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L-Type Calcium Channel Disease

Yanfei Ruan, Raffaella Bloise, Carlo Napolitano, and Silvia G. Priori

Introduction

In recent years, the progress of molecular genetics of inherited arrhythmogenic diseases portrays an unexpected complexity of clinical phenotypes associated with mutations in several genes that control cardiac excitability. Among the most recent findings, the voltage-gated L-type cardiac calcium channel (Cav1.2) has been involved in the pathogenesis of Timothy syndrome (TS). TS is a variant of the long QT syndrome (also LQT8) and it is a rare and severe genetic disorder characterized by a spectrum of complex phenotypes including QT interval prolongation, congenital heart defects, syndactyly, and distinctive dysmorphic features. So far TS is the only inherited arrhythmogenic disorder linked to cardiac calcium channel mutations. In this chapter, we will briefly review the structure, physiology, and pathophysiology of the cardiac Cav1.2 encoded by the *CACNA1c* gene.

L-Type Calcium Channel

Structure of the Cardiac Cav1.2 Channel

Cav1.2 constitutes the pore-forming protein (α_1 subunit) responsible for the voltage-dependent L-type Ca^{2+} channel in the heart. However, the channel may be considered as a macromolecular complex, made up of α_1, α_2/δ, and β subunits. The α_1 subunit is a protein of about 2000 amino acidic residues and consists of four homologous domains (I–IV), each one formed by transmembrane spanning segments (S1–S6), and a membrane-associated loop between S5 and S6[1] (Figure 10–1). The α_1 subunit forms the ion-selective pore, the voltage sensor, the gating machinery, and the binding sites for channel-modulating drugs. The positively charged S4 of each domain serves as the voltage sensors for channel activation. It is thought that the S4 moves outward and rotates under the influence of the electric field so as to induce a conformational change that opens the pore.[2] The pore-conducting calcium ions are composed of S5 and S6 and the loop between them. The pore is asymmetric: the outside pore is constructed by the pore loop, which contains highly conserved glutamate residues (EEEE) for calcium ion selectivity.[3] The inside pore is composed of the S6 segments, which include the receptor sites for L-type Ca^{2+} channel antagonist drugs.[1]

The β, α_2, and δ subunits appear to have a regulatory effect. Interestingly, the α_2 and δ subunits are encoded by a single gene that is translated as a precursor polypeptide and is posttranslationally cleaved into the two subunits. The transmembrane δ subunit anchors the α_2 protein to the membrane via a single putative transmembrane segment. This association is mediated by disulfide bridges. A range of functional effects has been identified for the associated subunits, especially the β subunit, including ligand binding, increasing peak currents, and modulation of activation and inactivation (increasing the rate of both voltage and Ca^{2+}-dependent inactivation) rates.[4,5]

Calcium Channel Function

Cav1.2 is the major calcium channel expressed in the ventricular myocytes. It produces a voltage-dependent inward Ca^{2+} current (I_{Ca}) that activates

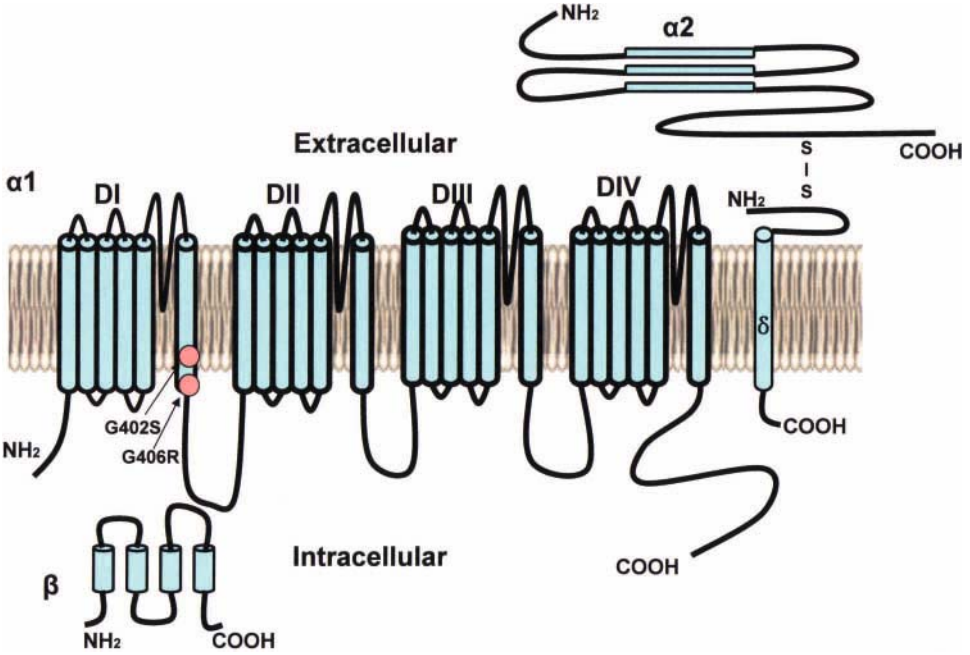

FIGURE 10–1. Predicted topology of Cav1.2, showing the location of the mutations. (From Splawski *et al.*[15])

