



# L-Type $\text{Ca}^{2+}$ Channels and Cardiac Arrhythmias

# 10

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## Abstract

L-type  $\text{Ca}^{2+}$  channels (LTCC) are voltage-gated  $\text{Ca}^{2+}$  channels with particular importance for cardiac function. They mediate  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum and thus are essential for excitation–contraction coupling. Furthermore, LTCCs play a key role in pacemaker and conductive tissue. Taken together, it is not surprising that LTCCs are associated with cardiac arrhythmias. The members of the family of voltage-gated  $\text{Ca}^{2+}$  channels differ in coding genes, expression pattern, and physiological as well as pharmacological properties. Cardiac LTCCs are defined by the pore-forming subunits  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$ , respectively. The expression and function of LTCCs is modulated by auxiliary  $\text{Ca}_v\beta$  and  $\text{Ca}_v\alpha_2\text{-}\delta$  subunits, which are also encoded by several genes. Furthermore, LTCC subunits are subject to splicing, cleavage, various protein–protein interactions (e.g., with calmodulin), and modulation by phosphorylation (e.g., by protein kinase A). This chapter focuses on the role of LTCCs in congenital and acquired cardiac arrhythmias. We review LTCC mutations associated with rhythm disturbances and LTCC dysregulations caused by pathologic

immune system activation. Furthermore, we address the dysfunction of LTCCs involved in the pathogenesis of atrial fibrillation, rhythm disturbances associated with heart failure, and age-related alterations of the heartbeat.

## Keywords

Voltage-dependent calcium channels · Arrhythmia · Cardiac disease · Congenital abnormality · Immune system disease · Heart failure · Atrial fibrillation

## Abbreviations

ACHB	Autoimmune congenital heart block
AF	Atrial fibrillation
AID	Alpha-interaction domain
AP	Action potential
APD	Action potential duration
AV	Atrioventricular
BrS	Brugada syndrome
CaM	Calmodulin
CC-AAb	Agonistic $\text{Ca}_v1.2$ autoantibody
CDI	$\text{Ca}^{2+}$ -dependent inactivation
CICR	$\text{Ca}^{2+}$ -induced $\text{Ca}^{2+}$ release
CPVT	Catecholaminergic polymorphic ventricular tachycardia
DCM	Dilated cardiomyopathy

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EAD	Early after depolarization
ERS	Early repolarization syndrome
HF	Heart failure
HVA	High-voltage activated
LQTS	Long QT syndrome
LTCC	L-type $\text{Ca}^{2+}$ channel
LVA	Low-voltage activated
miR	MicroRNA
$\text{Na}_v$	Voltage-gated $\text{Na}^+$ channel
NCX	$\text{Na}^+/\text{Ca}^{2+}$ exchanger
RyR	Ryanodine receptor
SA	Sinoatrial
SAN	Sinoatrial node
SANDD	SAN Dysfunction and deafness
SCD	Sudden cardiac death
SQTS	Short QT syndrome
SR	Sarcoplasmic reticulum
TS	Timothy syndrome
VDI	Voltage-dependent inactivation
VF	Ventricular fibrillation
VGCC	Voltage-gated $\text{Ca}^{2+}$ channel
VT	Ventricular tachycardia
VWA	von Willebrand factor A

## 10.1 Introduction

L-type voltage-gated  $\text{Ca}^{2+}$  channels (LTCCs) are voltage-gated  $\text{Ca}^{2+}$  channels (VGCCs) ubiquitously expressed in cardiac tissue, but also in smooth muscle, pancreas, adrenal gland, or brain tissue. LTCCs mediate entry of calcium ions ( $\text{Ca}^{2+}$ ) into cardiomyocytes, by this initiating excitation–contraction coupling. Furthermore, LTCCs are critically involved in shaping cardiac action potentials (AP) [1]. In sinoatrial and atrioventricular nodes, LTCCs are involved in diastolic depolarization, thereby participating in the AP firing and regulating automaticity [2, 3]. Accordingly, loss-of-function mutations in LTCCs can lead to sinus bradycardia, sick sinus syndrome, and dysfunction of atrioventricular conduction [4–7]. In the working myocardium and Purkinje system, depolarizing L-type  $\text{Ca}^{2+}$  currents determine the amplitude of the AP plateau, counterbalanced by repolarizing  $\text{K}^+$  currents. Overlapping with an increase of  $\text{K}^+$

outward-currents, inactivation of LTCCs drives the AP into the repolarizing phase and, thus, affects its duration (APD) [3]. A disturbed interplay of depolarizing and repolarizing currents and its influence on APD may result in susceptibility for life-threatening ventricular [8] and atrial [9] arrhythmias. Correspondingly, mutations in LTCCs are associated with arrhythmic disorders such as long QT, short QT, Brugada, and early-repolarization syndromes [10]. Furthermore, LTCCs are subject to pro-arrhythmic remodeling in diseased and/or aged hearts [11, 12].

This chapter focuses on the role of LTCCs in congenital and acquired cardiac arrhythmias. We review LTCC mutations associated with rhythm disturbances, LTCC dysregulations caused by pathologic immune system activation and dysfunction of LTCCs involved in the pathogenesis of atrial fibrillation (AF), rhythm disturbances associated with heart failure (HF), and age-related alterations of the heart beat (Table 10.1, Fig. 10.1).

## 10.2 Molecular Properties of LTCCs

VGCCs conduct  $\text{Ca}^{2+}$  upon surface-membrane depolarization. VGCCs are divided into high-voltage activated (HVA) and low-voltage activated (LVA) channels according to their activation potential thresholds [13]. Furthermore, HVA VGCCs are characterized by higher channel conductance and slower channel inactivation compared to LVA VGCCs [13]. Based on their biophysical and pharmacological properties, HVA VGCCs are further divided into L-, P/Q-, N-, and R-type channels. LTCCs are sensitive to antagonistic drugs classified as 1,4-dihydropyridines (e.g., amlodipine), phenylalkylamines (e.g., verapamil), and 1,5-benzothiazepines (e.g., diltiazem) [13]. LVA VGCCs consist of the subfamily of T-type  $\text{Ca}^{2+}$  channels, which can pharmacologically be discriminated against LTCCs by the specific LTCC agonist (*S*)-(–)-Bay K 8644 and the T-type preferring antagonist mibefradil [14]. In cardiomyocytes, both L- and T-type VGCCs are

**Table 10.1** Synopsis of rhythm disturbances and channelopathies related to L-type Ca<sup>2+</sup> channels

Gene/protein	Phenotype/channelopathy	Pathophysiological effects	Exemplifying references
<i>Genetics</i>			
<i>CACNA1C</i> (Ca <sub>v</sub> 1.2)	LQT8 (including TS)	Gain-of-function, $I_{CaL}$ ↑	[86, 91, 92, 293]
	BrS3, BrS3/SQT4	Loss-of-function, $I_{CaL}$ ↓	[129, 130]
	SQT4	Loss-of-function, $I_{CaL}$ ↓	[138]
	ERS2	Loss-of-function, $I_{CaL}$ ↓ <sup>a</sup>	[130]
	SAN dysfunction	Loss-of-function, $I_{CaL}$ ↓ <sup>a,b</sup>	[7]
<i>CACNA1D</i> (Ca <sub>v</sub> 1.3)	SANDD syndrome	Loss-of-function, $I_{CaL}$ ↓	[6]
<i>CACNB2b</i> (Ca <sub>v</sub> β <sub>2b</sub> )	BrS4, BrS4/SQT5	Loss-of-function, $I_{CaL}$ ↓	[129, 130]
	ERS3	Loss-of-function, $I_{CaL}$ ↓ <sup>a</sup>	[130]
	SNPs in addition to BrS3, BrS4, ERS3 mutations	Gain-of-function, $I_{CaL}$ ↑ <sup>c</sup>	[130]
<i>CACNA2D1</i> (Ca <sub>v</sub> α <sub>2</sub> δ-1)	BrS9	Loss-of-function, $I_{CaL}$ ↓ <sup>a</sup>	[130]
	SQTS6	Loss-of-function, $I_{CaL}$ ↓	[145]
	ERS4	Loss-of-function, $I_{CaL}$ ↓ <sup>a</sup>	[130]
<i>CALM1-3</i> (CaM)	LQT14-16	Loss-of-function of CaM, reduced CDI of $I_{CaL}$	[68, 123]
<i>Autoimmunity</i>			
Ca <sub>v</sub> 1.2	VT, SCD in DCM	Agonistic antibodies, $I_{CaL}$ ↑	[156, 157]
	Idiopathic cardiac arrest	Antagonistic antibodies, $I_{CaL}$ ↓	[180]
Ca <sub>v</sub> 1.2 and Ca <sub>v</sub> 1.3	ACHB	Ro/SSA antibodies, $I_{CaL}$ ↓	[154, 174]
<i>Inflammation</i>			
Ca <sub>v</sub> 1.2	LQTS <sup>d</sup>	IL-1, $I_{CaL}$ ↑	[190]
	AF <sup>d</sup>	IL-1, $I_{CaL}$ ↓	[193, 194]
	LQTS <sup>d</sup>	IL-6, Ca <sub>v</sub> 1.2 phosphorylation, $I_{CaL}$ ↑	[187]
	AF <sup>d</sup>	TNFα, $I_{CaL}$ ↓	[195]
	AF <sup>d</sup>	MIF, $I_{CaL}$ ↓	[196]
<i>Remodeling</i>			
Ca <sub>v</sub> 1.2 and Ca <sub>v</sub> 1.3	Age-related SSS	miR-1976 ↑, $I_{CaL}$ ↓	[200]
Ca <sub>v</sub> 1.2 and Ca <sub>v</sub> 1.3, Ca <sub>v</sub> β <sub>1</sub> , Ca <sub>v</sub> β <sub>2</sub> , Ca <sub>v</sub> α <sub>2</sub> δ-1	AF	$I_{CaL}$ ↓	[207, 218]
Ca <sub>v</sub> 1.2, Ca <sub>v</sub> β <sub>2a</sub>	HF occurrence of rhythm disturbances ↑	Single LTCC activity ↑, channel hyperphosphorylation, reduced number of functional channels	[253, 255, 267, 273]

