Calcium Revisited
New Insights Into the Molecular Basis of Long-QT Syndrome

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The first clinical description of long-QT syndrome (LQTS) occurred in 1957 when Drs Anton Jervell and Fred Lange-Nielsen postulated that syncopal/seizure episodes and high propensity for sudden cardiac death (SCD) observed in a subset of children with sensorineural deafness and otherwise unexplained heart rate–corrected QT interval prolongation on ECG stemmed from a novel congenital disorder.1 This was followed by independent descriptions of a syndrome characterized by a cardiac-only phenotype consisting of prolonged QT intervals and an increased risk for syncope, seizures, and SCD in the absence of sensorineural deafness by Drs Cesarino Romano and Owen Ward, in 1963 and 1964, respectively.2,3

During the past 5 decades, insights gleaned from a multitude of clinical, epidemiological, and molecular studies have demonstrated that LQTS is a collection of not only genetically and phenotypically diverse disorders of cardiac repolarization that encompasses the aforementioned predominantly autosomal-dominant, nonsyndromic Romano–Ward syndrome now simply referred to as LQTS4 and autosomal-recessive, multisystem, Jervell–Lange Nielsen syndrome but also exceedingly rare multisystem LQTS subtypes such as Timothy syndrome (TS) characterized by QT prolongation and an increased risk of SCD along with an array of extracardiac manifestations.

Although mutations in calcium (Ca2+)–handling proteins, including the CACNA1C-encoded L-type Ca2+ channel (LTCC), RYR2-encoded ryanodine receptor-2 intracellular calcium release channel (RyR2), and many other auxiliary interacting proteins, are central to the pathogenesis of inherited cardiac arrhythmia syndromes, such as catecholaminergic ventricular tachycardia and Brugada syndrome, until recently, the contribution of dysfunctional Ca2+ handling to the pathogenesis of LQTS was limited to the extremely rare and highly lethal multisystem TS. However, the unexpected recent discoveries of multiple nonsyndromic LQTS-causative mutations in CACNA1C,5–7 syndromic LQTS-causative mutations in CALM1-3-encoded Calmodulin8,9 and TRDN-encoded Triadin,10 and common genetic variation at several novel genetic loci that modulate QT interval duration in health11 that encode or reside near known Ca2+-handling proteins suggest a larger role for Ca2+ cycling in cardiac repolarization and the pathogenesis of LQTS.

In this review, we examine existing paradigms and recent advances that shape our current understanding of the molecular basis of LQTS with a focus on the molecular insights provided by recent discoveries linking genetic variation in Ca2+-handling proteins to the pathogenesis of LQTS and modulation of cardiac repolarization in health.

Molecular Basis of Cardiac Ca2+ Cycling
The carefully regulated flux of Ca2+ within and through cardiomyocytes governs both cardiac excitability and contractility. Not only does Ca2+ serve as the critical link between the electric stimuli generated by plasma membrane depolarization and mechanical contraction of the cardiomyocyte during excitation–contraction coupling (Figure 1)12 but also it generates a substantial inward/depolarizing current that modulates action potential (AP) duration and regulates several intracellular processes, including gene transcription. Although a detailed discussion of the major molecular players in cardiac excitation–contraction coupling/Ca2+ cycling is outside the scope of this review, a thorough review of the genetics, structure, and functionality of the Ca1.2 LTCC and RyR2 macromolecular complexes is contained within the Data Supplement to provide interested readers with sufficient background to appreciate how perturbations in these molecular constituents may contribute to the pathogenesis of LQTS.

Molecular Basis of LQTS
During the early-to-mid 1990s, a series of linkage analysis and positional cloning studies identified KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3) as genetic substrates for congenital LQTS.13–18 Subsequent epidemiological studies ascertained that disease-causative mutations in these 3 major genes account for an estimated 60% to 75% of genotype-positive LQTS cases (Table I in the Data Supplement).19,20

After the discovery of these 3 major LQTS-susceptibility genes, at least 14 additional minor LQTS-susceptibility genes have been described in the literature. The resulting LQTS subtypes can be further classified on the basis of whether they yield a nonsyndromic or multisystem clinical phenotype (Table I in the Data Supplement). Furthermore, because the

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majority of minor LQTS genes encode channel-interacting proteins that work in concert with the Na,1.5, Ca,1.2, K,11.1, and K,7.1 pore-forming α-subunits, a current-centric model has been proposed as a means of summarizing the genetic and electrophysiological basis of the major and minor LQTS-susceptibility genes (Figure 2).21

Because the molecular basis of the major and minor LQTS-susceptibility genes linked to the function of I Na, I Kr, and IKs currents have been described recently in detail elsewhere,22 the following sections focus on examining the ≈5% to 10% of multisystem and nonsyndromic LQTS cases that arise from Ca²⁺-handling protein dysfunction.

TS: Aberrant Ca²⁺-Dependent and Ca²⁺-Independent Intracellular Processes

TS is an extremely rare, largely sporadic form of multisystem LQTS characterized by severe QT prolongation on ECG (average heart rate–corrected QT interval ≈580 ms), high incidence of life-threatening cardiac arrhythmias/SCD, syndactyly, minor craniofacial/odontic abnormalities, and baldness at birth and variably expressed intermittent hypoglycemia, immunodeficiency, congenital heart defects, and developmental delay/neurocognitive impairments along the autism spectrum.23–25

Initially, TS was thought to arise from a single recurrent mutation, G406R, in the alternatively spliced CACNA1C exon 8a that accounts for ≈20% of the Ca v1.2 mRNA transcripts in the heart and brain and encodes the distal portion of the domain I transmembrane segment 6 (IS6) known to play a critical role in the voltage-dependent inactivation (VDI) of Ca v1.2 (Figure 3). However, shortly after the elucidation of the molecular basis of typical TS now referred to as type I TS (TS1), 2 cases of atypical or type II TS (TS2) were reported in the literature.28 Interestingly, both cases were characterized by extreme QT prolongation (average ≈640 ms) complicated by multiple episodes of cardiac arrest in the absence of syndactyly and harbored de novo mutations in the CACNA1C exon 8—containing heart- and brain-predominant Ca v1.2 splice variant.28 Interestingly, the exon 8 CACNA1C-G406R–positive TS2 proband displayed severe neurodevelopmental delay, craniofacial/odontic abnormalities, and possible nemaline skeletal myopathy during the first few months of life, whereas the exon 8 CACNA1C-G404S–positive TS2 proband seemed...
developmentally normal until suffering an out-of-hospital cardiac arrest at age 4. 28 It was postulated initially that the lack of syndactyly and the potentially more severe neurological/cardiac phenotypes anecdotally observed in TS2 versus TS1 were likely secondary to the differential tissue-specific expression of exon 8- and exon 8a–containing Cav1.2 splice variants. 28 Although this is likely the case for exon 8 G406R–mediated TS2, the recent identification of exon 8 G402S in an otherwise developmentally normal adolescent woman with borderline QT prolongation who presented with an out-of-hospital cardiac arrest29 coupled with the normal development of the initial G402S-positive TS2 proband before his first cardiac arrest28 suggest that (1) CACNA1C-G402S results in a milder clinical phenotype more akin to nonsyndromic LQTS and (2) the neurodevelopmental sequelae observed in the initial CACNA1C-G402S–positive TS2 proband are more likely secondary to arrhythmia-induced anoxic brain injury than underlying CACNA1C-mediated developmental delay.