upon depolarization and it is a crucial player in the maintenance of the plateau of the cardiac action potential; thus I_{Ca} modulation can greatly affect the action potential duration. Furthermore, Ca^{2+} ions play an important role in excitation–contraction coupling, since I_{Ca} triggers the release of the calcium ion from the sarcoplasmic reticulum and thereby elevates cytoplasmic Ca^{2+} to initiate contraction. Overall, any perturbation of this channel has a great potential of inducing an arrhythmogenic substrate.

Regulation and Tissue Distribution of the Cardiac Cav1.2 Channel

Besides voltage, many factors are important for regulation of cardiac Cav1.2 channel. Protein kinase A (PKA), protein kinase C (PKC), and Ca^{2+}-binding protein calmodulin constitute a key mechanism for controlling Ca^{2+} influx.[6–9] Furthermore, Cav1.2 channel activity is also enhanced by calcium, catecholamine[10] and Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII).[11,12] This latter interaction appears to have an important role for Timothy syndrome pathogenesis (see below).

Cav1.2 protein is expressed in several tissues including heart, the peripheral and central nervous system, liver, testis, spleen, connective tissue, and bone marrow. (see details at: http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.372570). On the basis of such widespread tissue distribution it is conceivable that mutations in this gene may perturb the function of several organs.

Furthermore, it must be emphasized that the *CACNA1c* gene (the gene encoding for Cav1.2) undergoes extensive alternative splicing, producing splice variants with distinct electrophysiological and pharmacological properties.[13] Cell-selective expression of Cav1.2 channels containing a specific alternatively spliced exon increases the functional variations for specific cellular activities in response to changing physiological signals.

Although the control pathways of such alternative splicing are unknown, this evidence highlights the complexity of regulation of the calcium current and it emphasizes the difficulty in predicting the clinical manifestation of mutants Cav1.2 proteins. This concept is well confirmed by the recent genetic findings linking Cav1.2 mutations to a severe inherited arrhythmogenic disease, the Timothy syndrome,[14,15] which will be described below.

Timothy Syndrome

Historical Notes

Until recently only anecdotal reports of QT interval prolongation, arrhythmias, and syndactyly were given in the literature. In 1992, Reichenbach reported and defined it as a novel clinical entity, a case of a male infant born at week 36 of gestation by cesarean section (because of intrauterine bradycardia) who died suddenly at age 5 months. Second degree atrioventricular (AV) block, QT interval prolongation and syndactyly were observed.[16] Subsequently Marks *et al.* reported additional three cases of long QT syndrome (LQTS) and syndactyly,[17] confirming the typical features of this disorder.

The first systematic description of the disease was jointly reported in a collaborative study by the group of Mark Keating and our group. In this study we described 13 cases of this distinctive form of LQTS, presenting with a complex disorder (Figure 10–2) (see below) with multiorgan involvement and defined the disease as Timothy syndrome (MIM:601005).[14]

Phenotype and Natural History of Timothy Syndrome

The initial clinical abnormalities of TS may manifest during gestation with bradycardia and 2:1 AV block, but diagnosis is often made within the first few days of life due to abnormal ventricular repolarization and soft tissue syndactyly of hands and toes. With few exceptions (possibly related to a different genetic substrate, see below), syndactyly has been observed in the majority of cases.

The QT interval is markedly prolonged in TS (mean value 600 msec); such extreme QT prolongation often causes 2:1 functional AV block. Remarkable abnormalities of T-wave morphology consisting of long and straight ST segment, negative T-wave, and macroscopic T-wave alternans are also evident.

The most frightful manifestations of the disease are represented by cardiac tachyarrhythmia [ventricular tachycardia (VT) or ventricular fibrillation (VF)], which occurs in 79% of patients and is the most frequent cause of mortality (Figure 10–3). Ten of the 17 patients described in the past decade died at a mean age of 2.5 years. In 12 of 17 patients life-threatening ventricular arrhythmias have been documented. The most severe variant of LQTS is probably TS.

