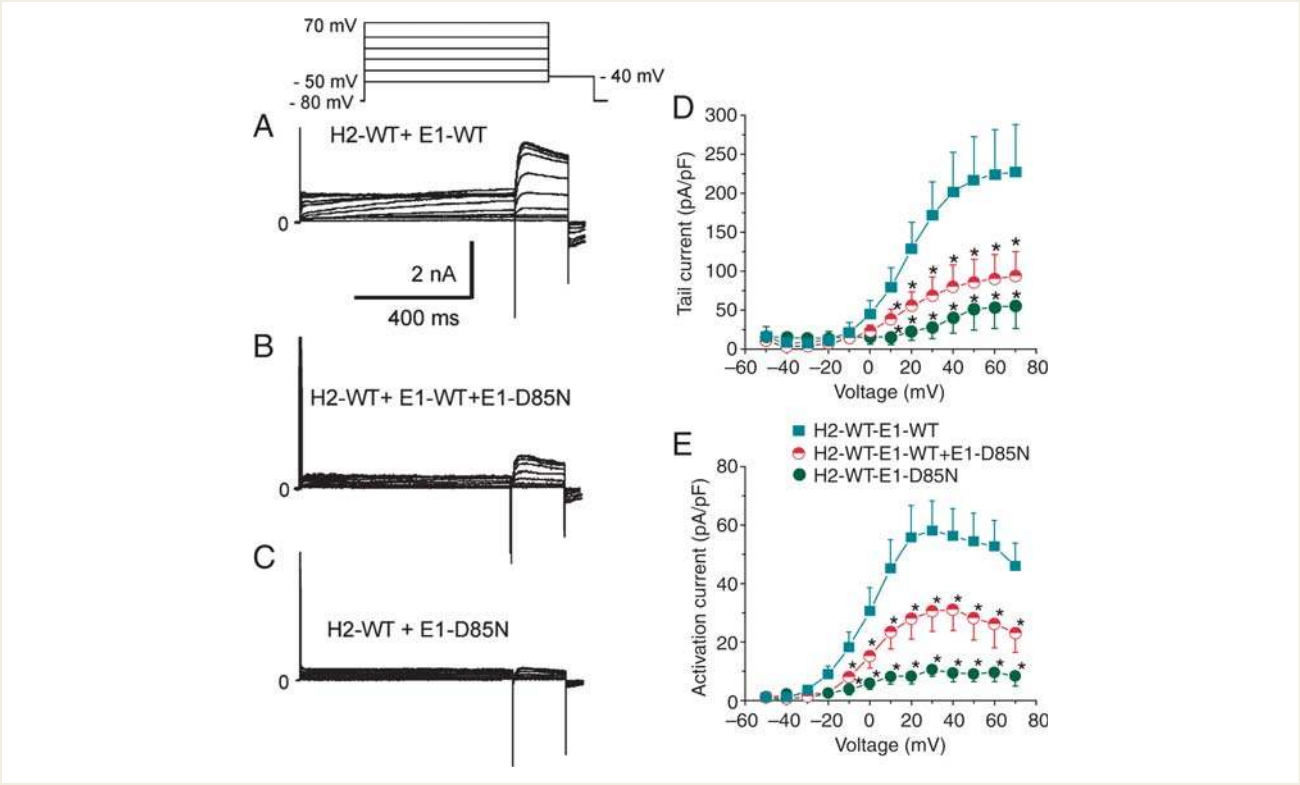

Figure 3 Functional co-expression of the *KCNE1*-D85N with *KCNH2* in human embryonic kidney cells (TSA-201). (*A*) Representative currents recorded from cells transfected with wild type (WT) *KCNH2* and *KCNE1*. (*B*) Heterozygous co-expression of *KCNE1*-D85N and *KCNH2*-WT. (*C*) Homozygous co-expression of *KCNE1*-D85N and *KCNH2*-WT. (*D*) *I–V* relationships of I_{Kr} tail current recorded at −40 mV. (*E*) *I–V* curve of I_{Kr} developing current of the *KCNE1*-D85N co-expressed with *KCNH2*-WT. *$P < 0.05$ vs. respective control (C); $n = 6$ to 8 for each group.

