

Cardiac sodium channel mutation associated with epinephrine-induced QT prolongation and sinus node dysfunction



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BACKGROUND Long-QT syndrome (LQTS) is an inherited arrhythmia characterized by prolonged ventricular repolarization and malignant tachyarrhythmias. LQT1, LQT2, and LQT3 are caused by mutations in *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3), which account for approximately 90% of genotyped LQTS patients. Most cardiac events in LQT1 patients occur during exercise, whereas patients with LQT3 tend to have arrhythmic events during rest or asleep.

OBJECTIVE The study aimed to identify a genetic mutation in a Japanese man who presented with sinus node dysfunction and prolonged QT interval on exercise and epinephrine stress tests, as well as to clarify the electrophysiological properties of mutant channels.

METHODS LQTS-related genes were screened in this patient. Electrophysiological functional assays were conducted with a heterologous expression system.

RESULTS We identified a heterozygous missense *SCN5A* mutation, V2016M, which changes the last amino acid of the cardiac sodium channel. Electrophysiological analyses revealed that the mutant channels exhibited a loss-of-function feature, decreased peak sodium current densities (wild type 175.2 ± 17.6 pA/pF; V2016M 97.2 ± 16.0 pA/pF; $P < .01$). In addition, the mutant channels

showed gain-of-function features: increased late sodium currents by protein kinase A activation (wild type $0.07 \pm 0.01\%$; V2016M $0.17 \pm 0.03\%$; $P < .05$) and impaired inactivation of sodium channels by protein kinase A or C activation.

CONCLUSION We identified an *SCN5A* mutation in a patient with sinus node dysfunction and epinephrine-induced QT prolongation, which was an atypical phenotype for LQT3. The electrophysiological properties of the mutant channels might be associated with the overlapping clinical features of the patient.

KEYWORDS *SCN5A*; Epinephrine; Long-QT syndrome; Protein kinase A; Protein kinase C; Adrenergic stimulation

ABBREVIATIONS **8-CPT-cAMP** = 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate; **BrS** = Brugada syndrome; **HEK** = human embryonic kidney; **LQTS** = long-QT syndrome; **OAG** = 1-oleoyl-2-acetyl-sn-glycerol; **PKA** = protein kinase A; **PKC** = protein kinase C; **QTc** = corrected QT interval; **WT** = wild type

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Introduction

Long-QT syndrome (LQTS) is an inherited arrhythmia characterized by prolonged ventricular repolarization and malignant arrhythmias leading to syncope, cardiac arrest,

and sudden death. The most common subtypes, LQT1, LQT2, and LQT3, which account for approximately 90% of genotyped LQTS patients (two-thirds of all patients with congenital LQTS), are caused by genetic mutations in the cardiac potassium channel genes (*KCNQ1* and *KCNH2*) and the sodium (Na^+) channel gene (*SCN5A*), respectively.^{1–3} Most of the cardiac events of LQT1 occur during exercise, and those of LQT2 are induced by auditory stimuli or emotional stress, whereas LQT3 patients tend to have arrhythmic events during sleep or at rest.⁴ Different therapeutic strategies are used according to the LQTS subtypes; therefore, exercise and epinephrine

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stress electrocardiogram tests have been conducted to predict the LQTS subtype.⁵

The subunit of voltage-gated cardiac Na⁺ channels, Nav1.5, encoded by the *SCN5A* gene plays a key role in the generation of excitation and conduction in cardiomyocytes. To date, numerous *SCN5A* mutations have been reported to be associated with a variety of cardiac arrhythmias. Gain-of-function *SCN5A* mutations lead to LQT3 and atrial fibrillation,⁶ whereas loss-of-function mutations cause Brugada syndrome (BrS),⁷ progressive cardiac conduction disease,⁸ sinus node dysfunction,⁹ atrial fibrillation,¹⁰ and dilated cardiomyopathy.¹¹ In addition, a number of *SCN5A* mutation carriers have been reported to present multiple phenotypes that were defined as an overlap syndrome of cardiac Na⁺ channelopathies. The overlap syndrome included both gain-of-function- and loss-of-function-type modulations in electrophysiological features.^{6,12}

In the present study, we identified a heterozygous missense *SCN5A* mutation, c.6046 G>A, p.V2016M, in a patient with epinephrine-induced QT prolongation and sinus node dysfunction. Electrophysiological functional assays were conducted with a protein kinase A (PKA) or protein kinase C (PKC) activator to mimic the effect of epinephrine. The mutant channels showed gain-of-function-type modulations by PKA or PKC activation, which might be associated with the clinical features of the atypical LQT3 patient.

Methods

DNA isolation and genetic mutation analyses

Genetic analyses were performed after written informed consent was obtained in accordance with the study protocol approved by the Kyoto University ethics committee. The investigation conformed to the principles outlined in the Declaration of Helsinki. Genomic DNA was isolated from peripheral blood lymphocytes and screened for the entire open-reading frames of the LQTS-related ion channel genes *KCNQ1*, *KCNH2*, *KCNE1-3*, and *SCN5A* by denaturing high-performance liquid chromatography with a WAVE system model 3500 (Transgenomic, Omaha, NE). Abnormal conformers were amplified by polymerase chain reaction, and sequencing was performed on an ABI PRISM 3100 genetic analyzer (Life Technologies, Foster City, CA). We also screened LQT1 through LQT13 causative genes (*KCNQ1*, *KCNH2*, *SCN5A*, *ANKB*, *KCNE1-2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, *SNTA1*, and *KCNJ5*), *CALM1-3*, and other genes related to catecholaminergic polymorphic ventricular tachycardia, BrS, and arrhythmogenic right ventricular cardiomyopathy using next-generation sequencing technology (MiSeq, Illumina, San Diego, CA).

