α1-Syntrophin Variant Identified in Drug-Induced Long QT Syndrome Increases Late Sodium Current

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Abstract

Drug-induced long-QT syndrome (diLQTS) is often due to drug block of \(I_{Kr}\), especially in genetically susceptible patients with subclinical mutations in the \(I_{Kr}\)-encoding KCHN2. Few variants in the cardiac NaV1.5 Na\(^+\) channel complex have been associated with diLQTS. We tested whether a novel SNTA1 (α1-syntrophin) variant (p.E409Q) found in a patient with diLQTS increases late sodium current \((I_{Na-L})\), thereby providing a disease mechanism. Electrophysiological studies were performed in HEK293T cells co-expressing human NaV1.5/nNOS/PMCA4b with either wild type (WT) or SNTA1 variants (A390V-previously reported in congenital LQTS; and E409Q); and in adult rat ventricular cardiomyocytes infected with SNTA1 expressing adenoviruses (WT or one of the two SNTA1 variants). In HEK293T cells and in cardiomyocytes, there was no significant difference in the peak \(I_{Na}\) densities among the SNTA1 WT and variants. However, both variants increased \(I_{Na-L}\) (% of peak current) in HEK293T cells (0.58 ± 0.10 in WT vs. 0.90 ± 0.11 in A390V, \(p = 0.048\); vs. 0.88 ± 0.07 in E409Q, \(p = 0.023\)). In cardiomyocytes, \(I_{Na-L}\) was significantly increased by E409Q, but not by A390V compared to WT (0.49 ± 0.14 in WT vs. 0.94 ± 0.23 in A390V, \(p = 0.099\); vs. 1.12 ± 0.24 in E409Q, \(p = 0.019\)). We demonstrated that a novel SNTA1 variant is likely causative for diLQTS by augmenting \(I_{Na-L}\). These data suggest that variants within the NaV1.5-interacting α1-syntrophin are a potential mechanism for diLQTS, thereby expanding the concept that variants within congenital LQTS loci can cause diLQTS.

Introduction

Drug-induced long-QT syndrome (diLQTS) is an acquired disorder, most often due to drug block of \(I_{Kr}\), such as antiarrhythmic agents.\(^{[1–3]}\) Recent data suggests that certain individuals have increased susceptibility to diLQTS because of reduced “repolarization reserve” due to subclinical mutations in the two most common congenital long QT syndrome (cLQTS) loci KCNH2 or KCNQ1, both of which encode K\(^+\) channels.\(^{[4, 5]}\) Addition of \(I_{Kr}\)-blocking drugs in these vulnerable populations may underlie increase in action potential (AP) duration through...
increased late cardiac sodium current ($I_{\text{Na-L}}$), leading to prolongation of QT interval associated with fatal ventricular tachyarrhythmias.[6] Increased $I_{\text{Na-L}}$ due to drug-induced decreased phosphoinositide 3-kinase (PI3K) signaling may also contribute to QT prolongation.[7] Variants in SCN5A, which encodes the cardiac Na$^+$ channel NaV1.5 and is the third most common cLQTs locus, however, have been only rarely associated with diLQTS.[8]

α1-syntrophin (SNTA1) encoded by SNTA1 is a dystrophin-associated protein and a potent regulator of voltage-gated Na$^+$ channels. SNTA1 is a component of the NaV1.5 channel macromolecular complex through an interaction with the pore-forming α subunit’s C terminus.[9–11] SNTA1 contains 4 protein interacting domains: a postsynaptic density protein-95/disc large/zona occludens-1 (PDZ) domain;[12] two pleckstrin homology (PH1 and PH2) domains;[13] and a syntrophin unique (SU) domain.[14] The PDZ domain of SNTA1 interacts with a PDZ binding motif comprised of the last 3 amino acids (serine-isoleucine-valine) of the NaV1.5 COOH-terminus; and SNTA1 also interacts with the plasma membrane Ca-ATPase (PMCA) 4b, thus forming a complex of all three proteins. This complex can inhibit neuronal nitric oxide synthase (nNOS),[10] reducing NOS-mediated NO production.[15] The physiological and clinical relevance of this interaction is highlighted by the previous identification in a patient with LQTS of a variant (A390V) within the PH2 domain of SNTA1 that led to disrupted binding between SNTA1 and to PMCA4b, thereby relieving inhibition of nNOS and resulting in increased pathogenic $I_{\text{Na-L}}$ through S-nitrosylation of NaV1.5, mediated by local accentuated NO production.[16] The increased $I_{\text{Na-L}}$ induces cardiac arrhythmias through prolonging AP duration and reducing repolarization reserve.[17, 18]

Previously, no variants in SNTA1 have been reported in patients with diLQTS. We found a novel SNTA1 variant (p.E409Q) found in a patient with diLQTS. Thus, we investigated whether this SNTA1 variant is pathogenic by increasing the $I_{\text{Na-L}}$. This discovery extends our understanding of how variants in cLQTs loci can increase susceptibility to diLQTS.

**Materials and Methods**

**Genetic Analysis**

Genetic analysis was obtained as a part of routine care for the patient and was performed in a commercial laboratory after obtaining written informed consent from the family. Consent was also given for further analysis of the biophysical properties of the subsequently identified mutation. Specific written consent is not required by either the Institutional Review Boards or either University of Virginia or Duke University Medical Centers for presentation of a case report.

**Subcloning and adenovirus production**

The cDNAs of SNTA1 (Genebank accession no. NM_003098.2) in the pIRES2EGFP and nNOS (Genebank accession no. NM_052799.1) in pcDNA3.1 were kind gifts from Jonathan C. Makielski (University of Wisconsin, Madison). The PMCA4b (Genebank accession no. AY560895) was subcloned into pcDNA3.1 (Addgene).