Data are compiled from human and animal studies

↑ indicates increase/upregulation; ↓, decrease/downregulation; ACHB, autoimmune congenital heart block; AF, atrial fibrillation; BrS, Brugada syndrome; CaM, calmodulin; CDI, Ca<sup>2+</sup>-dependent inactivation; DCM, dilated cardiomyopathy; ERS, early-repolarization syndrome; HF, heart failure;  $I_{CaL}$ , whole-cell L-type Ca<sup>2+</sup> current/current density; LQT, long QT syndrome (in the disease names); LQTS, long QT syndrome; LTCC, L-type Ca<sup>2+</sup> channels; miR, microRNA; SAN, sinoatrial node; SANDD, sinoatrial node dysfunction and deafness; SCD, sudden cardiac death; SNP, single-nucleotide polymorphism; SQT, short QT syndrome (in the disease names); SQTS, short QT syndrome; SSS, sick sinus syndrome; TS, Timothy syndrome; VT, ventricular tachycardia

<sup>a</sup>Proposed mechanism, no experimental measurements

<sup>b</sup>Accompanied by a titin mutation

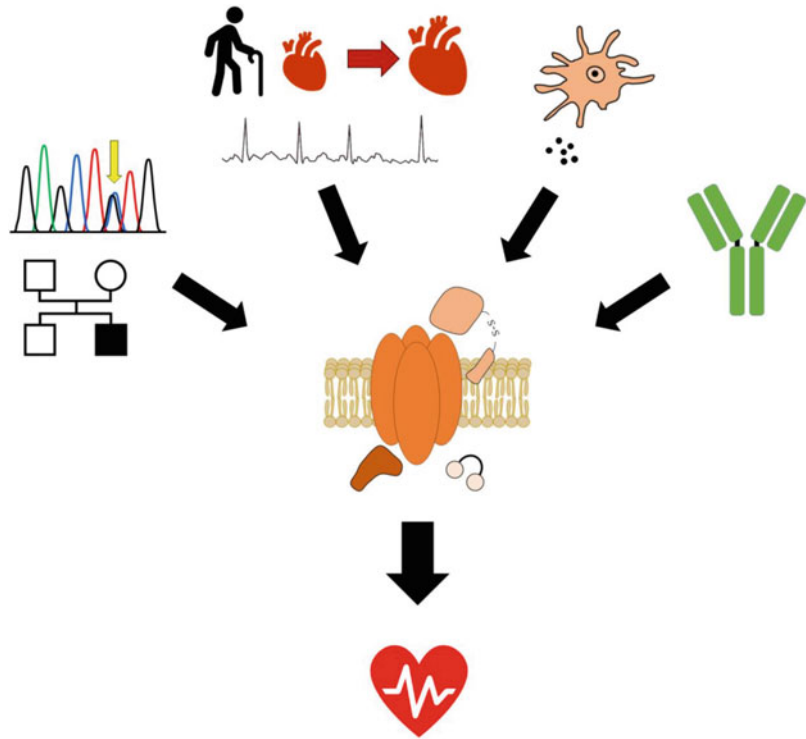
<sup>c</sup>Probably counteracted by a concomitant HERG channel mutation

<sup>d</sup>Proposed related disorder, no/little experimental evidence

expressed at significant levels [15–19]. LTCCs are the predominant VGCCs in the adult human heart [16–19], whereas T-type VGCCs seem to

prevail in the early stages of the heart development [16, 19]. Expression of T-type VGCCs in the adult myocardium depends on the species and

**Fig. 10.1** Schematic overview of mechanisms underlying rhythm disturbances associated with L-type  $\text{Ca}^{2+}$  channel (LTCC) dysfunction. From left to right: **LTCC mutations**, either de novo or hereditary, **aging and age-related diseases** such as heart failure or atrial fibrillation, **inflammatory diseases**, or **(auto-) antibodies** can affect LTCC expression and/or function and lead to alterations in electrical activity of the heart or even to cardiac arrhythmia



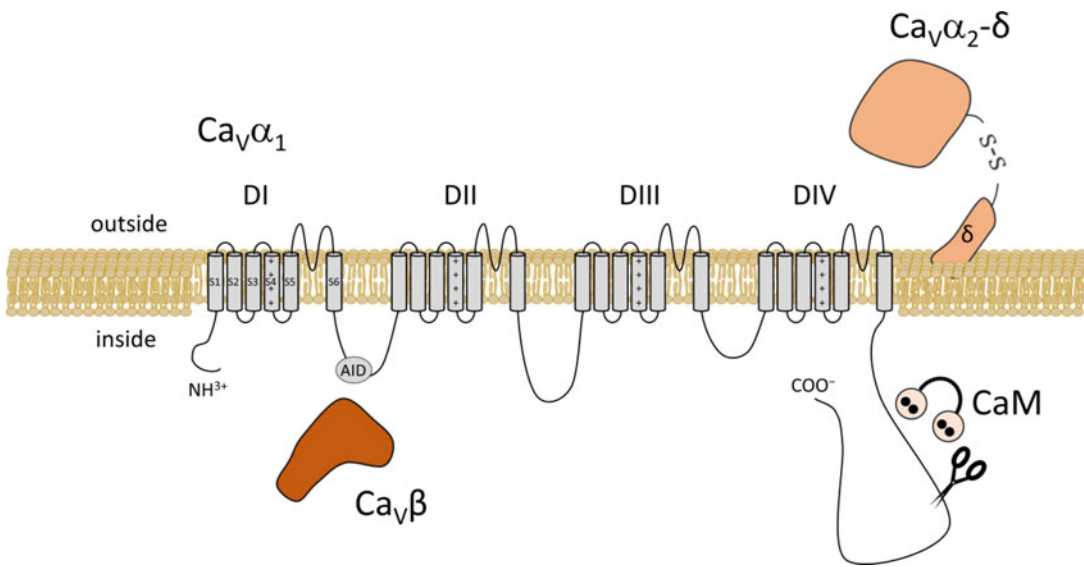
strongly decreases with increasing body size in mammals [20]. In diseased hearts, however, T-type VGCCs can be re-expressed [21].

LTCCs are defined by their transmembrane pore-forming subunit,  $\text{Ca}_v\alpha_1$  ( $\text{Ca}_v1.X$ ), which determines most properties of the ion channel complex, which consists of the pore and up to three auxiliary subunits,  $\text{Ca}_v\alpha_2\text{-}\delta$ ,  $\text{Ca}_v\beta$ , and  $\text{Ca}_v\gamma$  [13, 22]. Auxiliary  $\text{Ca}_v\alpha_2\text{-}\delta$  and  $\text{Ca}_v\beta$  subunits are constitutively bound to the  $\text{Ca}_v\alpha_1$  subunit, although different  $\text{Ca}_v\beta$  isoforms can dynamically alternate in a competitive manner [23, 24].  $\text{Ca}_v\alpha_2\text{-}\delta$  and  $\text{Ca}_v\beta$  modulate channel trafficking, gating properties, and interaction with other proteins. Cardiac LTCCs can also associate with  $\text{Ca}_v\gamma$  subunits in heterologous expression systems, but the role of this interaction in native heart tissues remains to be investigated [25]. Multiple genes encoding LTCC subunits and their alternative splicing provide molecular diversity of LTCCs [26]. In addition, the ubiquitous  $\text{Ca}^{2+}$  sensor protein calmodulin (CaM) can be considered essential for LTCCs [27, 28]. CaM associates with the  $\text{Ca}_v\alpha_1$  subunit with high

affinity and regulates channel activity and  $\text{Ca}^{2+}$ -dependent feedback, e.g.,  $\text{Ca}^{2+}$ -dependent inactivation (CDI).