Site-directed mutagenesis and cell preparation

The *SCN5A* cDNA was subcloned into pcDNA3.1 (Life Technologies). The *SCN5A* mutant plasmid was constructed by site-directed mutagenesis by use of a QuikChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA). Human embryonic kidney (HEK) 293 cells, cultured in a

35-mm dish, were transiently transfected with 0.5 µg of either pcDNA3.1-*SCN5A*-wild-type (WT) or mutant channels in combination with 0.5 µg of bicistronic plasmids encoding the enhanced green fluorescent protein and the human β1-subunit (pEGFP-IRES-hβ1).⁶

Electrophysiological functional assays

Macroscopic Na⁺ currents were recorded on green fluorescent protein-positive cells 48 to 72 hours after transfection by the whole-cell mode of patch-clamp techniques at 22°C as described elsewhere.⁶ From a holding potential of −120 mV, standard voltage-clamp protocols were used to examine the voltage dependence of steady-state activation and fast inactivation, recovery from fast inactivation, onset of slow inactivation, and closed-state inactivation. Late Na⁺ currents were determined by subtracting background currents measured in the presence of 30 µM tetrodotoxin (NacalaiTesque, Kyoto, Japan) from tetrodotoxin-free records. Late Na⁺ currents were evaluated using the mean value of the current amplitude during the depolarizing pulse step between 500 and 600 ms. To mimic the effect of epinephrine, a selective activator of PKA, 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate (8-CPT-cAMP, Wako, Osaka, Japan), and a selective activator of PKC, 1-oleoyl-2-acetyl-sn-glycerol (OAG, Wako), were used. PKA activator (200 µM 8-CPT-cAMP) or PKC activator (100 µM OAG) was added into the bath solution 10 minutes after whole-cell mode was achieved. Macroscopic Na⁺ current recordings were started 5 minutes after perfusion of the bath solution that included 8-CPT-cAMP or OAG.

Data analysis

The plots of voltage-dependent steady-state activation and fast inactivation were fitted by Boltzmann equation: $I/I_{\max} = 1/[1 + \exp(V - V_{1/2})/k]$, where $V_{1/2}$ is the voltage at which Na⁺ currents are half-maximally activated and k is the slope factor. The recovery from inactivation at −120 mV was fitted by a biexponential equation: $I/I_{\max} = A_f [1 - \exp(-t/\tau_f)] + A_s [1 - \exp(-t/\tau_s)]$, where A_f and A_s are fractions of fast and slow inactivation components and τ_f and τ_s are the time constants for fast and slow inactivation components, respectively. Time course of entry into the slow-inactivation state and development of closed-state inactivation were measured by a double-pulse method and fitted by a monoexponential equation: $I/I_{\max} = A [1 - \exp(-t/\tau)]$. Results were expressed as mean ± SEM, and statistical significance was analyzed with the Student *t* test. Statistical significance was assumed for $P < .05$.

Results

Clinical features

A 24-year-old man was admitted to a hospital because of dizziness and malaise. He had experienced syncope twice: The first episode occurred during a morning class in high-school at the age of 17 years and the second after a long-distance run when he was a college student. He had no family

history of sudden death. The electrocardiogram showed normal QT/corrected QT interval (QTc) (398/385 ms corrected by Bazett formula; heart rate 56 bpm) at baseline (Figure 1A). During the epinephrine stress test, the QT/QTc was extremely prolonged to 557/609 ms (heart rate 72bpm) (Figure 1A). During the exercise test, the QTc interval at

peak exercise was also prolonged: QTc 473 ms, heart rate 112 bpm at baseline; 608 ms, 173 bpm at peak exercise; 428 ms, 122 bpm at 1-minute recovery; and 411 ms, 102 bpm at 4-minute recovery. The proband had sinus bradycardia at rest (48 bpm), and further examination with an implantable loop recorder revealed several pauses that were up to 5.9 seconds