Mechanistically, the IS6 and α-interaction domain–containing I-II loop linker of the Cav1.2 α-subunit form a continuous α-helix–rich region that allow Cavβ auxiliary subunits to bind and modulate Cav1.2 gating in a manner that supports faster VDI.30,31 In addition, calmodulin (CaM)-mediated Ca2+-dependent inactivation (CDI) may also be dependent on Cavβ and the structural integrity of the Cavα 1.2 α-subunit IS6–α-interaction domain region suggesting that the Cavα 1.2 IS6–α-interaction domain/Cavβ complex serves as the common denominator by which VDI and CDI modulate Cav1.2 gating.32 Given the unique conformational flexibility imparted by glycine (G) residues and the common localization of TS-associated mutations to the distal IS6 region of Cavα 1.2, it is therefore not surprising that both TS1- and TS2-associated CACNA1C exon 8a (G406R) and exon 8 (G402S and G406R) mutations drastically reduce Cav1.2 VDI in vitro possibly via the alteration of Cavβ binding/function.25,28

Although subsequent studies, including those in TS-specific induced pluripotent stem cell (iPSC)–derived cardiomyocytes33 and cortical neurons,34 have reaffirmed the effect of CACNA1C-G406R on VDI, the effects of CACNA1C-G406R on CDI reported to date are widely discordant.28,35,36 Furthermore, several alternatives to the IS6–α-interaction domain/Cavβ-mediated reduction in VDI hypothesis have been proposed, including formation of aberrant A kinase anchor protein–Cavα 1.2 complexes37 and facilitation by aberrant/
excess calmodulin kinase II activity.\textsuperscript{38,39} As such, the precise mechanisms by which TS1- and TS2-causative mutations reduce Cav1.2 VDI and the potential contribution of impaired Cav1.2 CDI to the generation of the multisystem TS phenotype remain incompletely understood.

In the heart, the reduction of Cav1.2 VDI conferred by TS1- and TS2-causative \textit{CACNA1C} mutations is predicted in silico to accentuate the inward depolarizing current and prolong AP repolarization/duration.\textsuperscript{25,28} Although subsequent studies in both iPSC cardiomyocytes\textsuperscript{33} and a TS mouse model\textsuperscript{40} concluded that increased Ca\textsuperscript{2+} influx through mutant Cav1.2-TS channels during the plateau phase prolongs cardiac AP duration, both studies also demonstrated that TS ventricular myocytes are in a constant state of Ca\textsuperscript{2+} overload and thus prone to spontaneous ectopic Ca\textsuperscript{2+} release from sarcoplasmic reticulum (SR) and the generation of potentially arrhythmic delayed after-depolarizations (DADs). As such, the proarrhythmic state observed in TS likely arises from the generation of early after-depolarizations secondary to increased myocyte refractoriness and DADs secondary to spontaneous SR Ca\textsuperscript{2+} release during phase 2/3 and phase 4 of the cardiac AP, respectively. Interestingly, roscovitine (Seliciclib), a cyclin-dependent kinase inhibitor previously shown to enhance Cav1.2 VDI in heterologous expression systems via direct extracellular binding,\textsuperscript{41,42} rescued the electrophysiological perturbations observed in TS iPSC-derived cardiomyocytes,\textsuperscript{33} suggesting that agents with similar mechanisms of action may prove beneficial in the treatment of TS.

Although the contribution of increased Ca\textsuperscript{2+} influx to the proarrhythmic TS cardiac phenotype is relatively well understood, precisely how TS-causative \textit{CACNA1C} mutations generate a myriad of variably expressed extracardiac phenotypes, particularly in nonexcitable tissues, remains unclear. At present, there is evidence to suggest that Ca\textsuperscript{2+}-dependent processes likely underlie the intermittent hypoglycemia (excessive Ca\textsuperscript{2+}-mediated insulin release from pancreatic \(\beta\) cells)\textsuperscript{43} and craniofacial dysmorphism (mandibular chondrocyte/osteoblast hypertrophy via increased Ca\textsuperscript{2+}-dependent calcineurin/nuclear factor of activated T-cell transcription factors signaling)\textsuperscript{44} aspects of the multisystem TS phenotype. However, the

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\textbf{Figure 3.} Electrophysiological and clinical manifestations of putative long-QT syndrome (LQTS)–causative gain-of-function \textit{CACNA1C} mutations. \textbf{A}, The location of Timothy syndrome (TS; maroon circles), cardiac-only Timothy syndrome (COTS; orange circles), and LQTS (CACNA1C-LQTS; green circles)–causative \textit{CACNA1C} mutations are depicted on the Cav1.2 linear protein topology. Biogenic (small dash), kinetic (solid), and mixed (large dash) generalized electrophysiological manifestations of individual \textit{CACNA1C} mutations are indicated by contrasting circle outlines. \textbf{B}, Summary of the electrophysiological and clinical manifestations of TS, COTS, and LQTS. EP indicates electrophysiological; HCM, hypertrophic cardiomyopathy; and SCD, sudden cardiac death.

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\caption{Electrophysiological and clinical manifestations of putative long-QT syndrome (LQTS)–causative gain-of-function \textit{CACNA1C} mutations. A, The location of Timothy syndrome (TS; maroon circles), cardiac-only Timothy syndrome (COTS; orange circles), and LQTS (CACNA1C-LQTS; green circles)–causative \textit{CACNA1C} mutations are depicted on the Cav1.2 linear protein topology. Biogenic (small dash), kinetic (solid), and mixed (large dash) generalized electrophysiological manifestations of individual \textit{CACNA1C} mutations are indicated by contrasting circle outlines. B, Summary of the electrophysiological and clinical manifestations of TS, COTS, and LQTS. EP indicates electrophysiological; HCM, hypertrophic cardiomyopathy; and SCD, sudden cardiac death.}
\end{figure}
apparent lack of neurodevelopmental delay and other extra-
cardiac manifestations in the 2 CACNA1C-G402S-positive
TS2 cases,28,29 despite the fact that CACNA1C-G402S and
CACNA1C-G406R result in similar increases in Ca\textsuperscript{2+} influx
in vitro, suggests that perturbation of Ca\textsuperscript{2+}-independent pro-
cesses may also contribute to multiorgan dysfunction in TS.

Consistent with this theory, Krey et al\textsuperscript{45} demonstrated
that TS1 iPS-derived neurons displayed marked impairment
of dendrite formation, a common feature of neuro-
developmental/autism spectrum disorders, independent of
Ca\textsuperscript{2+} influx through mutant TS1-Ca\textsubscript{1.2} channels. Based on
an elegant series of experiments, Krey et al\textsuperscript{45} propose that
conformational changes in TS1-Ca\textsubscript{1.2} channels, unrelated
to their Ca\textsuperscript{2+}-permeating properties, lead to decreased bind-
ing/recruitment of Gem, a guanosine triphosphate-binding
protein, to the Ca\textsubscript{1.2} macromolecular complex which then precipitates
the loss of RhoA GTPase inhibition, aberrant cytoskeletal remodeling, and ultimately dendritic retraction
in TS1 iPS-derived neurons. As such, there is ample evi-
dence to suggest that electrophysiological perturbations in
L-type calcium current (I\textsubscript{Ca,L})\textsuperscript{3,40} underlie the proarrhythmic
component of the TS cardiac phenotype, whereas disruption
of downstream Ca\textsuperscript{2+}-dependent (eg, calcineurin/nuclear fac-
tor of activated T cells)\textsuperscript{44} and Ca\textsuperscript{2+}-independent (eg, Gem/
RhoA/ROCK)\textsuperscript{45} signaling cascades may underlie many of the
electrophysiological perturbations in the extracardiac
manifestations.