The human SNTA1 plasmid vector was mutated by using Quickchange II Site-Directed Mutagenesis (Agilent Technologies, Santa Clara, CA) and the following primers: for A390V-SNTA1, 5’ GTACCCCGAGGAGCTGGTTGGCTGAGACCCCGACG 3’ (forward) and 5’ GCTGGCGGGTCCAGGCAACCCCACTGGTGAC 3’ (backward); for E409Q-SNTA1, 5’GCCGCCGAGGGTGTGACGCGGTGTCTACAGCCTGCAC 3’ (forward) and 5’ GTGAGCCGGTGAGCAGCAGCAGTGGTCTACAGCCTGCAC 3’ (backward). The underlined and bolded nucleotides indicate the variants. These constructs were then subcloned into the pAdRFP adenovirus shuttle vector. Polymerase chain reactions and bacteria transformations were performed according to the manufacturer’s instructions. WT-SNTA1
and the mutants viruses were generated by using the AdEasy system (Agilent Technologies, Santa Clara, CA).[19] The adenoviral plasmid was packaged in HEK293 cells. The recombinant virus was isolated by multiple freeze/thaw cycles, further amplified and then purified and concentrated using Vivapure AdenoPACK 20 (Sartorius Stedim Biotech, Goettingen, Germany). The viral titer was determined and used at a multiplicity of infection (MOI) of 50–200. All constructs were confirmed by sequencing.

**HEK293T cell transfection and electrophysiology**

HEK293T cells was transfected with tetrodotoxin (TTX)-sensitive NaV1.5, nNOS, PMCA4b and the pIRES2EGFP plasmid vector expressing either human WT or SNTA1 mutants (A390V and E409Q) at a ratio of 4:4:4:1 at a confluency of 60% using Lipofectamine 2000 (Life Technologies). The cells were incubated at 37°C for 2 to 3 days before use. Transfected cells were identified by green fluorescent protein (GFP).

Na+ currents were recorded using the whole-cell voltage-clamp technique at room temperature (20–22°C) 48–72 hours after transfection, as previously described.[20] The bath solution containing (in mM, 300 mosm): NaCl 120, TEA-Cl 20, KCl 5.4, CaCl2 1.8, MgCl2 1, HEPES 10, D(+)-glucose 10, pH 7.4 adjusted with NaOH. The pipette solution containing (in mM, 290–295 mosm): CsCl 50, CsF 30, L-aspartic acid 50, EGTA 5, HEPES 10, NaCl 10, pH 7.3 adjusted with CsOH. Osmolarity was adjusted with sucrose for all solutions. Electrode resistance ranged from 2 to 4 MΩ. Standard step-pulse voltages were generated with Axopatch 200B amplifier using pClamp 9.0 software (Axon Instruments). Currents were filtered at 5 kHz and digitalized using an analog-to-digital interface (Digidata 1322A, Axon Instruments). To measure current amplitude data and voltage-dependence of steady-state activation, currents were elicited by a 50 ms pulse from a holding potential of -120 mV to test potentials between -100 mV and +60 mV in 5 mV increments. Current density (pA/pF) was calculated by normalization to cell capacitance. Conductance (G) was calculated by dividing the peak current for each voltage step by the driving force (Vm−V1/2), then normalizing to the peak conductance (Gmax). The data were fitted with the Boltzmann function of the form \[ \frac{G}{G_{\text{max}}/k} = 1 / \left[ 1 + \exp \left( \frac{V_{1/2} - V_{m}}{k} \right) \right] \]

where \(V_{1/2}\) is the voltage at which half of NaV1.5 channels are activated, \(k\) is the slope factor, and \(V_m\) is the membrane potential. Standard two-pulse protocols were used to generate the steady-state inactivation curves: from the holding potential -120 mV, cells were stepped to 500-ms preconditioning potentials varying between -140 mV and -20 mV (prepulse), followed by a 20 ms test pulse to -40 mV. Currents (I) were normalized to \(I_{\text{max}}\) and fit to a Boltzmann function of the form \[ \frac{I}{I_{\text{max}}} = 1 / \left[ 1 + \exp \left( \frac{V_{1/2} - V_{m}}{k} \right) \right] \]

where \(V_{1/2}\) is the voltage at which half of NaV1.5 channels is inactivated, \(k\) is the slope factor, and \(V_m\) is the membrane potential. Recovery from inactivation was analyzed by fitting data with the two exponential function: \[ \frac{I(t)}{I_{\text{max}}} = A_f \times \left[ 1 - \exp \left( -t/\tau_f \right) \right] + A_s \times \left[ 1 - \exp \left( -t/\tau_s \right) \right] \]

where values for \(A\) and \(\tau\) refer to amplitudes and time constants, respectively. \(I_{\text{Na-L}}\) was defined with 200-ms depolarization from -120 mV to -10 mV as the average between 145–150 ms after the initiation of the depolarization and reported as a percentage of peak current following digital subtraction of currents recorded in the presence and absence of 1 μmol/L TTX (AbcamBiochemicals) as previously described.[21] Curve fitting and data analysis were performed using Clampfit 10.5 software (Axon Instruments) and Origin 9.1 (Originlab Corporation).

**Isolation, culture, and adenoviral infection of adult rat ventricular myocytes**

Animals were handled according to National Institutes of Health’s *Guideline for the Care and Use of Laboratory Animals*. The study was approved by Duke University Animal Care and
Welfare Committee. Cardiomyocytes were isolated from 6- to 8-week-old Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) and cultured as described previously.[22, 23] The origin of Sprague-Dawley rats was to SASCO from ARS/Sprague Dawley in 1979/to Charles River in 1996. Rats were housed in cages in a sterilized room in which temperature and humidity ranges are controlled appropriate for the rats (20–26°C, 30–70% humidity; 10–15 fresh-air changes per hour provided by ventilation). A time-controlled lighting system was used to ensure a regular diurnal cycle, and timer performance is checked periodically to ensure proper cycling. Animals were fed palatable, non-contaminated, and nutritionally adequate food daily based on comprehensive treatments of the nutrient requirements of laboratory animals prepared by the National Research Council Committee on Animal Nutrition. The rats had access to potable, uncontaminated drinking water. Animals were anesthetized with tribromoethanol (250 mg/kg, intraperitoneal injection) and anti-coagulated with heparin following Duke University Animal Care & Use Program guidelines for systemic anesthetics in rats. We confirmed that the rats were completely anesthetized prior to removing their hearts. Hearts were removed and the aorta was cannulated to retrogradely perfuse the heart using a Langendorff apparatus (Radnoti Glass Technology, Inc) for about 10 minutes. The heart were first perfused with basal solution containing (in mM, from Sigma unless otherwise specified): NaCl 112, KCl 5.4, NaH2PO4·2H2O 1.7, NaHCO3 4.2, MgCl2·6H2O 1.63, HEPES 20.04, D(+)-glucose 5.4, taurine 10, L-carnitine 2, creatinine 2.3, glucose 5.4, taurine 10, L-carnitine 2, creatine 2.3, 2,3-butanedione monoxime (BDM) 10. After five minutes, the solution was switched to basal solution plus 150 u/ml Collagenase Type II (Worthington) and the heart was perfused until it was soft and boggy. The heart was then taken down from the Langendorff. Both ventricles were minced into small pieces, and then triturated in enzyme solution until all cell clumps were broken. The solution was filtered through sterile 190 μm nylon mesh and centrifuged at 300 rpm for 2 minutes. The cells were resuspended in perfusion solution with bovine serum albumin (BSA) at 5 mg/ml to quench the enzyme. Calcium tolerance was performed by gradually adding CaCl2 to a final concentration of 1 mM. For culture, cells were plated on laminin coated coverslips in plating medium of Minimal Essential Medium (MEM) with Earle’s Salts and L-glutamine, 10 mM BDM, 5% fetal bovine serum (Life Technologies) and 1% penicillin/streptomycin. After cells had adhered to the plates, the cells were washed once. Virus was resuspended in culture medium and the plating medium changed to culture medium into which the proper adenovirus had been added. Culture medium contained MEM with Earle’s Salts and L-glutamine, bovine serum albumin 0.1 mg/ml, BDM 10 mM, 1X insulin-selenium- transferrin supplement (Gibco), creatine 5 mM, taurine 5 mM, L-carnitine 2 mM, and blebbistatin 25 μM (Toronto Research Chemicals). All solutions were oxygenated in 95% O2/5% CO2 for at least 30 minutes. Cells were checked for RFP fluorescence 36–48 hours post infection. Rod-shaped, striated cells were analyzed for electrophysiology.