Several additional pathological (cardiac and extracardiac) phenotypes contribute to the TS phenotype:

1. Congenital heart disease (patent ductus arteriosus, ventricular septal defect, patent foramen ovalis, Tetralogy of Fallot) (55% of cases).
2. Hypertrophic cardiomyopathy,[18] cardiomegaly, and ventricular systolic dysfunction (30% of cases).
3. Facial dysmorphisms (91% of cases).
4. Predisposition to sepsis (50% of cases).
5. Metabolic (severe hypoglycemia) and immunological (recurrent infections) disturbances (40% of cases).
6. Neuropsychiatric involvement (autism, seizures, psychological developmental delays) (83%).

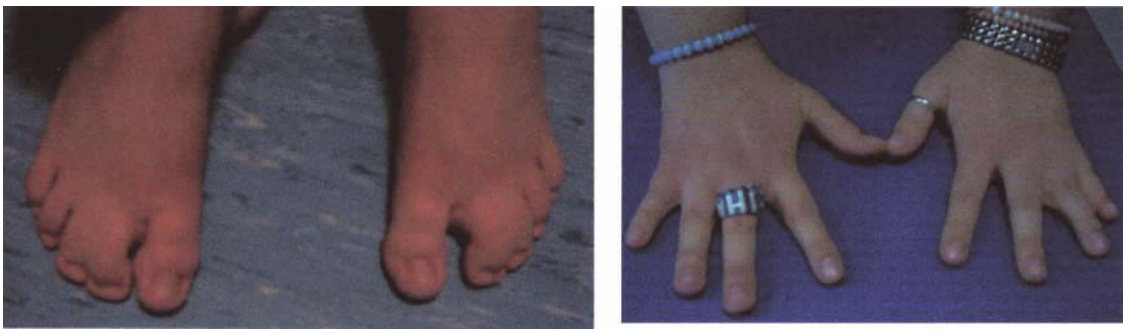

Figure 10–2. Syndactyly of feet (left panel) and hands (right panel) in patients with TS.

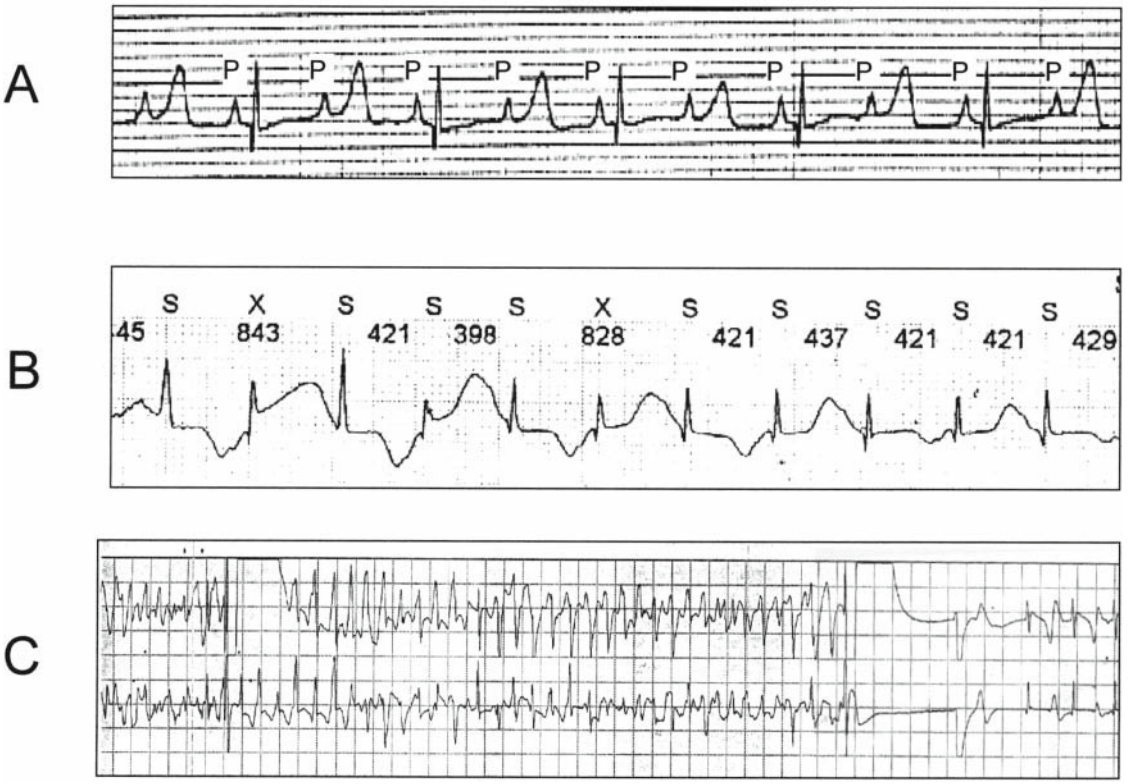

FIGURE 10–3. ECG recording in a TS patient. (A) Long QT and 2:1 atrioventricular block. (B) T-wave alternans. (C) Ventricular fibrillation.