Lastly, our current understanding of the molecular basis
of TS is further complicated by the recent identification
of 3 additional CACNA1C mutations (CACNA1C-G402R,
-11166T, and -A1473G), including 2 mutations that reside
outside of exon 8/8a, in patients with TS-like phenotypes
(Figure 3).46–48 Interestingly, all putative TS-causative muta-
tions described to date localize to the hydrophobic residue-
rich boundaries between the last transmembrane segment (S6)
and interdomain linkers of their respective Ca\textsubscript{1.2} domains/
repeats (Figure 3), suggesting that these regions may contri-
buting to a key tertiary/quaternary structure(s) within the Ca\textsubscript{1.2}
channel that regulate gating kinetics and function as a scaffold
for the interaction/binding of downstream signaling proteins.
At present, it remains to be seen if the novel phenotypes (eg,
hypotonia, joint hyperflexibility/contractures, clinodactyly,
etc)\textsuperscript{46,47} associated with these recently described TS-like cases
represent true genotype–phenotype correlations secondary to
unique electrophysiological perturbations (eg, increased I\textsubscript{Ca,L}
window current with CACNA1C-11166T)\textsuperscript{47} or are simply the result
of variable expressivity. Regardless, these findings sug-
gest that expanded CACNA1C sequencing should be consid-
ered for any patient with a TS-like phenotype and previous
negative exon 8/8a targeted screening.

**Nonsyndromic Long-QT Syndrome:**

**Evolving Role of the L-Type Ca\textsuperscript{2+} Channel**

In 2013, a combination of whole-exome sequencing, gene pri-
oritization, and candidate-based screening, unearthed 4 novel
putative disease-causative missense mutations in CACNA1C
in LQTS patients with isolated QT prolongation devoid of any
congenital heart defects or extracardiac manifestations that
define TS clinically.5 Interestingly, 3 out of 4 LQTS-causative
CACNA1C mutations (K834E, P857L, and P857R) localized
to the PEST (proline [P]-, glutamic acid [E]-, serine [S]-, and
threonine [T]-rich) domain of Ca\textsubscript{1.2}’s II-III linker (Figure IA in
the Data Supplement) that is believed to serve as a proteo-
lytic signal peptide that when unmasked marks the LTCC for
rapid degradation via calpain-mediated and ubiquitin-protea-
some–mediated mechanisms.5,46–51

Given the previous observation that deletion of the Ca\textsubscript{1.2}
LQTS’s 2 PEST domains (PEST1 in the I-II linker and the
aforementioned PEST3 in the II-III linker) individually
increased the stability and current density of heterologously
expressed Ca\textsubscript{1.2} channels,\textsuperscript{50} it is not entirely surprising that
in vitro functional characterization of P857R-CACNA1C
revealed a significant increase in both Ca\textsubscript{1.2} cell surface
expression and peak current density without significantly
altering Ca\textsubscript{1.2} channel kinetics.5 Collectively, these find-

ings suggest that rare CACNA1C mutations localizing to the
Ca\textsubscript{1.2} PEST3 domain may disrupt normal PEST3-mediated
Ca\textsubscript{1.2} degradation and impart a biogenic I\textsubscript{Ca,L} gain of function
that manifests clinically as an isolated LQTS phenotype
without any other cardiac or extracardiac abnormalities
(CACNA1C-LQTS).

After the initial whole-exome sequencing–aided discov-
yery by Boczek et al,\textsuperscript{5} screening of CACNA1C in 2 indepen-
dent LQTS cohorts identified 8 additional LQTS-causative
mutations shown to impart an I\textsubscript{Ca,L} gain of function in vitro
through a variety of electrophysiological mechanisms
(Figure 3).\textsuperscript{5,51} In addition, subsequent screening of genotype-
negative LQTS individuals with documented echocardi-
ographic evidence of hypertrophic cardiomyopathy and a
family history of hypertrophic cardiomyopathy–like pheno-
types led to the identification of a novel genetic hotspot in the
distal portion of the Ca\textsubscript{1.2} I-II linker (CACNA1C exon 12)
responsible for a newly described clinical entity, cardiac-only
TS (COTS), characterized by the concomitant but variably
expressed phenotypes of LQTS, hypertrophic cardiomyopa-
thy, congenital heart defects, and SCD in the absence of any
extracardiac symptoms (Figure 3).\textsuperscript{52} With these discoveries,
the initial hypothesis that TS and CACNA1C-LQTS may be
differentiated by distinct electrophysiological mechanisms
was turned on its head as several of the newly discovered
CACNA1C-LQTS/COTS mutations impacted an I\textsubscript{Ca,L}
gain of function via the slowing of VDI analogous to the effect
observed for the TS1- and TS2-causative CACNA1C muta-
tions.\textsuperscript{5,7} Furthermore, in silico action potential modeling
predicted that many CACNA1C-LQTS mutations result in a
more pronounced I\textsubscript{Ca,L} gain of function (AP duration pro-
longation and increased cytosolic Ca\textsuperscript{2+} concentration) than
the canonical TS exon 8a G406R-CACNA1C mutation when
the estimated percentage of affected Ca\textsubscript{1.2} transcripts are accounted for.\textsuperscript{7} These findings suggest that the stark pheno-
typic differences between TS, COTS, and CACNA1C-
LQTS cannot be explained by distinct electrophysiological
mechanisms readily appreciated in heterologous expression
systems, the degree of increased cytosolic Ca\textsuperscript{2+} influx, or
mutation localization to specific Ca\textsubscript{1.2} domains such as the
S6/interdomain linker boundaries as summarized in Figure 3.
As such, the extracardiac manifestations of TS syndrome
and the cardiac hypertrophy/congenital heart defects observed in
TS and COTS may be (1) independent of the Ca\textsuperscript{2+}-permeating
properties of mutant Ca\textsubscript{v}1.2 channels (eg, perturbation of aforementioned Ca\textsuperscript{2+}-dependent (calcineurin/nuclear factor of activated T cells) or Ca\textsuperscript{2+}-independent (GEM/RhoA/ROCK) signaling cascades that culminate in aberrant gene expression) and (2) modulated by as of yet unrecognized genetic/epigenetic mechanisms (eg, interindividual variation in splicing patterns, auxiliary subunit expression/function, or regulatory noncoding RNAs).\textsuperscript{53}

Calmodulinopathic LQTS: Impaired LTCC Ca\textsuperscript{2+}-Dependent Inactivation

Over the past several years, independent whole-exome sequencing studies by Crotti et al\textsuperscript{8}, Makita et al\textsuperscript{54}, and Reed et al\textsuperscript{9} linked heterozygous sporadic/de novo mutations in the identical \textit{CALM}1-, \textit{CALM}2-, and \textit{CALM}3-encoded CaM, a ubiquitous Ca\textsuperscript{2+} sensor essential for the Ca\textsuperscript{2+}-dependent regulation of an array of intracellular processes, to what was described initially as a rare multisystem disorder characterized by marked QT prolongation and recurrent cardiac arrest (often in infancy) accompanied by variously expressed congenital heart defects, seizures, and neurodevelopmental delays. However, the recent description of \textit{CALM}2-positive cases with a milder phenotype consisting of later onset cardiac events (>1 year of life) in the absence of discernible neurodevelopmental delay,\textsuperscript{54} raises the possibility that the phenotype initially observed in calmodulinopathic LQTS\textsuperscript{5} may (1) depend on the severity of the Ca\textsuperscript{2+} binding impairment/reduction in Ca\textsuperscript{2+} affinity conferred by mutant CaMs, (2) occur secondary to the poorly understood effects of the differential temporospatial/tissue-specific expression of the 3 \textit{CALM} genes (in the human heart \textit{CALM}1, \textit{CALM}2, and \textit{CALM}3 is expressed at \(\approx 1:2:5\) ratio),\textsuperscript{8} and (3) represent neurological sequelae of recurrent cardiac arrests early in life rather than an intrinsic manifestation of CaM mutations.