Cardiomyocyte electrophysiology

Na+ currents were recorded by using the whole-cell voltage clamp technique in cardiomyocytes, as described previously.[22, 23] Voltage-clamp experiments were performed at room temperature (22–24°C), 36–48 hours after infection of adult cardiomyocytes with adenovirus. Bath (Tyrode) solution contained (in mM): NaCl 140, KCl 5.4, CaCl2 1, MgCl2·6H2O 1, HEPES 5, glucose 10, pH 7.2 adjusted with NaOH. Once the cell was ruptured, solution was quickly changed to recording solution containing (in mM): NaCl 20, HEPES 20, CsCl 55, CaCl2 1, MgCl2 1, CsOH 10, 4-aminopyridine 2, D(+)-glucose 10, CdCl3 0.5, TEA-Cl 50, pH 7.35 adjusted with HCl. Internal solution contained (in mM): NaCl 5, CsF 120, HEPES 5, EGTA 10, GTP-Na sulfate 0.5, TEA-Cl 20, pH 7.35 adjusted with CsOH. Osmolarity was
adjusted to ~300 mOsm with sucrose for all solutions. Recordings were filtered at 5 kHz and digitally sampled at 20 kHz. The pulse protocol cycle time was 3 seconds to ensure full Na⁺ channel recovery. Current amplitude data for each cell were normalized to its cell capacitance (current density, pA/pF). To determine the voltage-dependence of steady-state activation, currents were elicited by a 50 ms pulse from a holding potential of -120 mV to test potentials between -100 mV and +60 mV in 5 mV increments. The sodium conductance (G) was calculated by dividing the peak current for each voltage step by the driving force (V_m - V_h) then normalized to the peak conductance (G_max). Data were fitted with the Boltzmann relationship, G / G_max = 1 / [1 + exp((V_1/2 - V_m) / k)] in which V_1/2 is the voltage at which half of NaV1.5 channels is activated, k is the slope factor and V_m is the membrane potential. Standard two-pulse protocols were used to generate the steady-state inactivation curves: from the holding potential -120 mV, cells were stepped to 500-ms preconditioning potentials varying between -140 mV and -20 mV (prepulse), followed by a 20 ms test pulse to -40 mV. Currents (I) were normalized to I_max and fit to a Boltzmann function of the form I / I_max = 1 / [1 + exp((V_m - V_1/2) / k)] in which V_1/2 is the voltage at which half of NaV1.5 channels is inactivated, k is the slope factor and V_m is the membrane potential. Recovery from inactivation was analyzed by fitting data with the two exponential function: I(t) / I_max = A_1 × [1 - exp(-t / τ_1)] + A_2 × [1 - exp(-t / τ_2)], where values for A and τ refer to amplitudes and time constants, respectively. I_{Na-L} was determined with 200-ms depolarization from -120 mV to -40 mV as the average between 190–200 ms after the initiation of the depolarization and reported as a percentage of peak current following digital subtraction of currents recorded in the presence and absence of 30 μmol/L TTX (Abcam Biochemicals) as previously described. The specific groups were blinded to an investigator when the measurements. These data were measured Curve fitting and data analysis were performed using Clampfit 10.5 software (Axon Instruments) and Origin 9.1 (Originlab Corporation).

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM). Statistical significance of differences between the groups was assessed using Student’s t-test. For experiments with deviations from normality, the nonparametric Mann-Whitney U test was used for comparison. Values of p ≤ 0.05 were considered statistically significant. Statistical analyses were carried out using SPSS Statistics software version 17.0 (IBM, Armonk, NY).

Results

Case report

A 36 year old previously healthy man with no family history of sudden cardiac death or LQTS suffered a witnessed cardiac arrest while exercising. At the time of his arrest his medications included amitriptyline, pseudoephedrine, and famotidine. His initial rhythm was ventricular fibrillation from which he was successfully resuscitated with an external countershock. Coronary angiography showed no evidence of coronary artery disease. Cardiac MRI showed an EF of 65% with no evidence of right ventricular dysplasia. ECGs in the post-arrest period were notable for prolonged QT intervals (repeated QTc measurements > 480 ms; the longest was 597 ms) (Fig 1A). ECGs obtained in the month after the arrest and in all follow up visits showed normal QT intervals (all QTc measurements < 440 ms) after discontinuation of amitriptyline, pseudoephedrine, and famotidine (Fig 1B).