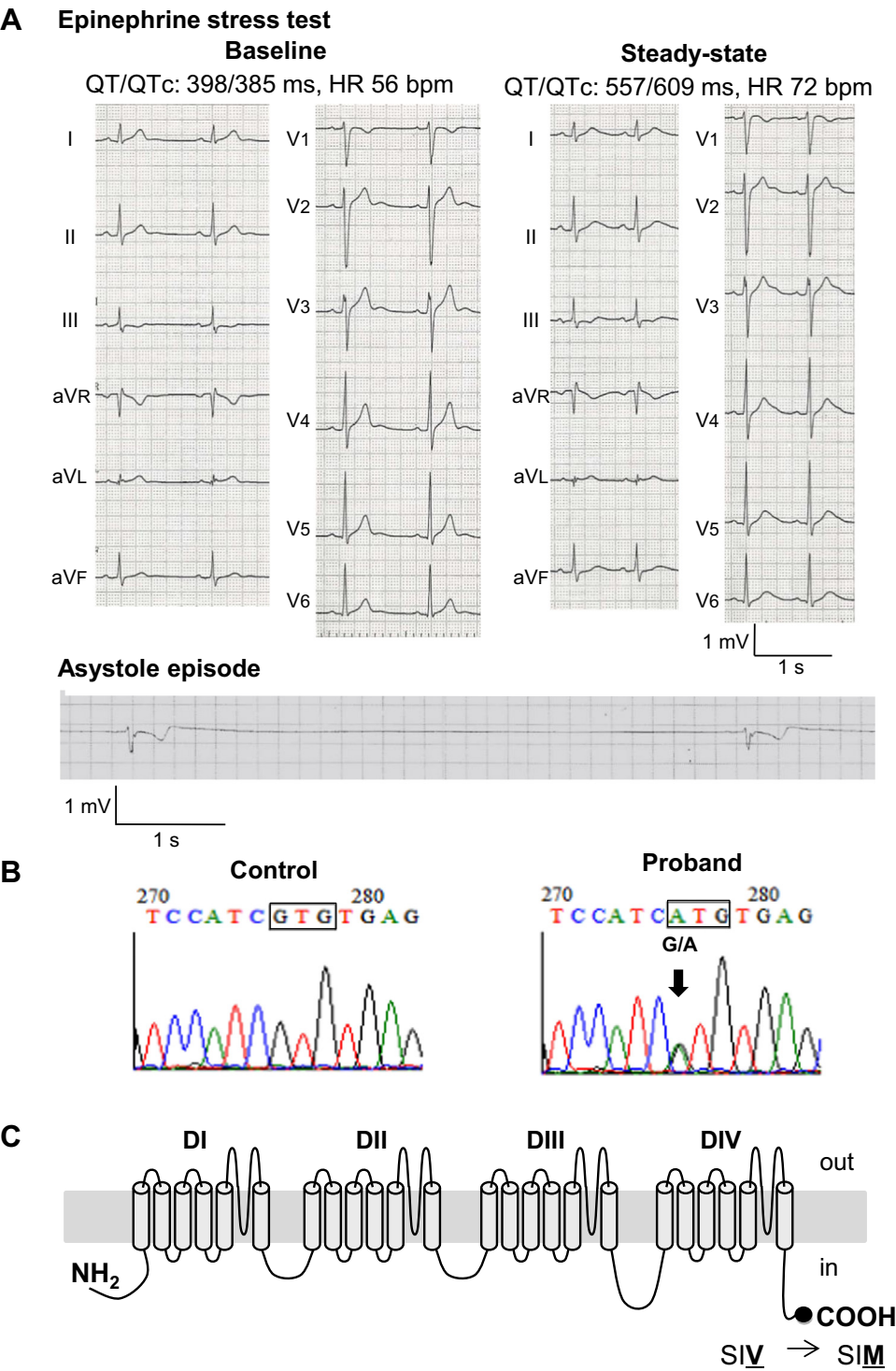


Figure 1 Clinical features and genetic analyses. **A:** Electrocardiograms obtained from the proband at baseline (left) and steady state (right) during the epinephrine stress test. A representative asystole episode recorded by an implantable loop recorder is shown below. **B:** DNA sequencing electropherograms of the *SCN5A* gene mutation (c.6046 G > A, p.V2016M). **C:** Topology of the α -subunit of the voltage-gated cardiac Na^+ channel, Nav1.5. The location of the detected mutation is shown as a circle. HR = heart rate; QTc = corrected QT interval.

(Figure 1A). The patient was diagnosed as having sinus node dysfunction, and a pacemaker was implanted. He also had asthma, and therefore, he was not treated with β -blockers. There have been no cardiac events for >5 years after the diagnosis. His family members did not agree to attend this study; therefore, we failed to acquire additional clinical information from the family members and proceed to genetic analysis.

Genetic analyses

As a result of genetic analyses in the index patient, we identified a missense *SCN5A* mutation, c.6046 G>A, p.V2016M; no other mutation in genes listed in the Methods section were found. Figures 1B and 1C show the results of sequencing and the position of the identified mutation in Nav1.5. The mutation was located at the very last amino acid of the last 3 residues of the Nav1.5 C-terminus (Ser-Ile-Val), which constitutes the PDZ domain-binding motif.

Functional analysis of *SCN5A*-V2016M

To assess the functional consequences of the *SCN5A*-V2016M, biophysical properties of Na^+ currents were determined in HEK293 cells expressing WT or mutant (V2016M) Na^+ channels. Figure 2A illustrates representative whole-cell current traces recorded from cells expressing WT or V2016M channels. As summarized in the current-voltage relations in Figure 2B, the peak current densities of V2016M channels were significantly smaller than those of WT channels (WT 175.2 ± 17.6 pA/pF; V2016M 97.2 ± 16.0 pA/pF at a test potential of -30 mV; $P < .01$) (Figure 2B). The mutant channels did not alter the voltage dependence of steady-state activation and fast inactivation of the Na^+ channels (Figure 3A) (Table 1). There were also no significant differences in parameters of other kinetic properties such as recovery from inactivation, onset of slow

inactivation, or closed-state inactivation (Figures 3B through 3D) (Table 1).

Effects of 8-CPT-cAMP on gating properties of WT and V2016M channels

In the index patient, exercise or epinephrine excessively prolonged QT intervals. Epinephrine is known to activate both PKA and PKC pathways. Therefore, we first conducted experiments to mimic the effect of PKA activation using a PKA activator, 8-CPT-cAMP. Figure 4A shows the current-voltage relationships after application of 8-CPT-cAMP. Compared with the results in Figure 2B, PKA activation increased peak current densities in both WT and V2016M channels. Table 1 summarizes the numerical data. Peak current densities in V2016M channels remained significantly smaller than those in WT even after PKA activation (WT 230.4 ± 24.7 pA/pF; V2016M 145.9 ± 17.7 pA/pF at a test potential of -30 mV; $P < .05$) (Figure 4A) (Table 1).

As shown in Figure 4B and Table 1, 8-CPT-cAMP induced a significant hyperpolarizing shift in the voltage dependence of steady-state activation and fast inactivation of WT channels, which was consistent with a previous report.¹³ In V2016M channels, 8-CPT-cAMP induced a hyperpolarizing shift in the voltage dependence of activation in the same manner as in WT channels; however, it did not shift the voltage dependence of fast inactivation (WT $V_{1/2}$ from -80.7 ± 1.3 to -86.7 ± 1.3 mV, $P < .05$, vs V2016M $V_{1/2}$ from -82.5 ± 0.8 to -81.7 ± 0.5 mV, $P = \text{NS}$) (Figures 3A and 4B) (Table 1).

8-CPT-cAMP enhanced recovery from inactivation in both WT and V2016M channels in a similar manner (Figures 3B and 4C) (Table 1). It did not change any kinetic parameters of recovery from inactivation, onset of slow inactivation, or closed-state inactivation on either WT or V2016M channels (Figures 4C through 4E) (Table 1). The diminished hyperpolarizing shift of fast inactivation in