Genetics of Timothy Syndrome

The ST-T wave morphology in TS patients resembles that of LQTS patients with sodium channel mutations (LQT3, see Chapter 11), suggesting the involvement of an inward current active during the plateau phase of the cardiac action potential. However, the screening of the *SCN5A* gene (as well as that of the other known LQTS genes) was negative. Thus the *CACNA1c* gene (encoding for Cav1.2) was considered a plausible candidate. In 2004, Splawski *et al.* identified the G1216A transition in exon 8A (an alternatively spliced exon), which caused the G406R amino acid transition in DI/S6 in TS patients.[14] Subsequently, Splawski *et al.* reported two individuals with a severe variant of TS but without syndactyly, which they named TS2.[15] Genetic analyses show G1216A and G1204A in exon 8, which caused a G406R and G402S amino acid transition, respectively (Figure 10–1).

By means of immunostaining experiments it was shown that exon 8A is expressed in the central nervous system, including hippocampus, cerebellum, and amygdala. Abnormalities of these brain regions have been implicated in autism, a typical feature of TS. Thus, TS may represent the first evidence for a genetic predisposition to behavioral disorders. Interestingly, the clinical relevance of transmembrane Ca^{2+} current alterations and behavioral abnormalities has been further supported by the association between allelic variants in the *CACNA1h* gene (Cav3.2, T-type calcium channel) in patients with autism spectrum disorder.[19] Although not directly related to TS pathogenesis these data strengthen the concept that Ca^{2+} dysfunction may be involved in severe neuropsychological disorders and indirectly the direct pathogenetic role of Cav1.2 in autism.

Exon 8A of *CACNA1c* is also expressed throughout the heart and the vascular system in develop-

ing digits and teeth. Thus, the expression pattern of exon 8A is consistent with the phenotypic abnormalities associated with TS.[14]

Exons 8 and 8A are mutually exclusive as they encode the same structural domain (DI/S6), but one of the two must be present to encode a functional channel. In the human heart it has been experimentally shown that 22.8% of Cav1.2 proteins contain exon 8A and 77.2% contain exon 8. In the brain, 23.2% contain exon 8A and 76.8% contain exon 8. Compared to exon 8A, exon 8 is very highly expressed in the heart and brain. Consistent with the expression, TS2 patients having a mutation in exon 8 appear to have a longer QTc than TS1, and a more severe pattern of arrhythmias. Interestingly, the TS2 patients reported so far do not show syndactyly, possibly because of the differential expression of exons 8 and 8A.[15]

Familial recurrence of the TS phenotype is rare. Indeed, the disease results from *de novo* mutation in most of the probands. Parent-to-offspring transmission of the phenotype has never been reported, probably because the high rate of malignancy associated with the disease prevents the majority of affected patients from reaching reproductive age. Familial recurrence in the offspring of normal parents has been reported in only three families and it was attributed to mosaicism. The evidence that a normal couple with no previous family history of TS may have children affected by this severe disease has a relevant impact for genetic counseling of TS patients/families.

Mechanism of Arrhythmogenesis

In the experiment of Splawski *et al.*,[14] the current–voltage (I/V) relationship and voltage dependence of activation were similar for wild-type (WT) and mutant channels. The difference between WT and G406R channels was the extent of inactivation. Inactivation of WT channel current was nearly complete in 300 msec, while G406R channels were only slightly inactivated during the same time frame. In the voltage dependence of inactivation curves, WT channel inactivation was complete at +20 mV. In contrast, inactivation was only 56% for the G406R channels (Figure 10–4). Thus, the likely mechanism of G406R mutation was assumed to be an increase of inward I_{Ca} due to loss of voltage-dependent inactivation.[14] The altered L-type calcium channel inactivation caused by the mutations was also simulated in a dynamic model of a human ventricular myocyte.[15] This model confirmed that the action potential is significantly prolonged as a consequence of this TS mutation and suggested that delayed afterdepolarizations and triggered activity are the final mechanisms for the onset of arrhythmias.

