This Review is part of a thematic series on Inherited Arrhythmogenic Syndromes: The Molecular Revolution, which includes the following articles:


The Cardiac Desmosome and Arrhythmogenic Cardiomyopathies: From Gene to Disease [Circ Res. 2010;107:700–714]

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Voltage–Dependent L-Type Calcium Channel

Phenotypical Manifestations of Mutations in the Genes Encoding Ryanodine Receptor and Calsequestrin

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Sodium Channel

Phenotypical Manifestations of Mutations in Genes Encoding Subunits of Cardiac Potassium Channels

Silvia Priori, Editor

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Voltage–Dependent L-Type Calcium Channel

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Abstract: The L-type cardiac calcium channel (LTCC) plays a prominent role in the electric and mechanical function of the heart. Mutations in the LTCC have been associated with a number of inherited cardiac arrhythmia syndromes, including Timothy, Brugada, and early repolarization syndromes. Elucidation of the genetic defects associated with these syndromes has led to a better understanding of molecular and cellular mechanisms and the development of novel therapeutic approaches to dealing with the arrhythmic manifestations. This review provides an overview of the molecular structure and function of the LTCC, the genetic defects in these channels known to contribute to inherited disorders, and the underlying molecular and cellular mechanisms contributing to the development of life-threatening arrhythmias. (Circ Res. 2011;108:607-618.)

Key Words: Timothy syndrome, Brugada syndrome, early repolarization syndrome, ventricular arrhythmias, electrophysiology

Transmembrane calcium current ($I_{Ca}$) in the human myocardium is conducted via a macromolecular complex that includes a pore-forming unit and a number of subunits that cooperate to control the entry of calcium ions. $I_{Ca}$ is a relatively “new kid on the block” in the arena of inherited arrhythmogenic diseases. Recent studies have identified genetic variants that are associated with electric abnormalities capable of causing a variety of clinical phenotypes, including Timothy, Brugada, and early repolarization syndromes. These genetic defects have also guided us toward a better understanding of molecular and cellular mechanisms, which serve as a cornerstone for the development of novel therapies for our patients. In this review, we attempt to provide a comprehensive overview of the molecular structure and function of the cardiac L-type calcium channel (LTCC), followed by a discussion of inherited disorders associated with genetic mutations in LTCC, as well as a discussion of underlying molecular and cellular mechanisms contributing to the development of cardiac arrhythmias.
Molecular Diversity and Genomic Organization of Cardiac Calcium Channels in the Heart

Voltage-gated calcium channels are macromolecular complexes consisting of an ion conducting protein (the \( \alpha \) subunit), and additional “accessory” peptides: \( \beta_1 \) to \( \beta_4 \) and \( \gamma \) subunits. These accessory subunits are not simply innocent bystanders. Indeed they modulate, gating, trafficking, and response to neurohormonal stimuli. Whereas the role of \( \alpha, \delta \) and \( \gamma \) subunits is less clear, the \( \beta \) subunit is known to increase the \( I_{Ca} \) density to enhance single channel open probability and to modulate inactivation\(^1\); it is also reported to activate \( Ca^{2+} \)/calmodulin-dependent protein kinase (CaMKII), leading to \( Ca^{2+} \) overload.\(^2\)

### Classification of Calcium Channels

Calcium channels were first identified by Fatt and Katz in 1953\(^3\) and classified according to their biophysical and pharmacological (block by specific toxins) properties in the 1980s\(^4\) (Table): neuronal (N-type) channel blocked by \( \omega \)-conotoxin; resistant (R-type) channel; P/Q-type (P indicates Purkinje) channel blocked by \( \omega \)-agatoxins; dihydropyridine-sensitive LTCC responsible for excitation–contraction coupling of in skeletal and cardiac muscle. Transient-type (T-type) calcium channel has been identified in neurons and pace-maker cells (Table).

The molecular architecture of voltage-gated calcium channels is complex. There are 11 known \( \alpha \) subunit (\( Ca_{V} \))-encoding genes in humans; these are divided into 3 major classes; \( Ca_{V}1.1 \) to 1.4 are those conducting the LTCC current expressed in smooth muscle, skeletal muscles and in the central nervous system. \( Ca_{V}2.1 \) to 2.3 are responsible for P-, N-, and R-type \( Ca^{2+} \) current and are in the central nervous system. Finally \( Ca_{V}3.1 \) to 3.3 produce the T-type calcium current in the central nervous system, bones and peacemaking cells.