### 10.2.1 $\text{Ca}_v\alpha_1$ Subunit

The  $\text{Ca}_v\alpha_1$  subunit is the primary subunit of VGCCs (Fig. 10.2), which encompasses an ion-conducting pore with a selectivity filter and voltage sensor, as well as activation and inactivation machineries. It provides sites for regulatory interactions and drug binding [1, 29]. Ten phylogenetically related genes encode different  $\text{Ca}_v\alpha_1$  subunits [1, 29]. Four of them, *CACNA1S*, *CACNA1C*, *CACNA1D*, and *CACNA1F*, encode the LTCC  $\text{Ca}_v\alpha_1$  subunits,  $\text{Ca}_v1.1\text{--}\text{Ca}_v1.4$ , respectively. While  $\text{Ca}_v1.1$  ( $\text{Ca}_v\alpha_{1S}$ ) is specific for skeletal muscle cells,  $\text{Ca}_v1.4$  ( $\text{Ca}_v\alpha_{1F}$ ) is essential for retinal photoreceptor function.  $\text{Ca}_v1.2$  ( $\text{Ca}_v\alpha_{1C}$ ) and  $\text{Ca}_v1.3$  ( $\text{Ca}_v\alpha_{1D}$ ) are the only LTCCs expressed in cardiac tissue and of particular functional relevance here.



**Fig. 10.2** Schematic structure of a  $\text{Ca}_V\alpha_1$  subunit with associated  $\text{Ca}_V\beta$ ,  $\text{Ca}_V\alpha_2\text{-}\delta$ , and calmodulin.  $\text{Ca}_V\alpha_1$  consists of four domains (DI–DIV), each comprised of six transmembrane segments (S1–S6). S4 segments are positively charged and serve as voltage sensors. S5 and S6 segments together with S5–S6 loops form the channel’s conducting pore. DI–DII loop contains the alpha-interaction domain (AID) enabling high-affinity interaction with  $\text{Ca}_V\beta$  subunit. The C-terminus of  $\text{Ca}_V\alpha_1$  can be either post-translationally cleaved ( $\text{Ca}_V1.2$  channels) or truncated due to alternative splicing ( $\text{Ca}_V1.3$  channels),

as symbolized by the scissors. The proximal part of the full-length C-terminus binds calmodulin (CaM). CaM consists of two lobes, each having two  $\text{Ca}^{2+}$ -binding sites. The distal part of the full-length C-terminus interacts with the proximal C-terminus and competes with CaM. Of note, the cleaved distal C-terminus of  $\text{Ca}_V1.2$  can also non-covalently bind to the channel’s proximal C-terminus.  $\text{Ca}_V\alpha_2\text{-}\delta$  subunit is anchored to the membrane through its  $\delta$  portion and interacts with extracellular loops of  $\text{Ca}_V\alpha_1$

The  $\text{Ca}_V\alpha_1$  subunit belongs to the family of four-domain cation channels, which also includes pore-forming subunits of voltage-gated  $\text{Na}^+$  channels ( $\text{Na}_V\text{s}$ ) [30].  $\text{Ca}_V\alpha_1$  protein with a length of approximately 2000 amino acids consists of four homologous domains (DI–DIV) connected by intracellular loops and having intracellular N- and C-termini [1]. Each domain contains six transmembrane segments (S1–S6). S5 and S6 are connected at their extracellular ends by the reentrant pore loops. Together, S5, S6, and the pore loops of DI to DIV form the ion-conducting pore of  $\text{Ca}_V\alpha_1$  [1, 31–33]. The lower (intracellular) thirds of S6, which are rich in hydrophobic amino acids, interact with each other, sealing the pore in the non-conducting (closed) state of the channel. The positively charged S4 segments serve as voltage sensors. Their outward movement in response to membrane depolarization

allows S6 segments to diverge thus opening the channel [34]. The intracellular DI–DII loop forms the inactivation gate, which docks to the cytoplasmic ends of S6 segments upon channel opening, leading to voltage-dependent inactivation (VDI) [35]. Furthermore, this DI–DII loop of HVA VGCCs contains the alpha-interaction domain (AID) (Fig. 10.2), which enables interaction with  $\text{Ca}_V\beta$  subunits [36]. The co-localization of AID and the inactivation gate in the DI–DII loop provides structural basis for profound regulation of VDI by  $\text{Ca}_V\beta$  subunits (see Sect. 10.2.2) [35].

The cytoplasmic C-terminus of  $\text{Ca}_V\alpha_1$  allows channel regulation by numerous protein–protein interactions [37, 38]. Particularly, it binds CaM and thus permits  $\text{Ca}^{2+}$ -dependent channel autoregulation [28]. Besides, distal C-termini of LTCC  $\text{Ca}_V\alpha_1$  contain auto-inhibitory domains [39–42]. However, LTCC  $\text{Ca}_V\alpha_1$  can exist in the

full-length form or in truncated forms, which lack the distal C-terminus. In  $\text{Ca}_v1.1$  and  $\text{Ca}_v1.2$  channels, the distal C-terminus can be post-translationally cleaved but remains non-covalently associated with the channel [39, 40]. A dissociated distal C-terminus of  $\text{Ca}_v1.2$  channels can translocate into the nucleus and regulate transcription [37]. In  $\text{Ca}_v1.3$  and  $\text{Ca}_v1.4$  channels, short and long C-termini arise from alternative splicing [42–45].

As mentioned above, from the family of LTCCs, only  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$  were found in the heart. While  $\text{Ca}_v1.2$  is ubiquitously expressed in all cardiac tissues,  $\text{Ca}_v1.3$  is preferentially expressed in sinoatrial (SA) and atrioventricular (AV) nodes, can be found in atria and Purkinje fibers, and is practically absent in ventricles [17–19, 46, 47].  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$  channels are often expressed in the same tissues but can have distinct functions due to differences in their gating properties.  $\text{Ca}_v1.3$  channels activate at more negative potentials than  $\text{Ca}_v1.2$ : the activation threshold of  $\text{Ca}_v1.3$  channels is at about  $-50$  mV, whereas  $\text{Ca}_v1.2$  channels open at voltages above  $-30$  mV [13, 46, 48, 49]. The low activation threshold of  $\text{Ca}_v1.3$  channels, which is slightly more positive than the activation threshold of T-type VGCCs, makes  $\text{Ca}_v1.3$  channels suitable to support slow conduction and pace-making activity and this correlates with  $\text{Ca}_v1.3$  expression patterns.

### 10.2.2 $\text{Ca}_v\beta$ Subunit

$\text{Ca}_v\beta$  subunits are cytosolic auxiliary subunits of HVA VGCCs (Fig. 10.2) and the main modulator of activation, inactivation, and membrane targeting of these channels [36]. Structurally,  $\text{Ca}_v\beta$  subunits consist of the so-called functional core flanked by variable N- and C-termini [36]. The  $\text{Ca}_v\beta$  core comprises conserved SH3 and GK domains, which are linked by a flexible, weakly conserved HOOK region. SH3 and GK domains evolved as interaction sites for protein–protein interactions [50, 51]. The SH3 domain of  $\text{Ca}_v\beta$ s can bind dynamin, promoting channel endocytosis [52]. The GK domain of  $\text{Ca}_v\beta$ s

interacts with the family of RGK proteins (Rem, Gem/kir, and Rad), which are strong inhibitors of HVA VGCCs [53]. It also contains a tiny hydrophobic groove, termed  $\alpha$ -binding pocket, which interacts with the AID of  $\text{Ca}_v\alpha_1$  [54]. Both SH3 and GK domains are required to reproduce multiple functional effects of  $\text{Ca}_v\beta$ , with the HOOK region being important for the modulation of VDI of HVA VGCCs [36].

Four  $\text{Ca}_v\beta$  subfamilies are known, each encoded by a distinct gene (*CACNB1–CACNB4*), but due to alternative splicing several variants of each  $\text{Ca}_v\beta$  isoform exist [36]. All  $\text{Ca}_v\beta$  subunits have been found in the mammalian heart, while  $\text{Ca}_v\beta_2$  is the most prominent. Among these,  $\text{Ca}_v\beta_{2b,c}$  are the most abundant, while  $\text{Ca}_v\beta_{2d,e}$  are only robust in young animals, and the expression levels of  $\text{Ca}_v\beta_{2a}$  seem to be the lowest [55–57].  $\text{Ca}_v\beta_2$  isoforms differ in their non-conserved N-terminal region and differentially affect channel activity and inactivation [57]. Besides, cardiac  $\text{Ca}_v\beta$  subunits can diverge in their subcellular localization: e.g., whereas  $\text{Ca}_v\beta_{1b}$ ,  $\text{Ca}_v\beta_2$  and  $\text{Ca}_v\beta_3$  were detected in the T-tubule sarcolemma,  $\text{Ca}_v\beta_{1a}$  and  $\text{Ca}_v\beta_4$  were found in the surface sarcolemma [58].

### 10.2.3 $\text{Ca}_v\alpha_2$ - $\delta$ Subunit

$\text{Ca}_v\alpha_2$ - $\delta$  subunits (Fig. 10.2) promote plasma membrane expression of HVA VGCCs, increase  $\text{Ca}^{2+}$  currents, shift activation to more hyperpolarized potentials, and accelerate inactivation [59–61]. There are four genetic variants,  $\text{Ca}_v\alpha_2$ - $\delta 1$ –4, encoded by *CACNA2D1–4* genes, respectively [61]. A precursor protein, translated from *CACNA2D*, is cleaved to yield two polypeptides,  $\alpha_2$  and  $\delta$ , which, however, remain connected by disulfide bridges.  $\text{Ca}_v\alpha_2$ - $\delta$  is an extracellularly glycosylated subunit anchored to the plasma membrane by a glycosylphosphatidylinositol attached to the C-terminus of  $\delta$ . The  $\alpha_2$  portion contains multiple domains, e.g., von Willebrand factor A (VWA) domain, commonly involved in extracellular protein–protein interactions. VWA domain is important for the physical interaction with

extracellular loops of LTCCs and the modulation of their biophysical properties [33, 59, 60].