Interestingly, all LQTS-causative \textit{CALM}1-3 mutations described to date localize at or immediately proximal to Ca\textsuperscript{2+}-coordinating residues of the C-terminal lobe (C-lobe) of CaM (Figure 4A) and impart a marked reduction in Ca\textsuperscript{2+} binding affinity.\textsuperscript{8,5,54} Despite the fact that CaM regulates a number of cardiac ion channels, the predominant effect of LQTS-causative \textit{CALM}1-3 mutations seems to be the loss of CDI resulting in unrestrained Ca\textsuperscript{2+} influx as the effects on RyR2 (reduced CaM binding) and Na\textsubscript{1.5} (increased \(I_{\text{Na}}\) late current) were mild and widely variable.\textsuperscript{5,55} In contrast, CaM mutations that produce a predominantly catecholaminergic ventricular tachycardia phenotype localize to both the N-terminal lobe (N-lobe; N54I) and C-lobe (N98S) and increase RyR2-binding affinity/single channel open probability and the incidence of spontaneous Ca\textsuperscript{2+} release from the SR with little to no effect on Ca\textsuperscript{2+} binding affinity (Figure 4A).\textsuperscript{57,58}

The fact that all LQTS-causative CaM mutations, including those that yield a LQTS/catecholaminergic ventricular tachycardia overlap phenotype (N98S, D132E, and Q136V), localize to the CaM C-lobe raises the possibility of a functional bipartition whereby the CaM N- and C-lobes are responsible for different sets of cellular functions. One example of partitioned CaM function potentially pertinent to the pathogenesis of calmodulinopathic LQTS is the presence of a dual-phase CDI unique to LTCCs (Figure 4B). In comparison to other voltage-gated calcium channel classes where the lower Ca\textsuperscript{2+} affinity N-lobe mediates CDI and the higher Ca\textsuperscript{2+} affinity C-lobe either potentiates Ca\textsuperscript{2+} entry during Ca\textsuperscript{2+}-dependent facilitation or plays no role in Ca\textsuperscript{2+}-dependent regulation,\textsuperscript{59,60} the LTCCs’ N-lobe and C-lobe underlie distinct slow and rapid components of CDI, respectively (Figure 4B).\textsuperscript{61} As such, the localization and function of LQTS-causative \textit{CALM} mutations as well as modest effects on RyR2 or Na\textsubscript{1.5} suggest that calmodulinopathic LQTS primarily arises from the impairment of C-lobe-mediated rapid CDI (Figure 4C).

However, given the ubiquitous nature of CaM, significant functional redundancy, and vast number of intracellular CaM targets, without more in-depth studies, it remains to be seen if more pervasive defects in ion channel regulation (eg, K\textsuperscript{+} channels, other voltage-gated Ca\textsuperscript{2+} channels, etc) and CaM-dependent signaling cascades contribute to the molecular basis of calmodulinopathic LQTS.

Triadin Knockout Syndrome: Remodeling of the Calcium Release Unit Molecular Architecture

Most recently, Altmann et al\textsuperscript{10} described a rare autosomal-recessive form of LQTS characterized by transient/consistent QT prolongation with extensive precordial (V\textsubscript{4}) T-wave inversions, severe and often \(\beta\)-blocker- and left cardiac sympathetic denervation-refractory exercise-induced cardiac events (eg, syncope, sudden cardiac arrest, and SCID) in early childhood, and mild-to-moderate proximal skeletal muscle weakness that arises secondary to either homozygous or compound heterozygous frameshift/null mutations in \textit{TRDN}-encoded triadin, a key structural component of the cardiac release unit (Figure 5A).

Although no functional studies were undertaken with the initial clinical and genetic description of Triadin knockout (TKO) syndrome, insights gleaned from ventricular arrhythmia-prone \textit{TRDN}-null mice suggest that complete ablation of triadin, as would be expected in TKO syndrome patients, significantly (1) disrupts the approximation of the T-tubule and the junctional SR within the cardiac dyad, (2) reduces the expression of key junctional SR proteins including RyR2, calsequestrin 2, and junctin, and (3) reduces the colocalization/juxtaposition of Ca\textsubscript{1.2}/RyR2 and RyR2/Calsequestrin2 in the cardiac release unit (Figure 5B).\textsuperscript{62} This radical remodeling of the cardiac release unit molecular architecture reduces SR Ca\textsuperscript{2+} release leading to impaired Ca\textsubscript{1.2} LTCC CDI, unstrained cytosolic Ca\textsuperscript{2+} influx via \(I_{\text{Ca,L}}\), and subsequent SR Ca\textsuperscript{2+} overload (Figure 5B).\textsuperscript{62-64} Interestingly, in \textit{TRDN}-null myocytes, Ca\textsubscript{1.2} activation and inactivation remain slower even when Ba\textsuperscript{2+}, which does not trigger SR Ca\textsuperscript{2+} release or Ca\textsubscript{1.2} CDI, is used as the charge carrier, suggesting that the loss of Triadin intrinsically alters Ca\textsubscript{1.2} gating by as of yet undefined mechanisms independent of SR Ca\textsuperscript{2+}-release–triggered Ca\textsubscript{1.2} CDI.\textsuperscript{52,65}

Collectively, the misappropriation of calsequestrin 2 in the SR,\textsuperscript{62,66} SR Ca\textsuperscript{2+} overload,\textsuperscript{62} and reduction in junction-mediated RyR2 inhibition at high SR luminal Ca\textsuperscript{2+} concentrations\textsuperscript{67} that arise secondary to remodeling of cardiac release unit molecular architecture in \textit{TRDN}-null
mice is hypothesized to increase the frequency of spontaneous ectopic Ca$^{2+}$ release from the SR leading to proarrhythmic DADs, particularly in the setting of β-adrenergic stimulation. In addition, the slowed Ca$^{2+}$ inactivation observed in TRDN-null mice theoretically could prolong action potential duration and create an electric substrate favorable for the generation of early after-depolarization–triggered ventricular arrhythmias, the predominant arrhythmogenic mechanism in most LQTS subtypes. Thus, in TKO syndrome, calmodulinopathic LQTS, and to a lesser extent in non-Ca$^{2+}$-mediated LQTS subtypes (eg, I$_{Na}$-mediated LQT3) the perturbation/modulation of a myriad of Ca$^{2+}$-dependent events, including disrupted Ca$^{1,2}$ kinetics/enhanced I$_{Na}$ current, increased spontaneous SR Ca$^{2+}$ release via RyR2, and promotion of SR Ca$^{2+}$ loading via NCX, likely contribute to an underlying proarrhythmic electrophysiological substrate capable of triggering DAD- and early after-depolarization–mediated arrhythmias.

Although it is not clear whether a DAD- or early after-depolarization–mediated mechanism is predominantly responsible for the ventricular arrhythmias observed in TRDN-null mice, treatment of TRDN-null myocytes with the DHP Ca$^{2+}$ channel blocker nifedipine abolished SR Ca$^{2+}$ overload and spontaneous SR Ca$^{2+}$ release, suggesting that DHPs and other class IV antiarrhythmics may one day play a role in the treatment of patients with TKO syndrome.

Ultimately, these observations in TRDN-null mice function to substantiate the hypothesized molecular basis of TKO syndrome in humans and provide a reasonable launching point for future inquiries aimed at elucidating the precise arrhythmogenic mechanism(s) that underlie the severe medically refractory adrenergically mediated cardiac events that plague TKO syndrome patients with the hope that these insights will catalyze novel therapeutic strategies.
In recent years, the association of QT prolongation/shortening with an increased risk of SCD in the general population and the barrier that drug-induced QT prolongation has presented to new drug development has served as an impetus for QT interval genome-wide association studies in several large population-based cohorts such as the Framingham Heart Study and the Cardiovascular Heart Study. Beginning in 2009, 3 large genome-wide association study meta-analyses, including the mammoth QT-IGC consortium expanded genome-wide association study meta-analysis of 76061 individuals of European descent, built on existing single cohort studies by collectively demonstrating that common variants in 6 established (NOS1AP, KCNQ1, KCNE1, KCNH2, SCN5A, and KCNJ2) and 29 novel genetic loci collectively explain ≈8% to 10% of heritable QT interval variability.