Recently Erxleben C *et al.* proposed a more complex model to explain the cellular phenotype

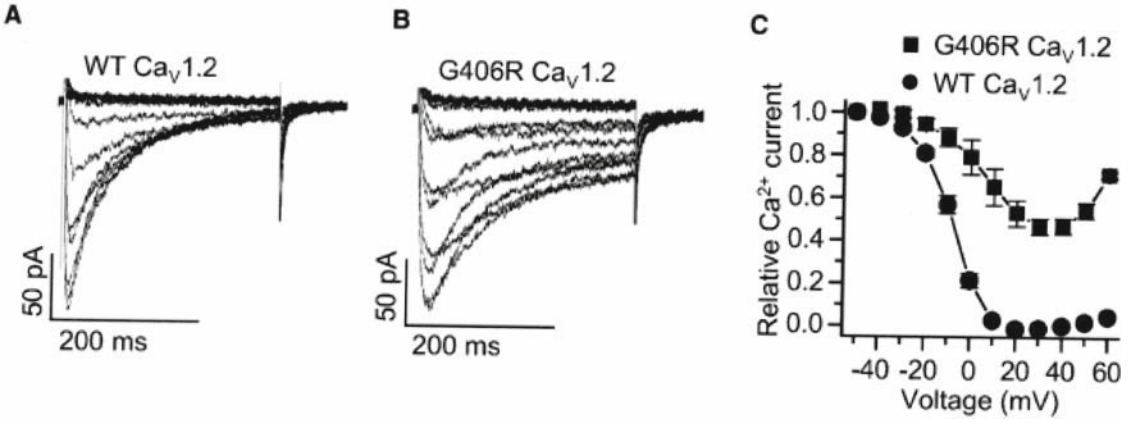

Figure 10–4. Wild-type (A) and G406R (B) Cav1.2 channel currents recorded from CHO cells in response to voltage pulses applied in 10 mV increments from −40 to +60 mV. (C) Voltage dependence of Ca^{2+} current inactivation for WT and G406R channels. (From Splawski *et al.*[14]).

caused by the G406R mutation.[20] By means of single channel recordings, they observed a decrease in unitary channel conductance and an increase in spontaneous "mode 2 gating" (which is characterized by increased open probability and longer channel activations). Such mode 2 gating could produce the apparent loss of macroscopic current inactivation at the whole cell level.

They further explored the role of G436R mutation (the rabbit homolog for human TS mutation G406R) on Cav1.2 channel phosphorylation. The presence of the TS mutation in rabbit Cav1.2 makes the protein much more sensitive to CaMKII (Ca^{2+}-calmodulin-dependent protein kinase II) and the channel is less easily dephosphorylated. Thus, the mutation is likely to be a kind of hyperphosphorylated state that in addition to the abovementioned electrophysiological effect can also create a cytotoxic effect due to chronic intracellular calcium overload.

Therapy for Timothy Syndrome

As pointed out previously, ventricular tachyarrhythmias (VT or VF) are the leading cause of death in TS. At present, most TS patients have been treated with β-blockers, since it is considered a generally effective therapy in patients with congenital long QT syndromes. However, no data are available concerning the effectiveness of this approach in the TS subgroup. Additional pharmacological therapies (mexiletine, calcium channel blockers) have been proposed in an attempt to shorten ventricular repolarization, restore 1:1 conduction, and reduce the risk of arrhythmias, but their use still has to be considered in a experimental evaluation phase. Therefore an implantable cardioverter defibrillator (ICD) is the most important tool to prevent sudden cardiac death in TS patients. The implant should be considered in all patients with confirmed diagnosis as soon as body weight allows the procedure.

Although prevention of cardiac arrhythmia is the primary goal of therapy, it is very important to note that TS patient may die of other causes. (1) Severe infections, probably the consequence of altered immune responses, are frequent in TS patients, and deaths have been reported despite aggressive antibiotic therapy. (2) Intractable hypoglycemia has also been reported as a cause of death. A close monitoring of glucose levels, especially in patients treated with β-blockers, is required since these drugs may mask hypoglycemic symptoms.

Finally, it is important keep in mind that severe ventricular arrhythmias have been reported in TS patients during induction of anesthesia: whether the increased susceptibility to arrhythmias is a nonspecific response related to adrenergic activation or is the consequence of the specific pharmacological activity of the drugs used is currently unknown.

Conclusion

Timothy syndrome is a genetic channelopathy with a complex clinical presentation. Mutations in Cav1.2 cause LQTS associated with dysfunction in multiple organ systems, including congenital heart disease, syndactyly, immune deficiency, hypoglycemia, and autism. Impaired gating caused by mutation is supposed to be the underlying mechanism. An implantable cardioverter defibrillator and β-blockers are the recommended treatments.

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