All isoforms  $\text{Ca}_V\alpha_2\text{-}\delta 1\text{--}4$  were found in the heart but only  $\text{Ca}_V\alpha_2\text{-}\delta 1$  is abundantly expressed in human ventricles [17, 18, 62–64]. In atria,  $\text{Ca}_V\alpha_2\text{-}\delta 1$  and  $\text{Ca}_V\alpha_2\text{-}\delta 2$  were found to be expressed at similarly high levels [17, 64].

### 10.2.4 $\text{Ca}_V\gamma$ Subunit

$\text{Ca}_V\gamma$  subunits are transmembrane proteins encoded by eight *CACNG* genes. They build a functionally heterogeneous family and can regulate other proteins besides VGCCs [65].  $\text{Ca}_V\gamma 1$  was identified as a part of the skeletal muscle LTCCs.  $\text{Ca}_V\gamma 4$ ,  $\text{Ca}_V\gamma 6$ ,  $\text{Ca}_V\gamma 7$ , and  $\text{Ca}_V\gamma 8$  were found in the human heart and they were able to associate with  $\text{Ca}_V 1.2$  and differentially modulate its properties in a heterologous expression system [25]. However, whether  $\text{Ca}_V\gamma$  regulates native cardiac LTCCs requires further studies. So far, there is no direct evidence of interaction between LTCCs and  $\text{Ca}_V\gamma$  in cardiomyocytes.

### 10.2.5 Calmodulin (CaM)

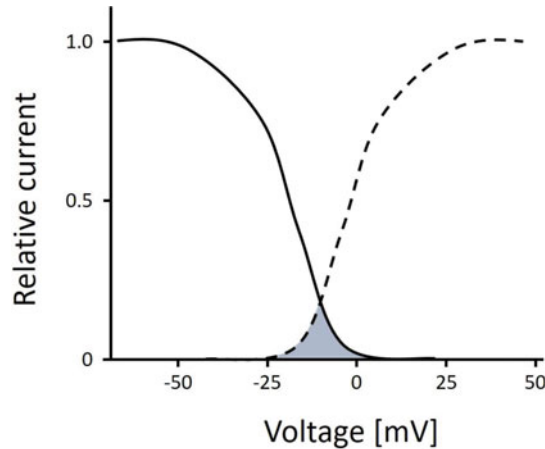
CaM is a universal  $\text{Ca}^{2+}$ -sensing protein regulating a vast number of proteins [27, 66]. It can be considered as an auxiliary subunit of various ion channels including LTCCs (Fig. 10.2) [27, 28]. In humans, CaM is encoded by three genes, *CALM1–3*, with divergent nucleotide but identical protein sequences [67]. All CaM genes are expressed in the heart, showing expression levels in the rank order *CALM3* > *CALM2* > *CALM1* [68]. CaM consists of two lobes, each composed of two EF hands with high affinity to  $\text{Ca}^{2+}$  [28]. In LTCCs, CaM is bound to the channel's proximal C-terminus but can be competitively displaced by the distal part of the C-terminus of the channel [41, 69–73]. The competition between CaM and the distal C-terminus is further regulated, e.g., by PKA phosphorylation in response to  $\beta$ -adrenergic stimulation [74]. CaM strongly enhances channel activity in its ( $\text{Ca}^{2+}$ -free) apo form and is responsible for CDI of the channel upon  $\text{Ca}^{2+}$  conduction [28]. Furthermore,

$\text{Ca}^{2+}$  binding to the channels' CaM is involved in internalization of the channels in response to their high activity [75].

## 10.3 LTCCs and AP

Interplay of various ion currents through the cardiomyocyte membrane regulates generation of cardiac APs [3]. LTCCs open upon membrane depolarization giving rise to inward, depolarizing currents. Activation of LTCCs is slow compared to that of  $\text{Na}_V$ s; therefore, LTCCs contribute little to the upstroke (phase 0) of the AP in ventricular, Purkinje, and atrial myocytes [3]. Instead, long-lasting LTCC currents together with opposing outward  $\text{K}^+$  currents shape the AP plateau (phase 2) in these cells. In the working myocardium,  $\text{Ca}^{2+}$  influx through LTCCs activates ryanodine receptors (RyRs) leading to  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR). This process is called  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR), and it underlies cardiac excitation–contraction coupling [76, 77]. Subsequent VDI and CDI of LTCCs result in accelerated membrane repolarization (phase 3). Regeneration of depolarizing currents in the case of insufficient repolarization reserve [78] can lead to a new membrane depolarization (early after depolarization, EAD) and thus trigger a premature AP [79, 80]. EADs are associated with fatal arrhythmias, such as Torsades de Pointes tachycardia, a specific form of polymorphic ventricular tachycardia with a high risk of sudden cardiac death (SCD) [79]. Overlap of LTCC activation and inactivation curves results in steady-state currents (so-called window currents, Fig. 10.3). Thus, LTCCs can reactivate and contribute to an increasing (late) inward current [79, 81]. Accordingly, increased LTCC window currents can increase the risk of EADs, while reduced window currents can decrease it.

In SA and AV nodes, LTCCs are essential drivers of the membrane depolarization during pace-making [82]. In the SA node, spontaneous APs are initiated by coupled “membrane-” and “ $\text{Ca}^{2+}$ -clock” mechanisms, which include depolarizing currents mediated by HCN channels (“funny” currents) and T-type VGCCs as well as



**Fig. 10.3** Schematic drawing of a so-called window current. The two curves depict steady-state inactivation (solid line) and activation (dashed line) of  $\text{Ca}^{2+}$  currents depending on the respective membrane potential. Within the potential range marked in gray (“permissive window of

voltage”), a large proportion of the  $\text{Ca}^{2+}$  channels is in the inactivated state, i.e., non-conducting, while another proportion is just activated. L-type  $\text{Ca}^{2+}$  channels may reactivate within this voltage range

$\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) activation in response to the spontaneous  $\text{Ca}^{2+}$  release through RyRs [82, 83] (see also Chaps. 4 and 6). Subsequent activation of  $\text{Ca}_v1.3$  channels further promotes membrane auto-depolarization. After the membrane potential reaches the activation threshold of  $\text{Ca}_v1.2$  channels, these LTCCs contribute to the upstroke of the AP. Besides its  $\text{Ca}^{2+}$ -conducting function,  $\text{Ca}_v1.3$  channels are an essential molecular determinant of the depolarizing, dihydropyridine-sensitive sustained  $\text{Na}^+$  currents in the SA node [84].  $\text{Ca}_v1.3$  strongly co-localizes with RyRs in SA node cells [85]. Thus,  $\text{Ca}_v1.3$  opening here can stimulate RyRs-mediated  $\text{Ca}^{2+}$  release and further activate depolarizing NCX currents.

#### 10.4 LTCC Mutations Associated with Cardiac Rhythm Disturbance

Ion channel mutations in general account for a variety of hereditary cardiac arrhythmias. For example, a large fraction of sudden unexplained deaths in the young are postmortem attributed to ion-channel mutations which led to cardiac

arrhythmias [86]. Primary channelopathies predisposing to sudden cardiac death include long QT syndrome (LQTS), short QT syndrome (SQTS), the Brugada Syndrome (BrS), and the catecholaminergic polymorphic ventricular tachycardia (CPVT) [87]. As discussed below, some LTCC mutations are associated with rhythm disturbances, although the major susceptibility genes revealed so far are *KCNQ1* ( $\text{K}_v7.1$ ), *KCNH2* ( $\text{K}_v11.1/\text{hERG}$ ), *SCN5A* ( $\text{Na}_v1.5$ ), and *RYR2* (ryanodine receptor 2) [86, 87].

The report of LTCC mutations causing the Timothy Syndrome (TS) by Splawski et al. in 2004 has highlighted the role of LTCCs for hereditary cardiac arrhythmias and up to now the number of identified mutations in LTCCs affecting cardiac rhythm strongly increased [10, 88–90]. Cardiac LTCC channelopathies can be conditionally classified as gain- or loss-of-function mutations [89]. LQTS mutations in  $\text{Ca}_v1.2$  channels typically show gain-of-function features, whereas loss-of-function  $\text{Ca}_v1.2$  mutations were found in BrS, SQTS, and early-repolarization syndrome (ERS) [10, 89]. Furthermore, loss of  $\text{Ca}_v1.3$  or  $\text{Ca}_v1.2$  function can lead to sinus node malfunction from bradycardia to sick sinus syndrome [6, 7].



### 10.4.1 Long QT Syndromes (LQTS)

On the cellular level, LQTS results from a prolongation of the ventricular AP due to an increase of depolarizing Na<sup>+</sup> and Ca<sup>2+</sup> currents or a decrease of repolarizing K<sup>+</sup> currents [91]. This favors EADs, which can lead to Torsades de Pointes arrhythmia [79]. Three major and at least 14 minor susceptibility genes were identified for congenital LQTS [92].

