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REVIEW

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Calmodulin Mutations in Human Disease

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ABSTRACT

Calcium ions (Ca^{2+}) are the basis of a unique and potent array of cellular responses. Calmodulin (CaM) is a small but vital protein that is able to rapidly transmit information about changes in Ca^{2+} concentrations to its regulatory targets. CaM plays a critical role in cellular Ca^{2+} signaling, and interacts with a myriad of target proteins. Ca^{2+}-dependent modulation by CaM is a major component of a diverse array of processes, ranging from gene expression in neurons to the shaping of the cardiac action potential in heart cells. Furthermore, the protein sequence of CaM is highly evolutionarily conserved, and identical CaM proteins are encoded by three independent genes (*CALM1-3*) in humans. Mutations within any of these three genes may lead to severe cardiac deficits including severe long QT syndrome (LQTS) and/or catecholaminergic polymorphic ventricular tachycardia (CPVT). Research into disease-associated CaM variants has identified several proteins modulated by CaM that are likely to underlie the pathogenesis of these calmodulinopathies, including the cardiac L-type Ca^{2+} channel (LTCC) $Ca_V1.2$, and the sarcoplasmic reticulum Ca^{2+} release channel, ryanodine receptor 2 (RyR2). Here, we review the research that has been done to identify calmodulinopathic CaM mutations and evaluate the mechanisms underlying their role in disease.

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Introduction

In 1957, Hodgkin and Keynes noted that Ca^{2+} ions microinjected into a squid giant axon were not free to move in the presence of an externally applied electric field[1] and postulated the existence of an intracellular agent capable of binding Ca^{2+}. Later work by Cheung *et al.* [2–4] as well as Kakiuchi and Yamazaki [5] converged on an endogenous protein factor identified in brain tissue that could enhance the Ca^{2+} sensitivity of particular enzymes. This protein was soon recognized to be calmodulin (CaM), and over the following decades it was identified as an indispensable component of signal transduction pathways that target a large array of structural proteins, receptors, enzymes, and ion channels [6,7]. By acting on these diverse targets, CaM impacts numerous cellular processes including cell differentiation and division, gene transcription, membrane fusion, and muscle contraction.

CaM is a ubiquitously expressed Ca^{2+}-sensor molecule found in all eukaryotic cells. CaM is a 17-kDa 149 amino acid protein that contains two distinct yet broadly similar regions, the N- and C-lobes, linked by a flexible helix. Each lobe of CaM contains two canonical Ca^{2+} binding motifs, termed EF hands. Each lobe of CaM exhibits a distinct Ca^{2+} binding affinity, and the K_D has been measured to be approximately 2.4 μM for the C-lobe, and 16 μM for the N-lobe [8]; however, the presence of a target protein may have a significant impact on these values. Interestingly, the N- and C-lobes of CaM can each contribute separable effects on a target protein, a concept that highlights the functional bipartition of CaM lobes. The first glimpse of this phenomenon was observed in *Paramecium*. Deficits in the Ca^{2+}-binding properties of CaM's N-lobe leads to sluggish behavior in paramecia, while the same type of deficit in the Ca^{2+}-binding properties in the C-lobe leads to hyperactive behavior [9]. Thus, each lobe of CaM is capable of a unique interaction with, and impact on, a target protein, amplifying the modulatory capability of this Ca^{2+} sensor. In this way, CaM confers a critical Ca^{2+} sensitivity to hundreds of substrates [10].

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In humans, CaM is encoded by three genes (*CALM1, CALM2*, and *CALM3*) that vary in nucleotide sequence, yet nonetheless encode identical protein products. Each CaM gene resides on a distinct chromosome, with *CALM1* located at chr14q31, *CALM2* at chr2p21 and *CALM3* at chr19a13. Many of the mechanisms underlying how these three distinct genes regulate CaM expression remain unknown. While all three genes appear to function across different tissues, the level of transcript expression may not be equal. In human heart, the relative abundance of *CALM3* has been shown to be largest, with all three genes producing significant levels of transcript across multiple developmental timepoints [11]; however, transcript levels measured in induced pluripotent stem cell (iPSC)-derived cardiomyocytes showed higher levels of *CALM1* [12]. Moreover, the expression of all three transcripts has been shown to increase over the course of development [11,13].

CaM is highly conserved across species and is essential for normal physiological function. It was therefore once thought that non-synonymous mutations in *CALM* genes were incompatible with life [14]. Starting in 2012, the discovery of disease-associated human CaM mutations overturned this idea [11,15] and initiated a wave of research into the mechanisms of disrupted CaM regulation.

CaM regulation in diverse cellular contexts

Either through activation of third-party enzymes or direct binding, CaM modulates a diverse range of targets that span across multiple organ systems, including the immune system, the nervous system, smooth muscle, and the heart.

CaM regulation in the immune system

In the immune system, Ca^{2+}/CaM is required to activate T lymphocytes, one of the major mechanisms of adaptive immunity [16,17]. Upon binding to the antigen-presenting cell, intracellular Ca^{2+} in T lymphocytes rises due to the opening of IP_3 receptors and store-operated Ca^{2+} channels, STIM and ORAI. The calcified CaM then binds to and activates calcineurin, which in turn dephosphorylates the transcription factor, nuclear factor of activated T-cells (NFAT). This modification permits NFAT to enter the nucleus and initiate the production of a cytokine responsible for activating downstream defense against the pathogen.

CaM regulation in smooth muscle

In smooth muscle, CaM controls both initiation and cessation of contraction. Ca^{2+}/CaM binds and activates myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin, and thus initiates smooth muscle contraction [18]. In addition, Ca^{2+}/CaM also activates Ca^{2+}/CaM-dependent kinase II (CaMKII) which phosphorylates MLCK, lowering its sensitivity to Ca^{2+} and thus promoting muscle relaxation. In contrast, within skeletal muscle, CaM modulates the contraction rather than initiating or ceasing it. Ca^{2+}/CaM activates MLCK, which in turn phosphorylates the regulatory light chain of myosin, making it more sensitive to Ca^{2+} and thus generating more force [19]. Moreover, CaMKII and CaMKIV play a major role in muscle plasticity. These two enzymes encode frequency-dependent muscle usage by transducing the changes in frequency and magnitude of Ca^{2+} transients into changes in protein expression [20]. It is through dysregulation of this mechanism that CaM mutations in *Drosophila* can lead to skeletal muscle dysfunction [21].

CaM regulation in the brain

In the nervous system, Ca^{2+}/CaM-dependent enzymes regulate neural development, memory, and learning. The classic example includes long-term potentiation in hippocampal CA1 neurons, which involves phosphorylation of AMPA and NMDA receptors by CaMKII [22]. Calcineurin, a Ca^{2+}/CaM-dependent phosphatase, is also proposed to be involved in long-term depression in hippocampal neurons [23,24] and to control the polymerization of actin in dendrites, which ultimately leads to dendritic growth or retraction [25,26].

CaM regulation in the heart

In the heart, Ca^{2+}/CaM-dependent enzymes shape electrical and Ca^{2+} activities of cardiomyocytes

both during physiological and pathological responses. For instance, responding to the rise in intracellular Ca^{2+}, CaMKII phosphorylates numerous Ca^{2+} signaling proteins includingRyR2, phospholamban, sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), and L-type Ca^{2+} channels [27,28]. During pathologic adaptation such as in heart failure, calcineurin dephosphorylates numerous targets including the transcription factor NFAT, which leads to altered expression levels of proteins involved in cardiac hypertrophy [29,30].