Three types of calcium channels are expressed in the human heart: T-type, P-type, and L-type. T-type calcium channels encompass 3 different homolog \( \alpha \) subunits: \( Ca_{V}3.1 \) (gene: \( CACNA1G \)), \( Ca_{V}3.2 \) (gene: \( CACNA1H \)), and \( Ca_{V}3.3 \) (gene: \( CACNA1I \)). \( Ca_{V}3.1 \) and \( Ca_{V}3.2 \) are expressed in the sinus node and contribute to peacemaking activity\(^5\) but also in ventricular myocytes during fetal life.\(^6\) The P-type calcium channels are found in Purkinje cells in cerebellum but also in low levels in the heart, where its expression is altered during heart failure.\(^7,8\) \( Ca_{V}1.3 \) is an L-type calcium channel expressed in both atria and ventricle of the fetal heart, but only

### Table. Five Subclasses of Calcium Channels With Distinct Electrophysiological and Pharmacological Properties: L, T, N, P, Q, and R

<table>
<thead>
<tr>
<th>Channel Type</th>
<th>Gated By</th>
<th>Protein</th>
<th>Gene</th>
<th>Tissue Distribution</th>
<th>Drug Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>High voltage</td>
<td>( Ca_{V}1.1 )</td>
<td>( CACNA1S )</td>
<td>Skeletal muscle</td>
<td>DHP, PAA, BTZ</td>
</tr>
<tr>
<td>L</td>
<td>High voltage</td>
<td>( Ca_{V}1.2 )</td>
<td>( CACNA1C )</td>
<td>Heart, brain, aorta, lung, fibroblast</td>
<td>Dihydropyridines (DHP) (nifedipine)</td>
</tr>
<tr>
<td>L</td>
<td>High voltage</td>
<td>( Ca_{V}1.3 )</td>
<td>( CACNA1D )</td>
<td>Brain, endocrine, atria, and ventricle of fetal heart</td>
<td>DHP, PAA, BTZ</td>
</tr>
<tr>
<td>L</td>
<td>High voltage</td>
<td>( Ca_{V}1.4 )</td>
<td>( CACNA1F )</td>
<td>Supraventricular tissues of adult heart</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>High voltage</td>
<td>( Ca_{V}2.2 )</td>
<td>( CACNA1B )</td>
<td>Brain</td>
<td>( \omega )-CgTXGVI (smaller, 100 nmol/L)</td>
</tr>
<tr>
<td>P/Q</td>
<td>High voltage</td>
<td>( Ca_{V}2.1 )</td>
<td>( CACNA1A )</td>
<td>Brain, heart</td>
<td>( \omega )-Aga-IVA (larger, 100 nmol/L)</td>
</tr>
<tr>
<td>R</td>
<td>Intermediate voltage</td>
<td>( Ca_{V}2.3 )</td>
<td>( CACNA1E )</td>
<td>Brain</td>
<td>( \omega )-CTX-MVIIC (larger, 100 nmol/L)</td>
</tr>
<tr>
<td>T</td>
<td>Low voltage</td>
<td>( Ca_{V}3.1 )</td>
<td>( CACNA1G )</td>
<td>Heart, brain, bone (osteoclasts)</td>
<td>Ni(^{2+} ) (smaller, 30 nmol/L)</td>
</tr>
<tr>
<td>T</td>
<td>Low voltage</td>
<td>( Ca_{V}3.2 )</td>
<td>( CACNA1H )</td>
<td></td>
<td>Ni(^{2+} ), Mibebradil</td>
</tr>
<tr>
<td>T</td>
<td>Low voltage</td>
<td>( Ca_{V}3.3 )</td>
<td>( CACNA1I )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
in supraventricular tissues (SA node, AV node, and atria) of
the adult heart.9 Although no mutations of Ca1.3 have as yet
been associated with human disease, deletion of Ca1.3 has
been reported to cause sinus bradycardia, AV block, as well
as hearing impairment.10

Of specific interest to this review is the L-type calcium
channel and more specifically the CaV1.2 channel encoded by
the CACNA1c gene, which maps to chromosomal locus
12p13.3. CaV1.2 is the most widely expressed calcium
channel in the heart where it plays a prominent role in
defining the electric milieu.11

Genetic Diversity of the Cardiac Calcium Channel
CACNA1c Subunit

CACNA1c has a very complex genomic architecture and
transcriptional splicing. It spans >500 kb and undergoes
extensive alternative splicing. There are 12 alternative
splicing loci that generate 42 different variants. Alternative
splicing occurs in the N-terminal, DI-II and DII-DIII linkers,
between S5 and S6 in DI and DII, and in the C-terminal
region.12 Interestingly some of these splice variants are
physiologically expressed and have been associated with a
diversity of biophysical properties: (1) depolarizing shift of
inactivation for alternatively spliced exons 31 to 33 encoding
the DIV S3-S4 linkers12; (2) faster inactivation kinetics,
strong voltage dependence, and no Ca2+-dependent inactiva-
tion13 for exon 41 to 42 variants in the C terminus;3
dihydropyridine sensitivity for exon 21 to 22 variants in the
DIII-S2 variant13 and for exon 9 in IS6;4 and (4) protein
kinase C sensitivity for the N-terminal variant resulting from
alternative splicing of exon 1 to 2,15 CACNA1c splice variant
relative abundance presents interindividual variability16 and
disease-specific variability. The splice variant containing
exon 31 is more abundant in normal human hearts with a
switch to the exon 32 variant in failing hearts.17 Preferential
deletion of exon 9 and inclusion of exon 33 has been observed
in rats with myocardial infarction.18

These observations support the notion that CaV1.2 is
responsible for a significant portion of the electric heteroge-
neity under physiological and pathophysiological conditions.
It follows also that the identification of CACNA1c mutations
may bring about interpretation hurdles both at the pathophy-
siological and clinical level.