The patient was prescribed β-blocker therapy and underwent implantable cardioverter-defibrillator (ICD) implantation. He received one ICD shock for polymorphic ventricular tachycardia after re-initiation of vigorous exercise while he was only intermittently compliant with β-blocker therapy.
Genetic testing and sequence homologies for the \textit{SNTA1} variants

Genetic testing with a 30-gene arrhythmia panel revealed a novel heterozygous missense variant (c.1225 \textgreater C; p.Glu409Gln, p.E409Q) in \textit{SNTA1}. The pedigree is shown in Fig 1C. This variant is within a residue highly conserved across species (Fig 1D), and is near to the originally reported A390V \textit{SNTA1} variant associated with LQTS,[16] which we used as a positive control. The p.E409Q \textit{SNTA1} variant is not present in normal population databases including the Exome Aggregation Consortium (ExAC),[24] the NHLBI ESP Exome Variant Server (EVS),[25] and the 1000 Genomes Project.[26] Multiple in-silico analyses predicted the \textit{SNTA1} variant, p.E409Q, to be pathogenic: PolyPhen-2 (prediction = probably damaging, score = 1.000),[27] MutationTaster2 (prediction = disease-causing, probability value = 0.999),[28] and SIFT (prediction = damaging, score = 0).[29]

Biophysical properties of Na\textsubscript{\textit{V}}1.5 Co-expressed with PMCA4b, nNOS, and SNTA1

We recorded voltage-gated Na\textsuperscript{+} currents in HEK293T cells, in which we transiently co-expressed components of the Na\textsuperscript{+} channel macromolecular complex necessary for regulation by \textit{SNTA1}. Specifically, we expressed human Na\textsubscript{\textit{V}}1.5 (with a C373Y mutation rendering the channel sensitive to TTX), nNOS, and PMCA4b with WT SNTA1 or either of the two SNTA1 mutants. Previous studies have shown that the TTX-sensitive mutation does not affect any permeation properties.[30] Table 1 shows the summary data for the 3 groups. Representative
traces of whole-cell currents (Fig 2A) and I-V curves (Fig 2B) show that neither mutant affected peak $I_{Na}$ current density nor the kinetics of activation compared to WT-SNTA1 (Fig 2C). The $k$ of inactivation was significantly reduced only in E409Q-SNTA1 ($p < 0.001$). There was no significant difference in rate of fast recovery from inactivation using a two-pulse protocol among WT and the mutants, but the rate of slow recovery was significantly prolonged in A390V-SNTA1 compared to WT-SNTA1 ($p = 0.035$) (Fig 2D). Focusing only on the mutants, there was no significant difference in all parameters of activation, inactivation and recovery (Table 1).

**SNTA1 variants increase late $I_{Na}$ in HEK293T cells**

The $I_{Na-L}$ was measured using a long depolarization pulse (200 ms at -10 mV from a holding potential of -120 mV) and background was subtracted by administration of TTX (1 μM). Representative traces are shown in Fig 3A and the data are summarized in Table 1. $I_{Na-L}$ (% of peak current) was significantly increased with both mutants compared to WT-SNTA1 (0.58±0.097 in WT vs. 0.899±0.110* in A390V-SNTA1, $p = 0.048$; vs. 0.883±0.065* in E409Q-SNTA1, $p = 0.023$) (Fig 3B). There was no significant difference in $I_{Na-L}$ between A390V-SNTA1 and E409Q-SNTA1 ($p = 0.903$).

**Biophysical properties of sodium currents in adult rat cardiomyocytes**

To confirm these results, we also recorded voltage-gated Na$^+$ currents in cultured adult rat cardiomyocytes 36–48 h after they were infected with WT or either of the two SNTA1 variants. Because the patient is heterozygous for the SNTA1 variant, we expressed the WT or the variants without knocking down the endogenous Snta1 in the rat cardiomyocytes, thus more accurately recapitulating the patient’s condition in which the WT and variant were both present. Table 2 shows the summary data for the 3 groups. Representative traces of whole-cell currents were shown in Fig 4A. I-V curves (Fig 4B) show that neither mutant affected peak $I_{Na}$ current density (WT-SNTA1 vs. A390V-SNTA1, $p = 0.895$; vs. E409Q-SNTA1, $p = 0.929$). In addition, there was no significant difference in the current voltages between the variants (A390V-SNTA1 vs. E409Q-SNTA1, $p = 0.994$). The kinetics of activation and steady-state
inactivation of WT and mutants were shown in Fig 4C and 4D. There was no significant difference in kinetics of activation and steady-state inactivation between WT and either of the SNTA1 variants. Compared to WT-SNTA1, the rates of recovery from inactivation appeared to be slightly delayed in E409Q-SNTA, but there was no statistical significance (Fig 4E).

SNTA1 Variant in Drug-Induced Long QT Syndrome

Fig 2. Electrophysiological data of Na\textsubscript{v}1.5 in HEK293T cells coexpressing PMCA4b, nNOS, and either WT or SNTA1 mutants. (A) Representative traces of inward Na\textsuperscript{+} current for the 3 groups tested. (B) I-V curve. (C) Activation (G/G_{max}). (D) Inactivation (I/I_{max}). (E) Recovery (P2/P1).

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The $I_{\text{Na-L}}$ was measured using a long depolarization pulse (200 ms at -40 mV from a holding potential of -120 mV) and background was subtracted after TTX (30 μM) was applied to the bath. Representative traces are shown in Fig 5A and the data are summarized in Table 2. Compared to WT-STNA1, $I_{\text{Na-L}}$ (% of peak current) was significantly increased with E409Q mutant, but not the A390V mutant (0.49±0.14 in WT-SNTA1 vs. 0.94±0.23 in A390V-SNTA1, $p = 0.099$; vs. 1.12±0.24 in E409Q-SNTA1, $p = 0.019$) (Fig 5B). There was no significant difference in $I_{\text{Na-L}}$ between A390V-SNTA1 and E409Q-SNTA1 ($p = 0.903$).