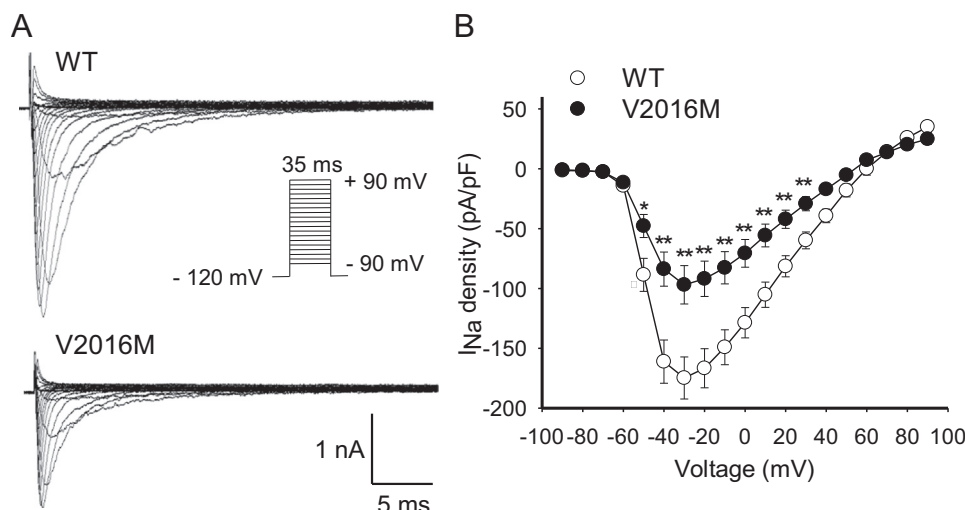


Figure 2 Macroscopic Na^+ currents of V2016M channels. **A:** Currents were elicited by the protocol shown in the inset. Representative whole-cell current traces of wild-type (WT) and V2016M channels are shown. **B:** Average current-voltage relationship in WT (open circles) and V2016M channels (closed circles). The current was normalized to cell capacitance to give a measure of peak current densities. * $P < .05$, ** $P < .01$.

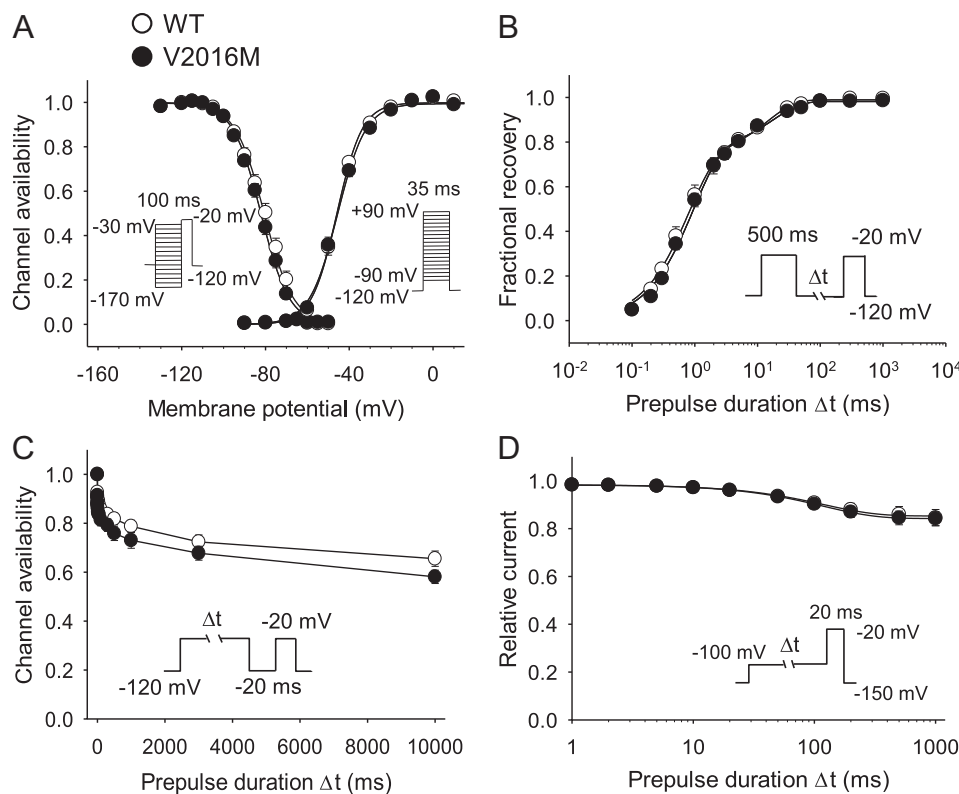


Figure 3 Gating properties for V2016M channels. **A:** Voltage dependence of relative Na^+ conductance activation and steady-state inactivation in wild-type (WT, open circles) and V2016M (closed circles) channels was determined by means of the voltage protocols. Curves were fit with the Boltzmann equation, $I/I_{\max} = 1/[1 + \exp((V - V_{1/2})/k)]$ to determine the membrane potential for half-maximal inactivation or activation ($V_{1/2}$) and the slope factor k . **B:** Time course of recovery from inactivation was elicited with a double-pulse protocol. Data were fit with a biexponential equation: $I/I_{\max} = A_f [1 - \exp(-t/\tau_f)] + A_s [1 - \exp(-t/\tau_s)]$, where A_f and A_s are fractions of fast and slow inactivation components and τ_f and τ_s are the time constants of fast and slow inactivation components, respectively. **C:** Onset of slow inactivation. Time course of entry into the slow inactivation state was obtained by a double-pulse protocol. Curves were fit with a single exponential equation: $I/I_{\max} = y_0 + A [1 - \exp(-t/\tau)]$. **D:** Closed-state inactivation. The transfer rate of Na^+ channels from closed state to inactivated closed state without an intervening opening state was measured by a double-pulse protocol. Time course for development of closed-state inactivation was fit with a single exponential equation: $I/I_{\max} = y_0 + A [1 - \exp(-t/\tau)]$.

V2016M channels in the presence of 8-CPT-cAMP was considered to be associated with a gain-of-function type modulation on Nav1.5.

Effects of OAG on gating properties of WT and V2016M channels

In the next series of experiments, we evaluated the effect of PKC activation on peak Na^+ current densities and kinetic properties using a PKC activator, OAG. OAG did not alter the peak current densities in either WT or V2016M channels (Figure 5A) (Table 1). Therefore, similar to baseline, in the presence of OAG, the peak current densities of V2016M channels were significantly smaller than WT (WT 159.9 ± 31.3 pA/pF; V2016M 93.5 ± 17.2 pA/pF at a test potential of -30 mV; $P < .05$) (Figure 5A) (Table 1).

Figure 5B shows steady-state activation and fast-inactivation curves of WT and V2016M channels in the presence of OAG. Although OAG had no effect on steady-state activation of WT and V2016M channels, it induced a significant hyperpolarizing shift in the voltage dependence of fast inactivation in WT channels, but not in V2016M channels (WT $V_{1/2}$ from -80.7 ± 1.3 to -87.8 ± 1.6 mV,

$P < 0.05$, vs V2016M $V_{1/2}$ from -82.5 ± 0.8 to -82.0 ± 2.1 mV, $P = \text{NS}$) (Figures 3A and 5B) (Table 1).