LQTS caused by mutations in the pore-forming subunit of Ca<sub>v</sub>1.2 channels (*CACNA1C*-LQTS) is historically termed LQT8. Given the strong expression of Ca<sub>v</sub>1.2 in various tissues, gain-of-function Ca<sub>v</sub>1.2 channelopathies can result in multi-organ pathologies. LQT8 was thus subdivided into the multisystem Timothy syndrome (TS) and LQT8 without extra-cardiac symptoms as more and more *CACNA1C* mutations and associated effects became known [10].

TS is a rare multisystem disorder characterized by QT prolongation, cardiac arrhythmias (bradycardia, AV block, ventricular tachyarrhythmia), congenital heart defects, and extra-cardiac manifestations such as syndactyly, facial abnormalities, immune system dysfunction, intermittent hypoglycemia, and neuropsychiatric disorders [88, 93, 94]. TS patients are at high risk of SCD and often die during childhood [88, 93, 95]. TS is a dominant genetic disorder, resulting from de novo *CACNA1C* mutations. However, it can also be inherited from an asymptomatic parent who carries a mosaic *CACNA1C* mutation [88, 96]. The particular phenotype depends on the respective *CACNA1C* mutation and can vary among patients with the same mutation [88, 93–95, 97, 98]. Classical TS (TS-1) is most commonly caused by a recurrent missense mutation (G406R) in the alternatively spliced exon 8a, which is widely expressed and represents approx. 20% of Ca<sub>v</sub>1.2 transcripts in cardiac and neuronal tissue [88]. The analogous mutation in the mutually exclusive exon 8, responsible for nearly 80% of cardiac Ca<sub>v</sub>1.2 channels, leads to an atypical TS (TS-2) without

syndactyly but a more severe cardiac phenotype [93].

The G406R mutation alters Ca<sub>v</sub>1.2 activity at various levels: it drastically inhibits channel inactivation (particularly VDI), shifts channel activation to more negative potentials, and promotes a channel gating mode with very long openings [88, 93, 99, 100]. The mutation occurs at the cytoplasmic end of the sixth transmembrane segment of the channel's domain I (DI/S6), which is involved in the voltage-dependent regulation of the channel activation and inactivation and is conserved in VGCCs across various species [34, 35, 93, 101]. Moreover, G406R introduces a new consensus site for CaMKII enabling phosphorylation of S409, which could promote long channel openings ([99]; but: [102]). Furthermore, G406R leads to enhancement of coupled channel gating, by altering the interaction with the scaffolding protein AKAP150 [103, 104].

Some TS cases were reported to be caused by mutations other than the typical G406R mutation. Interestingly, many of them are also located at the cytoplasmic end of an S6 segment similar to G406R, e.g., G402S, G402R, S405R, E407A in DI [93, 95, 105], I1166T in DIII [106, 107], or A1473G in DIV [108]. Besides, a TS-associated mutation was reported in a DII/S4–S5 linker, S643F [109]. Of note, S4–S5 linkers appear to interact with S6 segments [34]. Electrophysiologically, gain of Ca<sub>v</sub>1.2 function was confirmed for G402S (impairment of inactivation) [93] and I1166T (increased window current due to shifted activation potentials) [106, 107]. S643F mutation showed mixed loss- and gain-of-function features: a reduction of peak current density was opposed by shifting the activation toward more depolarized potentials and increasing late currents due to the drastic reduction of VDI [109].

Although initially LQT8 was equated to TS, later studies expanded the spectrum of phenotypes associated with LQT8 [110–113]. For example, one patient with idiopathic QT prolongation, bradycardia, and autism spectrum disorder carried an E1115K mutation in the selectivity filter in the DIII/S5-S6 loop [113]. When expressed in a recombinant system the mutation caused AP prolongation and turned

the LTCC into a dihydropyridine-sensitive but nonselective cation channel.

Some *CACNA1C* mutations result in LQTS without obvious extra-cardiac attributes [10, 90, 107, 110]. A whole-exome sequencing of 102 unrelated “genotype-negative/phenotype-positive” LQTS patients suggested that *CACNA1C* mutations may be responsible for 1% of LQTS cases [110]. *CACNA1C* mutations leading to isolated LQTS were found at various cytoplasmic linkers and both N- and C-terminus and showed gain-of-function features like reduced channel inactivation, increased current density, increased window current, or negative shift of activation potential range [10]. Interestingly, many of the  $\text{Ca}_v1.2$  mutations concentrate in the DII–DIII linker at a PEST sequence, which serves as a signal for protein degradation [110, 114]. Genetic analyses of several families with multiple cardiac abnormalities, such as LQTS, hypertrophic cardiomyopathy, or congenital heart defects, identified recurrent R581C/H mutations in  $\text{Ca}_v1.2$  channels [111, 115, 116]. R581C/H mutations lie within the intracellular DI–DII loop, which is responsible for VDI regulation and binding of  $\text{Ca}_v\beta$  subunits. These mutations show an increase of window and late currents indicating gain of function [111, 117].

LQTS can also be associated with gain of LTCC function due to mutations in the CaM genes *CALM1–3* (LQT14–16, respectively) [68, 118–123]. These CaM mutations led to a reduction of  $\text{Ca}^{2+}$  affinity to the EF hands in the C-lobe of CaM, resulting in the loss of CDI of  $\text{Ca}_v1.2$  channels [68, 118, 120, 122, 124, 125]. Crystallographic and NMR investigations suggest that altered interaction between CaM and  $\text{Ca}_v1.2$  may also be involved [126].

#### 10.4.2 Brugada, Early-Repolarization, and Short QT Syndromes

BrS and ERS belong to the continuous spectrum of so-called J-wave syndromes. They are diagnosed based on the ECG patterns in structurally normal hearts and are associated with an increased risk of SCD due to polymorphic

ventricular tachycardia (VT) and ventricular fibrillation (VF) [127]. The mechanisms underlying BrS and ERS are not fully understood [127, 128]. Besides, BrS and ERS appear to be multifactorial rather than pure Mendelian disorders [129].

LTCCs came into focus, when it was observed that the  $\text{Ca}^{2+}$  channel antagonist verapamil can induce BrS-like patterns in APs and ECGs of canine right ventricular wedges [130]. Subsequent studies revealed that mutations in *CACNA1C* ( $\text{Ca}_v1.2$ ), *CACNB2b* ( $\text{Ca}_v\beta_{2b}$ ), and *CACNA2D1* ( $\text{Ca}_v\alpha_2\delta-1$ ) account for a significant fraction of BrS and ERS cases and are often accompanied by shortening of the QT interval [89, 131, 132].

The latter observation led to reassessment of  $\text{Ca}_v1.2$  subunit genes as candidate susceptibility genes for SQTS [133]. SQTS is a rare inherited cardiac disease, characterized by a high risk of developing AF and VT leading to syncope or SCD [134, 135]. The short QT interval results from a shortening of APD due to accelerated membrane depolarization. Reduced effective refractory period and increased transmural dispersion predispose to re-entry arrhythmias [91, 136].

In BrS patients, at least eleven *CACNA1C* mutations were identified of which four lead to concomitant BrS/SQTS [131, 132, 137, 138]. Additionally, two ERS-, one SQTS-, and one ERS/SQTS-associated mutations were identified in *CACNA1C* [132, 133, 139, 140]. The majority of the mutations were located in cytoplasmic regions of the channel, particularly in the distal C-terminus. Nine mutations were studied in heterologous expression systems and showed a decrease of  $\text{Ca}^{2+}$  current density [131, 132, 138–141]. In particular, the splicing-error mutation R632R could be a subject to nonsense-mediated mRNA decay [142]. One BrS patient was shown to carry the missense mutation E1115K [132] converting  $\text{Ca}_v1.2$  into a nonselective monovalent cation channel associated with LQTS, as discussed above [113]. This BrS patient, however, additionally showed a HERG channel mutation known to be associated with BrS and thus probably compensating for the  $\text{Ca}_v1.2$  LQTS mutation [132]. *CACNA1C* genetic variants present at low

frequencies in the general population may also predispose to BrS and ERS [132, 143]. A T1787M variant, found in 0.8% of an African population, was identified in three unrelated patients with BrS, ERS, or idiopathic VF. T1787M is located in the distal C-terminus and strongly increased its auto-inhibitory effect [143].

Auxiliary Ca<sub>v</sub>β and Ca<sub>v</sub>α<sub>2</sub>-δ subunits, which modulate surface expression and gating properties of Ca<sub>v</sub>1.2 channels, are also associated with malignant ventricular arrhythmias. Genetic testing identified nine BrS-related and three ERS-related distinct amino acid changes in the Ca<sub>v</sub>β<sub>2b</sub> subunit [131, 132, 144–146]. Among these, one BrS mutation led to concomitant SQTS [131, 132]. Two BrS mutations in the Ca<sub>v</sub>β<sub>2b</sub> subunit were studied in whole-cell patch-clamp experiments and showed loss-of-function features such as decreased current density or increased rates of channel inactivation [132, 146]. In the *CACNA2D1* gene, four BrS-, one ERS-, and one SQTS-associated mutations were reported [132, 146, 147]. The SQTS mutation showed reduced current density in patch-clamp experiments [147].