Interestingly, a surprising number of these novel genetic loci localize within or near genes that encode intracellular Ca²⁺-handling proteins, including ATP2A2-encoded SR ATPase 2a calcium pump, SERCA-regulator PLN-encoded phospholamban, and the SLC8A1-encoded NCX1 Na⁺/Ca²⁺ exchanger whose roles in excitation–contraction coupling were discussed previously and are summarized in Figure 1. Furthermore, mutational analysis of the 6 genes (ATP2A2, CAV1, CAV2, SLC8A1, SRL, and TRPM7), in closest proximity to the novel genetic loci with the strongest statistical significance and highest biological plausibility in an international cohort of 298 unrelated LQT1-LQT3 negative LQTS probands, identified potentially pathogenic frameshift mutations in ATP2A2-encoded SR ATPase 2a and the TRPM7-encoded transient receptor channel melastatin 7 Mg²⁺/Ca²⁺ channel known to mediate Ca²⁺ influx and regulate expression of other Ca²⁺-handling proteins. Although much work remains to fully understand the physiological ramifications of common and rare genetic variation in these newly identified genetic loci, the above studies highlight a larger than anticipated role for Ca²⁺ flux in cardiac repolarization/modulation of QT interval in health and hint at a potentially even greater role for Ca²⁺-handling proteins in the pathogenesis of congenital and acquired LQTS.

**Concluding Remarks**

The discovery and subsequent investigation of rare and common genetic variation in Ca²⁺-handling proteins has illuminated a previously underappreciated and rapidly expanding role for Ca²⁺ signaling in cardiac repolarization and the pathogenesis of nonsyndromic and multisystem forms of congenital LQTS. Undoubtedly, these discoveries will provide a rich foundation for future inquiries, aided by the continued use of next-generation sequencing technologies and patient-specific iPSC-derived cardiomyocytes, aimed at (1) determining the
genetic substrates that underlie the roughly 15% to 20% of LQTS cases that remain genetically elusive, (2) elucidating the novel genetic/epigenetic determinants that underlie the variable phenotypic expressivity observed in most LQTS subtypes and the dramatic phenotypic divergences observed between CACNA1C-mediated TS, CACNA1C-mediated COTS, and CACNA1C-LQTS, (3) developing a deeper understanding of the pathophysiological mechanisms of LQTS-causative mutations in Ca2+-handling proteins in hopes that such insights will lead to the development of individualized, genotype-guided therapeutic strategies with improved efficacy and safety profiles, and (4) further investigating the potential contribution of an increased burden of common genetic variation in Ca2+-handling proteins to the pathogenesis of drug-induced LQTS. Because we continue to revisit the role of Ca2+ in cardiac repolarization and the pathogenesis of nonsyndromic and multisystem forms of LQTS, there is renewed hope that insights gleaned from these efforts will lead to the development of novel approaches to the diagnosis, risk stratification, and treatment of LQTS patients, particularly those with high-risk LQTS subtypes such as TS, calmodulinopathic LQTS, and Tako-Tsubo syndrome who are among those at greatest risk of succumbing to potentially ventricular arrhythmias.

Sources of Funding

This work was supported by the Windland Smith Rice Sudden Comprehensive Sudden Cardiac Death Program (to Dr Ackerman). Dr Giudicessi thanks the Mayo Clinic Internal Medicine Residence and Clinician Investigator Training Programs for fostering an outstanding environment for physician-scientist training.

Disclosures

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References


9 Giudicessi and Ackerman Calcium and LQTS


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SUPPLEMENTAL MATERIAL

Genetics and Structure of the L-Type Ca\textsuperscript{2+} Channel Macromolecular Complex

Although three types of voltage-gated calcium channels, including the CACNA1G-encoded nodal T-type Ca\textsubscript{v}3.1 and the CACNA1A-encoded neuronal P/Q-type Ca\textsubscript{v}2.1 pore-forming \(\alpha_1\)-subunits, are expressed in the human heart, the CACNA1C-encoded Ca\textsubscript{v}1.2 \(\alpha_1\)-subunit, which conducts the “long lasting” L-type Ca\textsuperscript{2+} current (\(I_{\text{CaL}}\)), represents the most prevalent and only class of voltage-gated calcium channels currently linked to the pathogenesis of LQTS or any other heritable arrhythmia syndrome.\textsuperscript{1, 2}

Human CACNA1C spans >500 kb of chromosome 12 and is comprised of 55 exons, of which 19 are subject to alternative splicing, providing the theoretical substrate for the generation of a staggering number of Ca\textsubscript{v}1.2 splice variants each with the potential to impart their own unique functional consequences.\textsuperscript{3} Interestingly, those Ca\textsubscript{v}1.2 splice variants studied to date display significant differences in channel kinetics\textsuperscript{3-6}, dihydropyridine affinity\textsuperscript{7, 8}, and regulatory properties\textsuperscript{5, 9} that explain many of the functional characteristics that differentiate smooth muscle- and cardiac-specific Ca\textsubscript{v}1.2 \(\alpha_1\)-subunit isoforms (Supplemental Table 2). Although the adult cardiac Ca\textsubscript{v}1.2 reference isoform is defined traditionally as a combination of the presence of mutually exclusive exons 1a, 8, and 32 along with the absence of 75 nucleotides following exon 9\textsuperscript{10, 11}, quantitative analysis of mRNA expression in human hearts demonstrated substantial inter-individual variation in overall CACNA1C expression levels and splicing patterns across the majority of the 12 identified splicing loci (Supplemental Table 2).\textsuperscript{12} Collectively, these findings suggest that variable splicing patterns can influence profoundly Ca\textsubscript{v}1.2 function and that the electrophysiological and clinical phenotypes associated with CACNA1C mutations may depend
on the Ca\textsubscript{v}1.2 splice variant(s) in which they reside as well as the degree to which particular Ca\textsubscript{v}1.2 splice variants are expressed in a given host.

Structurally, the ~220kDa Ca\textsubscript{v}1.2 α1-subunit contains ~2100 amino acids divided into four structural repeats (domains I-IV) each composed of six helical transmembrane domains (S1-S6) linked by three intracellular loops that collectively form a pseudo-tetrameric structure akin to voltage-gated sodium channels (Supplemental Figure 1a).\textsuperscript{10} In addition, several intracellular Ca\textsubscript{v}1.2 structural domains play a critical role in optimizing channel activity. First, the I-II loop linker α-interaction domain (AID) facilitates β\textsubscript{2} subunit binding that enhances Ca\textsubscript{v}1.2 trafficking and modulates voltage-dependent inactivation (VDI).\textsuperscript{13} Secondly, the amino (N)-terminal spatial Ca\textsuperscript{2+}-transforming element\textsuperscript{14} and the carboxy (C)-terminal “IQ”-motif\textsuperscript{15,16} containing Ca\textsuperscript{2+}-inactivation region bind calmodulin (CaM) and mediate calcium-dependent inactivation (CDI), a crucial negative feedback mechanism for the prevention of intracellular Ca\textsuperscript{2+} overload. Thirdly, the C-terminus also facilitates β-adrenergic modulation via the binding of the A kinase anchor protein 15 (AKAP15)/protein kinase A (PKA) complex to atypical leucine zipper motifs\textsuperscript{17} referred to as the AKAP binding domain (ABD). Lastly, the far distal portion of C-terminus is cleaved proteolytically \textit{in vivo} to generate an auto-inhibitory peptide containing prominent PKA phosphorylation sites and the ABD, which significantly reduces channel activity/voltage-dependent activation by non-covalently binding to the truncated Ca\textsubscript{v}1.2 channels (Supplemental Figure 1a).