In WT channels, OAG also significantly enhanced recovery from inactivation (Figures 3B and 5C) (Table 1), onset of slow inactivation (Figures 3C and 5D) (Table 1), and closed-state inactivation (Figures 3D and 5E) (Table 1); however, in V2016M channels, we observed no enhanced development of slow inactivation and closed-state inactivation. Taken together, there were 3 kinetic changes seen in the WT channels in the presence of OAG: (1) a hyperpolarizing shift of fast inactivation, and enhanced state shifts of (2) slow inactivation and (3) closed-state inactivation. In SCN5A-V2016M channels, these were all abolished, which indicates a gain-of-function type of modulation on Nav1.5.

Late Na^+ currents in WT and V2016M channels in the presence of PKA or PKC activator

Figure 6A shows representative tetrodotoxin-subtracted whole-cell currents for WT and V2016M channels. There was no significant difference in late Na^+ currents between WT and V2016M channels at baseline or in the presence of OAG (Figure 6B). However, in the presence of 8-CPT-cAMP, late Na^+ currents were significantly increased in

Table 1 Biophysical properties of WT and V2016M channels

	Baseline		8-CPT-cAMP		OAG	
	WT	V2016M	WT	V2016M	WT	V2016M
Normalized peak I_{Na^+} (pA/pF)	(n = 14) −175.2 ± 17.6	(n = 12) −97.2 ± 16.0 [*]	(n = 10) −230.4 ± 24.7	(n = 9) −145.9 ± 17.7 [†]	(n = 8) −159.9 ± 31.3	(n = 7) −93.5 ± 17.2 [†]
Steady-state activation	(n = 14)	(n = 12)	(n = 10)	(n = 9)	(n = 8)	(n = 7)
$V_{1/2}$	−45.7 ± 0.7	−44.9 ± 0.8	−50.1 ± 1.9 [‡]	−50.3 ± 1.1 [§]	−45.8 ± 1.4	−45.4 ± 1.2
k	5.8 ± 0.3	6.6 ± 0.2 [†]	4.8 ± 0.4 [‡]	5.8 ± 0.3 [‡]	5.4 ± 0.6	6.2 ± 0.4
Steady-state fast inactivation	(n = 12)	(n = 11)	(n = 8)	(n = 9)	(n = 11)	(n = 11)
$V_{1/2}$	−80.7 ± 1.3	−82.5 ± 0.8	−86.7 ± 1.3 [§]	−81.7 ± 0.5 [*]	−87.8 ± 1.6 [§]	−82 ± 2.1 [†]
k	−6.5 ± 0.2	−6.7 ± 0.2	−6.5 ± 0.2	−7 ± 0.6	−7.4 ± 0.1 [§]	−7.5 ± 0.5
Recovery from inactivation	(n = 13)	(n = 12)	(n = 8)	(n = 9)	(n = 8)	(n = 7)
A_f	0.77 ± 0.03	0.76 ± 0.03	0.81 ± 0.05	0.86 ± 0.02 [‡]	0.88 ± 0.02 [§]	0.88 ± 0.02 [§]
A_s	0.22 ± 0.02	0.22 ± 0.03	0.19 ± 0.05	0.13 ± 0.03 [‡]	0.11 ± 0.02 [§]	0.11 ± 0.02 [§]
τ_f (ms)	9.8 ± 1.4	10.2 ± 0.9	14.5 ± 3.0	15.4 ± 2.9 [‡]	25.9 ± 3.5 [§]	19.9 ± 3.0 [§]
τ_s (ms)	181.5 ± 15.6	186.9 ± 18.9	583.8 ± 171.8 [§]	479.9 ± 88.3 [‡]	357.9 ± 83.6 [§]	383.6 ± 70.1 [§]
Onset of slow inactivation	(n = 9)	(n = 12)	(n = 9)	(n = 9)	(n = 8)	(n = 8)
A	0.27 ± 0.03	0.41 ± 0.02	0.23 ± 0.03	0.27 ± 0.02	0.25 ± 0.04	0.22 ± 0.02
τ (ms)	1131.3 ± 225.4	1208.8 ± 325.2	1678.8 ± 209.6	1685 ± 353.8	844.1 ± 170.0 [‡]	1561.9 ± 196.8 [*]
Closed-state inactivation	(n = 10)	(n = 10)	(n = 8)	(n = 9)	(n = 7)	(n = 7)
A	0.13 ± 0.03	0.14 ± 0.03	NA	0.11 ± 0.04	0.53 ± 0.06 [§]	0.21 ± 0.06 [*]
τ (ms)	97.9 ± 13.4	116.7 ± 17.9	NA	123.7 ± 21.5	145.0 ± 11.4	131.1 ± 10.6

Data are mean ± SEM. Parameters were obtained from fitting individual experiments illustrated in Figures 3 through 5. n = number of tested cells.

8-CPT-cAMP = 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate; A = fractional amplitude; k = slope factor; NA = not applicable; OAG = 1-oleoyl-2-acetyl-sn-glycerol; T = time constant; $V_{1/2}$ = midpoint potential; WT = wild type.

* $P < .01$ vs WT;

[†] $P < .05$ vs WT.

[‡] $P < .05$ vs baseline.

[§] $P < .01$ vs baseline.

^{||}These parameters were not applicable because the development of closed-state inactivation could not be fitted by a monoexponential equation

V2016M channels compared with WT channels (WT 0.07 ± 0.01%; V2016M 0.17 ± 0.03%; $P < .05$) (Figure 6B). Gain-of-function effects of the mutation on late Na^+ currents may explain in part the epinephrine-induced QT prolongation seen in the index patient as well.