Discussion

We describe a young asymptomatic female with mild QT prolongation at rest. Epinephrine and adenosine challenges clearly unmask long QTc intervals. Genetic analysis revealed a rare polymorphism in *KCNE1* (D85N: heterozygous frequency: 1–2.5%). Functional expression of D85N alone has been shown to reduce I_{Ks} by ~50%.[27] In other studies[18,28] there were no significant differences between WT and D85N co-expressed with *KCNQ1*-WT; however, the channels activated more slowly and deactivated to a greater extent after a long diastolic pause.[18]

A recent study reported that the allele frequency of *KCNE1*-D85N is significantly higher in LQTS patients than in control subjects.[20] The authors conclude that D85N is a disease-causing gene variant that functions by interacting with *KCNQ1* as well as *KCNH2*. Studies examining the effect of *KCNE1*-D85N on *KCNQ1* current have yielded variable results and studies probing its effect on KCNH2 current are sparse. *KCNE1*-D85N homozygously expressed has been reported to cause an ~50% reduction in KCNQ1 current in *Xenopus oocytes*.[27] In Chinese hamster ovary (CHO) cells, homozygous expression of D85N reduced *KCNQ1* current by 28% compared with wild type.[20] Our results with co-expression of *KCNE1*-D85N with *KCNQ1* in TSA201 cells indicate no effect on I_{Ks}, consistent with previous studies suggesting little or no reduction in I_{Ks}.

Previous studies have reported that the D85N variant of *KCNE1* homozygously expressed reduces *KCNH2* current or I_{Kr} by 31 to 36% in CHO cells.[20] These investigators did not examine the effect of D85N heterozygously expressed. With heterologous homozygous expression in TSA201 cells, we found that D85N reduces *KCNH2* current by 85% and that a dominant-negative loss of function is observed when the variant is heterozygously expressed. To our knowledge this is the first demonstration of a dominant-negative effect of *KCNE1*-D85N to reduce I_{Kr}. The lack of effect of D85N to suppress I_{Ks} and its potent effect to reduce I_{Kr} are consistent with the LQT2 phenotype observed in our patient.

An adenosine challenge was reported by Viskin *et al.*[23] to provoke a transient bradycardia followed by sinus tachycardia and thus to unmask subclinical LQTS (mostly LQT2). Compared with controls, patients with latent LQTS, like our patient, display a prominent increase in QTc interval both during bradycardia and tachycardia as well as accentuated bifurcation of the T wave. The parameter that best distinguished between control and LQTS groups was found to be the QTc interval during maximal bradycardia. A QTc >380 ms strongly implied LQTS. In our particular case, QTc was 408 at maximal bradycardia. During the tachycardia phase her QTc increased to 620 ms.

In normal individuals, infusion of catecholamine reduces action potential duration via an increase in I_{Ks}, thus abbreviating the QT

