Calmodulin Mutations and Life-Threatening Cardiac Arrhythmias: Insights from the International Calmodulinopathy Registry

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ABSTRACT

Aims: Calmodulinopathies are rare life-threatening arrhythmia syndromes, caused by mutations in any of the 3 genes (CALM 1-3) that encode identical calmodulin proteins, affecting mostly young individuals. We established the International Calmodulinopathy Registry (ICalmR) to understand the natural history, clinical features, and response to therapy of patients with a CALM-mediated arrhythmia syndrome.

Methods and Results: A dedicated Case Report File was created to collect demographic, clinical and genetic information. ICalmR has enrolled 74 subjects, with a variant in the CALM1 (n=36), CALM2 (n=23) or CALM3 (n=15) genes. Sixty-four (86.5%) were symptomatic and the 10-year cumulative mortality was 27%. The two prevalent phenotypes are long QT syndrome (LQTS; CALM-LQTS, n=36, 49%) and catecholaminergic polymorphic ventricular tachycardia (CPVT; CALM-CPVT, n=21, 28%). CALM-LQTS patients have extremely prolonged QTc intervals (594±73 ms), high prevalence (78%) of life-threatening arrhythmias with median age at onset of 1.5 years (IQR 0.1-5.5 years) and poor response to therapies. Most ECGs show late onset peaked T waves. All CALM-CPVT patients were symptomatic with median age of onset of 6.0 years (IQR 3.0-8.5 years). Basal ECG frequently shows prominent U waves. Other CALM-related phenotypes are idiopathic ventricular fibrillation (IVF, n=7), sudden unexplained death (SUD, n=4), overlapping features of CPVT/LQTS (n=3), and predominant neurological phenotype (n=1). Cardiac structural abnormalities and neurological features were present in 18 and 13 patients, respectively.

Conclusion: Calmodulinopathies are largely characterized by adrenergically-induced life-threatening arrhythmias. Available therapies are disquietingly insufficient, especially in CALM-LQTS. Combination therapy with drugs, sympathectomy, and devices should be considered.

Keywords: Calmodulin; Cathecolaminergic polymorphic ventricular tachycardia; Idiopathic Ventricular Fibrillation; Long QT Syndrome; Sudden Death.
INTRODUCTION

A severe form of long QT syndrome (LQTS) with early occurrence of life-threatening arrhythmias caused by mutations in one of the 3 genes (CALM1-2-3) encoding calmodulin (CaM) was first reported in 2013. CaM is a ubiquitous, multifunctional Ca$^{2+}$ binding protein. The clinical presentation was similar among the affected individuals, including a markedly prolonged QTc, episodes of T wave alternans, 2:1 functional atrio-ventricular block, cardiac arrest in the first year(s) of life, and need for an implantable cardioverter defibrillator (ICD) despite optimal medical therapy. Since then, scattered reports on few patients and isolated families emerged. The phenotype most frequently shown by patients with CALM mutations was LQTS, but some had catecholaminergic polymorphic ventricular tachycardia (CPVT) or idiopathic ventricular fibrillation (IVF). CALM mutations were also identified in autopsy-negative sudden unexplained deaths (SUD) in young individuals. Given the wide spectrum of the clinical manifestations associated to CALM mutations, the term “Calmodulinopathy” was coined.

Given the apparent rarity and lethality of the CALM-mediated arrhythmogenic diseases, with a heterogeneous genetic background and variable clinical phenotype, we thought that an international cooperative effort was the only feasible way to begin to understand the natural history, clinical features, and response to therapy of these patients. As done 40 years ago for LQTS, we (LC, PJS and MJA) established the International Calmodulinopathy Registry (ICalmR) and here we report the initial findings.
METHODS

Subjects and ascertainment

The study population (n=74) comes from two sources, the International Calmodulinopathy Registry (ICalmR) and the published literature. The ICalmR is a worldwide multicenter clinical observational Registry established in 2015 with the goal of recruiting patients with a pathogenic variant in the CALM1, CALM2 or CALM3 genes regardless of the phenotype (LQTS, CPVT, IVF, other). To enrol patients we have involved most of the centers and key investigators expert in arrhythmic diseases of genetic origin and having published the first description of CALM-LQTS patients, we have also been contacted by investigators who had encountered even a single case. Including the two Coordinating Centers (Istituto Auxologico Italiano IRCCS, Milan, Italy, and Mayo Clinic, Rochester, USA), 22 centers from Europe, North America, and Asia have joined the Registry by contributing 58 (78%) genotyped patients with CALM-mediated arrhythmia syndromes. A dedicated Case Report File was created to collect demographic, clinical and genetic information. The Ethics Committees of the coordinating and enrolling centers approved the study. The patient data were released in an anonymous form.

We used published literature as an additional source of 16 cases (22%), for whom high quality clinical and genetic details were available. In total, here we report data on 74 patients with a calmodulinopathy.

Genetic characterization

The pathogenic/likely pathogenic CALM variants included in the Registry were identified through whole exome sequencing (WES), targeted next-generation sequencing (NGS), or Sanger sequencing, in 51%, 29% and 20% of the index patients, respectively. None of the patients enrolled had additional clinically relevant pathogenic variants in other arrhythmia-susceptibility genes.