**Discussion**

**Principal findings**

This is the first report to our knowledge of a SNTA1 variant in a patient with diLQTS and a first demonstration that SNTA1 affects $I_{\text{Na-L}}$ in adult rat cardiomyocytes. We provide functional data demonstrating a gain-of-function for a novel SNTA1 variant, p.E409Q, leads to diLQTS by augmenting $I_{\text{Na-L}}$. Overall, the effects of the p.E409Q variant on Na⁺ currents are
generally similar to the effects of the p.A390V variant that was the original LQT mutation described in\textit{SNTA1}.\textsuperscript{16}

Syntrophin mutation and late sodium currents

\textit{SNTA1} is now a well-established a Na\textsubscript{V}1.5 channel interacting protein (NaChIP) in complex with nNOS and PMCA4b.\textsuperscript{[31]} Mutations lead to gain-of-function modulations of Na\textsubscript{V}1.5 (increased $I_{Na-L}$) and cLQTS. Ueda et al. reported a missense mutation (p.A390V) within the PH2 domain of \textit{SNTA1} disrupted its binding with PMCA4b, thereby disinhibiting nNOS, which caused S-nitrosylation of Na\textsubscript{V}1.5 and a resultant increase in $I_{Na-L}$.\textsuperscript{[16]} The novel variant tested here, p.E409Q lies outside of the PH2 domain. Thus, the data showing that p.E409Q affects Na\textsubscript{V}1.5 currents similarly to p.A390V in both cardiomyocytes, and in a heterologous system in which all the key components of the macromolecular complex are present, suggest that E409Q affects PMCA4b interaction similarly to A390V. A schematic depicting this proposed interaction and the consequent mechanism for increased $I_{Na-L}$ is shown in Fig 6.

Recent cohort-based studies in patients with cLQTS reported that increased $I_{Na-L}$ due to mutations in \textit{SNTA1} is a pathogenic mechanism for cLQTS and a subset of channelopathic sudden infant death syndrome (SIDS),\textsuperscript{[32, 33]} and the mutations also represented gain-of-function of $I_{Na}$. Our data also showed that two \textit{SNTA1} variants increase $I_{Na-L}$ without causing a significant difference in peak $I_{Na}$ compared to wild type \textit{SNTA1}.

The absence of an effect on peak current (recorded in the absence of TTX) is an important result from our studies that helps add confidence to our measurements of $I_{Na-L}$, which is a generally challenging analysis given its relatively small amplitude. Because peak current was unchanged, it is unlikely that observed increase in the small amplitude $I_{Na-L}$ (for which we used TTX to effectively isolate $I_{Na-L}$ from other currents) simply reflects a change in the peak

| Table 2. Summary of electrophysiological data in the adult rat cardiomyocytes. |
|-------------------------------|-----------------|-----------------|-----------------|
|                                | SNTA1-WT        | SNTA1-A390V     | SNTA1-E409Q     |
| Peak $I_{Na}$ at -40mV, pA/pF | -6.8±0.8 (13)   | -6.6±1.1 (7)    | -6.6±1.6 (14)   |
| Activation                     |                 |                 |                 |
| $V_{1/2}$, mV                  | -44.5±1.2 (13)  | -47.9±1.8 (7)   | -44.1±1.4 (14)  |
| $k$, pA/mV                     | 4.4±0.1 (13)    | 4.3±0.2 (7)     | 4.0±0.2 (14)    |
| Inactivation                   |                 |                 |                 |
| $V_{1/2}$, mV                  | -83.1±1.1 (12)  | -83.6±0.6 (7)   | -81.3±1.5 (10)  |
| $k$, pA/mV                     | 4.8±0.2 (12)    | 4.9±0.3 (7)     | 4.7±0.2 (10)    |
| Recovery (P2/P1)               |                 |                 |                 |
| tau, ms                        | 10.9±1.6 (11)   | 25.5±8.4 (5)    | 7.1±1.3 (8)     |
| tau fast recovery, ms          | 8.8±1.7 (11)    | 23.3±8.6 (5)    | 6.8±1.4 (8)     |
| tau slow recovery, ms          | 119.7±20.6 (11) | 97.2±42.7 (5)  | 89.3±31.6 (8)   |
| $I_{Na-L}$, %                  | 0.49±0.14 (8)   | 0.94±0.23 (5)   | 1.12±0.24* (6)  |

* P-value < 0.05 versus SNTA1-WT.
current. Rather, it likely reflects a true change in inactivation properties of the channel as influenced by the channel’s macromolecular complex. Two additional factors add to our confidence in accurately measuring this small amplitude $I_{\text{Na-L}}$. First, we performed the experiments in both HEK cells and myocytes, and obtained consistent results. Second, the analysis of the $I_{\text{Na-L}}$. 

Fig 4. Electrophysiological data of Na+ current in adult rat cardiomyocytes which were infected with either WT or SNTA1 mutants. (A) Representative traces of inward Na+ current for the 3 groups tested. (B) I-V curve. (C) Activation (G/Gmax). (D) Inactivation ($I_{\text{max}}$). (E) Recovery (P2/P1). doi:10.1371/journal.pone.0152355.g004
amplitude was performed while blinded to the SNTA1 genotype, thus avoiding any unintentional bias.

While generally consistent with previous studies of SNTA1 variants, our results are slightly different in a subset of electrophysiologic parameters. In HEK293T cells, the E409Q mutant showed a reduced $k$ of inactivation whereas previous studies demonstrated a reduced $V_{1/2}$ but not $k$ of inactivation. In addition, we tested the electrophysiologic properties of SNTA1 mutants in adult rat cardiomyocytes using adenoviral expression. In our data, the recovery from inactivation appeared to be different in comparing HEK293T cells and cardiomyocytes. The reasons for this are not known, but it could be that there are additional regulatory components in the native myocytes that are regulating the interaction of SNTA1 and SCN5A. Alternatively, the stoichiometry of the transfected/infected components may be different between the two systems. Nevertheless, these data, querying the roles of wild type and mutant SNTA1 proteins in their native environment and in an adult cardiomyocyte add new information beyond the previous studies.

**Fig 5.** Late Na⁺ current in adult rat cardiomyocyte infected with adenoviruses expressing either the WT or one of the two SNTA1 mutants. (A) Representative late Na⁺ currents in with WT and the SNTA1 variants (Fig 5A). (B) E409Q-SNTA1 significantly increased $I_{Na-L}$ compared to that of WT-SNTA1. * P<0.05 versus WT-SNTA1. $I_{Na}$ indicates sodium current; TTX, tetrodotoxin. Mean and standard error of mean (SEM) are shown in the graph.