Like most cardiac voltage-gated ion channels, the optimum function and regulation of Ca\textsubscript{v}1.2 depends on a macromolecular complex composed of multiple auxiliary proteins (Supplemental Figure 1b). At present, the Ca\textsubscript{v}1.2 α1-subunit, CACNA2D1-encoded α\textsubscript{2}δ-subunit, and CACNB2-encoded β\textsubscript{2}-subunit, assembled in a 1:1:1 ratio, is necessary and sufficient
to recapitulate the $I_{\text{CaL}}$ current in vitro (Supplemental Figure 1b). Functionally, the α2δ-subunit, which is comprised of two disulfide-linked proteins (α2 and δ) derived from a single post-translationally cleaved transcript/precursor protein, increases the speed of CaV1.2 activation and inactivation, whereas the aforementioned β2-subunit predominantly promotes trafficking of CaV1.2 to the cell surface. As such, both the α2δ and β2 subunits function to increase $I_{\text{CaL}}$ current density, albeit by distinct mechanisms. The role of a third class of auxiliary proteins, the CACNG4, 6, 7, and 8-encoded transmembrane-spanning glycoprotein γ subunits, in the heart remains unclear. However, the recent discovery of γ subunits in cardiac tissue coupled with in vitro evidence that γ subunits modulate CaV2.1 voltage-dependence in a β-subunit dependent fashion suggest that contrary to popular belief, γ subunits may contribute to the function of the cardiac CaV2.1 macromolecular complex in vivo.

Genetics and Structure of RyR2 Intracellular Ca$^{2+}$ Release Channel Macromolecular Complex

Currently, three mammalian ryanodine receptor isoforms (RyR1-3) have been identified, but only RyR2 is expressed at appreciable levels in the human heart. Although direct perturbation of RyR2 function is not linked currently to LQTS pathogenesis, defective RyR2 function predominantly results in catecholaminergic polymorphic ventricular tachycardia (CPVT), but is also anecdotally linked to a trio of cardiomyopathies [arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy (HCM), and nonischemic dilated cardiomyopathy] reviewed in detail elsewhere.

Structurally, the RYR2 gene encodes a single 560kDa monomer comprised of a vast cytosolic N-terminal domain connected to a smaller C-terminal domain by six SR-spanning transmembrane domains that assemble to form the massive homo-tetrameric RyR2 channel.
Similar to the LTCC, the RyR2 channel functions as a macromolecular complex dependent on an array of cytoplasmic and luminal SR auxiliary interacting proteins for proper function. The cytoplasmic N-terminal domain serves as a binding site-containing scaffold for a multitude of auxiliary interacting proteins that modulate Ca\(^{2+}\)-release from the C-terminal pore region including the channel-stabilizing/tetramer-coupling FK506-binding protein 12.6 (FKBP12.6)/calstabin 1\(^{26}\), Ca\(^{2+}\)-dependent Ca\(^{2+}\) release inhibiting CaM\(^{27}\), and Ca\(^{2+}\)-release inhibiting sorcin\(^{28}\) (Supplemental Figure 1d). Furthermore, the N-terminal domain also contains three highly conserved leucine/isoleucine zipper (LIZ) motifs that provide binding sites for phosphatases [protein phosphatases 1 (PP1) and 2A (PP2A)]\(^{29}\) and PKA\(^{30}\) as well as Ca\(^{2+}\)/CaM-dependent protein kinase II (CaMKII) phosphorylation sites\(^{31}\) that augment the probability the RyR2 channel assumes the open conformation thereby increasing (PKA and CAMKII) or decreasing (PP1 and PP2A) channel sensitivity to Ca\(^{2+}\)-dependent activation (Supplemental Figure 1d).

Within the SR lumen, RyR2 activity is modulated in response to luminal Ca\(^{2+}\) concentrations by a complex consisting of the low affinity Ca\(^{2+}\) storage protein, calsequestrin2, which is encoded by CASQ2 and three structural SR-spanning transmembrane proteins, TRDN-encoded triadin, JCN-encoded junctin, and JPH2-encoded junctophilin-2, which maintain the structural and functional integrity of the LTCC-RyR2 calcium release unit [CRU,(Supplemental Figure 1d)].\(^{32, 33}\) When SR luminal Ca\(^{2+}\) concentrations are high, calsequestrin2 oligomerizes to function as a Ca\(^{2+}\) buffer. However, as RyR2 opens and SR luminal Ca\(^{2+}\) concentrations decline, calsequestrin2 reverts to its monomeric form and via an interaction with triadin inhibits RyR2 activity contributing to the termination of Ca\(^{2+}\) release that occurs with each heartbeat.\(^{34}\) A summary of the cytoplasmic and luminal RyR2 auxiliary interacting proteins is depicted visually.
in Supplemental Figure S1 and the role of PKA, CaMKII, and other regulatory pathways in the regulation of RyR2 and Ca\textsubscript{v}1.2 during EC coupling are reviewed in more complete detail elsewhere.\textsuperscript{1}

SUPPLEMENTAL REFERENCES


57. Altmann HM, Tester DJ, Will ML, Middha S, Evans JM, Eckloff BW and Ackerman MJ. Homozygous/Compound Heterozygous Triadin Mutations Associated With Autosomal-
### Supplemental Table 1 | Genetic basis of non-syndromic and multisystem long QT syndrome subtypes.