Discussion

In the present study, we first reported an *SCN5A* mutation associated with epinephrine-induced QT prolongation, which is an atypical clinical feature of LQT3. Epinephrine-induced QT prolongation is a key clinical marker of LQT1, which is caused by mutations in *KCNQ1*, but we failed to find any mutation in *KCNQ1* or in *KCNE1* or *AKAP9*, which are associated with I_{Ks} channels.¹⁴

Functional analyses on resting V2016M channels revealed a loss-of-function-type modulation on Nav1.5, significantly reduced peak current densities (Figure 2). In contrast, V2016M channels in the presence of PKA or PKC activators exhibited both gain- and loss-of-function types of modulations. Among the gain-of-function features were (1) a loss of the hyperpolarizing shift of steady-state fast inactivation by PKA activation, (2) increased late Na^+ currents by PKA activation, and (3) impaired inactivation gating by PKC activation. On the other hand, peak Na^+ current densities in mutant channels remained decreased significantly compared with those in WT channels even in the presence of PKA or

PKC stimulation, which resulted in loss-of-function features. These results suggest that the *SCN5A*-V2016M mutation could be associated with the overlapping clinical features of epinephrine-induced LQTS and sinus node dysfunction.

The PDZ domain-binding motif, which consists of the last 3 residues (Ser-Ile-Val) of the Nav1.5 C-terminus, is a conserved domain that interacts with syntrophin, synapse-associated protein 97 (SAP97), and protein tyrosine phosphatase 1.^{15–17} Those proteins have been reported to regulate peak Na^+ current densities via the expression level of Nav1.5 or channel availability. The *SCN5A*-V2016M mutation identified in the proband altered the last amino acid of Nav1.5 within the SIV motif.

The same V2016M mutation was recently identified in a patient with BrS by Shy et al.¹⁸ In their report, cell surface expression and peak Na^+ currents of V2016M channels were reduced when the constructs were expressed in HEK293 cells, probably because of a disrupted interaction with syntrophin and dystrophin. In addition, they generated knock-in mice that lacked the SIV domain (Δ SIV), which displayed reduced Nav1.5 expression and peak Na^+ currents at the lateral myocyte membrane. The homozygous Δ SIV mice displayed a significantly prolonged PR interval and QRS duration compared with WT littermates. They concluded that the SIV motif plays an important role in the correct expression of Nav1.5 in the lateral myocyte membrane, which is associated with cardiac conduction. Further

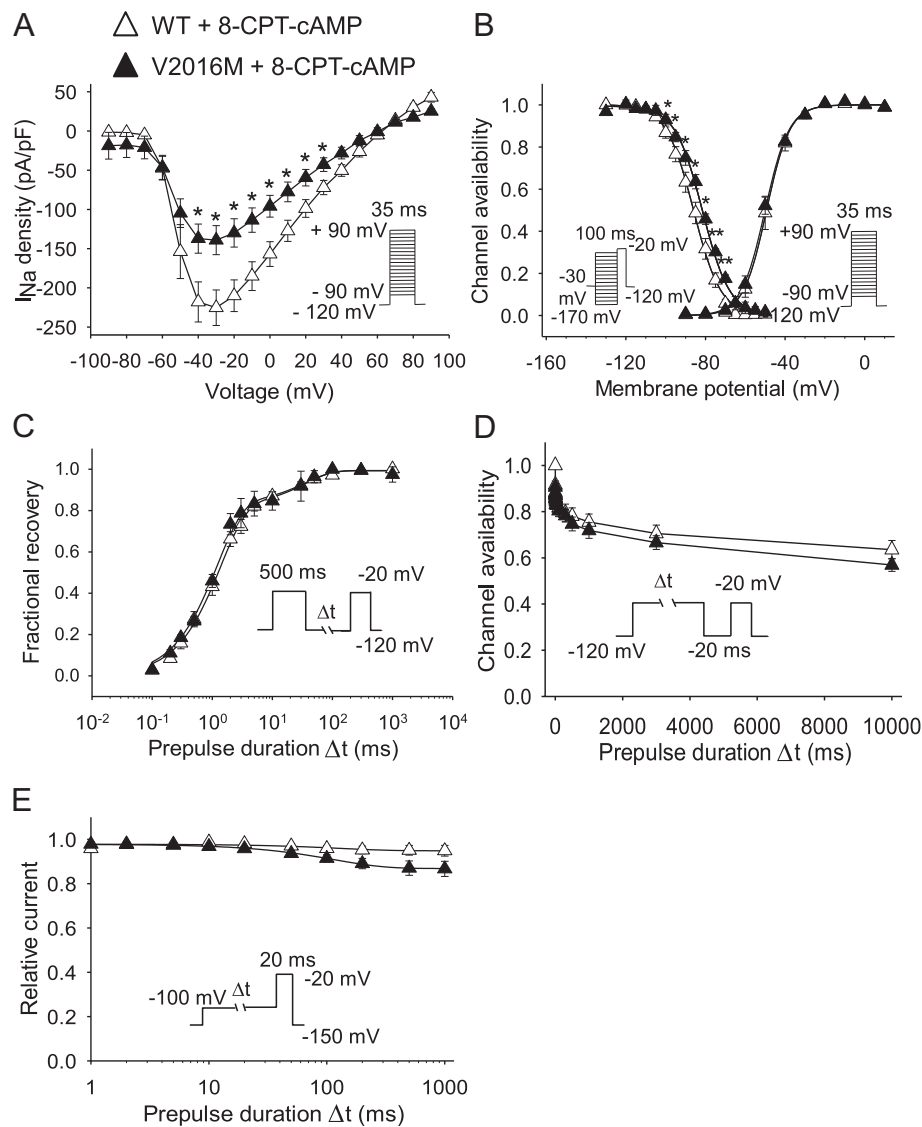


Figure 4 PKA activation on wild-type (WT) and V2016M channels. The effect of protein kinase A (PKA) activation on Na^+ current densities and kinetic properties were evaluated with a PKA activator, 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate (8-CPT-cAMP). **A:** Average current-voltage relationships in WT (open triangles) and V2016M (closed triangles) channels. The current was normalized to cell capacitance to give a measure of Na^+ current densities. **B:** Voltage dependence of relative Na^+ conductance activation and steady-state fast inactivation. **C:** Time course of recovery from inactivation. **D:** Onset of slow inactivation. **E:** Closed-state inactivation. * $P < .05$, ** $P < .01$, significant difference between groups.

investigation will be needed to elucidate the mechanism underlying different clinical features between our case and the previous report.