CaM regulation of Ion channels

CaM regulation of voltage-gated calcium channels

A critical form of CaM modulation is the direct binding of apoCaM or Ca^{2+}/CaM to targets, particularly ion channels. By physically associating with ion channels, CaM is capable of imparting multifunctional Ca^{2+}-dependent regulatory processes on channel biophysical properties. For the Ca_V1 and Ca_V2 families of voltage-gated Ca^{2+} channels (VGCCs), at low cytosolic levels of Ca^{2+}, apoCaM is bound to the IQ motif within the carboxy-tail of the channel, positioning CaM as a resident Ca^{2+} sensor. Upon Ca^{2+} elevation, Ca^{2+} binding to this resident CaM initiates either of two distinct forms of feedback regulation. Ca^{2+}-dependent facilitation (CDF) occurs within select cannels, where Ca^{2+} entry through the channel initiates an increase in current amplitude [31]. Alternatively, Ca^{2+} binding to CaM often results in Ca^{2+} dependent inactivation (CDI), where a rise in cytosolic Ca^{2+} reduces the Ca^{2+} influx through the channels. As each lobe of CaM is able to impart its own independent form of channel regulation, each channel type may exhibit one or both forms of regulation. $Ca_V2.2$ and $Ca_V2.3$ each display CDI driven by Ca^{2+} binding to the N-lobe of CaM [32], while $Ca_V2.1$ exhibits CDI in response to Ca^{2+} binding to the N-lobe of CaM, and CDF in response to C-lobe Ca^{2+} binding [31,33]. Finally, the L-type channels ($Ca_V1.2$, $Ca_V1.3$, and $Ca_V1.4$) each display CDI in response to Ca^{2+} binding to either the N- or C-lobe of CaM, with distinct kinetics corresponding to signaling from each lobe [34–41]. The exception to this CaM regulatory scheme is the $Ca_V1.1$ channel, where apoCaM binds poorly to the carboxy-tail of the channel [42], resulting in a lack of CDI or CDF in most physiological systems [43]. Nonetheless, fusion of CaM to the channel enhances trafficking and open probability [44], generalizing much of the mechanism of CaM regulation across the L-type channel family. Functionally, CDF has been shown to be an important element in synaptic plasticity [45], CDI can reduce Ca^{2+}-dependent and activity-dependent toxicity, as well as tune the temporal activity of Ca^{2+} flux to shape action potentials (APs) in excitable cells, especially those in cardiac tissue [14].

Unlike Ca_V1-2 channels, Ca_V3 channels lack a carboxy-terminal IQ motif, eliminating the Ca^{2+}/CaM regulatory scheme described above. Despite this, several reports have described a role for CaM in the function of these channels, including binding to the gating brake of the I–II linker of $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ channels [46], activity-dependent association of CaM with the $Ca_V3.1$ channel [47], and Ca^{2+}-dependent regulation of the $Ca_V3.3$ channel [48], providing a potential role for calmodulin within this channel family.

CaM regulation of voltage-gated sodium channels

Parallel to its modulation of VGCCs, apoCaM also binds to select voltage-gated sodium channels [49,50]. In particular, apoCaM has been shown to bind to the C-tail of sodium channels in an analogous manner to its binding with voltage-gated Ca^{2+} channels [51]. In contrast to the dual-lobe regulation of VGCCs, it is the C-lobe that tethers apoCaM to $Na_V1.5$ [52], and disruption of this interaction both reduces the peak open probability of the channel and increases the amount of late sodium current [53]. Moreover, it has been shown that the N-lobe of Ca^{2+}/CaM drives CDI in the skeletal muscle isoform of Na channels ($Na_V1.4$), however the same process does not appear to exist in the cardiac $Na_V1.5$ channels [50].

CaM regulation of potassium channels

Slowly activating delayed rectifier K channels (KCNQ1/KCNE1) that generate I_{Ks} currents in

cardiac myocytes are also proposed to be modulated by CaM. Like for VGCCs, CaM appears to constitutively associate with KCNQ channels and is required for voltage-dependent opening. ApoCaM may help in channel folding, assembly, and trafficking of this channel to the plasmalemma [54,55]. However, the impact of Ca^{2+}/CaM dependent regulation on I_{KS} remains under debate. Despite this, it has been established that CaM association with the channel is critical to modulate the channel's activity [56]. Moreover, neuronal M-currents (KCNQ2/KCNQ3) were proposed to be regulated by Ca^{2+}/CaM- either by the promotion of channel assembly and trafficking [57] or by acute current suppression in response to a rise in the intracellular Ca^{2+}. Finally, [58]the Ca^{2+} activated SK potassium channel represents one of the first channels in which CaM was identified as the Ca^{2+} sensor [59]. ApoCaM pre-associates with the cytosolic tail of SK1,2 and 3 channels [60], with each domain channel capable of binding, resulting in four CaM proteins per channel. For SK2 channels, it has been shown that the N-lobe of CaM drives the Ca^{2+} dependence of the channel [61].

CaM regulation of intracellular ion channels

In addition to regulating ion channels on the plasma membrane, CaM also modulates the properties of intracellular ion channels such as ryanodine receptors. For the skeletal form of the ryanodine receptor (RyR1), CaM increases the open probability of the channels at submicromolar Ca^{2+} concentrations, i.e. in the apoCaM state, while the C-lobe of CaM inhibits channel opening under calcified conditions [62]. Conversely, both apoCaM and Ca^{2+}/CaM decrease the open probability of the cardiac isoform of RyR (RyR2) [62,63], while only the C-lobe of Ca^{2+}/CaM increases the termination threshold of spontaneous Ca^{2+} release from the sarcoplasmic reticulum [64]. Another type of intracellular Ca^{2+} release channel is the inositol 1,4,5 triphosphate receptor (IP_3R). Both apoCaM and Ca^{2+}/CaM decrease the sensitivity of the channel to IP_3, and thus inhibit the IP_3-induced Ca^{2+} release in type I IP_3Rs [65,66].

Importantly, CaM in its apo *versus* calcified form can exert opposite modulatory effects on channels. Mutations in the EF hands of CaM can alter the Ca^{2+} binding affinity without altering the structure of CaM in the Ca^{2+}-free state, thus keeping the apoCaM modulation intact despite disrupted Ca^{2+}/CaM modulation. Thus, mutations that alter distinct properties of CaM may have unique effects on different targets.

Described here are only a few examples of a vast array of targets of calmodulation. A more comprehensive list can be found elsewhere [67,68].

LQTS-associated CaM mutations

Long-QT syndrome (LQTS) is characterized by an abnormal prolongation of the QT interval of an ECG, resulting from a prolongation of the cardiac action potential. Multiple forms of LQTS have been identified and are classified based on the gene associated with the phenotype. The majority of these genes are either ion channels, or proteins that modulate ion channels. In 2013, Crotti *et al.* identified a new form of LQTS, where infants exhibiting severe LQTS and recurrent cardiac arrest were found to harbor mutations in CaM (Figure 1) [11]. These mutations span a broad phenotypic spectrum with various degrees of penetrance and severity. In addition to frequent ventricular fibrillations brought on by adrenergic stimulation, the patients also exhibited neurological and/or developmental delay and seizure. Whole-exome sequencing of these probands revealed *de novo* CaM mutations, D130G and F142L in *CALM1*, as well as D96V in *CALM2*. Patients harboring these mutations displayed severe clinical pathology early in life. Moreover, these patients were resistant to conventional treatments for LQTS such as beta blockers, sodium channel blockers, calcium channel blockers, and implantable cardioverter defibrillators.