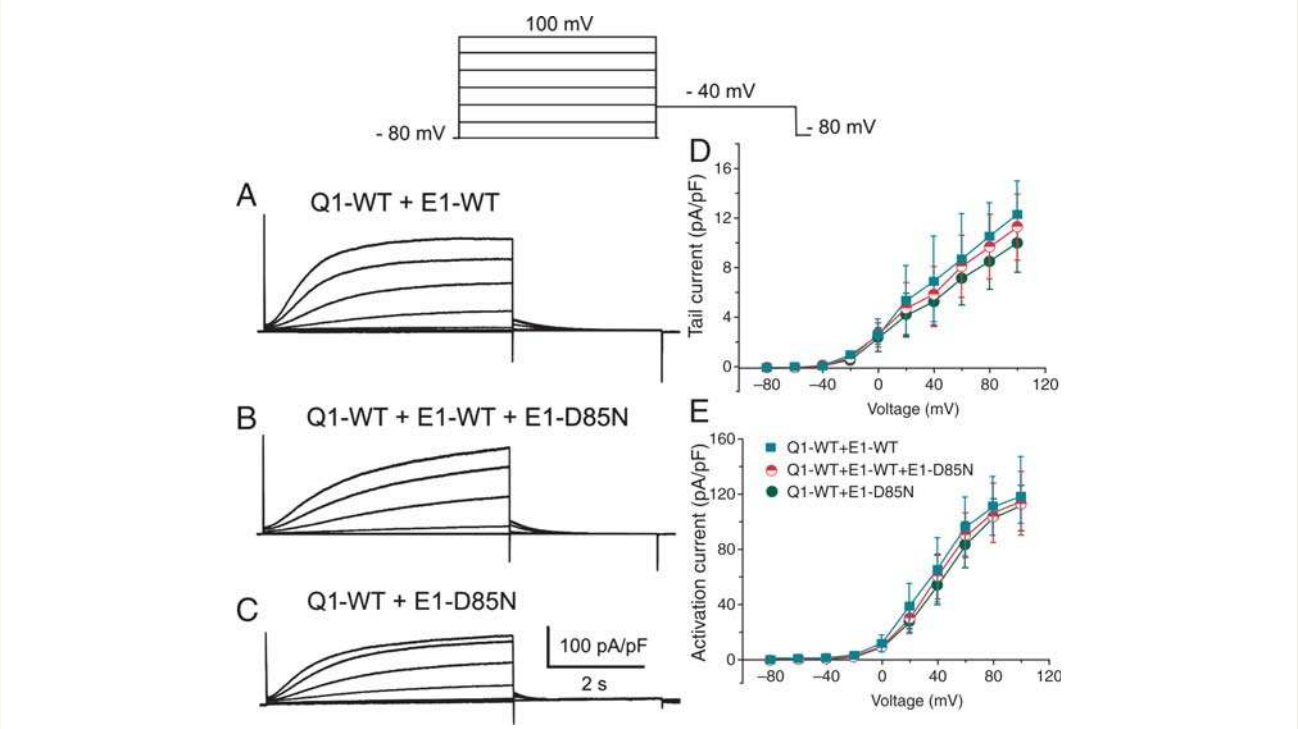

Figure 4 Functional co-expression of *KCNQ1* with *KCNE1*-D85N in TSA201 Cells. (*A*) Representative current traces of *KCNQ1* wild-type (Q1-WT) co-expression with the *KCNE1* wild type (E1-WT). (*B*) Heterozygous co-expression with the E1-WT and *KCNE1*-D85N (E1-D85N) plus Q1-WT. (*C*) Homozygous co-expression of E1-D85N and Q1-WT. (*D*) *I–V* relationships of I_{Ks} tail current recorded at −40 mV following co-expression of WT or D85N KCNE1 with *KCNQ1*. (*E*) *I–V* relationship of I_{Ks} developing current. Each data point represents the mean ± SEM of $n = 6$ to 12 cells for each experimental group.

interval. A defect in I_{Ks} is responsible for the failure of epinephrine to abbreviate the QT, leading to a paradoxical prolongation secondary to the sympathetic stimulation.[29] This accounts for the increase in QTc interval during the tachycardia phase of the adenosine challenge. While a △QT (*not QTc*) of more than 30 ms is considered diagnostic of LQT1, in LQT2 △QT did not prolong but rather abbreviated by only −4 ms during low-dose epinephrine test.[30,31] Mutation-negative patients had an average ΔQT of −23 ms during infusion of low-dose epinephrine. Our patient had a △QT of −20 ms, with an increase to QTc of 37 ms (from 530 to 567 ms). Thus, both adenosine and epinephrine tests are in agreement with the LQT2 phenotype vs.LQT1.

Single-nucleotide polymorphisms have an important role in modifying the disease phenotype of affected individuals with arrhythmia-causing mutations. Single-nucleotide polymorphisms such as *KCNH2*-K897T or *SCN5A*-H558R have been found to aggravate the clinical phenotype of LQT2 or Brugada syndromes, respectively, when associated with other disease-causing mutations in those genes.[32,33] D85N can theoretically similarly modulate the LQTS phenotype of patients with *KCNQ1* loss-of-function mutations by further reducing I_{Ks}[27] or by having a direct effect on I_{Kr}.[20]

Our patient differs from the previous cases in that there were no other mutations or polymorphisms in other LQT genes detected that could have an additive modifying effect on the I_K current. Instead, we hypothesize that the loss of function of I_{Kr} due to the D85N polymorphism was directly responsible for the LQT2 ECG phenotype displayed by the proband.

We found a common polymorphism (H558R) in *SCN5A*; however, this polymorphism was not found to have any effect on the I_{Na} by itself[34] and is unlikely to have contributed to the LQT phenotype.

Refraining from competitive sports will hopefully prevent the adrenergic challenge such an activity imposes and over the long term will increase her resting heart rate. As D85N is known to be associated with drug-induced LQT, it is important that in addition to the above measures the patient refrain from use of drugs with QT interval prolonging actions.

Study limitation

Our expression studies were performed without co-transfection of *KCNE2* along with *KCNH2*. The role of this subunit in modifying KCNH2 function continues to be debated. Relevant to this issue is the observation that the results of the functional studies correlate well with the clinical phenotype.

Clinical implications

LQT is a syndrome displaying a wide array of phenotypes. Identifying affected individuals can be a major challenge. This case demonstrates the importance of clinical provocative and genetic testing. Although the role of SNP in the diagnosis and management of

LQTS is yet to be defined, in our view it is important for molecular diagnosis to be performed. Identifying the genetic predisposition can aid in the management of LQT and help identify individuals who under specific precipitating factors may be at risk of more severe ventricular arrhythmias.

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