The nomenclature of the reported genetic variants conforms to the latest Human Genome Variation Society guidelines. We specifically numbered amino acid positions based on assignment of the initiating methionine start codon as position 1. The CALM acronym refers to any of the three
calmodulin genes, while the CaM acronym refers to the calmodulin protein. Variant classifications were made according to the American College of Medical Genetics Guidelines (ACMG)\textsuperscript{19}. Genetic variants were specifically analyzed focusing on: presence or absence in publicly available exome/genome databases (1,000 Genomes, Exome Variant Server, ExAC, gnomAD)\textsuperscript{20–22}, amino acid conservation across species (in 100 vertebrates), protein domain affected by the amino acid change, previous literature description and functional characterization.

**Phenotype characterization**

The data of each enrolled patient included demographics, personal and family histories, fetal-perinatal period information, comorbidities, ECG parameters, individual phenotypic features, cardiac events information, therapies. Based on phenotype and clinical judgment, the patients were classified with one of the following major conditions: LQTS\textsuperscript{23}, CPVT\textsuperscript{24}, IVF\textsuperscript{24}, overlap between LQTS/CPVT, and SUD in young individuals. LQTS and CPVT were the two most prevalent subgroups, henceforth referred to as *CALM*-LQTS and *CALM*-CPVT. IVF is defined as a resuscitated cardiac arrest victim, preferably with documentation of VF, in whom known cardiac, respiratory, metabolic and toxicological etiologies have been excluded through clinical evaluation\textsuperscript{23}. SUD is defined as a natural, unexpected fatal event occurring within one hour from the onset of symptoms in an apparently healthy subject, with no known familial disease. In all SUD cases, an autopsy was performed and obvious causes were excluded; no ECG was available in these cases, and only molecular autopsy allowed the diagnosis of calmodulinopathy.

Subjects were considered symptomatic if they suffered at least one cardiac event, such as syncope (fainting spell with transient, but complete, loss of consciousness), aborted cardiac arrest (ACA) requiring resuscitation, or sudden cardiac death (SCD) occurring before age 20. Seven first-degree relatives of three probands who died before diagnosis, in the absence of genetic testing, were assumed to be positive for the same *CALM* variant identified in their family and were consequently included. One patient, with marked neonatal sinus bradycardia and a cardiogenic shock soon after birth, was considered as symptomatic. Appropriate ICD discharges for VT/VF were counted and
considered together with ACA and SCD as major arrhythmic events (MAEs). A perinatal presentation was defined as the occurrence of symptoms in the period ranging from approximately the 28th week of gestation to the 28th day after birth.

**Statistical analysis**

Continuous data are presented as mean and standard deviation (SD) or as median and interquartile range (IQR, 25th-75th percentile), and analyzed with the Student t test or with the Mann-Whitney test. Categorical variables were presented as absolute (n) and relative frequencies (%), and compared among phenotype groups with the Fisher exact test or χ² test as appropriate. Event-free survival is described by Kaplan-Meier cumulative estimates, with the comparison between subgroups performed by the log-rank test. Two-sided p-values <0.05 were considered statistically significant. SPSS Statistics version 23 (IBM Co, Armonk, NY) was used for computation.
RESULTS

Registry population

The ICalmR has enrolled 74 subjects with a pathogenic or likely pathogenic variant in either CALM1, CALM2, or CALM3 from 51 different families.

Genetic features

Thirty-five single nucleotide substitutions leading to 28 distinct amino acid changes were identified in the 74 CALM-positive patients (36 CALM1, 23 CALM2 and 15 CALM3 patients) included in the Registry. Among these 35 variants, 11 (31%) were in CALM1, 16 (46%) in CALM2 and 8 (23%) in CALM3. These genetic variants are schematically depicted in Figure 1 and summarized in Supplemental Table 1. They constitute a homogeneous group of missense variants with recurrent and similar features. Specifically, amino acid changes always involved highly conserved residues (degree of conservation among 100 vertebrates ≥99%), all were classified as pathogenic/likely pathogenic\(^{19}\) and none were present in publicly available exome/genome databases including more than 140,000 individuals (accessed September 2018)\(^{20-22}\).

The majority of variants affected amino acid residues in the EF-hand Ca\(^{2+}\) binding loop III and IV [28/35 nucleotide substitutions (80%)], and most of them affected one of the four amino acid residues principally involved in Ca\(^{2+}\) binding (Asp, Asp, Asp/Asn and Glu, at positions 1, 3, 5 and 12, respectively, from the beginning of each 12-residue loop) [25/28 nucleotide substitutions (89%)]. Interestingly, while most of the variants were unique by type and gene location, 9 were present in more than one index case and among these, 3 (p.Asn98Ser, p.Asp130Gly and p.Phe142Leu) appeared to be relative hot-spots, identified in 10, 5, and 4 families, respectively. Of note, while p.Asp130Gly and p.Phe142Leu were always associated with the LQTS phenotype, the p.Asn98Ser had phenotypic variability, including LQTS, CPVT, IVF and SUD. Despite the relatively small numbers of cases, a significant association (p=0.001) was observed between location of mutation and phenotype (Supplemental Figure 1). Indeed, a pathogenic variant in EF-hand IV was found in the majority (17/32, 53%) of CALM-LQTS index cases but in only 1 of the 9
CALM-CPVTs (11%). Conversely, variants identified in CALM-CPVT index cases were mostly located either in EF-hand III (n=5, 56%), or in the inter-EF hand I-II linker (n=3, 33%).