Since this first description, numerous mutations in CaM have been described in patients with LQTS (Figure 1, Table 1). In 2014, Makita *et al.* performed whole-exome sequencing on over 200 LQTS patients and discovered *de novo* CaM mutations, all within the *CALM2* gene [69]. Three cases (N98S, N98I, and D134H) had a diagnosis of LQTS alone, while the two other cases (D132E and Q136P) had a mixed diagnosis of LQTS and CPVT. The onset of symptoms occurred in early

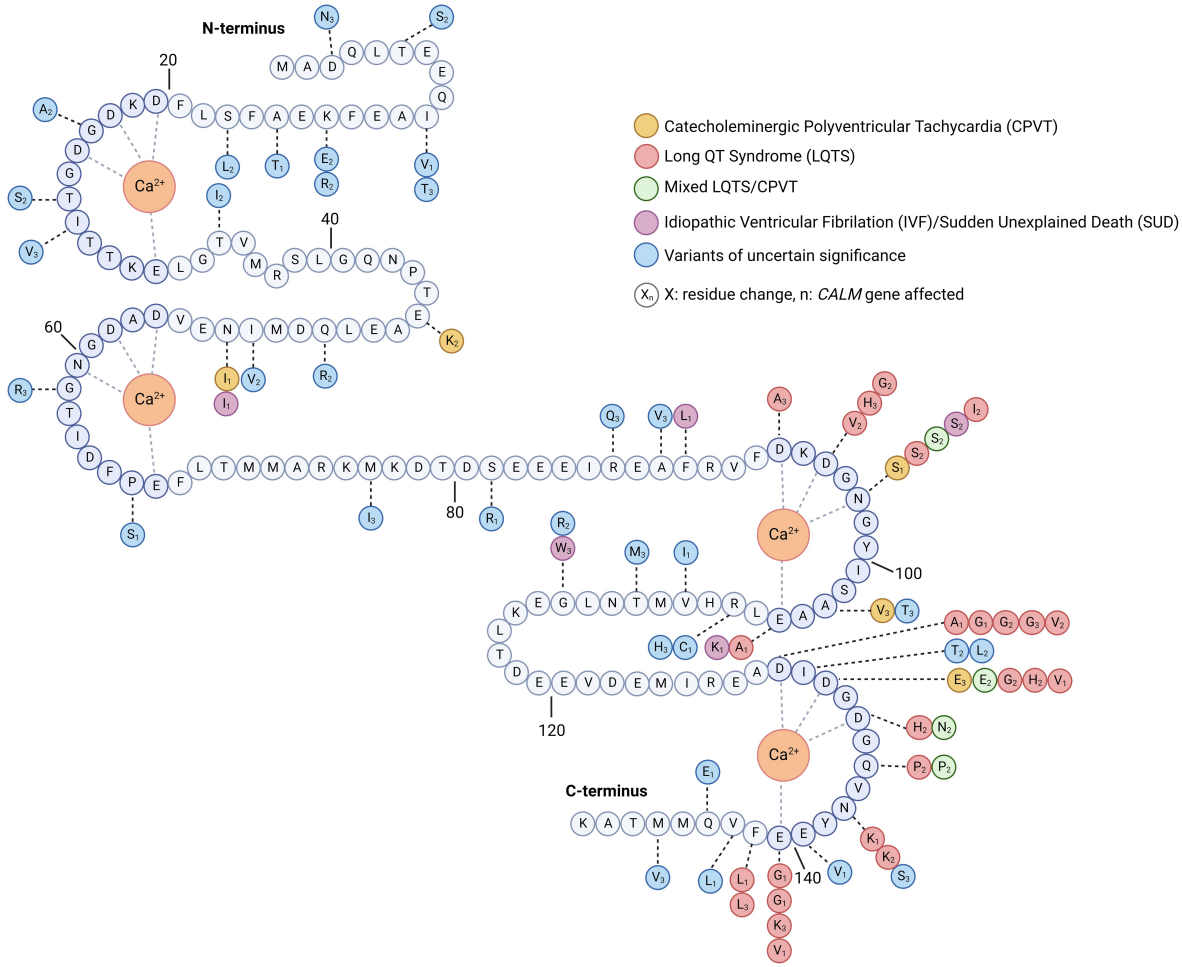

Figure 1. Missense Mutations Identified Within Human Calmodulin. Schematic featuring the amino acid sequence of CaM, with the location of identified pathogenic mutations indicated. Calcium ions (Orange) are coordinated within each of the four EF hands (shaded blue). Mutations in residues identified within patients suffering from CPVT are in Orange, LQTS residues are in red, mixed CPVT and LQTS are in green, IVF and/or SUD are in purple, and variants of uncertain significance that are logged in Gnomad are in blue. Numbers within each mutation circle indicate the specific *CALM* gene where the mutation was identified. Figure created with BioRender.com.

childhood, and these symptoms were relatively well controlled by treatment with beta blockers and sodium channel blockers. Moreover, none of these patients were reported to exhibit developmental delays or neurological symptoms. Interestingly, the same D130G mutation identified originally in *CALM1* [11] was subsequently identified as producing LQTS when present in both *CALM3* [70,71] *and CALM2* [72]. Thus, all three *CALM* genes are able to produce LQTS, leading to the designation of LQT type 14, 15 and 16 corresponding to mutations within *CALM1*, *CALM2* and *CALM3*, respectively. Moreover, this same D130 residue has been identified in *CALM2* with different changes at the same amino acid, such that D130V [72], and D130A [73] have both been associated with LQTS. Likewise, the mutation E141G in *CALM1* was identified as associated with LQTS by two groups [72,74], and subsequently the same locus with a distinct E141V mutation was identified [71]. As the number of known CaM mutations continues to grow, additional clusters of mutations continue to be identified, sometimes with as many as four distinct residue changes at a single locus (Figure 1). The majority of these calmodulinopathy hotspots occur within or directly adjacent to the EF-hand motifs, demonstrating the critical nature of Ca^{2+} binding to CaM.

While all three *CALM* genes have been implicated in the same overall LQTS phenotype, differences in severity and age of onset point to possible differences between the genes. *CALM1* mutations

Table 1. Summary of identified missense CaM mutations.