### 10.4.3 SA and AV Node Dysfunctions

LTCCs are involved in the slow auto-depolarization (Ca<sub>v</sub>1.3) and in the generation of the upstroke (Ca<sub>v</sub>1.2 and Ca<sub>v</sub>1.3) of APs in SA and AV nodes [82]. *CACNA1D* (Ca<sub>v</sub>1.3)-deficient mice showed SA node (SAN) bradycardia with irregular cellular cycles, disturbed AV conduction, and congenital deafness [4, 5, 46, 148]. Later, a similar phenotype was observed in two consanguineous inbred Pakistani families [6]. The disorder, termed sinoatrial node dysfunction and deafness (SANDD) syndrome, is characterized by severe hearing loss, bradycardia, increased heart-rate variability, and variable degree of AV block [6]. SANDD patients were homozygous carriers of a 3 base pairs insertion in the alternatively spliced exon 8b of *CACNA1D*. The 8b isoform of Ca<sub>v</sub>1.3 channels is predominant in the SAN [6]. This mutation leads to a glycine insertion (403\_404insGly) at the

cytoplasmic end of the inner-pore lining helix DI/S6. This region is conserved in human LTCCs, and associated with pathological gain-of-function mutations at homologous position not only in Ca<sub>v</sub>1.3 (G403R and G403D) but also in Ca<sub>v</sub>1.2 (G402S known from TS) and Ca<sub>v</sub>1.4 (G359R) [93, 149, 150]. In contrast, the 403\_404insGly mutation results in non-conducting Ca<sub>v</sub>1.3 channels, although the channels are expressed in the surface membrane and exhibit ON-gating currents, indicating mobility of the voltage sensor [6]. The glycine insertion occurs at the cytoplasmic end of the pore, at the interaction site with S4–S5 linkers, which transmit movements of voltage-sensing S4 segments to the pore-forming S6 segments [34]. Measurements of Ca<sub>v</sub>1.3 gating currents suggested that the 403\_404insGly mutation can disturb this interaction by uncoupling S4 movements from the channel opening [6]. However, it is also possible that the mutation obstructs ion permeation. Recent studies of five supposedly unrelated Pakistani families with SANDD syndrome revealed that four of them were carriers of the 403\_404insGly mutation, which was likely inherited from a common distant ancestor [151]. In the fifth family, SANDD syndrome was associated with a homozygous A376V mutation in the exon 8b of *CACNA1D*. A376V is located in the pore loop (DI/S5–S6 linker). The alanine at this position is conserved across LTCCs in various tissues. It was proposed that the substitution by valine could affect the shape of the inner part of the pore and thus affect Ca<sup>2+</sup> conduction.

Ca<sub>v</sub>1.2 channels could be involved in familial SAN dysfunction as well [7]. Genetic analysis of a Korean family with sinus bradycardia, early repolarization, and AF revealed compound heterozygosity of *CACNA1C* and titin mutations, predicted to be deleterious and perhaps increasing the severity of early repolarization [7]. Additional age-dependent loss of Ca<sub>v</sub>1.2 channels in the SAN (see Sect. 10.5.2.1 for more details) imposes a risk of the affected family members to develop sick sinus syndrome [7, 152].

## 10.5 LTCCs in Acquired Arrhythmias

### 10.5.1 Autoimmune and Inflammatory LTCC Channelopathies and Cardiac Arrhythmias

The immune system plays an important role in the pathogenesis of cardiac arrhythmias [153–155]. An exemplary mechanism is the production of autoantibodies and inflammatory cytokines which can affect ion channels involved in the generation of cardiac APs [153, 154].

#### 10.5.1.1 Antibodies Activating LTCCs

Autoimmune LTCC channelopathies play an important role in ventricular arrhythmias in dilated cardiomyopathy (DCM) [156]. Agonistic autoantibodies directed against  $\text{Ca}_v1.2$  channels (CC-AAbs,  $\text{Ca}^{2+}$  channel autoantibodies) were identified in up to 50% of patients with idiopathic DCM [157–160]. In the prospective studies, patients positive for CC-AAbs had a several-fold higher risk of ventricular tachycardia and SCD than patients without these antibodies [158, 160]. CC-AAbs can bind to the  $\text{Ca}_v1.2$  N-terminus and increase  $\text{Ca}^{2+}$  currents [159]. The arrhythmogenicity of CC-AAbs was confirmed in an animal model [158]: in isolated rat hearts, application of the purified CC-AAbs led to ventricular tachycardia and ventricular premature beats. Consistently, on the level of ventricular myocytes, the antibodies led to AP prolongation and occurrence of EADs. Summing up, CC-AAbs are an independent predictor of SCD in DCM resulting from ventricular arrhythmias [158, 160]. CC-AAbs were found in patients with ischemic cardiomyopathy (ICM) at a similar frequency as in DCM patients [160]. In ICM patients, CC-AAbs were associated with a several-fold increased risk of both SCD and non-SCD. Binding of CC-AAbs to the intracellular part of  $\text{Ca}_v1.2$  and similar prevalence of CC-AAbs in ICM and DCM suggest that CC-AAbs can develop secondary to myocyte damage [154, 158, 161].

Several autoantibodies with targets other than LTCCs have been shown to increase LTCC-

mediated currents in DCM. Autoantibodies against adenine nucleotide translocators cross-react with LTCCs, increase  $\text{Ca}^{2+}$  influx, and lead to progressive cardiomyocyte damage [157]. Monoclonal antibodies against cardiac troponin I can strongly enhance LTCC currents in murine myocytes, likely by an indirect mechanism [162]. However, this effect has not yet been reproduced with human samples [163]. Autoantibodies against  $\beta_1$  adrenoceptors from DCM patients prolonged APD by increasing LTCC currents via activation of cAMP/PKA signaling [164].

#### 10.5.1.2 Antibodies Inhibiting LTCCs

Inhibition of LTCCs by anti-Ro/SSA autoantibodies plays an important role in autoimmune congenital heart block (ACHB) [154, 156]. Of note, anti-Ro antibodies are present in healthy people, too. Nonetheless, risk for ACHB is significantly enhanced in anti-Ro antibody positive individuals with an incidence of about 5% compared to 1:11,000 in general. ACHB is thought to be caused by transplacental transfer of maternal antibodies, leading to inflammation and eventually fibrosis of the fetal AV node, but also of SAN and His bundle [165–167]. Ro/SSA and La/SSB antigens are intracellular ribonucleoproteins, which become surface exposed in apoptotic cells [168]. Interaction with maternal anti-Ro and anti-La antibodies impairs clearance of fetal apoptotic cardiomyocytes by healthy proliferating cardiomyocytes and provokes inflammation and fibrosis, normally not elicited in physiological apoptosis [169, 170]. However, this hypothesis alone cannot explain many features of ACHB, such as targeting the conduction system [171, 172]. Furthermore, in vitro perfusion of animal or human fetal hearts with Ro/SSA antibodies, which are prevalent antibodies in ACHB, led to sinus bradycardia and AV block within minutes [173–175]. Ro/SSA antibodies directly inhibit L-type ( $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$ ) and T-type ( $\text{Ca}_v3.1$  and  $\text{Ca}_v3.2$ ) channels in isolated cardiac cells and heterologous expression systems [174–180]. Of note, a study in rabbit SAN cells supported antibody specificity toward VGCCs:

while human antibodies of mothers whose children showed CHB inhibited L- and T-type  $\text{Ca}^{2+}$  currents, delayed rectifier  $\text{K}^+$ , and hyperpolarization-activated “funny” currents remained unaffected [177]. Since AP generation in AV and SA nodes depends on inward  $\text{Ca}^{2+}$  currents, VGCC inhibition explains the acute rhythm disturbances induced by Ro/SSA antibodies. While both  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$  inhibition may cause AVB, studies on expression patterns, electrophysiological properties, and cellular functions suggest that SAN dysfunction is more likely due to an inhibition of  $\text{Ca}_v1.3$  channels [5, 156, 181]. The antibody recognition site was identified in the DI/S5-S6 extracellular loop of  $\text{Ca}_v1.3$  and  $\text{Ca}_v3.1$  channel pores, respectively [179, 180]. Regarding chronic effects of Ro/SSA antibodies it was proposed that cross-linkage of antibody–antigen complexes leads to channel internalization [156, 171]. As fetal hearts show lower expression of VGCCs and lower SR capacity, particularly here VGCC downregulation can lead to severe  $\text{Ca}^{2+}$  dysregulation and abnormal apoptosis, initiating inflammation and fibrosis. Translocation of SSA/Ro and SSB/La antigens to the surface of affected cells may play an important role here.

Recently, an observational study identified a novel anti- $\text{Ca}_v1.2$  antibody in patients with idiopathic cardiac arrest [182]. The antibody was shown to target the ion selectivity and permeability filter segment in the  $\text{Ca}_v1.2$  domain DIII and to be pro-arrhythmic by shortening the APD due to  $\text{Ca}^{2+}$  current inhibition. Of note, it cannot be excluded that antibody generation may be secondary to cardiac arrest rather than being causal for this event.