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**SNTA1: A novel susceptibility gene for diLQTS**

The voltage gated cardiac sodium channel is known to be responsible to a subgroups of LQTS (LQT3). Other subgroups of LQTS (LQT9, LQT10 and LQT12) also affect the β subunit of Na\(_\text{v}1.5\) or the NaChIPs, such as caveolin-3 and SNTA1, leading to increased \(I_{\text{Na-L}}\). Our patient was diagnosed with diLQTS, an acquired or iatrogenic disease, which has been described most commonly as caused by repolarization abnormalities due to block of potassium channels. The notched T wave shown in Fig 1A is consistent with the pattern observed after \(I_{\text{Kr}}\) block. \(I_{\text{Kr}}\) (hERG channel) blocking drugs, prokinetics or antiarrhythmic drugs, decrease a patient’s "repolarization reserve" and then can prolong the QT interval. The patient studied here, was exposed to three drugs (the tricyclic antidepressant amitriptyline; pseudoephedrine; and the H\(_2\) receptor antagonist famotidine) before his episode of aborted sudden death, two of which (amitriptyline and pseudoephedrine) are known to cause QT interval prolongation. Thus, we conclude that this episode of diLQTS resulted from an underlying genetic susceptibility due to a mutation in a cLQTS locus (SNTA1) exacerbated by a reduction in the repolarization reserve caused by amitriptyline and pseudoephedrine. Consistent with our hypothesis, Itoh et al. reported that diLQTS had a similar positive mutation rate compared to cLQTS and QT prolongation by \(I_{\text{Kr}}\)-blocking agents was excessive in diLQTS subjects with SNTA1 variants.
the underlying genetic background. They concluded that certain individuals may have increased susceptibility to diLQTS because of reduced "repolarization reserve" due to subclinical mutations in the cLQTS loci KCNH2 or KCNQ1, both of which encode K⁺ channels. Our results expand this concept to include susceptibility due to a variant in a SNTA1. In previous reports regarding pathologic SNTA1 variants, the affected patients presented with a cLQTS phenotype in contrast to the index patient here, who had an aborted sudden death event in the setting of medications and who showed normal QT intervals on ECGs after discontinuation of the drugs. In addition, the proband’s family members carrying the same variants also showed normal QTc interval (Fig 1C) and no evidence of symptoms. A reasonable explanation for these observations is that these individuals might harbor genetic modifier protecting from the cLQTS, but in the absence of whole exome sequencing we cannot be certain.

The connection between a reduced repolarization reserve via K⁺ channel mutations and susceptibility due to a mutation in the NaV1.5 macromolecular complex has been suggested by several recent reports. Wu et al. demonstrated that endogenous late $I_{Na}$ contributed to the reverse rate dependence of $I_{Kr}$ inhibitor-induced increases in action potential duration and beat-to-beat variability of repolarization, which are proarrhythmic.[6] Recently, Yang et al. showed some $I_{Kr}$ blockers with torsades liability, such as dofetilide, increase $I_{Na-L}$ through inhibition of phosphoinosotide 3-kinase (PI3K) pathway.[5] Exposure to dofetilide generated arrhythmogenic afterdepolarizations and ≥ 15-fold increases in $I_{Na-L}$, and a downstream effector for the PI3K pathway inhibited these effects.5 Many anti-cancer drugs that target the PI3K signaling have been developed, and inhibition of the PI3K pathway has been reported as the cause of a diLQTS in which alterations in several ion currents contribute to arrhythmogenic drug activity.[39] Lin and Cohen et al demonstrated cardiac myocytes of mice with diabetes exhibited an increase in action potential duration (APD) by altering $I_{Na-L}$, which was reversed by expression of constitutively active PI3K.[40] While the specific drugs used by the proband in this study have not been shown to affect PI3K, neither were drugs such as dofetilide until these recent studies. Thus, an alternative mechanism that could explain this proband’s arrhythmia is through inhibition of the PI3K pathway or that the SNTA1 variant renders the channel complex more susceptible to changes in PI3K metabolites.

Nevertheless, those studies firmly identify the NaV1.5 cardiac sodium channel as an important mediator of diLQTS and fit with prior studies, such as Makita et al., which observed that subclinical mutations (L1825P) in the LQTS-related gene SCN5A might predispose certain individuals to diLQTS when treated with the prokinetic drug cisapride, a K⁺ channel blocking agent.[41] S1103Y, a common SCN5A variant, has been associated with a predisposition to abnormal cardiac repolarization and acquired arrhythmia when cardiac potassium channel blocking medications, such as amiodarone, were administered.[8] [42] In this context, our data are consistent with those reports and extend the model beyond NaV1.5 mutations to NaChIPs.

Beyond diLQTS and cLQTS, $I_{Na-L}$ in cardiomyocytes can be increased by acquired conditions such as heart failure.[42] Whether variants in SNTA1 increase the risk of arrhythmias in heart failure patients has not been tested, but would fit with a previous study showing that the S1103Y polymorphism in SCN5A confers an increased risk of arrhythmogenesis in patients with heart failure.[43] In summary, our study demonstrated that a novel SNTA1 variant, p. E409Q, increased the $I_{Na-L}$ and is a potential mechanism for acquired lethal ventricular arrhythmias.

**Clinical implications**

This study may provide additional motivation not only for genetic screening in patients experiencing diLQTS, but it suggests that a broad panel of cLQTS loci should be tested—not
only KCNQ1 or KCHAN2. Identification of variants in cLQTS loci in a patient suffering from diLQTS may be motivation for cascade screening and consequent advice to affected family members to avoid known QT prolonging drugs. Further, for those patients suffering diLQTS in the setting of SNTA1 or variants in other NaChIPs, treatment with agents targeting $I_{\text{Na-L}}$ such as ranolazine may be a reasonable strategy,[44] particularly in cases when concomitant treatment with a QT prolonging agent is deemed necessary.

Conclusions
In conclusion, we demonstrated a novel SNTA1 variant, E409Q-SNTA1, leads to diLQTS by augmenting $I_{\text{Na-L}}$. These data suggest the variant of the NaV1.5-interacting α1-syntrophin is a potential mechanism for the genetic susceptibility in patients with diLQTS, thereby expanding, beyond K+ channel loci, the concept that variants within cLQTS can cause diLQTS.

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Author Contributions
Conceived and designed the experiments: J-IC GSP. Performed the experiments: J-IC CW. Analyzed the data: J-IC GSP. Contributed reagents/materials/analysis tools: J-IC CW MJT GSP. Wrote the paper: J-IC MJT GSP.