Structure–Function of Cardiac L-Type Channels

Cardiac calcium channels are composed of a central pore-
forming CaV1.2 subunit (α1) and a set of ancillary subunits
(α2, δ, and β) (Figure 1). The α1 subunit contains 4
homologous domains (I to VI) of 6 transmembrane segments
(S1 to S6), each connected by intracellular linkers (I–II,
II–III, and III–IV loops) and to the intracellular N and C
terminals. CaV1.2 channels are clustered in the t-tubules in
close proximity to the sarcoplasmic reticulum Ca2+-release
channels ryanodine receptor 2 to form the so-called “calcium-
release units,”19 This spatial interaction allows optimal coupling
for the calcium-induced calcium release process.

ICa activates in response to voltage but the biophysics of
the current is not completely understood because of the
complexity of molecular and genomic components. ICa acti-
vation at physiological extracellular calcium concentration is
approximately -30 mV and it has slower kinetic than INa.20
ICa inactivation has 2 components: voltage- and Ca2+-
dependent inactivation. The Ca2+ mediated component of
inactivation is linked to intracellular calcium concentration
([Ca2+]i). The interdomain loop I–II of CaV1.2 is important
in controlling this process via CaV1.2 inactivation. Interest-
ingly, Timothy syndrome mutations (see below), are located
in this region. Additional elements that affect inactivation and
channel kinetics are in the S6 segments and in the
C-terminus.21 Regional and sex-related differences in ICa
density have been reported in animal models22 and may affect
the propensity for early afterdepolarization development.
Interestingly, higher current density in apex versus base in
females but not in males22 has been postulated to account for
the higher propensity of increased risk of cardiac events
among females with long QT syndrome (LQTS) type 2
(LQT2).23

Genetic Disorders Associated With Cardiac
Calcium Channel Mutations

Timothy Syndrome

Cardiologists consider Timothy syndrome (TS) a rare variant
of the Long QT syndrome (LQT8) but its complex phenotype
involving central nervous system and metabolic abnormali-
ities justifies a separate classification. In 1992, Reichenbach24
reported a case of intrauterine bradycardia secondary to AV
block caused by pronounced QT interval prolongation. The
male infant also showed hand and feet syndactyly. He died
suddenly at 5 months and the authors proposed the clinical
phenotype to be a novel clinical entity: the “heart and hand syndrome.”

Phenotype and Natural History

TS is characterized by multisystem dysfunction and developmental defects causing dysmorphic facial features including round face, flat nasal bridge, receding upper jaw, and thin upper lip (A through C) and webbing of the toes and fingers (syndactyly) (D and E). ECG shows severe QT interval prolongation causing 2:1 atrioventricular block seen as 2 atrial beats (P-waves) for each ventricular beat (QRS complex). Right, ECG shows alternating T-wave polarity (arrows), indicating severe cardiac repolarization abnormality (F). Ventricular tachycardia recorded from a TS patient by an implanted automatic defibrillator (G). Reprinted from Splawski et al25 with permission from Elsevier Inc.

Autism and autism spectrum disorder is a common manifestation of TS (83% in our series).28 It is well known that CACNA1c is expressed in the central nervous system; recent data suggest it is implicated in brain stem function29 and behavioral control such as “fear learning,”30 whereas population genetics studies have identified an association of specific single-nucleotide polymorphisms (eg, rs7959938 and rs1006737) and the risk of bipolar disorders and schizophrenia.29,31 Although there is no direct evidence for CACNA1c to cause autism, it is conceivable that, when mutated, it may cause a wide range of neuro-developmental and psychiatric disorders.

Few TS patients have survived to puberty thus far; average survival is 2 to 3 years; this Figure partially explains the low prevalence of the disease, which is almost entirely attributable to the sporadic mutations with few exceptions (see below).