Mutation	CALM gene	Clinical Phenotype	Reference	Variant ID (Gnomad/ ClinVar)	Polyphen	SIFT	ACMG Classification
D3N	3	Uncertain	Gnomad[75]	19–47109087-G-A	Benign	tolerated	
T6S	2	Uncertain	Gnomad[75]	2–47397890-G-C	Benign	tolerated	
I10T	3	Uncertain	Gnomad[75]	14–90866433-A-G	Benign	deleterious	
I10V	1	Uncertain	Gnomad[75]	19–47109109-T-C	Benign	tolerated	
K14E	2	Uncertain	Gnomad[75]	2–47389796-T-C	Benign	deleterious	
K14R	2	Uncertain	Gnomad[75]	2–47389796-T-C	Benign	tolerated	
A16T	1	Uncertain	Gnomad[75]	14–90867614-G-A	Possibly damaging	deleterious	
S18L	2	Uncertain	Gnomad[75]	2–47389783-G-A	Benign	tolerated	
G24A	2	Uncertain	Gnomad[75]	2–47389765-C-G	Benign	deleterious	
T27S	2	Uncertain	Gnomad[75]	2–47389756-G-C	Benign	tolerated	
I28V	3	Uncertain	Gnomad[75]	19–47111501-A-G	Benign	deleterious	
T35I	2	Uncertain	Gnomad[75]	2–47389732-G-A	Possibly damaging	tolerated	
E46K	2	CPVT	Crotti et al 2019[73]				Likely Pathogenic
Q50R	2	Uncertain	Gnomad[75]	2–47389687-T-C	Benign	tolerated	
I53V	1	Uncertain	Nykamp et al 2017[76]	VCV000475380.4			
N54I	1	CPVT	Nyegaard et al 2012[15]	VCV000039757.5			
N54I	1	CPVT, IVF/SUD	Crotti et al 2019[73]	VCV000039757.5			Pathogenic
G62R	3	Uncertain	Gnomad[75]	19–47111744-G-A	Probably damaging	deleterious	
P67S	1	Uncertain	Gnomad[75]	14–90870226-C-T	Benign	tolerated	
M77I	3	Uncertain	Gnomad[75]	19–47111791-G-A	Benign	tolerated	
S82R	1	Uncertain	Gnomad[75]	14–90870273-T-G	Benign	tolerated	
R87Q	3	Uncertain	Mirsadeghi et al 2021[77]				
A89V	3	Uncertain	Gnomad[75]	19–47111826-C-T	Benign	deleterious	
F90L	1	IVF/SUD	Marsman et al 2013[85]	VCV000183232.2			Pathogenic
D94A	3	LQTS	Nykamp et al 2017[76]	VCV000409871.6			Likely Pathogenic
D96G	2	LQTS	Crotti et al 2019[73]				Likely Pathogenic
D96H	3	LQTS	Chaix et al 2016[83]	VCV000409870.6			Pathogenic
D96V	2	LQTS	Crotti et al 2013[11]	VCV000183233.5			Pathogenic
N98I	2	LQTS	Makita et al 2014[69]	VCV000096721.4			Likely Pathogenic
N98S	1	CPVT	Nyegaard et al 2012[15]	VCV000039758.7			Pathogenic
N98S	2	LQTS	Makita et al 2014[69]	VCV000096720.7			Pathogenic
N98S	2	Mixed LQTS + CPVT, SUD	Jiménez-Jáimez et al, 2016[78]	VCV000096720.7			Pathogenic
A103V	3	CPVT	Gomez-Hurtado et al, 2016[84]	VCV000579223.4			Likely Pathogenic
A103T	3	Uncertain	Gnomad[75]	19–47112124-G-A	Possibly damaging	deleterious	
E105A	1	LQTS	Takahashi et al 2016[104]				Likely Pathogenic
E105K	1	Atypical	Crotti et al 2019[73]	VCV000388500.2			Likely Pathogenic
R107C	1	Uncertain	Gnomad[75]	14–90870756-C-T	Benign	tolerated	
R107H	3	Uncertain	Gnomad[75]	19–47112137-G-A	Benign	tolerated	
V109I	1	Uncertain	Gnomad[75]	14–90870762-G-A	Benign	tolerated	
T111M	3	Uncertain	Gnomad[75]	19–47112149-C-T	Possibly damaging	deleterious	
G114 R	2	Uncertain	Brohus et al 2020[79]				*Likely pathogenic (in-silico analysis)*
G114W	3	IVF	Crotti et al 2019[73]				Likely Pathogenic
D130A	1	LQTS	Crotti et al 2019[73]				Likely Pathogenic
D130G	1	LQTS	Crotti et al 2013[11]	VCV000183230.3			Pathogenic
D130G	2	LQTS	Boczek et al 2016[72]	VCV000812710.7			Pathogenic
D130G	3	LQTS	Reed et al 2015[70]	VCV000812676.1			Pathogenic
D130V	2	LQTS	Boczek et al 2016[72]				Likely Pathogenic
I131T	2	Uncertain	Gnomad[75]	2–47388891-A-G	Benign	tolerated	
I131L	2	Uncertain	Gnomad[75]	2–47388892-T-G	Benign	tolerated	
D132E	2	Mixed LQTS + CPVT	Makita et al 2014[69]	VCV000096722.3			Pathogenic
D132E	3	CPVT	Crotti et al 2019[73]	VCV000423164.2			Pathogenic
D132H	2	LQTS	Pipilas et al 2016[80]				Likely Pathogenic
D132G	2	LQTS	Zahavich et al 2018[81]	VCV000955533.6			Likely Pathogenic

(*Continued*)

Table 1. (Continued).

Mutation	CALM gene	Clinical Phenotype	Reference	Variant ID (Gnomad/ ClinVar)	Polyphen	SIFT	ACMG Classification
D132V	1	LQTS	Pipilas et al 2016[80]	VCV000948239.5			Likely Pathogenic
D134H	2	LQTS	Makita et al 2014[69]	VCV000096723.1			Likely Pathogenic
D134N	2	Mixed LQTS + CPVT	Crotti et al 2019[73]	VCV000448971.4			Likely Pathogenic
Q136P	2	Mixed LQTS + CPVT	Makita et al 2014[69]	VCV000096724.1			Likely Pathogenic
Q136P	2	LQTS	Crotti et al 2019[73]	VCV000096724.1			Likely Pathogenic
N138K	2	LQTS	Crotti et al 2019[73]	VCV000488476.2			Likely Pathogenic
N138K	3	LQTS	Kato et al 2022[91]				
N138S	3	Uncertain	Gnomad[75]	19–47112230-A-G	Benign	tolerated	
E140V	1	Nervous system abnormality	Retterer et al 2016[105]	VCV000418107.2			
E141G	1	LQTS	Boczek et al 2016[72]	VCV000974612.1			Pathogenic
E141G	2	LQTS	Crotti et al 2019[73]				Pathogenic
E141K	3	LQTS	Wren et al 2019[71]				Likely Pathogenic
E141V	1	LQTS	Wren et al 2019[71]	VCV000974613.1			Pathogenic
F142L	1	LQTS	Crotti et al 2013[11]	VCV000183231.4			Pathogenic
F142L	3	LQTS	Chaix et al 2016[83]				Pathogenic
V143L	1	Uncertain	Gnomad[75]	14–90870762-G-A	Benign	deleterious	
Q144E	1	Uncertain	Gnomad[75]	14–90871041-C-G	Benign	deleterious	
M146V	3	Uncertain	Gnomad[75]	19–47112396-A-G	Benign	deleterious	

have been described as having a higher likelihood of identification among patients younger than 10 years old [72]. This finding aligns well with a later age of symptom onset reported for patients with mutations reported in *CALM2* [69] as compared to *CALM1* [11]. Thus, it may be possible that relative expression levels of *CALM1* and *CALM2* at different timepoints during development, or distinct tissue distribution of the *CALM* genes, may impact the severity and onset of symptoms. Moreover, while the majority of reported missense mutations within CaM are located within *CALM1* and *CALM2* genes, LQTS-associated mutations have also been observed in *CALM3*. The first of these mutations described was a D130G mutation, which produced severe LQTS with 2:1 AV block and fetal bradycardia in an infant [82]. In addition, the patient presented somewhat younger than most calmodulinopathy patients, with symptoms identified at birth. Additional *CALM3* mutations associated with LQTS, F142L and D96H, were identified in patients presenting with fetal bradycardia [83]. The patients each survived for over a decade and a half at the time the article was published, with both responding suitably to traditional LQTS therapies including beta blockers and pacemakers. Thus, there may be some subtle differences in phenotype dependent on the specific *CALM* gene affected. However, the *de-novo* nature of most CaM mutations also brings along the possibility of mosaicism, where the mutation occurs at a point in development such that expression occurs only in a subset of cells or tissues. This has been identified in some parents of calmodulinopathy patients, where mosaicism in the parent prevented overt symptoms but enabled genetic transmission of the mutation [71].