References
1. Zipes DP. Proarrhythmic effects of antiarrhythmic drugs. The American journal of cardiology. 1987; 59 (11):26E–31E. Epub 1987/04/30. PMID: 2437787.
2. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the Ikr potassium channel. Cell. 1995; 81(2):299–307. doi: http://dx.doi.org/10.1016/0092-8674(95)90340-2 PMID: 7736582
3. Roden DM. Drug-induced prolongation of the QT interval. The New England journal of medicine. 2004; 350(10):1013–22. Epub 2004/03/05. doi: 10.1056/NEJMra032426 PMID: 14999113.
4. Roden DM. Repolarization reserve: a moving target. Circulation. 2008; 118(10):981–2. Epub 2008/09/04. doi: 10.1161/circulationaha.108.798918 PMID: 18763886.
5. Yang T, Chun YW, Stroud DM, Mosley JD, Knollmann BC, Hong C, et al. Screening for acute Ikr block is insufficient to detect torsades de pointes liability: role of late sodium current. Circulation. 2014; 130 (3):224–34. Epub 2014/06/05. doi: 10.1161/circulationaha.113.007765 PMID: 24895457; PubMed Central PMCID: PMCPMC4101031.
6. Wu L, Ma J, Li H, Wang C, Grandi E, Zhang P, et al. Late sodium current contributes to the reverse rate-dependent effect of Ikr inhibition on ventricular repolarization. Circulation. 2011; 123(16):1713–20. Epub 2011/04/13. doi: 10.1161/circulationaha.110.000661 PMID: 21482963; PubMed Central PMCID: PMCPMC4028960.
7. Lu Z, Wu CY, Jiang YP, Ballou LM, Clausen C, Cohen IS, et al. Suppression of phosphoinositide 3-kinase signaling and alteration of multiple ion currents in drug-induced long QT syndrome. Science translational medicine. 2012; 4(131):131ra50. Epub 2012/04/28. doi: 10.1126/scitranslmed.3003623 PMID: 22539774; PubMed Central PMCID: PMCPMC3492828.
8. Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. Science. 2002; 297(5585):1333–6. Epub 2002/08/24. doi: 10.1126/science.1073569 PMID: 12193783.
9. Gee SH, Madhavan R, Levinson SR, Caldwell JH, Sealock R, Froehner SC. Interaction of muscle and brain sodium channels with multiple members of the syntrophin family of dystrophin-associated proteins. The Journal of neuroscience: the official journal of the Society for Neuroscience. 1998; 18 (1):129–37. Epub 1998/01/24. PMID: 9412493.
Bhat HF, Adams ME, Khanday FA. Syntrophin proteins as Santa Claus: role(s) in cell signal transduction. Cellular and molecular life sciences: CMLS. 2013; 70(14):2533–54. Epub 2012/12/25. doi: 10.1007/s00018-012-1233-9 PMID: 23263165.

11. Adams ME, Mueller HA, Froehner SC. In vivo requirement of the alpha-syntrophin PDZ domain for the sarcosomal localization of nNOS and aquaporin-4. The Journal of cell biology. 2001; 155(1):113–22. Epub 2001/09/26. doi: 10.1083/jcb.200106158 PMID: 11571312; PubMed Central PMCID: PMCPMC2150783.

12. Zhao C, Yu D-H, Shen R, Feng G-S. Gab2, a New Pleckstrin Homology Domain-containing Adapter Protein, Acts to Uncouple Signaling from ERK Kinase to Elk-1. Journal of Biological Chemistry. 1999; 274(28):19649–54. doi: 10.1074/jbc.274.28.19649 PMID: 10391903.

13. Ahern GP, Hsu S-F, Klyachko VA, Jackson MB. Induction of Persistent Sodium Current by Exogenous and Endogenous Nitric Oxide. Journal of Biological Chemistry. 2000; 275(37):28810–5. doi: 10.1074/jbc.M003090200 PMID: 10833522.

14. Williams JC, Armesilla AL, Mohamed TMA, Schomburg S, et al. The Sarcolummal Calcium Pump, α-1 Syntrophin, and Neuronal Nitric-oxide Synthase Are Parts of a Macromolecular Protein Complex. Journal of Biological Chemistry. 2006; 281(33):23341–8. doi: 10.1074/jbc.M513414200 PMID: 16735509.

15. Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, et al. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(27):9355–60. doi: 10.1073/pnas.0801294105 PMID: 18591664; PubMed Central PMCID: PMC2442127.

16. Luo J, Deng Z-L, Luo X, Tang N, Song W-X, Chen J, et al. A protocol for rapid generation of recombinant adenoviruses using the AdEasy system. Nature protocols. 2007; 2(5):1236–47. PMID: 17546019.

17. Shryock JC, Song Y, Rajamani S, Antzelevitch C, Belardinelli L. The arrhythmogenic consequences of increasing late Ina in the cardiomyocyte. Cardiovascular research. 2013; 99(4):800–11. Epub 2013/06/12. doi: 10.1093/cvr/cvr145 PMID: 23752976; PubMed Central PMCID: PMCPMC3841414.

18. Belardinelli L, Giles WR, Rajamani S, Karagueuzian HS, Shryock JC. Cardiac late Na+ current: Proarrhythmic effects, roles in long QT syndromes, and pathological relationship to CaMKII and oxidative stress. Heart rhythm: the official journal of the Heart Rhythm Society. 2015; 12(4):440–8. doi: http://dx.doi.org/10.1016/j.hrthm.2014.11.009.

19. Luo J, Deng Z-L, Luo X, Tang N, Song W-X, Chen J, et al. A protocol for rapid generation of recombinant adenoviruses using the AdEasy system. Nature protocols. 2007; 2(5):1236–47. PMID: 17546019.

20. Wang C, Wang C, Hoch EG, Pitt GS. Identification of novel interaction sites that determine specificity between fibroblast growth factor homologous factors and voltage-gated sodium channels. The Journal of biological chemistry. 2011; 286(27):24253–63. doi: 10.1074/jbc.M111.245803 PMID: 21566136; PubMed Central PMCID: PMC3129206.

21. Cronk LB, Ye B, Kaku T, Tester DJ, Vatta M, Makielski JC, et al. Novel mechanism for sudden infant death syndrome: Persistent late sodium current secondary to mutations in caveolin-3. Heart rhythm: the official journal of the Heart Rhythm Society. 2015; 12(4):440–8. doi: http://dx.doi.org/10.1016/j.hrthm.2014.11.009.