The onset of cardiac tachyarrhythmia (ventricular tachycardia [VT] or fibrillation [VF]) is the cause of death in 79% of cases. No specific triggers for cardiac arrhythmias are known. However, life-threatening arrhythmias have been frequently reported during anesthesia.25,26 Nonarrhythmic death is also possible in TS: sepsis possibly caused by reduced immunity and intractable hypoglycemia has been anecdotally reported as possible causes of death.27

Genetics of Timothy Syndrome

The lack of familial clustering of the disease seemed to be a peculiar feature of the disease. For this reason, the initial hypothesis was that of chromosomal rearrangements. However, extensive karyotype investigations did not identify any significant alterations, therefore sporadic mutations or recessive inheritance was postulated. Through careful scrutiny of the ST-T wave morphology we hypothesized the involvement of an inward current and undertook a collaborative genetic screening program in TS patients with the Keating group. In 2004, we identified the G1216A transition in an alternatively spliced exon (8A), which causes the G406R missense mutation in DI/S6 segment (Figure 3).25 The same nucleotide

Figure 3. Predicted topology of the Ca,1.2 (α1c) subunit with associated β2 and α2δ subunits showing the location of mutations associated with BrS, BrS+SQTS, ERS, TS, and idiopathic ventricular fibrillation (IVF) probands. AID indicates α subunit interaction domain; BID, β subunit interaction domain. SH3 indicates Src homology domain; GK, guanylate kinase-like domain. Larger symbols with numbers denote multiple probes with the same mutation. Modified from Burashnikov et al51 with permission from the American Heart Association. (Illustration Credit: Cosmocyte/Ben Smith.)

Figure 2. Clinical manifestations of TS. TS is characterized by multisystem dysfunction and developmental defects causing dysmorphic facial features including round face, flat nasal bridge, receding upper jaw, and thin upper lip (A through C) and webbing of the toes and fingers (syndactyly) (D and E). ECG shows severe QT interval prolongation causing 2:1 atrioventricular block seen as 2 atrial beats (P-waves) for each ventricular beat (QRS complex). Right, ECG shows alternating T-wave polarity (arrows), indicating severe cardiac repolarization abnormality (F). Ventricular tachycardia recorded from a TS patient by an implanted automatic defibrillator (G). Reprinted from Splawski et al25 with permission from Elsevier Inc.
variation was detected in all patients included in this initial cohort, suggesting an unusual molecular homogeneity. In 2005, Splawski et al reported 2 additional cases (G406R and G402S) associated with the typical multifaceted phenotype but without syndactyly.24 One patient was carrier of the G406R mutation, whereas a novel variant, G402S, was found in the second case. Interestingly, both variants were present in exon 8 at variance with the first reported G406R occurring in the alternatively spliced exon 8A. The lack of syndactyly was explained by the differential expression of the 2 alternative exons.

The rare familial recurrence of TS has been linked to parental mosaicism. A mosaic is defined as an organism carrying cells with variable genotype at one or more loci. Thus apparently healthy parents may harbor a mutation only in the germline (or in few tissues) and be able to generate an affected offspring. If a CACNA1c mutation carrier expresses a low percentage of mutant cells, the clinical manifestation are variable and can be mild and atypical (depending on the specific tissue expressing the mutant channel).

Mosaicism was initially suggested in the original publication from Reichenbach et al24 and genetically demonstrated by Splawski et al in 2004 and 2005.25,26 The demonstration of mosaic CACNA1c mutation carriers has important implications: first of all, there is the possibility of incomplete penetrance or mild/atypical manifestations; second, phenotypically healthy parents may generate multiple affected offspring because of germine mutations.

Thus, genetic testing should be carried out on DNA extracted from multiple tissues (at least those easily testable: blood, sperm, saliva, skin) even if the parents of the proband are clinically unaffected. Finally, it is important to consider that the combination of syndactyly and QT prolongation may occur in a different inherited arrhythmogetic disorder, such as the Andersen–Tawil syndrome. This disorder (also referred to as LQT7) has autosomal dominant inheritance and it is characterized by QT prolongation, ventricular arrhythmias, periodic paralysis and facial dimorphisms. Differential diagnosis is important because Andersen–Tawil syndrome is usually a benign condition that rarely causes sudden death.32

J Wave Syndromes

Because they share a common arrhythmic platform related to amplification of transient outward current (I_o),-mediated J waves, and because of similarities in ECG characteristics, clinical outcomes and risk factors, congenital and acquired forms of Brugada (BrS) and early repolarization (ERS) syndromes have been grouped together under the heading of J wave syndromes.33 Recent studies have implicated loss of function mutations in all 3 subunits of the cardiac LTCC in the generation and accentuation of electrocardiographic J waves associated with these syndromes, as discussed below.34,35

Brugada Syndrome

BrS, an inherited cardiac arrhythmia syndrome associated with a relatively high risk of VF, was first described as a new clinical entity in 1992.10 The ECG features of the Brugada patient includes an accentuated J wave displaying a real or apparent right branch bundle block and ST segment elevation in the right precordial leads (V_1-V_3).36