CPVT-associated CaM mutations

The first set of CaM mutations associated with CPVT was discovered in 2012 by Nyegaard *et al.* [15]. The study reported a case of dominant inherited CPVT-like syndrome in a Swedish family with a history of ventricular arrhythmias, syncope, and sudden death, typically brought on by physical exertion and/or emotional stress during childhood. Despite these CPVT-like symptoms, genetic screening showed no mutations in *RYR2* or *CASQ2*, the typical genetic loci for CPVT-associated mutations. Linkage analysis identified the first human CaM mutation, N54I, in the *CALM1* gene dominantly inherited with 100% penetrance in this family. Having identified a mutation in *CALM1* as a likely cause of this CPVT phenotype in a single family, the researchers probed whether mutations in *CALM1* might underlie CPVT in a group of patients who had previously tested negative for all other known arrhythmia-associated genes. Sequencing of this

cohort revealed a *de novo* N98S mutation in *CALM1*. Both the N54I and N98S mutations produced a phenotype that was unresponsive to beta blockers, the conventional treatment of CPVT, indicating a potentially new mechanism of CPVT pathogenesis. In the following year, targeted sequencing in over 100 patients with genotype-negative LQTS, CPVT, IVF, and sudden death in the young (SUDY) identified an A103V mutation within *CALM3* which was associated with CPVT [84]. Thus, mutations in CaM became a newly recognized cause of CPVT.

Interestingly, while the N98S mutation in *CALM1* was associated with CPVT in one patient [15], the same point mutation in *CALM2* was associated with LQTS in another patient, as described earlier [69]. Moreover, the phenotypic divergence is complicated by a sometimes mixed phenotype of CPVT and LQTS, as observed for D132E and Q136P mutations in the *CALM2* gene. Although the mechanism behind this intriguing phenomenon is yet unknown, it has been hypothesized that the different clinical phenotypes could stem from cell type-specific expression levels of *CALM1* vs. *CALM2*, or different genetic backgrounds [69]. Another consideration may be the age of onset of the symptoms. While LQTS onset is earlier in life (i.e. during infancy) with more severe symptoms, the CPVT phenotype seems to surface during late childhood with slightly milder symptoms.

IVF/SCD-associated CaM mutations

A second heritable CaM mutation was discovered in 2014 in a Moroccan family with a history of idiopathic ventricular fibrillation (IVF) manifesting in childhood and adolescence [85]. Upon exome sequencing of members of this family, Marsman and colleagues discovered a heterozygous F90L mutation in *CALM1* in the affected individuals. However, there were also individuals in the family carrying the F90L mutation without presentation of symptoms. Since this first description, several other CaM mutations have been associated with IVF (Figure 1). Given that IVF is one of the most common causes of sudden cardiac death (SCD), it is not surprising that SCD has been associated with calmodulinopathies. Moreover, there is considerable overlap between these phenotypes. Both LQTS and CPVT are associated with distinct forms of ventricular fibrillation and can cause SCD, thus this category of mutations may not be entirely distinct from those described above.

Overall, CaM mutations span a broad genotypic and phenotypic spectrum. For example, mutations have been discovered in all three *CALM* genes, affecting both the N- and C-lobes of CaM, and can be either hereditary or *de novo* in nature. Moreover, in addition to various degrees of penetrance, calmodulinopathies also display different levels of expressivity among affected individuals. While some mutations lead to severe symptoms emerging during infancy, others lead to milder symptoms that do not manifest until childhood or adolescence. Furthermore, the same mutation can manifest as CPVT in one individual and LQTS in another. Thus, uncovering the mechanisms underlying the different phenotypes of this array of CaM mutations has begun to offer insights into both disease pathophysiology and the development of therapies.

Mechanistic studies of LQT-associated CaM mutations

As the number of known calmodulinopathic mutations has grown, a general pattern has emerged that has informed studies into the structure–function relationship of the protein. The majority of the documented LQTS-associated CaM mutations, including CaM D96V, D130G, and F142L, reside in or near the Ca^{2+}-binding EF hand motifs of the C-lobe (Figure 1). This observation points to a potential disruption of the Ca^{2+} binding affinity of the C-lobe of CaM. This prediction has been confirmed experimentally. While the Ca^{2+} binding affinity of the N-lobe remains intact [86], CaM D96V, D130G, and F142L cause a 13.6-, 53.6-, and 5.4-fold reduction in Ca^{2+} binding affinity of the C-lobe, respectively, [11]. The greater reduction in Ca^{2+}-binding displayed by CaM D96V and D130G as compared to CaM F142L likely stems from the fact that D96 and D130 act as Ca^{2+}-coordinating residues of the EF hands, while F142 resides one position adjacent to the Ca^{2+} binding loop. Moreover, the locus of the LQTS mutations on CaM (Figure 1) leads one to postulate that the LQTS phenotype is associated

with target proteins that are modulated by the C-lobe of CaM.

While CaM has numerous targets, the known importance of CaM regulation of the LTCC Ca_V1.2 in controlling the cardiac action potential (AP) positions these channels as likely targets for LQTS associated calmodulinopathy mutations [14,87]. The mechanism for this Ca_V1.2 mediated LQTS is illustrated in Figure 2. As a resident Ca^{2+} sensor, CaM is bound to the C-tail of the Ca_V1.2 channel (Figure 2a). A mutation in the C-lobe of CaM reduces Ca^{2+} binding (Figure 2a, **red**). Upon Ca^{2+} entry through the channel, Ca_V1.2 normally exhibits robust CDI (Figure 2b, **black**); however, CDI is significantly blunted in channels harboring a mutant CaM (Figure 2b, **red**). This loss of inactivation results in added Ca^{2+} entry during the plateau phase of the cardiac AP, significantly prolonging the duration of the AP (Figure 2c, **red**) as compared to cardiomyocytes harboring a WT CaM (Figure 2c, **black**). This AP prolongation at the level of the ventricular myocyte corresponds to a prolongation of the QT interval as seen on a patient ECG (Figure 2d). Moreover, the increased influx of Ca^{2+} through Ca_V1.2 under these conditions also leads to increased calcium-induced calcium release (CICR) from the SR, further enhancing the propensity for arrhythmia in response to these mutations.

This mechanism has been evaluated in a cardiomyocyte expression system. Overexpression of mutant CaM (CaM D96V, CaM D130G, and CaM F142L) in adult Guinea pig ventricular myocytes (aGPVMs) severely blunted CDI of the Ca^{2+} current from these cells (Figure 3a) [87]. Likewise, similar deficits in CDI were demonstrated in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) derived from a patient harboring the CaM F142L mutation [12] and the D130G mutation [88] (Figure 3b). Consistent with the proposed impact of reduced inactivation of Ca_V1.2 (Figure 2), overexpression of mutant CaM within aGPVMs produced severe prolongation of the AP and proarrhythmic events including electrical alternans and after depolarizations, as well as increased Ca^{2+} transient magnitude and duration, increased diastolic Ca^{2+}, and increased SR load [87]. In addition, aGPVMs expressing LQTS-associated CaM displayed increased beat-to-beat heterogeneity in both AP and Ca^{2+} transient morphology, a critical element in creating a proarrhythmic condition. In concordance with these findings, Yin *et al.* observed slower Ca^{2+} current decay and similar Ca^{2+} cycling