22. Wang C, Hennessey JA, Kirkton RD, Wang C, Graham V, Puranam RS, et al. Fibroblast growth factor homologous factor 13 regulates Na+ channels and conduction velocity in murine hearts. Circulation research. 2011; 109(7):775–82. doi: 10.1161/CIRCRESAHA.111.247957 PMID: 21817159; PubMed Central PMCID: PMC3383600.

23. Hennessey JA, Wei EQ, Pitt GS. Fibroblast growth factor homologous factors modulate cardiac calcium channels. Circulation research. 2013; 113(4):381–8. doi: 10.1161/CIRCRESAHA.113.301215 PMID: 23804213; PubMed Central PMCID: PMC3813963.

24. Exome Aggregation Consortium (ExAC) C, MA (URL: http://exac.broadinstitute.org) [June 2015].

25. Exome Variant Server NESPE, Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [June 2015].

26. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012; 491(7422):56–65. Epub 2012/11/07. doi: 10.1038/nature11632 PMID: 23128226; PubMed Central PMCID: PMCPMC3498066.

27. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7(4):248–50. Epub 2010/04/01. doi: 10.1038/nmeth0410-248 PMID: 20354512; PubMed Central PMCID: PMCPMC2855889.

28. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Meth. 2014; 11(4):361–2. doi: 10.1038/nmeth.2890 http://www.nature.com/nmeth/journal/v11/n4/abs/nmeth.2890.html#supplementary-information.
29. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4(7):1073–81. Epub 2009/06/30. doi:10.1038/nprot.2009.86 PMID: 19561590.

30. Satin J, Kyle JW, Chen M, Bell P, Cribs LL, Fozzard HA, et al. A mutant of TTX-resistant cardiac sodium channels with TTX-sensitive properties. Science. 1992; 256(5060):1202–5. Epub 1992/05/22. PMID: 1375397.

31. Oceandy D, Cartwright EJ, Emerson M, Prehar S, Baudoin FM, Zi M, et al. Neuronal Nitric Oxide Synthase Signaling in the Heart Is Regulated by the Sarcolemmal Calcium Pump 4b. Circulation. 2007; 115(4):483–92. doi:10.1161/circulationaha.106.643791 PMID: 17242280.

32. Cheng J, Van Norstrand DW, Medeiros-Domingo A, Valdivia C, Tan BH, Ye B, et al. Alpha1-syntrophin mutations identified in sudden infant death syndrome cause an increase in late cardiac sodium current. Circulation Arrhythmia and electrophysiology. 2009; 2(6):667–76. doi:10.1161/CIRCEP.109.891440 PMID: 20009079; PubMed Central PMCID: PMC2810855.

33. Wu G, Ai T, Kim JJ, Mohaptra B, Xi Y, Li Z, et al. alpha-1-syntrophin mutation and the long-QT syndrome: a disease of sodium channel disruption. Circulation Arrhythmia and electrophysiology. 2008; 1(3):193–201. doi:10.1161/CIRCEP.108.769224 PMID: 19684871; PubMed Central PMCID: PMC2726717.

34. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell. 1995; 80(5):805–11. Epub 1995/03/10. PMID: 7889574.

35. Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, et al. SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. Circulation. 2007; 116(2):134–42. Epub 2007/06/27. doi:10.1161/circulationaha.106.659086 PMID: 17060380.

36. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation. 2006; 114(20):2104–12. Epub 2006/10/25. doi:10.1161/circulationaha.106.639268 PMID: 17060380.

37. Vicente J, Johannesen L, Mason JW, Crumb WJ, Pueyo E, Stockbridge N, et al. Comprehensive T wave Morphology Assessment in a Randomized Clinical Study of Dofetilide, Quinidine, Ranolazine, and Verapamil. Journal of the American Heart Association. 2015; 4(4). doi:10.1161/jaha.114.001615 PMID: 25870186.

38. Itoh H, Sakaguchi T, Ding WG, Watanabe E, Watanabe I, Nishio Y, et al. Latent genetic backgrounds and molecular pathogenesis in drug-induced long-QT syndrome. Circulation Arrhythmia and electrophysiology. 2009; 2(5):511–23. doi:10.1161/CIRCEP.109.862649 PMID: 19843919.

39. Ballou LM, Lin RZ, Cohen IS. Control of Cardiac Repolarization by Phosphoinositide 3-Kinase Signaling to Ion Channels. Circulation research. 2015; 116(1):127–37. doi:10.1161/circresaha.116.303975 PMID: 25552692.

40. Lu Z, Jiang YP, Wu CY, Ballou LM, Liu S, Carpenter ES, et al. Increased persistent sodium current due to decreased PI3K signaling contributes to QT prolongation in the diabetic heart. Diabetes. 2013; 62(12):4257–65. Epub 2013/08/27. doi:10.2337/db13-0420 PMID: 23974924; PubMed Central PMCID: PMCPmc3837031.

41. Makita N, Horie M, Nakamura T, Ai T, Sasaki K, Yokoi H, et al. Drug-Induced Long-QT Syndrome Associated With a Subclinical SCN5A Mutation. Circulation. 2002; 106(10):1269–74. doi:10.1161/01.cir.0000027139.42087.b6 PMID: 12208804.

42. Valdivia CR, Chu WW, Pu J, Foell JD, Haworth RA, Wolff MR, et al. Increased late sodium current in myocytes from a canine heart failure model and from failing human heart. Journal of molecular and cellular cardiology. 2005; 38(3):475–83. Epub 2005/03/01. doi:10.1016/j.yjmcc.2004.12.012 PMID: 15733907.

43. Sun AY, Koontz JL, Shah SH, Piccini JP, Nilsson KR Jr., Craig D, et al. The S1103Y cardiac sodium channel variant is associated with implantable cardioverter-defibrillator events in blacks with heart failure and reduced ejection fraction. Circulation Cardiovascular genetics. 2011; 4(2):163–8. Epub 2011/04/19. doi:10.1161/circgenetics.110.958652 PMID: 21498565; PubMed Central PMCID: PMCPMC3086077.

44. Hund TJ, Mohler PJ. Nav channel complex heterogeneity: new targets for the treatment of arrhythmia? Circulation. 2014; 130(2):132–4. Epub 2014/06/05. doi:10.1161/circulationaha.114.010867 PMID: 24895456; PubMed Central PMCID: PMCPMC409268.