The ECG characteristics and clinical outcomes of the BrS are influenced by heart rate, autonomic tone as well as other conditions and agents that accentuate the manifestation of the J wave, which is inscribed as a result of a transmural voltage gradient caused by a prominent action potential notch in epicardium but not endocardium.33,37 Sodium channel blockers like procainamide, pilsicainide, propafenone, and flecainide unmask the prominent J waves via an outward shift in the balance of currents active in the early phases of the action potential.37–39 Overall rate of cardiac arrest/VF in BrS is 8% to 12%. Few variables have proven to be useful metrics for risk stratification; the presence of a spontaneous coved-type ST segment elevation and history of syncope appear to be clearly associated with risk of events.40 Inducibility with programmed electric stimulation, QRS fragmentation and the type of underlying mutation are still under debate.40–43

BrS has been associated with mutations in 8 distinct genes, which encode cardiac ion channels or proteins that modulate channel activity. Mutations in SCN5A (Na_1.5, BrS1) have been reported in 11% to 28% of BrS probands, CACNA1C (Ca_1.2, BrS3) in 6.7%, CACNB2b (Ca_2b, BrS4) in 4.8% and mutations in Glycerol-3-phosphate dehydrogenase 1-like enzyme gene (GPD1L, BrS2), SCN1B (β_1-subunit of sodium channel, BrS5), KCNE3 (MiRP2; BrS6), and SCN3B (β_3-subunit of sodium channel, BrS7) are much more rare.34,44–50 These genetic defects lead to either a loss of function of sodium (I_Na) or I_o, or a gain of function of I_o. It is noteworthy that mutations in the calcium channel genes often gives rise to BrS associated with shorter than normal QT interval, in some cases qualifying them for a combined diagnosis of BrS and short QT (BrS+SQT).34

Recent studies have identified CACNA2D1 (Ca_2.δ) as a new BrS-susceptibility gene.51 Missense mutations predicting p.S709N and p.D550Y alteration of the α_2δ subunit of the LTCC were identified in 3 BrS probands. Functional expression studies indicate that the p.D550Y mutation coupled with a p.Q917H rare polymorphism in CACNA2D1 reduces I_o to 25% of normal (Barajas et al, unpublished observation). Our most recent yields in a cohort of 205 probands diagnosed with a J wave syndrome indicate that 12.3% BrS and BrS+SQT probands display mutations in α1, β2, or α2δ subunits of LTCC. With inclusion of rare polymorphisms, the yield increased to 17.9%. The genotype of approximately 60% to 65% of BrS probands remains to be identified.

Although loss-of-function mutations in the LTCC are known to predispose to a phenotype consisting of BrS with an abbreviated QTc, the majority of BrS probands thus far identified with calcium channel mutations present with normal QTc intervals. This apparent paradox has been attributed to the copresence of genetic variations in other ion channel genes that are known to lead to prolongation of
QTc in 86% (12 of 14) of patients displaying characteristics of BrS and a normal QTc.51

Early Repolarization Syndrome
An early repolarization (ER) pattern, consisting of a J point elevation, a notch or slur on the QRS (J wave), and tall/symmetrical T waves, is generally found in healthy young males and is traditionally regarded as totally benign.52,53 The pathogenicity of an ER pattern and its association with potentially life-threatening arrhythmias emerged from observations in the coronary-perfused wedge preparation in 2000.33,54 Many case reports and experimental studies have long suggested a critical role for the J wave in the pathogenesis of idiopathic ventricular fibrillation (IVF) (see Antzelevitch and Yan33 for references). Several recent studies have provided a definitive association between ER and IVF.55–59

Because of its high prevalence in the general population ER cannot be considered a specific marker for sudden cardiac death (SCD), but the high incidence of ER in patients with IVF suggests that ER is a predisposing factor. As is discussed in the next section, a transient J wave augmentation secondary to a shift of the balance of currents active in the early phases of the action potential caused by any one of a number of risk factors may set the stage for the development of VF in patients with ER, thus precipitating the early repolarization syndrome (ERS).

A classification scheme for ERS recently proposed on the basis of available data associates risk with spatial localization of the ER pattern.33 Type 1 is associated with ER pattern predominantly in the lateral precordial leads; this form is very prevalent among healthy male athletes and is thought to be largely benign. Type 2, displaying an ER pattern predominantly in the inferior or inferolateral leads, is associated with a moderate level of risk; In a community-based general population study of 10,864 middle-aged subjects, Tikkanen et al reported that J-point elevation of >0.2 mV in the inferior leads is associated with a markedly elevated risk of death from cardiac arrhythmia (adjusted relative risk, 2.92; 95% CI, 1.45 to 5.89; P=0.01).56 Type 3, displaying an ER pattern globally in the inferior, lateral and right precordial leads, appears to be associated with the highest level of risk and is often associated with electrical storms.53 BrS represents a fourth variant in which ER is limited to the right precordial leads.