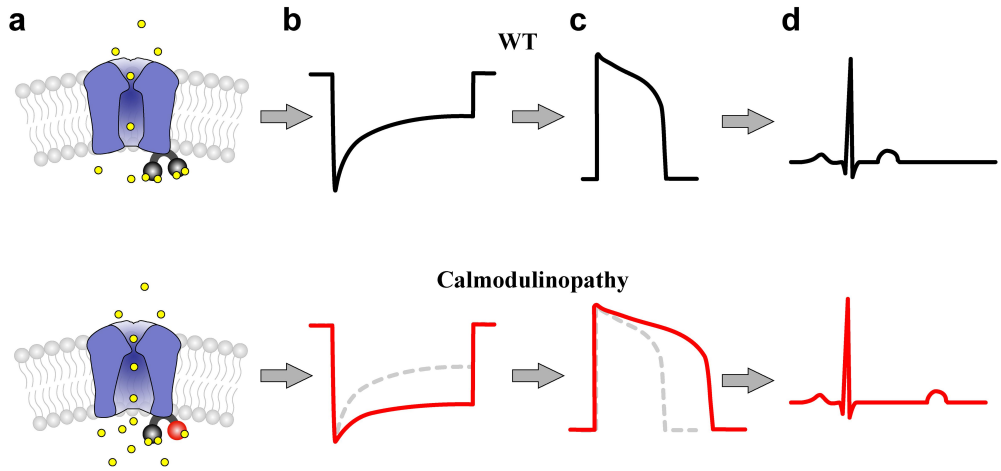

Figure 2. Proposed Impact of a Reduction in Ca_V1.2 CDI in the Heart. (*a*), Cartoon illustrating Ca_V1.2 channels pre-associated with WT (top) *vs*. mutant (bottom) calmodulin. A mutation in the EF-hand of the C-lobe of CaM (bottom, red) reduces the ability of CaM to bind Ca^{2+} (yellow) (*b*), Illustration of Ca_V1.2 Ca^{2+} current in response to a step depolarization from channels harboring a WT (top, black) *vs*. calmodulinopathic (bottom, red) CaM. The CaM mutation causes a reduction in CDI (bottom, red), as compared to WT CaM (top, black; bottom dashed gray). (*c*), The resulting cardiac AP for WT (top, black) *vs*. mutant (bottom, red) CaM. Excess calcium due to loss of channel inactivation leads to the characteristically prolonged action potential, as seen in the red trace. WT AP (top, black) is reproduced in the bottom panel as the dashed gray line for comparison. (d), ECG schematic illustrating the effect of CaM for WT (top, black) *vs*. calmodulinopathic (bottom, red) conditions. The prolonged action potential results in a pathologically extended QT interval (bottom).

disturbances upon overexpression of LQTS-associated mutant CaM in fetal mouse ventricular myocytes [89]. Thus, evidence suggests that disruption of CDI of $Ca_V1.2$ channels by mutant CaM may be sufficient to produce an LQTS phenotype.

However, Ca^{2+} channels are only one of many potential targets of CaM that may be disrupted in calmodulinopathies, and additional targets have been considered. Particular focus has been aimed at ion channels with known CaM interactions, and which have been identified as substrates of genetic forms of LQTS. As such, the cardiac sodium channel, $Na_V1.5$, has been considered as a potential pathogenic element in the LQTS phenotype of calmodulinopathies [89]. Modulation of apoCaM binding via mutations within the C-tail of the channel has been shown to be causative of LQTS [53], making $Na_V1.5$ a potential target in the pathogenesis of calmodulinopathies despite the lack of CDI identified within these channels [50]. However, heterologous expression of a human

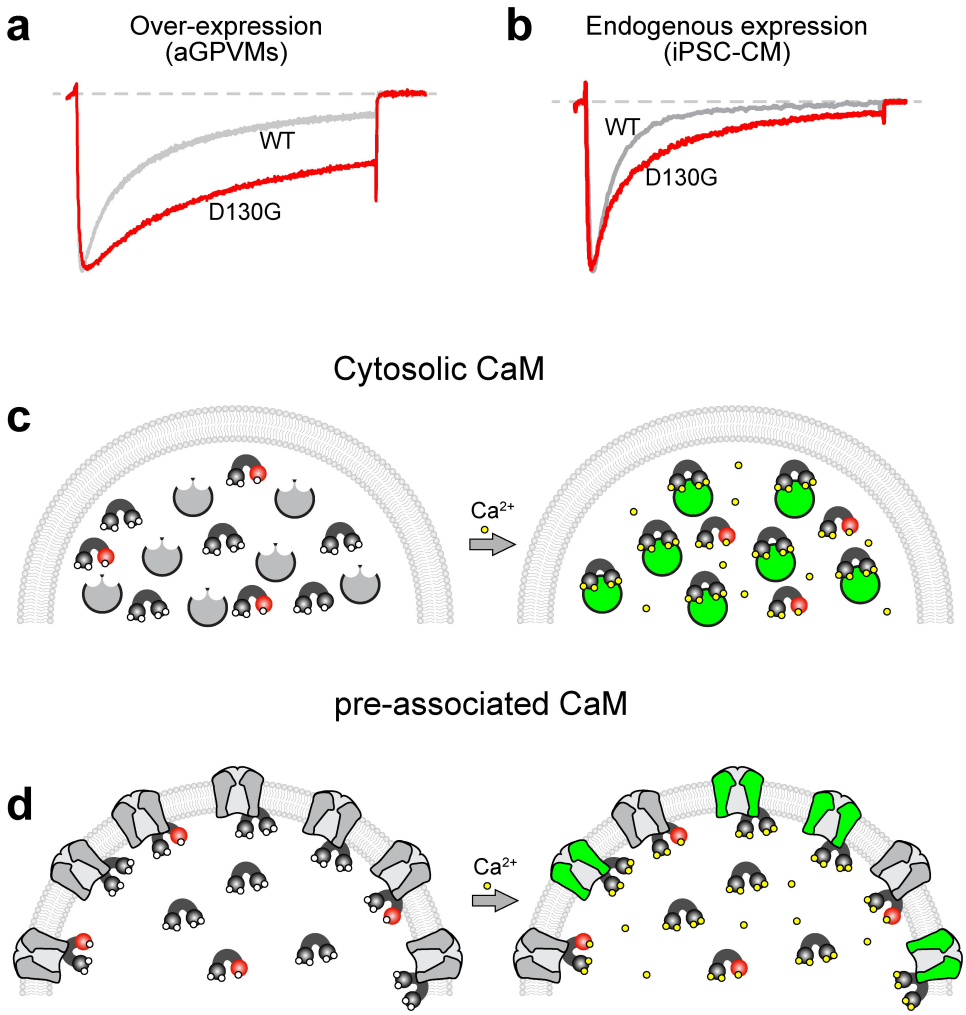

Figure 3. The Implications of Mixed Mutant and WT CaM Expression. (*a*), Exemplar Ca^{2+} current in response to a 30 mV depolarizing step recorded from aGPVMs overexpressing CaM WT (gray) *vs*. CaM D130G (red). The D130G mutations causes a loss of CDI, as seen by the lesser decay of the Ca^{2+} current (red) as compared to WT (gray). This figure is reproduced from Limpitikul *et. al*. (2014) [87] with permission. (*b*), Exemplar Ca^{2+} current recording in response to a 30 mV depolarizing step from iPSC-CMs derived from a patient harboring a D130G CaM mutation (red) as compared to iPSC-CMs from an unaffected individual (gray) demonstrating a reduction in CDI. Data is reproduced from Limpitikul *et. al*. (2017) [88] with permission. (*c*), Cartoon illustrating the impact of mutant CaM under conditions where CaM remains cytosolic until Ca^{2+} is bound, resulting in Ca^{2+}/CaM binding to the target. Green indicates a modulated target protein. Mutant CaM (red) inhibits Ca^{2+} (yellow) from binding, thus preventing mutant CaM from interacting with the target protein. (*d*), Cartoon illustrating the impact of mutant CaM under conditions where CaM is pre-associated to its target in the apo state. Mutant CaM (red) inhibits Ca^{2+} (yellow) from binding, thus preventing the associated target protein from undergoing Ca^{2+} dependent modulation (target remains gray).

adult splice variant of $Na_V1.5$ along with an LQTS-associated CaM demonstrated no significant difference in persistent sodium current under both low and high Ca^{2+} conditions [89]. A slight increase in persistent current was observed upon co-expression of CaM D130G with the fetal variant of $Na_V1.5$ under high Ca^{2+} conditions in tsA201 cells, but this finding was not confirmed in mouse ventricular myocytes. Overall, this study concluded that $Na_V1.5$ was not the primary target of LQTS-associated CaM mutations. In contrast, work in a heterologous expression system raised the possibility that mild enhancement of $Na_V1.5$ late current was possible alongside overexpression of the CaM mutant E141G [72]. Thus, mutant CaM acting on $Na_V1.5$ may represent a contributory pathway in the pathogenesis of LQTS in the context of calmodulinopathies, but may not be sufficient on its own to explain the full phenotype.