### 10.5.1.3 Inflammatory Cytokines and LTCCs

Systemic and cardiac inflammation is associated with development of atrial and ventricular arrhythmias [153, 183, 184]. Higher levels of circulating inflammatory factors are associated with an increased AF risk in the general population as well as in patients after cardiac surgery, AF ablation, or cardioversion [185, 186]. Of note, anti-inflammatory treatments can potentially

decrease the risk of postoperative and post-ablation AF [183]. It has also been shown that the risk of ventricular arrhythmias and SCD in apparently healthy subjects and in patients with either cardiac or systemic inflammatory disease correlates with higher serum cytokine levels [184]. Particularly, pro-inflammatory cytokine levels were related to a QT prolongation and cytokine inhibitors shortened QT intervals.

Inflammation promotes structural and electrical remodeling of cardiac tissue. On the cellular level, pro-inflammatory cytokines, such as  $\text{TNF}\alpha$ , IL-1, and IL-6, can regulate ion channels and  $\text{Ca}^{2+}$ -handling proteins [187]. For example, they can inhibit depolarizing  $\text{K}^+$  currents, thus prolonging ventricular AP [187, 188]. Reported effects of cytokines on LTCCs vary because of different experimental conditions and models. In murine ventricles, IL-6 augmented LTCC currents by SHP2/ERK-mediated phosphorylation of the distal  $\text{Ca}_v1.2$  C-terminus, thus contributing to the observed APD prolongation [189]. However, no changes in the LTCC current were reported in ventricles of rats and guinea pigs [190, 191]. IL-1 was shown to be able to either stimulate or inhibit LTCC currents via different pathways. It increased LTCC currents by a lipoxigenase pathway in guinea pig ventricular myocytes, and here LTCC upregulation was consistent with the measured prolongation of APD [192]. In other studies on rat and murine ventricular myocytes, IL-1 inhibited LTCCs via G-proteins, NO- (but not cGMP), and ROS/PKC-dependent pathways, respectively [193–195].  $\text{TNF}\alpha$  effects on ventricular LTCC currents were studied in various animal models and showed either no effect on LTCCs or current inhibition at  $\text{TNF}\alpha$  concentrations exceeding those at pathophysiological conditions [187, 195]. Studies of cytokine effects on atrial LTCCs showed channel downregulation, which is consistent with AF pathogenesis (see Sect. 10.5.2.2 for more details). In murine atrial myocytes, IL-1 reduced  $\text{Ca}_v1.2$  protein levels by inhibiting expression of an mRNA-binding quaking protein [196]. In rabbit cardiomyocytes from pulmonary veins, which are critical in AF initiation,  $\text{TNF}\alpha$  significantly reduced LTCC

currents [197]. Together with the upregulation of atrial  $K^+$  currents by  $TNF\alpha$ , this could shorten APD in a pro-arrhythmogenic manner and contribute to AF vulnerability [197]. Similarly, MIF (macrophage migration inhibitory factor), another cytokine associated with the pathogenesis of AF, decreased LTCC current density by lowered  $Ca_v1.2$  protein expression, impaired LTCC function, and activation of c-Src kinases [198]. Recombinant MIF reduced LTCC currents in human atrial myocytes from patients with sinus rhythm but not in patients with AF. In contrast, application of an antibody against MIF increased LTCC currents in atrial myocytes from AF but not sinus rhythm patients.

In summary, although the effects of inflammation on LTCCs are poorly understood, it appears that inflammatory cytokines can alter LTCC currents through various mechanisms, so that ventricular (LQTS) and atrial (AF) arrhythmias are promoted.

## 10.5.2 LTCCs in Age- and Remodeling-Related Arrhythmias

### 10.5.2.1 SAN Aging and Dysfunction

Decline of SAN function, reflected in the progressive reduction of intrinsic pacemaker activity and maximum heart rates, is an inherent feature of cardiac aging, eventually leading to sinus node dysfunction in some individuals [199]. Besides vital loss of SAN cells and tissue remodeling, reduced excitability of SA myocytes is an intrinsic cause of age-dependent deterioration of SAN function. LTCC currents and subsequently triggered local  $Ca^{2+}$  release regulate slow diastolic depolarization and upstroke of SAN AP [82, 181, 200]. In SAN of aged mammals, LTCC expression and consecutive currents were reduced in comparison to younger adults [152, 201, 202]; but: [203]. MicroRNAs (miRs) can post-translationally regulate expression of ion channels. Significant upregulation of circulating miR-1976 was observed in patients with age-related sick sinus syndrome compared to age-matched healthy controls [202].  $Ca_v1.2$  and  $Ca_v1.3$  channels were found to be direct targets of

miR-1976. In transgenic mice, miR-1976 led to the inhibition of  $Ca_v1.2$  and  $Ca_v1.3$  expression in SAN and to slowed intrinsic cardiac rhythm. Furthermore, miR-1976 level was progressively increased in blood plasma and SAN tissue of aging rabbits and negatively correlated with protein levels of  $Ca_v1.2$  and  $Ca_v1.3$  in SAN [202].

### 10.5.2.2 Atrial Fibrillation

Prevalence of AF depends on age: compared to men aged 65–69 years the AF prevalence is two-fold increased at an age of 75–79 years and more than fivefold compared to men aged 55–59 years [204]. While often cardiac diseases like coronary artery disease or heart failure increase the risk of developing AF, there are also genetic and hereditary components [205–207]. Until 2018 over 30 genetic loci have been identified as significantly associated with AF, although none of them were related to genes coding for VGCCs [207]. With regard to aging,  $Ca_v1.2$  LTCCs were found to be downregulated in atria of humans, dogs, and sheep, reflected by mRNA, protein, or peak current levels [12, 208]. In patients, downregulation is associated with AF [12, 209], atrial dilation, or other structural diseases predisposing to AF [210, 211]. In animal models of AF, atrial tachypacing reduces LTCC expression and currents in early stages of AF as well as in persistent AF [209]. Downregulation of LTCCs could be initially adaptive to oppose  $Ca^{2+}$  overload resulting from atrial tachycardia [212]. Indeed, the  $Ca^{2+}$  channel antagonist verapamil prevented atrial contractile dysfunction and attenuated electric remodeling caused by short-term AF in patients and animal models [213–217]. Furthermore, verapamil and other drugs lowering intracellular  $Ca^{2+}$  load enhanced cardioversion success in patients with persistent AF [218]. On the other hand, reduction of LTCC currents in AF can be pro-arrhythmic, because it is associated with shortened APD, abolished AP plateau, and attenuation of rate-dependent AP accommodation [219, 220]. A diminished effective refractory period due to shorter APD results in a decreased wavelength of conduction, which permits more re-entrant circuits and thus facilitates AF [221].

Multiple mechanisms may be involved in the regulation of LTCCs in AF. In AF animal models, LTCC currents start to decline within several hours or days of atrial tachypacing, depending on the species [219, 222, 223]. Current reduction paralleled mRNA expression of the pore-forming subunit (*CACNA1C*) as well as  $\text{Ca}_V\beta$  subunits (*CACNB2a*, *CACNB2b*, and *CACNB3*). In human AF patients, reduced mRNA expression was reported for  $\text{Ca}_V1.2$  [224–228]; but: [229],  $\text{Ca}_V1.3$  [227],  $\text{Ca}_V\beta_1$  [229],  $\text{Ca}_V\beta_2$  [228], and  $\text{Ca}_V\alpha_2\delta-1$  [227, 229]. In cultured canine atrial myocytes, it was shown that tachypacing-induced  $\text{Ca}^{2+}$  load initiated LTCC downregulation by activating  $\text{Ca}^{2+}$ -dependent calmodulin–calcineurin–NFAT signaling within a few hours of atrial tachypacing [230].

Several studies pointed to the role of miRs in the pathogenesis of AF [231]. MiR-328 was found to be upregulated in canine and murine AF models and human AF patients [232]. Induction of miR-328 expression promoted AF in dogs and mice, and this effect could be antagonized by a specific antisense inhibitor oligonucleotide (AMO-328). In murine atrial myocytes, miR-328 caused APD shortening and reduction of LTCC currents as observed in experimental and clinical AF before. Downregulation of  $\text{Ca}_V1.2$  and  $\text{Ca}_V\beta_1$  protein was seen in atria of AF patients, in dogs treated with tachypacing, as well as in murine and neonatal rat atria infected with miR-328. In rats, this effect could be reversed by AMO-328. Other miRs (miR-21 and miR-208b) were also found to be upregulated in AF and to decrease LTCC currents by targeting *CACNA1C* and *CACNB2* expression [228, 233].

Activation of proteolytic mechanisms can decrease LTCC protein expression independent of mRNA reduction [226, 234]. The  $\text{Ca}^{2+}$ -dependent protease calpain can degrade surface LTCCs on the minute timescale, regulating channel turnover [235]. Expression and activity of atrial calpain was found to be upregulated in AF patients as well as AF animal models and negatively correlated with LTCC protein levels [234, 236].

Phosphorylation of pore and auxiliary LTCC subunits can affect channel gating as well. The

role of phosphorylation in chronic human AF, however, is controversial [237]. One group reported unchanged levels of  $\text{Ca}_V1.2$  and  $\text{Ca}_V\beta_{2a}$  expression but a more than twofold reduction of LTCC currents, which was associated with the increased activity of protein phosphatases [238]. In contrast, another group observed a reduced  $\text{Ca}_V1.2$  protein expression, which was accompanied by a compensatory increase in single-channel activity due to higher phosphorylation [239].