The dynamic nature of J wave manifestation in ERS is well recognized. The amplitude of J waves, which may be barely noticeable during sinus rhythm, may become progressively accentuated with increased vagal tone and bradycardia and still further accentuated following successive extrasystoles and compensatory pauses giving rise to short long short sequences that precipitate VT/VF.33,58,60

Data relative to the genetic and molecular basis for ERS is very limited. Haissaguerre et al were the first to associate a missense mutation in KCNJ8 (S422L) with ERS.61 Functional expression data in support of this as a disease-causing genetic variant was recently provided by Medeiros-Domingo et al.62 However, the gold standard for demonstrating a change in sensitivity to adenosine triphosphate (ATP) involves the study of inside-out patches with the internal membrane exposed to increasing concentrations of ATP. These data unfortunately are still not available. The prospect of a gain of function in the ATP-sensitive potassium current (IK-ATP) as the basis for ERS is supported by the observation that pinacidil, an IK-ATP opener, induces both the electrocardiographic and arrhythmic manifestation of ERS in LV wedge preparations.33

Our recent study designed to identify mutations in the LTCC identified 4 individuals with ERS among 205 J wave syndrome probands with mutations in highly conserved residues of CACNA1C, CACNB2 and CACNA2D1.51 Preliminary expression studies indicate that these mutations are associated with a loss of function of ICa,L supporting the thesis that all 3 are ERS-susceptibility genes (Barajas et al, unpublished observation).

Association of Site of Mutation With Channel Dysfunction
The predicted topology of the 3 subunits of LTCC showing the location of the mutations thus far identified is illustrated in Figure 3. Six of the 9 mutations in Ca1.2 a1 subunit associated with the J wave syndromes were in either the N or C terminus. It is interesting that no mutations were detected in any of the transmembrane regions of Ca1.2. Consistent with this finding is the demonstration by Soldatov and colleagues of voltage-gated mobility of the C-and N-cytoplasmic tails of Ca1.2 and their important regulatory role in voltage- and Ca2+-dependent inactivation.63,64 In addition, it has been shown that cleavage of the C terminus of native Ca1.2 channels results in a proteolytic fragment that is able to act as a repressor of Ca1.2 promoter activity.65,66 Thus, mutations in the C terminus could have very significant effects on the regulation of expression level and on function of the Ca1.2 channel. Another mutation of great interest is p.E1115K because it is located in the region of the calcium ion selectivity site, giving rise to multiple SCD in the family.51

Most recently, Navedo et al67 demonstrated that a cluster of Ca1.2 channels organize to facilitate coordinated openings and closings. Fluorescence resonance energy transfer analysis of enhanced green fluorescent protein– and red fluorescent protein–tagged Ca1.2 channels suggest that coupled gating between these channels may involve transient interactions between 2 to 6 adjacent channels of adjacent channels via their C termini. This finding provides further insight into the mechanisms underlying channel dysfunction associated with C terminus mutations.

Molecular and Cellular Pathophysiology of Calcium Channel Mutations
Timothy Syndrome
Timothy syndrome arises from sporadic missense mutations (G406R and G402R) affecting the I–II linker of Ca1.2,
which is believed to act as the inactivation gate. By disrupting inactivation, these mutations lead to a gain of function of \( I_{Ca} \), which is responsible for the clinical phenotype.\(^{25}\) Barrett and Tsien found that the TS mutation G406R selectively slows voltage-dependent inactivation while sparing or slightly speeding the kinetics of \( Ca^{2+}/H_1 \) dependent inactivation.\(^{68}\) \( Ca^{2+}/H_1 \) dependent inactivation did not proceed to completeness but was observed to level off at approximately 50%, consistent with a change in gating modes. Thiel et al.\(^{69}\) found a voltage-dependent inactivation loss in the cells infected with the mutant channels when intracellular \( Ca^{2+}/H_1 \) was buffered. In the presence of normal \( Ca^{2+} \) solution, CaMKII activity was increased and the cells exhibit prolonged action potentials and (ER). Inhibition of CaMKII activity reversed the proarrhythmic phenotypes to normal.\(^{69}\) G406R may create a CaMKII consensus site at Ser-409 and that Ser-409 phosphorylation leads to an increased channel activity (“mode 2” gating). It is unknown, however, whether or not Ser.409 is a CaMKII target.