Total adrenergic signaling in the heart is activated via both α - and β -adrenergic receptors, which results in stimulation of PKC and PKA signaling pathways, respectively. β -Adrenergic signaling is considered to play a key role in regulating Na^+ channels.^{19,20} In our study, late Na^+ currents were increased in V2016M channels under PKA activation. In addition, PKA stimulation induced a significant hyperpolarizing shift of steady-state fast inactivation in WT channels but not in V2016M channels. These functional changes under PKA stimulation resulted in a gain-of-function type of modulation in V2016M channels, which might be associated with the epinephrine-induced QT prolongation. PKA stimulation has also been reported to

augment late Na^+ currents in mutant Na^+ channels (Δ KPQ, D1790G, and R1623Q).^{21–23} One of these 3 *SCN5A* mutations, D1790G, is located in the C-terminal domain. Its effect on augmentation of late Na^+ currents was reportedly diminished by the copresence of *SCN5A*-S36A and -S525A mutations, which lost phosphorylation sites in the N-terminus and in the cytoplasmic I-II loop, respectively.²¹ Thus, interactions among the C-terminus and the residues 36 and 525 may contribute to the functional consequences of PKA stimulation, although it remains unknown whether the mutation in the C-terminus discussed here is involved in the PKA-dependent inactivation.

α -Adrenergic receptors were recently reported to play an important role in Ca^{2+} handling of myocytes.^{24–26} In this study, we conducted electrophysiological functional assays to investigate α -adrenergic effects on regulation of cardiac

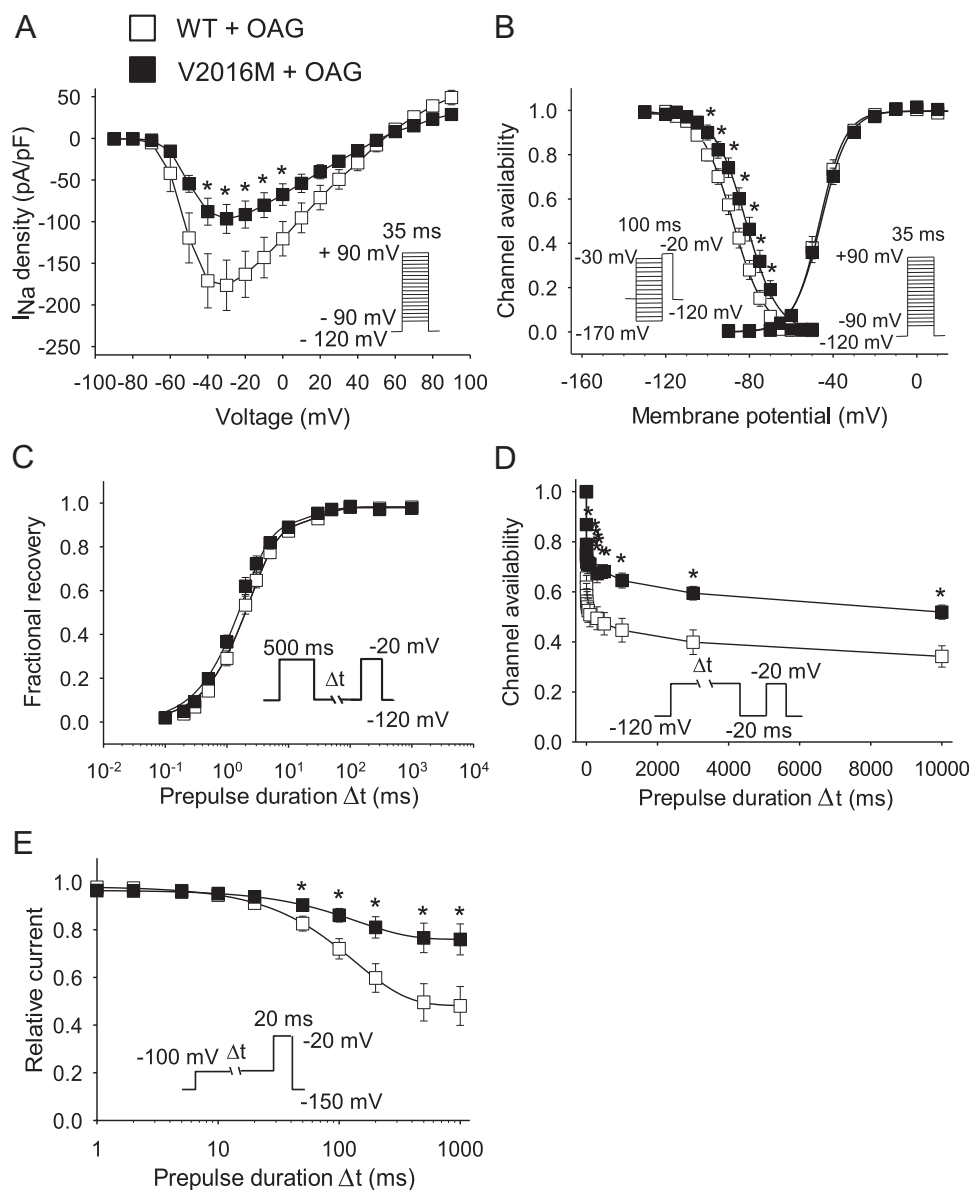


Figure 5 Protein kinase C (PKC) activation on wild-type (WT) and V2016M channels. The effect of PKC activation on Na⁺ current densities and kinetic properties were evaluated with a PKC activator, OAG (1-oleoyl-2-acetyl-sn-glycerol). **A:** Average current-voltage relationships in WT (open squares) and V2016M (closed squares) channels. The current was normalized to cell capacitance to give a measure of Na⁺ current densities. **B:** Voltage dependence of relative Na⁺ conductance activation and steady-state inactivation. **C:** Time course of recovery from inactivation. **D:** Onset of slow inactivation. **E:** Closed-state inactivation. **P* < .05, significant difference between groups.

Na⁺ channels using a PKC activator, OAG. PKC activation has been reported to have loss-of-function effects on cardiac Na⁺ channels, such as enhancing inactivation and reducing peak Na⁺ currents.^{27–29} Consistent with previous studies, PKC activation displayed loss-of-function effects on WT channels, characterized by hyperpolarized fast inactivation, enhanced slow inactivation, and closed-state inactivation. However, these effects were abolished in V2016M channels, thus exerting a gain-of-function effect.