<table>
<thead>
<tr>
<th>Gene (Genotype)</th>
<th>Acronym</th>
<th>OMIM</th>
<th>Protein</th>
<th>Functional Effect</th>
<th>Mode of Inheritance</th>
<th>Frequency</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-syndromic Long QT Syndrome (Major)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNQ1 (LQT1)</td>
<td>LQT1</td>
<td>192500</td>
<td>Kv7.1</td>
<td>Reduced ( I_{K_s} )</td>
<td>AD; AR</td>
<td>~30-35%</td>
<td>35, 36</td>
</tr>
<tr>
<td>KCNH2 (LQT2)</td>
<td>LQT2</td>
<td>613688</td>
<td>Kv11.1</td>
<td>Reduced ( I_{K_s} )</td>
<td>AD</td>
<td>~25-30%</td>
<td>37</td>
</tr>
<tr>
<td>SCN5A (LQT3)</td>
<td>LQT3</td>
<td>603830</td>
<td>Nav1.5</td>
<td>Increased ( I_{Na} )</td>
<td>AD</td>
<td>~5-10%</td>
<td>38</td>
</tr>
<tr>
<td>Non-syndromic Long QT Syndrome (Minor)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKAP9 (AKAP9-LQTS)</td>
<td>LQT11</td>
<td>611820</td>
<td>Yotiao</td>
<td>Reduced ( I_{K_s} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>39</td>
</tr>
<tr>
<td>CAV3 (CAV3-LQTS)</td>
<td>LQT9</td>
<td>611818</td>
<td>Caveolin 3</td>
<td>Increased ( I_{Na} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>40</td>
</tr>
<tr>
<td>KCNE1 (KCNE1-LQTS)</td>
<td>LQT5</td>
<td>613695</td>
<td>MinK</td>
<td>Reduced ( I_{K_s} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>41</td>
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<tr>
<td>KCNE2 (KCNE2-LQTS)</td>
<td>LQT6</td>
<td>613693</td>
<td>MiRP1</td>
<td>Reduced ( I_{K_s} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>42</td>
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<tr>
<td>KCNJS (CNSJ-LQTS)</td>
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<td>613485</td>
<td>Kir3.4</td>
<td>Reduced ( I_{K_Ach} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>43</td>
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<tr>
<td>SCN4B (SCN4B-LQTS)</td>
<td>LQT10</td>
<td>611819</td>
<td>Nav1.5 β4-subunit</td>
<td>Increased ( I_{Na} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>44</td>
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<tr>
<td>SNTAI (SNTAI-LQTS)</td>
<td>LQT12</td>
<td>612955</td>
<td>Syntrophin-α1</td>
<td>Increased ( I_{Na} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>45</td>
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<tr>
<td>CACNA1C (CACNA1C-LQTS)</td>
<td>LQT8</td>
<td>N/A</td>
<td>Cav1.2</td>
<td>Increased ( I_{Ca,L} )</td>
<td>AD</td>
<td>~1-2%</td>
<td>46</td>
</tr>
<tr>
<td>Cardiac-Only Timothy Syndrome</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CACNA1C</td>
<td>COTS</td>
<td>N/A</td>
<td>Cav1.2</td>
<td>Increased ( I_{Ca,L} )</td>
<td>AD</td>
<td>~1%</td>
<td>47</td>
</tr>
<tr>
<td>Jervell and Lange-Nielson Syndrome</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KCNQ1</td>
<td>JLNS1</td>
<td>220400</td>
<td>Kv7.1</td>
<td>Reduced ( I_{K_s} )</td>
<td>AR</td>
<td>Very rare</td>
<td>48</td>
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<tr>
<td>KCNE1</td>
<td>JLNS2</td>
<td>612347</td>
<td>MinK</td>
<td>Reduced ( I_{K_s} )</td>
<td>AR</td>
<td>Very rare</td>
<td>49</td>
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<td>Ankyrin-B Syndrome</td>
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<tr>
<td>ANKB</td>
<td>LQT4/ABS</td>
<td>6000919</td>
<td>Ankyrin B</td>
<td>Aberrant ion channel/transporter localization</td>
<td>AD</td>
<td>&lt;1%</td>
<td>50</td>
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<tr>
<td>Andersen-Tawil Syndrome</td>
<td></td>
<td></td>
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<tr>
<td>KCN2</td>
<td>LQT7/ATS</td>
<td>170390</td>
<td>Kir2.1</td>
<td>Reduced ( I_{K_s} )</td>
<td>AD</td>
<td>&lt;1%</td>
<td>51</td>
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<tr>
<td>Timothy Syndrome</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CACNA1C exon 8a</td>
<td>TS1</td>
<td>601005</td>
<td>Cav1.2</td>
<td>Increased ( I_{Ca,L} ) (slowed VDI)</td>
<td>Sporadic; AD mosaicism</td>
<td>Very rare</td>
<td>52, 53</td>
</tr>
<tr>
<td>CACNA1C exon 8</td>
<td>TS2</td>
<td>601005</td>
<td>Cav1.2</td>
<td>Increased ( I_{Ca,L} ) (slowed VDI)</td>
<td>Sporadic</td>
<td>Very rare</td>
<td>54</td>
</tr>
<tr>
<td>Calmodulinopathic LQTS</td>
<td></td>
<td></td>
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<tr>
<td>CALM1</td>
<td>LQT14</td>
<td>616247</td>
<td>Calmodulin 1</td>
<td>Increased ( I_{Ca,L} ) (defective CDI)</td>
<td>Sporadic</td>
<td>~1-2%</td>
<td>55</td>
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<tr>
<td>CALM2</td>
<td>LQT15</td>
<td>616249</td>
<td>Calmodulin 2</td>
<td>Increased ( I_{Ca,L} ) (defective CDI)</td>
<td>Sporadic</td>
<td>~1%</td>
<td>55</td>
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<tr>
<td>CALM3</td>
<td>LQT16</td>
<td>N/A</td>
<td>Calmodulin 3</td>
<td>Likely increased ( I_{Ca,L} ) (defective CDI)</td>
<td>Sporadic</td>
<td>&lt;1%</td>
<td>56</td>
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<tr>
<td>Triadin Knockout Syndrome</td>
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<tr>
<td>TRDN</td>
<td>TKOS</td>
<td>N/A</td>
<td>Triadin</td>
<td>Likely increased ( I_{Ca,L} ) (disruption of CRU)</td>
<td>AR; Sporadic</td>
<td>~2%</td>
<td>57</td>
</tr>
</tbody>
</table>

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; ABS, Ankyrin-B syndrome; ATS, Andersen Tawil syndrome; COTS, cardiac-only Timothy syndrome; LQTS, long-QT syndrome; and TS, Timothy syndrome.
## Supplemental Table 2 | Distribution and function of key Ca\textsubscript{v}1.2 splice variants in the human heart

<table>
<thead>
<tr>
<th>Alternatively spliced exon</th>
<th>Ca\textsubscript{v}1.2 Topology</th>
<th>Cardiac Expression*</th>
<th>Canonical Isoform\textsuperscript{#}</th>
<th>Functional Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>N-terminus</td>
<td>69%</td>
<td>Smooth Muscle</td>
<td>PKC activation</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>N-terminus</td>
<td>31%</td>
<td>Cardiac</td>
<td>PKC inhibition</td>
<td>9</td>
</tr>
<tr>
<td>8\textsuperscript{a}</td>
<td>IS6</td>
<td>80%</td>
<td>Cardiac</td>
<td>Lower affinity for DHP</td>
<td>7</td>
</tr>
<tr>
<td>8a\textsuperscript{a}</td>
<td>IS6</td>
<td>20%</td>
<td>Smooth Muscle</td>
<td>Higher affinity for DHP</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>I-II loop</td>
<td>96.5%</td>
<td>Cardiac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9\textsuperscript{*}</td>
<td>I-II loop</td>
<td>3.5%</td>
<td>Smooth Muscle</td>
<td>↑ I\textsubscript{CaL}; altered channel kinetics</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>IIS6</td>
<td>76.5%</td>
<td>Smooth Muscle; Cardiac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15(-73 nt)</td>
<td>IIS6</td>
<td>23.5%</td>
<td>-</td>
<td>Frameshift; inactive Ca\textsubscript{v}1.2 hemi-channel</td>
<td>3</td>
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<tr>
<td>21</td>
<td>IIIS2</td>
<td>88%</td>
<td>Cardiac</td>
<td>Lower affinity for DHP</td>
<td>4</td>
</tr>
<tr>
<td>22</td>
<td>IIIS2</td>
<td>12%</td>
<td>Smooth Muscle</td>
<td>Higher affinity for DHP</td>
<td>4</td>
</tr>
<tr>
<td>31</td>
<td>IVS3</td>
<td>19.5%</td>
<td>Cardiac (fetal)</td>
<td>Increased expression during heart failure/cardiac hypertrophy</td>
<td>11</td>
</tr>
<tr>
<td>32</td>
<td>IVS3</td>
<td>80.5%</td>
<td>Cardiac (adult)</td>
<td>Lower affinity for DHP</td>
<td>11</td>
</tr>
</tbody>
</table>

\*Expression derived from the average expression of 65 patients undergoing heart transplantation and five healthy donors who died of non-cardiac causes. \#Canonical assignment of alternatively spliced exons based on prior cloning efforts. \$There has been significant confusion in regards to the mutually exclusive exon 8/8a nomenclature; we have elected to use the nomenclature initially used in Splawski et al.\textsuperscript{52}

Abbreviations: DHP, dihydropyridine; I\textsubscript{CaL}, L-type calcium current; IS6, domain I transmembrane segment 6; IIS6, domain II transmembrane segment 6; IIIS2, domain III transmembrane segment 2; IVS3, domain IV transmembrane segment 3.
**Supplemental Table 2** | Genetic basis of non-syndromic and multisystem long QT syndrome subtypes.