**J Wave Syndromes**

Transmural differences in early phases of the action potential (phases 1 and 2) are responsible for inscription of the electrocardiographic J wave.\(^{70,71}\) The ventricular epicardium commonly displays action potentials with a prominent \( I_{to} \) mediated notch or spike and dome. A prominent \( I_{to} \) mediated action potential notch in ventricular epicardium but not endocardium produces a transmural voltage gradient during ERS that registers as a J wave or J point elevation on the ECG.\(^{72}\)

Conditions and agents that influence \( I_{to} \) kinetics or ventricular activation sequence can modify the manifestation of the J wave on the ECG. Because of its slow recovery from inactivation, \( I_{to} \) is increased following slowing of heart rate, resulting in an increase in the magnitude of the J wave.\(^{73,74}\)

Augmentation of net early repolarizing current, attributable either to a decrease of inward current or to an increase of outward current, accentuates the notch leading to augmentation of the J wave or appearance of ST segment elevation. A further increase in net repolarizing current can result in partial or complete loss of the action potential dome, leading to a transmural voltage gradient that manifests as greater ST segment elevation.\(^{73–75}\)

**Brugada Syndrome**

In regions of the myocardium exhibiting a prominent \( I_{to} \), such as the right ventricular outflow tract epicardium, accentuation of the action potential notch by a reduction of calcium or sodium channel current or an increase in outward current, results in a transmural voltage gradient that leads to coved ST segment elevation (Figure 4B). Under these conditions, there

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**Figure 4. Cellular basis for electrocardiographic and arrhythmic manifestation of BrS.** Each panel shows transmembrane action potentials from 1 endocardial (top) and 2 epicardial sites together with a transmural ECG recorded from a canine coronary-perfused right ventricular wedge preparation. **A,** Control. **B,** Combined calcium and sodium channel block with terfenadine (5 \( \mu \)mol/L) accentuates the epicardial action potential notch creating a transmural voltage gradient that manifests as a ST segment elevation or exaggerated J wave in the ECG. **C,** Continued exposure to terfenadine results in all-or-none repolarization at the end of phase 1 at some epicardial sites but not others, creating a local epicardial dispersion of repolarization (EDR), as well as a transmural dispersion of repolarization (TDR). **D,** Phase 2 reentry occurs when the epicardial action potential dome propagates from a site, where it is maintained to regions where it has been lost giving rise to a closely coupled extrasystole. **E,** Extrastimulus (S1–S2=250 ms) applied to epicardium triggers a polymorphic VT. **F,** Phase 2 reentrant extrasystole triggers a brief episode of polymorphic VT. Reprinted from Antzelevitch and Yan\(^{53}\) with permission from Elsevier Inc.
is little in the way of an arrhythmogenic substrate. However, a further outward shift of the currents active during the early phase of the action potential can lead to loss of the action potential dome, thus creating a dispersion of repolarization between epicardium and endocardium as well as within epicardium, between the region where the dome is lost and regions at which it is maintained (Figure 4C).

When \( I_{\text{to}} \) is prominent, because it is in the right ventricular epicardium, \(^{74-76}\) an outward shift of current causes phase 1 of the action potential to progress to more negative potentials at which the \( I_{\text{Ca}} \) fails to activate, leading to an “all-or-none” repolarization and loss of the dome (Figure 4C). Because loss of the action potential dome is usually heterogeneous, the result is a marked abbreviation of action potential at some sites but not others. The epicardial action potential dome can then propagate from regions where it is maintained to regions where it is lost, giving rise to a very closely coupled extrasystole via a mechanism that we have termed phase 2 reentry (Figure 4D).\(^{77}\) The extrasystole produced via a mechanism that we have termed phase 2 reentry often result in an R-on-T phenomenon precipitating life-threatening arrhythmias (Figure 4E and 4F).

### Early Repolarization Syndrome

An outward shift of current may extend beyond the action potential notch thus leading to an elevation of the ST segment akin to early repolarization. Activation of \( I_{\text{K-ATP}} \) or depression of \( I_{\text{Ca}} \) can affect such a change.\(^{51}\) Figure 5 shows an example of ER produced by calcium channel current inhibition. Transmural gradients generated in response to \( I_{\text{Ca}} \) block could manifest in the ECG as a diversity of ER patterns including J point elevation, slurring of the terminal part of the QRS and mild ST segment elevation. The ER pattern could facilitate loss of the dome and lead to the development of ST segment elevation, phase 2 reentry, and VT/VF.

The ability to recapitulate the ECG and arrhythmic manifestations of ERS using agents that inhibit \( I_{\text{Ca}} \) or augment \( I_{\text{K-ATP}} \) is consistent with the fact that ERS has been found to be associated with mutations in genes encoding the \( I_{\text{K-ATP}} \) (\( KCNJC8 \)), as well calcium channels (\( CANCA1c, CACNB2, CANCA2D1 \)).