β -Blocker therapy was assumed to be ineffective or contraindicated in LQT3 patients based on studies with a small number of LQT3 cases.^{4,30,31} However, more recent clinical and experimental data clearly demonstrated the efficacy of β -blockers for LQT3 patients, except those with

cardiac events in the first year of life.^{32–34} The gain-of-function-type modulation of mutant channels under PKA or PKC stimulation in our study might be a novel mechanism, which may explain in part why β -blockers are very effective in some LQT3 patients.

Conclusion

In the present study, we identified an *SCN5A* mutation in a patient with sinus node dysfunction and exercise/epinephrine-induced QT prolongation, which was an atypical phenotype for LQT3. The results of biophysical assays strongly suggest that V2016M is a functional mutation that can increase susceptibility to the overlapping clinical features of the patient.

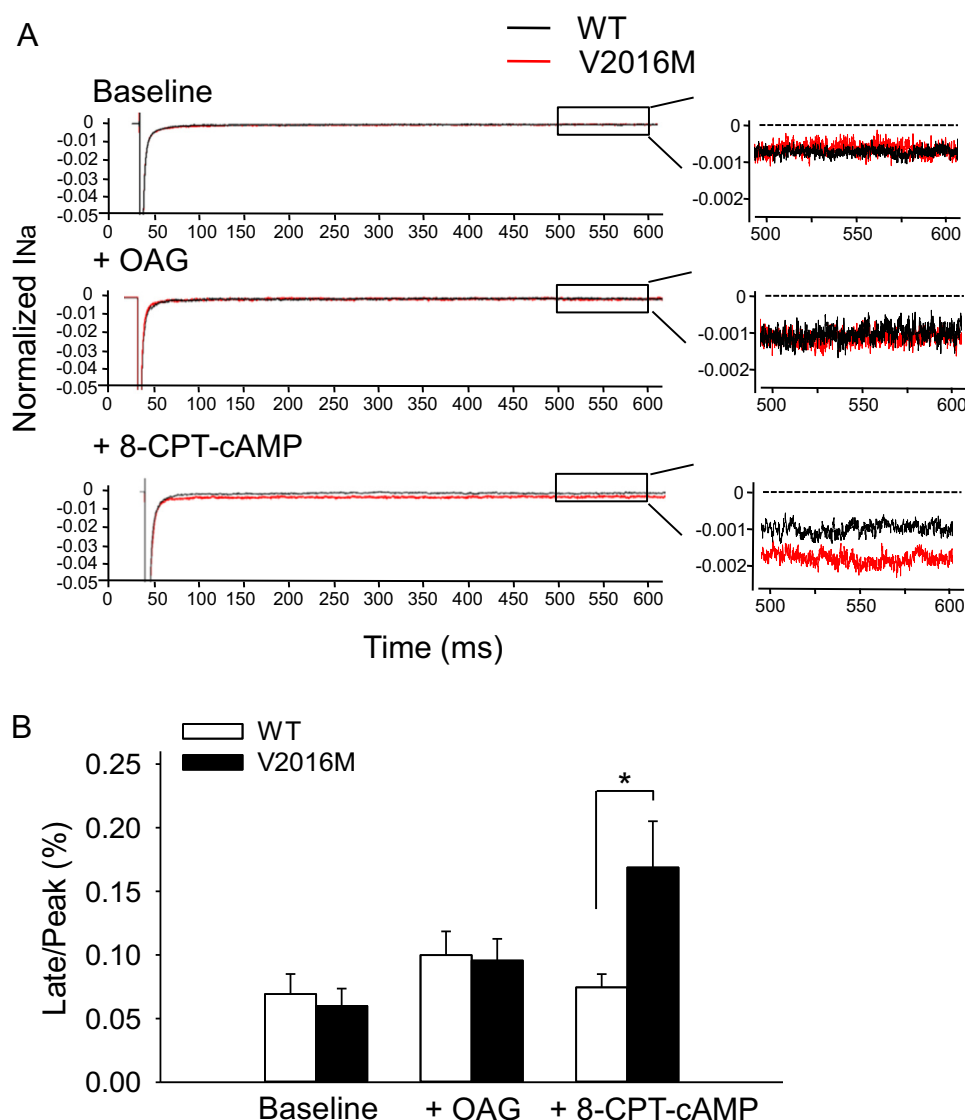


Figure 6 Effect of 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate (8-CPT-cAMP) and 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) on late Na^+ currents in wild-type (WT) and V2016M channels. **A:** Representative tetrodotoxin-subtracted whole-cell currents for WT (black line) and V2016M (red line). Late Na^+ currents were measured over an 800-ms pulse at -20 mV and normalized to the peak current. Inset shows late Na^+ currents between 500 ms and 600 ms. **B:** The percentage of late Na^+ currents relative to peak Na^+ currents of WT and V2016M channels at baseline and in the presence of 8-CPT-cAMP or OAG. Data are mean $\pm$ SEM for $n = 4$ to 6 cells. * $P < .05$.

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CLINICAL PERSPECTIVES

Previous clinical studies indicated that cardiac events in LQT3 patients tended to occur during sleep or at rest. β -Blockers have been shown to be effective to prevent them in some LQT3 patients. In our study, we identified an *SCN5A* mutation in a patient with sinus node dysfunction and epinephrine-induced QT prolongation, which was an atypical phenotype for LQT3. Functional analyses revealed that V2016M channels exhibited overlapping electrophysiological properties, with both gain- and loss-of-function types of modulations. These electrophysiological properties were thought to be associated with the overlapping clinical features of the patient. The gain-of-function-type modulation of the mutant channels under PKA or PKC stimulation might be a novel mechanism predisposing to exercise- or epinephrine-induced LQTS. This finding may explain in part why β -blockers are very effective in some LQT3 patients. Additional genetic testing on *SCN5A* will be necessary for patients with exercise-induced LQTS who are negative for *KCNQ1*, *KCNE1*, or *AKAP9* for future therapeutic management.