Besides  $\text{Ca}_V1.2$ ,  $\text{Ca}_V1.3$  channels may also be involved in AF pathogenesis. Reduction of *CACNA1D* mRNA was reported in atrial samples of patients with persistent AF [227].  $\text{Ca}_V1.3$ -deficient mice showed significant reduction of LTCC currents in atria but not ventricles and an increased vulnerability to atrial arrhythmias and AF [5, 148, 240]. Although still controversial, several clinical studies observed an association between PR interval prolongation and risk of AF [241].  $\text{Ca}_V1.3$ -deficient mice showed PR prolongation, too [5, 240]. In atria of AF patients, reduced  $\text{Ca}_V1.3$  expression was accompanied by decreased levels of ankyrin-B [242]. Using a murine model, the authors showed that ankyrin-B mediates membrane targeting of atrial  $\text{Ca}_V1.3$  by interacting with its C-terminus and that ankyrin-B deficiency leads to reduced  $\text{Ca}^{2+}$  current density and enhanced AF vulnerability.

### 10.5.2.3 Heart Failure

According to the European Society of Cardiology, heart failure (HF) is a clinical syndrome characterized by typical symptoms caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures [243]. Of note, there is no agreed single classification of the causes of HF, and many patients have several different pathologies—cardiovascular and non-cardiovascular—underlying this disease. Among the various cardiovascular pathologies are coronary artery disease, myocardial infarction, hypertension, valve disease, AF, and DCM. Prevalence of HF is estimated to be 1–2% among adults in developed countries, but steeply rises with age, reaching 70% and more among persons

>70 years of age. Although treatment options clearly improved during the last few decades, HF is still associated with a high mortality [244]. Data collected in Europe between 2011 and 2013 showed 12-month all-cause mortality rates for hospitalized (acute) and ambulatory (chronic) HF of 24% and 6%, respectively [245]. About a half of the deaths is sudden, with a large fraction of them presumably due to ventricular arrhythmias [246, 247]. AF and HF often coexist, sharing risk factors and promoting development and deterioration of each other [248]. In chronic HF, AF is an independent predictor of mortality [245].

#### 10.5.2.3.1 Alterations of Ventricular LTCCs in HF

Electrophysiological remodeling promoting arrhythmias in HF is manifold, including ion-channel and  $\text{Ca}^{2+}$ -handling alterations [249–252]. LTCC expression (detected as dihydropyridine binding sites, *CACNA1C* mRNA, or  $\text{Ca}_V1.2$  protein level) and current density were mostly reported to be unchanged in human failing ventricular myocytes [253–259]. Nonetheless, some studies described reduction of currents or LTCC expression [260–263], although HF etiology and/or stage of HF may play a role [250, 253]. Furthermore, a shift in the expression of  $\text{Ca}_V1.2$  splice variants was observed in human failing myocytes [264–266]. Expression of the auxiliary  $\text{Ca}_V\alpha_2\text{-}\delta$  subunit was not altered in human HF [257, 267]. Studies on expression of  $\text{Ca}_V\beta$  subunits in human HF have been conflicting and inconclusive [268]. In a comprehensive analysis of human specimens from failing ventricles, we found an increased expression of  $\text{Ca}_V\beta_2$  subunits at both mRNA and protein level, while that of  $\text{Ca}_V\beta_1$  and  $\text{Ca}_V\beta_3$  appeared to be unchanged or even reduced ( $\text{Ca}_V\beta_3$  protein) [257]. Overexpression of  $\text{Ca}_V\beta_2$  subunits in adult mouse hearts over a period of 6 weeks caused LTCC current alterations similar to those observed in human HF [269]. On the background of constitutive cardiac  $\text{Ca}_V1.2$  overexpression, this furthermore led to a significantly increased occurrence of cardiac arrhythmias including supraventricular and

ventricular extrasystoles and AV block. Expression of the RGK protein Rad was found to be decreased in failing human ventricles both at mRNA and protein levels [270, 271]. Of interest, overexpressing a dominant negative mutant of Rad caused ventricular arrhythmia in mice [272]. Given the inhibitory effect of Rad on  $\text{Ca}_V1.2$ , increased LTCC activity might have been pro-arrhythmic here [272, 273]. Very recently, a study found that Rad inhibits  $\text{Ca}_V\beta_2$ -mediated stimulation of  $\text{Ca}_V1.2$  [274]. Rad phosphorylation following  $\beta$ -adrenergic stimulation led to a decrease of its affinity for  $\text{Ca}_V\beta_2$  subunits and by this relieved constitutive inhibition of  $\text{Ca}_V1.2$ . This study sheds new light on the role of  $\beta$ -adrenergic signaling and  $\text{Ca}_V\beta_2$ - $\text{Ca}_V1.2$  interaction for LTCC stimulation and thus promotion of probably pro-arrhythmic conditions.

The group of Stefan Herzig found activity of single LTCCs to be significantly increased in human failing ventricular myocytes [275]. Although this was supported by later studies [255, 256], these data are in an apparent contradiction to the abovementioned findings on reduced or rather unchanged  $\text{Ca}_V1.2$  expression and whole-cell LTCC current density. However, there is loss and remodeling of the T-tubule system, which plays an important role in the pathogenesis and arrhythmogenesis in HF [252, 276]. T-tubule degradation in human and rat HF was accompanied by redistribution of LTCCs from their native positions in T-tubules to the sarcolemma crest [277, 278]. Targeting of LTCCs to T-tubules is controlled by the scaffolding BIN-1 protein [279]. Its downregulation in HF leads to diminished abundance of  $\text{Ca}_V1.2$  channels in the periphery and, specifically, T-tubules of the failing myocytes despite unchanged overall cellular expression [258]. Besides, BIN-1 organizes dyads formed by LTCCs and RyRs [280]. In HF, the abovementioned microdomain remodeling of LTCCs is related to an increased occurrence of orphaned RyRs, i.e., RyRs not coupled to LTCCs [280–282]. Such RyRs can lead to spontaneous  $\text{Ca}^{2+}$  release promoting  $\text{Ca}^{2+}$ -dependent arrhythmias [280]. Dislocated crest LTCCs showed increased single-channel activity in HF,



whereas the activity of the remaining T-tubule LTCCs was not changed [275, 278]. The underlying mechanism was proposed to be channel hyper-phosphorylation due to dephosphorylation defects on the one hand and increased phosphorylation on the other hand [255, 256, 275, 278]. An increased expression of  $\text{Ca}_v\beta_{2a}$  subunits, which are subject to PKA and CaMKII phosphorylation, can also play a role [257, 283, 284]. A channel's basal hyper-phosphorylation is consistent with a compromised ability of  $\beta$ -adrenergic stimulation to (further) augment LTCC activity in HF [255, 256, 262, 275]. Switch to the high-activity gating mode and disorganization with RyRs can result in a slowed inactivation of LTCC currents and thus promote EADs [278]. Accordingly, whole-heart computer simulations demonstrated that this microdomain remodeling of LTCCs promotes EADs in endocardial myocytes, which can trigger reentrant arrhythmias.

So far, research has focused on  $\text{Ca}_v1.2$  LTCCs in HF. However, recently re-expression of the fetal  $\text{Ca}_v1.3$  subunit was reported in the human failing ventricles on both mRNA and protein level [263]. The putative role of  $\text{Ca}_v1.3$  channels in HF and ventricular arrhythmias requires further studies.

#### 10.5.2.3.2 Alterations of Atrial LTCCs in HF

Remodeling of atrial  $\text{Ca}_v1.2$  LTCCs in HF is similar to that in AF [209]. In HF animal models, atrial LTCC current density was reported to be reduced [285–289]. In human HF, atrial LTCC currents were either decreased [211, 262] or showed no difference compared to control [259, 290, 291]. Besides, disruption of atrial T-tubules in HF can lead to a decreased number of functional LTCCs [292, 293]. Loss of T-tubules caused by HF was more dramatic in atria than in ventricles [292, 293]. Moreover, single-channel current amplitudes of T-tubular LTCCs were largely decreased in HF [294]. Taken together, animal models of HF indicate reduction of functional LTCCs creating substrate for AF [209], while human data are conflicting.

## 10.6 Conclusion and Limitations

LTCCs are critically involved in shaping cardiac action potentials. By this capability, dysfunction of LTCCs, either congenital or acquired, can lead to life-threatening cardiac rhythm disturbances (Table 10.1, Fig. 10.1). Though LTCC mutations are rare, a growing number of LTCC genetic variants are identified in and related to arrhythmogenic syndromes. While the association between *CACNA1C* mutations and Timothy syndrome is well established, additional research is required to validate causality of arrhythmias by new LTCC mutations, many of them identified in single patients only. Functional studies with cultured cells allow for comparison of mutant and wild-type LTCCs, but limited by the fact that these cells cannot fully reconstitute the conditions in native cardiomyocytes. LTCCs are also involved in the pathogenesis of acquired cardiac diseases. Despite the fact that human data are not easy to obtain and that comparison with animal models is hampered by differences in ion channel expression patterns as well as structural and electrical properties of the myocardium, great progress has been made in understanding LTCC (dys-)regulation in atrial fibrillation, heart failure, and other arrhythmogenic conditions, including aging.

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