Morrissey et al showed that ventricular Kir6.1 (pore-forming unit of the \( I_{\text{K-ATP}} \) channel) is more strongly expressed in epicardial ventricular myocytes.\(^{78}\) This heterogeneous transmural distribution of Kir6.1, if present in humans, can contribute to the development of ST segment elevation observed in patients with BrS and ERS carrying mutations in \( KCNJ8 \) or \( SUR2A \). The presence of an additional repolarization force during the early phases of the epicardial action potential, attributable to a gain of function of \( I_{\text{K-ATP}} \), can generate an early repolarization pattern in the ECG by causing depression of the epicardial action potential dome.\(^{33}\) The heterogeneous distribution of \( I_{\text{K-ATP}} \) channels may be unmasked by a reduction in calcium channel activity (attributable to mutations or calcium channel blocking drugs). The transmural distribution of \( I_{\text{to}} \) may also accentuate the effects of reduced \( I_{\text{Ca}} \). Moreover, a further outward shift in the balance of current caused by augmented vagal influence (\( I_{\text{K-ATP}} \) activation), bradycardia (greater availability of \( I_{\text{to}} \)), or mild ischemia (more \( I_{\text{K-ATP}} \)), can lead to heterogeneous loss of the action potential dome, thus creating a dispersion of repolarization that can facilitate the development of phase 2 reentry and polymorphic VT.\(^{33,79}\)

### Approach to Therapy of Timothy and J Wave Syndromes Associated With Calcium Channel Mutations

#### Timothy Syndrome

Ventricular tachyarrhythmias (VT or VF) is the leading cause of death in TS and should be a primary target for therapy. Although no data are specifically available for the TS cohort, \( \beta \)-blockers are used based on the evidence that they are effective in most LQTS.\(^{80}\) Additional drug therapy (mexilene, calcium channel blockers) has been proposed in an attempt to abbreviate ventricular repolarization, restore 1:1 conduction, and thus reduce the risk of arrhythmias, but their use has to be considered investigational. Ranolazine has been reported to suppress atrial and ventricular arrhythmias associated with TS in experimental studies as well as in one case report.\(^{81,82}\) Overall the lack of firm data on effectiveness of drug therapy suggest the use of an implantable cardioverter defibrillator (ICD) in all patients with a confirmed diagnosis and QTc \( \geq \)500 ms. Careful monitoring and aggressive therapy of infections (altered immune response) and blood glucose levels is also mandatory.

#### J Wave Syndromes

The mainstay of therapy for the J wave syndromes is the implantation of an ICD.\(^{49,83-85}\) However, ICD implantation is
problematic in infants and young children. A pharmacological approach to therapy, based on a rebalancing of currents active during the early phases of the epicardial action potential so as to reduce the magnitude of the action potential notch and/or restore the action potential dome, has been a focus of basic and clinical research. Because the presence of a prominent transient outward current, \( I_{to} \), is central to the mechanism underlying the J wave syndromes, the most rational approach to therapy, regardless of the ionic or genetic basis for the disease, is to partially inhibit \( I_{to} \). The only agent on the market with significant \( I_{to} \) blocking properties is quinidine.

Brugada Syndrome

Experimental studies have shown quinidine to be effective in restoring the epicardial action potential dome, thus normalizing the ST segment and preventing phase 2 reentry and polymorphic VF in experimental models of BrS. Clinical evidence of the effectiveness of quinidine in normalizing ST segment elevation in patients with the BrS has been reported. The effects of quinidine to prevent inducible and spontaneous VF has been reported by Belhassen and Viskin. A prospective registry for asymptomatic Brugada patients has been created with the aim of tracking the effectiveness of empirical therapy with quinidine. The development of a more cardioselective and \( I_{to} \)-specific blocker would be a most welcome addition to the limited therapeutic armamentarium currently available.

Agents that boost \( I_{Ca} \), including \( \beta \) adrenergic agents such as isoproterenol or the phosphodiesterase III inhibitor cilostazol, are useful as well. Isoproterenol, sometimes in combination with quinidine, has been shown to be effective in normalizing ST segment elevation in patients with the BrS and in controlling electrical storms, particularly in children.

Early Repolarization Syndrome

Data relative to an approach to therapy of arrhythmic complications of early repolarization syndrome are scarce owing to the recent emergence of this syndrome. Experimental studies suggest an approach to therapy similar to that used in BrS. Quinidine and \( \beta \)-adrenergic agonists have recently been shown to be effective in suppressing arrhythmic events in patients with ERS.

Conclusions

The LTCC is widely distributed and plays a critical physiological role in many organs, including the heart. Once thought to be rare, genetic mutations in the subunits of the LTCC are now recognized to be relatively common and to be associated with a wide variety of cardiac arrhythmia syndromes including Timothy, Brugada, and early repolarization syndromes, as well as other clinical phenotypes. Mutations of LTCC genes were identified only recently. Over the past 6 years, more than 25 mutations in the \( \alpha 1 \), \( \beta 2 \), and \( \alpha 26 \) subunits have been identified. Although we have made significant progress in the identification of genetic variations contributing to sudden death syndromes and the ionic and cellular mechanisms responsible for the associated arrhythmias, we are clearly at the tip of the iceberg. With further advances in our understanding of the function of LTCC in health and disease, we hope to develop better therapies for these syndromes.

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None.

References


Peer-reviewed research articles and reviews on early repolarization and Brugada syndrome.


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