<table>
<thead>
<tr>
<th>Gene (Genotype)</th>
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<th>OMIM</th>
<th>Protein</th>
<th>Functional Effect</th>
<th>Mode of Inheritance</th>
<th>Frequency</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-syndromic Long QT Syndrome</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Major)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>KCNQ1 (LQT1)</td>
<td>LQT1</td>
<td>192500</td>
<td>Kv7.1</td>
<td>Reduced I_Ks</td>
<td>AD; AR</td>
<td>~30-35%</td>
<td>35, 36</td>
</tr>
<tr>
<td>KCNH2 (LQT2)</td>
<td>LQT2</td>
<td>613688</td>
<td>Kv11.1</td>
<td>Reduced I_Ki</td>
<td>AD</td>
<td>~25-30%</td>
<td>37</td>
</tr>
<tr>
<td>SCN5A (LQT3)</td>
<td>LQT3</td>
<td>603830</td>
<td>Nav1.5</td>
<td>Increased I_Na</td>
<td>AD</td>
<td>~5-10%</td>
<td>38</td>
</tr>
<tr>
<td>Non-syndromic Long QT Syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(Minor)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AKAP9 (AKAP9-LQTS)</td>
<td>LQT11</td>
<td>611820</td>
<td>Yotiao</td>
<td>Reduced I_Ks</td>
<td>AD</td>
<td>&lt;1%</td>
<td>39</td>
</tr>
<tr>
<td>CAV3 (CAV3-LQTS)</td>
<td>LQT9</td>
<td>611818</td>
<td>Caveolin 3</td>
<td>Increased I_Na</td>
<td>AD</td>
<td>&lt;1%</td>
<td>40</td>
</tr>
<tr>
<td>KCN21 (KCN21-LQTS)</td>
<td>LQT5</td>
<td>613695</td>
<td>MinK</td>
<td>Reduced I_Ki</td>
<td>AD</td>
<td>&lt;1%</td>
<td>41</td>
</tr>
<tr>
<td>KCN22 (KCN22-LQTS)</td>
<td>LQT6</td>
<td>613693</td>
<td>MIRP1</td>
<td>Reduced I_Ki</td>
<td>AD</td>
<td>&lt;1%</td>
<td>42</td>
</tr>
<tr>
<td>SCN4B (SCN4B-LQTS)</td>
<td>LQT10</td>
<td>611819</td>
<td>Nav1.5 β4-subunit</td>
<td>Increased I_Na</td>
<td>AD</td>
<td>&lt;1%</td>
<td>44</td>
</tr>
<tr>
<td>SNTA1 (SNTA1-LQTS)</td>
<td>LQT12</td>
<td>612955</td>
<td>Syntrophin-α1</td>
<td>Increased I_Na</td>
<td>AD</td>
<td>&lt;1%</td>
<td>45</td>
</tr>
<tr>
<td>CACNA1C (CACNA1C-LQTS)</td>
<td>LQT8</td>
<td>N/A</td>
<td>Cav1.2</td>
<td>Increased I_Ca,L</td>
<td>AD</td>
<td>~1-2%</td>
<td>46</td>
</tr>
<tr>
<td>Cardiac-Only Timothy Syndrome</td>
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<tr>
<td>CACNA1C</td>
<td>COTS</td>
<td>N/A</td>
<td>Cav1.2</td>
<td>Increased I_Ca,L</td>
<td>AD</td>
<td>~1%</td>
<td>47</td>
</tr>
<tr>
<td>Jervell and Lange-Nielson Syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNQ1</td>
<td>JLNS1</td>
<td>220400</td>
<td>Kv7.1</td>
<td>Reduced I_Ks</td>
<td>AR</td>
<td>Very rare</td>
<td>48</td>
</tr>
<tr>
<td>KCNE1</td>
<td>JLNS2</td>
<td>612347</td>
<td>MinK</td>
<td>Reduced I_Ki</td>
<td>AR</td>
<td>Very rare</td>
<td>49</td>
</tr>
<tr>
<td>Ankyrin-B Syndrome</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>ANKB</td>
<td>LQT4/ABS</td>
<td>600919</td>
<td>Ankyrin B</td>
<td>Aberrant ion channel/transporter localization</td>
<td>AD</td>
<td>&lt;1%</td>
<td>50</td>
</tr>
<tr>
<td>Andersen-Tawil Syndrome</td>
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<tr>
<td>KCN2</td>
<td>LQT7/ATS</td>
<td>170390</td>
<td>Kir2.1</td>
<td>Reduced I_Ki</td>
<td>AD</td>
<td>&lt;1%</td>
<td>51</td>
</tr>
<tr>
<td>Timothy Syndrome</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CACNA1C exon 8α</td>
<td>TSI</td>
<td>601005</td>
<td>Cav1.2</td>
<td>Increased I Ca,L  (slowed VDI)</td>
<td>Sporadic; AD mosaicism</td>
<td>Very rare</td>
<td>52, 53</td>
</tr>
<tr>
<td>CACNA1C exon 8</td>
<td>TS2</td>
<td>601005</td>
<td>Cav1.2</td>
<td>Increased I Ca,L  (slowed VDI)</td>
<td>Sporadic</td>
<td>Very rare</td>
<td>54</td>
</tr>
<tr>
<td>Calmodulinopathic LQTS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CALM1</td>
<td>LQT14</td>
<td>616247</td>
<td>Calmodulin 1</td>
<td>Increased I_Ca,L  (defective CDI)</td>
<td>Sporadic</td>
<td>~1-2%</td>
<td>55</td>
</tr>
<tr>
<td>CALM2</td>
<td>LQT15</td>
<td>616249</td>
<td>Calmodulin 2</td>
<td>Increased I_Ca,L  (defective CDI)</td>
<td>Sporadic</td>
<td>~1%</td>
<td>55</td>
</tr>
<tr>
<td>CALM3</td>
<td>LQT16</td>
<td>N/A</td>
<td>Calmodulin 3</td>
<td>Likely increased I_Ca,L  (defective CDI)</td>
<td>Sporadic</td>
<td>&lt;1%</td>
<td>56</td>
</tr>
<tr>
<td>Triadin Knockout Syndrome</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>TRDN</td>
<td>TKOS</td>
<td>N/A</td>
<td>Triadin</td>
<td>Likely increased I_Ca,L  (disruption of CRU)</td>
<td>AR; Sporadic</td>
<td>~2%</td>
<td>57</td>
</tr>
</tbody>
</table>

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; ABS, Ankyrin-B syndrome; ATS, Andersen Tawil syndrome; COTS, cardiac-only Timothy syndrome; LQTS, long-QT syndrome; and TS, Timothy syndrome.
Supplemental Figure 1 | Ca\textsubscript{v}1.2 L-type calcium channel (LTCC) and ryanodine receptor-2 (RyR2) linear protein topologies and macromolecular complexes. a) Ca\textsubscript{v}1.2 α1-subunit linear protein topology illustrating the four repeat domains each composed of six transmembrane domains (S1-S6) divided into the voltage sensing (S1-S4) and pore-forming (S5 and S6) regions as well as the intracellular amino (N)- and carboxy (C)-terminal regions that contain a number of protein-protein interaction domains/motifs including the N-terminal spatial Ca\textsuperscript{2+}-transforming element (NSCaTE), I-II linker α-interaction domain (AID), the proximal C-terminal CaM-binding IQ and EF hand-containing Ca\textsuperscript{2+}-inactivation (CI) region, and the distal A kinase anchor protein (AKAP)/protein kinase A (PKA) complex-binding AKAP binding domain (ABD) within the C-terminal auto-inhibitory peptide region. b) Artistic rendering of the Ca\textsubscript{v}1.2 macromolecular complex with α\textsubscript{2}δ, β\textsubscript{2}, γ, CaM, and AKAP/PKA complex interacting proteins bound. c) Ryanodine receptor-2 (RyR2) linear protein topology illustrating the six sarcoplasmic reticulum-spanning transmembrane domains and large cytosolic N-terminal domain containing binding sites for CaM-dependent kinase II (CaMKII), FK506-binding protein 12.6/calstabin 1, CaM (IQ motif), and multiple leucine/isoleucine zipper (LIZ) motifs that mediate binding of adaptor proteins that target protein phosphatases PP1
and PP2 as well PKA to RyR2. d) Artistic rendering of the RyR2 macromolecular complex with PP1, PP2, AKAP/PKA complex, FKBP12.6, CaMKII, CaM, sorcin, and triadin/junctin/calsequestrin-2 complex bound.