Finally, potassium channels may participate in the mechanisms underlying LQTS in calmodulinopathies. The small-conductance Ca^{2+}-activated K^+ (SK) channel has been identified as a possible element of pathogenesis. Co-expression of LQTS-associated mutant CaM in HEK 293 cells demonstrated a reduction in activation of SK2 channels [90], pointing to a possible role of these channels in arrhythmogenesis. Furthermore, I_{KS} may play a role in modulating the pathogenesis of select mutations. Recent work has shown that a somewhat milder LQTS phenotype results from an N138K CaM mutation, and may be caused by a moderate loss of $Ca_V1.2$ CDI, combined with an enhancement of current through the KCNQ1 channel [91]. Thus, combinatorial effects of mutations on multiple CaM targets may further impact the severity or presentation of calmodulinopathy phenotypes.

Mechanistic studies of CPVT-associated CaM mutations

As with LQTS-associated CaM mutations, numerous studies have aimed to elucidate the mechanism underlying the CPVT phenotype of calmodulinopathy patients. In the case of CaM N98S, the mutated residue itself participates in Ca^{2+} binding of the third EF hand motif, and the mutation causes a 1.6–3.3-fold reduction in Ca^{2+} binding affinity of C-lobe CaM, with no effect on the N-lobe [15,86]. On the other hand, the mutation N54I lies outside the Ca^{2+}-binding portion of the CaM EF hands and does not alter Ca^{2+} binding affinity of either the N- or C-lobe [15,86]. Nonetheless, both mutations caused an increase in Ca^{2+} wave frequency with a slightly smaller amplitude in adult mouse ventricular myocytes incubated with either mutant or WT CaM [86]. These results are similar to those obtained by an established CPVT mouse model (Casq2 knockout) [92], demonstrating a mechanistic overlap between previously studied forms of CPVT and calmodulinopathies.

As CPVT is known to involve RyR2 dysregulation [93], it seems reasonable that disruption of CaM binding to RyR2 might represent a potential pathological mechanism. Indeed, it has been shown that the N98S mutation produced a significant reduction in binding affinity of apoCaM to one of the CaM binding domains (CaM binding domain 2: CaMBD2) of RyR2; however, binding was restored following calcification of CaM [15]. At the same time, there was no disruption in the binding between CaM N54I and CaMBD2 either at low or high Ca^{2+} concentrations. Since both apoCaM and Ca^{2+}/CaM decrease the open probability of RyR2[63], Nyegaard *et al.* hypothesized that CaM N98S may contribute to CPVT via a diminished ability to inhibit RyR2 due to decreased apoCaM binding. However, since there was no change in the binding of CaM N54I to RyR2, they proposed that this mutant CaM might act through another mechanism to cause CPVT. Similarly, the binding affinity of mutant CaM to full-length RyR2 was examined in SR vesicles extracted from porcine cardiomyocytes [86]. Interestingly, both CaM N54I and CaM N98S were shown to exhibit enhanced binding to RyR2 as compared to WT CaM, when evaluated in the apo state (30 nM Ca^{2+}). However, binding was equivalent between mutant and WT CaM in the calcified state (30 μM Ca^{2+}). The apparent discrepancy with previous study may stem from the use of a shortened RyR2 peptide containing only the CaMBD2 binding domain [15] as compared to full-length RyR2 [86]. However, these are not the only studies to identify variable effects of mutant

CaM on RyR2 binding. The CPVT-associated mutation A103V did not appear to increase affinity for RyR2 [84]. However, a later study observed a significant decrease in binding to RyR2 for both A103V and CaM N54I [94]. Thus, measured changes in binding affinity between CaM and RyR2 have not indicated a consistent mechanism leading to CPVT.

Despite the lack of consensus in terms of binding affinity changes, the impact of mutant CaM on RyR2 function appears to be more consistent. Generally, CaM mutants associated with CPVT have increased Ca^{2+} release from the SR. Both N54I and N98S CaM mutations induced higher spark frequency and longer spark duration in permeabilized ventricular myocytes preincubated with CaM at low (50 nM) Ca^{2+} concentrations. This effect was amplified upon addition of cAMP to mimic adrenergic stress. Corresponding to the high Ca^{2+} efflux from RyR2, the SR content was also lower in cells with mutant compared to WT CaM [86]. Furthermore, CaM N54I enhanced the open probability of RyR2 in both low and high Ca^{2+} conditions, and CaM N98S increased the open probability of RyR2 in the presence of Ca^{2+}. Moreover, CPVT-associated mutation A103V promoted spontaneous Ca^{2+} wave and spark activity by enhancing SR Ca^{2+} release, even when expressed at a ratio of 1:3 mutant to WT CaM, demonstrating an ability to disrupt Ca^{2+} dynamics even in the presence of WT CaM [84], as would occur in a calmodulinopathy patient. Likewise, Hwang *et al.* demonstrated an increase in Ca^{2+} wave frequency in cardiac cells even when mutant CaM was expressed with WT CaM at a ratio of 1:8. Overall, increased spontaneous Ca^{2+} release from the SR appears to be a common mechanism underlying the pathogenesis of CPVT in calmodulinopathies, consistent with other forms of CPVT that are associated with increased Ca^{2+} leak through RyR2.

The impact of CaM pre-association

In all reported cases, calmodulinopathy patients harbor a heterozygous mutation in CaM, sometimes with somatic mosaicism. Given that there are three different *CALM* genes (*CALM1, 2*, and *3*) encoding the identical protein, how can a relatively small amount of mutant CaM produce severe disease phenotypes while in the presence of relatively abundant WT CaM? To resolve this question, one can consider the binding state of apoCaM, which can either exist in a free form floating in the cytoplasm, or can pre-associate with a target protein. The latter option provides an avenue by which a relatively small amount of mutant CaM can lead to the large pathological effects observed on cellular function (Figure 3c,d)

First, consider the case where apoCaM is distributed throughout the cell (Figure 3c**, left**), with a fraction of the population harboring a mutation that reduces the Ca^{2+}-binding affinity (Figure 3c**, red**). Upon the rise of intracellular Ca^{2+}, only WT CaM fully binds the ion and is thus able to bind to the Ca^{2+}/CaM effector site on the target (Figure 3c**, right**). Because there is an abundance of WT CaM, all target proteins are effectively modulated (Figure 3c**, green**), with mutant CaM unable to compete for the target protein. Thus, when the primary effect of the mutation is a loss of Ca^{2+} binding to CaM, targets without pre-association are unlikely to represent pathogenic elements. Examples of CaM targets that follow this model include CaMKII, MLCK, and calcineurin.

On the other hand, consider the case where apoCaM is pre-associated with its target (Figure 3d). If apoCaM can bind to its target at least as well as WT CaM, i.e. the mutations do not diminish the binding affinity of apoCaM to its target, then a fraction of targets will be occupied by mutant CaM even at basal Ca^{2+} levels (Figure 3d**, left**). Upon the rise of intracellular Ca^{2+}, mutant CaM is unable to fully bind Ca^{2+}, leaving the fraction of target proteins associated with these mutant proteins unmodulated (Figure 3d**, right, gray**). This fraction of unmodulated targets results in an overall decrease in the magnitude of Ca^{2+}/CaM regulation. Thus, pre-association enables a smaller amount of mutant CaM to have a larger effect on cellular function. Indeed, multiple ion channels exhibit such apoCaM pre-association, including the $Ca_V1.2$ channel that has been strongly implicated in the pathogenesis of calmodulinopathic LQTS [87,95]. Moreover, it has been demonstrated that for most calmodulinopathic mutations, apoCaM binding to

the channel is preserved, or in some cases enhanced, validating the ability of the mutant CaM to pre-associate with the channel [87,96]. As a result of this pre-association, it has been shown that small amounts of mutant CaM, even in the presence of an overabundance of WT CaM, can reduce the CDI of $Ca_V1.2$ channels in heterologous systems [87]. This translates to a marked effect of endogenously expressed mutant CaM on CDI when measured in iPSC-derived cardiomyocytes derived from calmodulinopathy patients (Figure 3b) [12,88,89]. Interestingly, this pre-association also means that the effect of any CaM mutation can be modulated if the mutation also alters the binding affinity between apoCaM and its target. This appears particularly relevant for select mutations such as F142L, which have been shown to bind $Ca_V1.2$ with higher affinity as compared to WT CaM [87,96], thus increasing the number of channels harboring a mutant CaM and thereby enhancing the pathogenic impact of the mutation. Conversely, mutation Q136P appears to reduce apoCaM binding to $Ca_V1.2$, thus likely reducing the pathogenic burden of the mutation [97]. Thus, in addition to impacting the Ca^{2+} binding affinity of CaM, pathogenesis may be modulated by other changes to the protein structure resulting in altered interactions with the target of interest.

Potential effects outside the heart

CaM is ubiquitously expressed and modulates multiple targets across various organ systems. Alterations in the levels of CaM protein have been implicated in several disease states, including heart failure [98], schizophrenia [99], and Parkinson's disease [100–103]. Thus, pathogenic CaM mutations may be predicted to impair cellular function in tissues beyond the heart. In fact, multiple reports include extra-cardiac symptoms among calmodulinopathy patients. In the original description of LQTS-associated calmodulinopathy, multiple patients were reported to suffer from seizures and neuro-developmental delay [11]. Subsequent reports have identified similar effects. The E105A mutation in *CALM1* is associated with both LQTS and developmental delay [104], and neurological features were identified in 13 calmodulinopathy patients in an international calmodulinopathy registry [73]. Likewise, CaM variant E140V was detected in a patient with a described abnormality of the nervous system [105]. Although it remains possible that some of these neurological symptoms could be caused by hypoxic brain injuries secondary to cardiac arrest, in at least one case cardiac influence was ruled out [73]. Thus, mutant CaM may directly impact neuronal targets, leading to these neurological symptoms.

To date, few studies have considered the functional impact of mutant CaM on the brain; however, ion channels that pre-associate with apoCaM may be considered potential candidates for neurological pathogenicity in calmodulinopathy patients. In fact, both RyR2 and $Ca_V1.2$, which have been shown to be critical to the pathogenesis of calmodulinopathic CPVT and LTS respectively, are also highly expressed in the brain. Thus, similar pathogenic mechanisms involving these two proteins may well underly the neurological phenotypes of calmodulinopathies. Moreover, the complement of ion channels in the brain includes a variety of channels not found in the heart. In particular, multiple voltage-gated Ca^{2+} channels that are known to pre-associate with apoCaM are found in the brain. Given the greater abundance of C-lobe CaM mutations (Figure 1) channels with strong C-lobe CaM regulation may be of interest. In particular, $Ca_V1.3$ channels exhibit both N- and C-lobe CaM mediated CDI. On the other hand, $Ca_V2.1$ is known to display N-lobe mediated CDI, and C-lobe mediated CDF [32]. Thus, the potential impact of mutant CaM on these channels may actually be in the opposite direction, causing a disruption of facilitation rather than inactivation. Overall, the potential impact of these and other channels in the context of disease-associated CaM variants remains unknown and may describe an interesting future direction in calmodulinopathy research.

Therapeutic implications

Treatment options for calmodulinopathies have remained limited as most patients are refractory to conventional therapies. For those patients suffering from LQTS, beta-adrenergic blockers, Ca^{2+} channel blockers (CCBs), and Na^+ channel

blockers have often failed to alleviate symptoms [73]. The lack of efficacy by CCBs, is worth further consideration. Given that LQTS in calmodulinopathy patients appears to result largely from a loss of inactivation of the $Ca_V1.2$ LTCC, it might be reasonable to expect CCBs to provide some symptom relief due to their inhibition of LTCCs. However, the CCB verapamil failed to correct the LQTS phenotype of one patient, instead causing electrical instability, resulting in discontinuation of the drug [11]. Subsequently, a combination of a beta-blocker with verapamil reduced the occurrence of cardiac arrest, but still did not correct the QT prolongation [106]. This lack of efficacy may stem directly from the inactivation deficits produced by the mutant CaM. CCBs are known to be use-dependent blockers, where the drug binds preferentially to channels in the open and inactivated states [107,108]. Thus, as mutant CaM reduces channel inactivation, it may also reduce CCB block. Such a mechanism has been shown for mutations on the $Ca_V1.2$ channel itself, where loss of inactivation correlated with decreased efficacy of CCBs [109]. Thus, currently available CCBs may not be adequate for the treatment of calmodulinopathic LQTS.

Similar challenges have been found in the treatment of calmodulinopathic CPVT. Beta-adrenergic blockers, typically a first-line therapy for CPVT, have often been ineffective in the context of calmodulinopathies [15,69]. As a result, additional options have been sought. The sodium channel blocker flecainide was shown to suppress the Ca^{2+} wave frequency in permeabilized myocytes incubated with CaM N54I and N98S, providing hope for use of this drug in calmodulinopathy patients [86]. In fact, flecainide was shown to be efficacious in multiple CPVT disease models [110], providing evidence that its effect might be mutation-independent. Another potential candidate that has been suggested for the treatment of calmodulinopathic CPVT is the benzothiazepine derivative, K201, also called JTV519 [111,112]. K201 has been shown to stabilize the closed state of RyR2, thereby restoring channel function in a RyR-centric disease model [113]. Additionally, a related compound S107, also called rycal, has been shown to stabilize RyR2-calstabin2 interactions and thereby reduce diastolic SR Ca^{2+} leak, as well as reduce arrhythmias, infarct size, and ventricular remodeling in a rat ischemia–reperfusion model [114,115]. While it remains to be seen if these therapies can provide long-term benefit in patients, the research indicates that treatment options may exist for the CPVT phenotype of calmodulinopathies.

More broadly, targeted ablation of alleles containing disease-associated CaM mutations has been proposed as a potential avenue for therapy. Multiple frameshift mutations have been identified that can result in the near-complete loss of a CaM allele without apparent clinical deficits [116], leaving open the possibility that a single *CALM* gene could be targeted without detrimental side effects. CRISPR/Cas9 deletion of a *CALM* gene harboring a deleterious mutation has been evaluated in iPSC derived cardiomyocytes derived from calmodulinopathy patients [88,117]. In these studies, ablation of a single *CALM* gene harboring either a D130G [88] or an N98S [117] mutation restored normal CDI and AP morphology in iPSC derived cardiomyocytes. Thus, a genetic approach to the treatment of calmodulinopathies may provide a path forward for the treatment of LQTS in these patients.

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