In 27/29 (93%) CALM-LQTS and in 7/9 CALM-CPVT (78%) patients whose family members were genetically screened, the culprit variant was de novo. In the remaining two CALM-LQTS cases and in one IVF case, germline mosaicism was present in one of the parents.

Interrogation of the gnomAD database (September 2018) for variants in the three main transcripts of the calmodulin genes revealed a much lower than expected number of missense and loss-of-function variants, indicating that the CALM genes are intolerant to such variations, while they result more tolerant to synonymous variation, as indicated by the relative constraint metrics (i.e. statistics based on observed/expected variant comparisons). Among the exonic nonsynonymous variants reported in the gnomAD datasets (n=29), 21 were located outside the EF-hand domains, and none of the remaining 8 involved the principal Ca\(^{2+}\) binding residues. Therefore, the presence of genetic variants within the EF-hands differs significantly between index cases of the Registry and the general population (80% vs 28%, p<0.0001). The different location of variants in our cases and in the general population is shown in Figure 1.

**General Clinical features**

The clinical characteristics of the study population [51% males, median age 9.0 years (IQR 4.5-15.0)] are summarized in Table 1. The ethnic-geographic background was heterogeneous with 55% of subjects having European ancestry. Most patients (n=64, 86.5%) were symptomatic and, by age 5, 50% had already experienced an arrhythmic event (Figure 2A). Males had a significantly higher probability of becoming symptomatic than females (p=0.02; Figure 2B). Triggers of cardiac events were adrenergic stimuli in 81% of patients, irrespective of the phenotype and mostly (62%) associated with exertion. MAEs (ACA, SCD/SUD, ICD appropriate discharges) occurred in 50 (68%) subjects at a median age at first event of 5.0 years (IQR 2.0-10.0). Twenty (27%) had SCD at a mean age of 5.7±4.6 years [median 4.8, IQR (1.6-9.8)]. As shown in Figure 2C, the 2-, 5-, and 10-year cumulative survival was 88%, 84% and 73%, respectively. Three children with CALM-
LQTS diagnosed in the neonatal period died a non-arrhythmic death, at 8 days, 6 months, and 5 years, due to ICD implant complications, heart failure and infection post-cardiac surgery, and hypoglycemia, respectively.

The two most prevalent phenotypes were LQTS (n=36) and CPVT (n=21), observed in 77% of the entire study population (Table 2).

**CALM-LQTS**

The thirty-six CALM-LQTS patients (49% of total cohort) exhibited an extremely prolonged mean QTc (594±73 ms) and a high prevalence (78%) of life-threatening arrhythmias occurring very early in life. There were 10 SCD, mostly before age 3. In addition to the marked QT interval prolongation, a typical repolarization morphology, characterized by a late onset peaked T wave, was observed in 19/23 (83%) CALM-LQTS patients. This ECG pattern resembles that of LQT3 and Timothy Syndrome (Supplemental Figure 2). However, at variance with LQT3, only in 1 of the 6 CALM-LQTS cases acute oral mexiletine significantly shortened QTc. The onset of spontaneous major arrhythmias was recorded only in 7 cases: it was always an abrupt onset of VF while a pause-dependent TdP was never observed (Figure 3C). In 58% of CALM-LQTS patients there was a perinatal presentation, consisting in a variable combination of striking QT prolongation (QTc 628±62 ms), sinus bradycardia, 2:1 atrioventricular block (AVB) (Figure 3A), T wave alternans (Figure 3B), and/or MAEs (Figure 3C). This early onset was almost exclusive of the LQTS phenotype (91%) (Supplemental Table 2). Among CALM-LQTS patients, 13 (41%) also had cardiac structural abnormalities (Table 3, Supplemental Table 3).

**CALM-CPVT**

All 21 CALM-CPVT patients (13 from a single large family) were symptomatic for adrenergically-induced cardiac events (10 with MAEs) starting in childhood, with CPVT-like features of stress test-induced ventricular ectopies, ranging from isolated PVCs to NSVT and VT/VF (Supplemental Figure 3). Ectopic beats were most often reported as polymorphic and rarely and inconsistently as truly bidirectional. Only 1 patient showed perinatal onset with severe
fetal bradycardia ≤90 bpm at 28 weeks gestational age (Supplemental Table 2). In comparison with CALM-LQTS, CALM-CPVT patients were older at onset (median age 6 vs 1.5 years, p=0.005) and had fewer MAEs (48% vs 78%, p=0.04), with sudden death occurring in 14% of them (Table 2). Two unrelated subjects, with the same CALM mutation, had a patent ductus arteriosus (PDA) as associated cardiac abnormality (Supplemental Table 3). In 6 of the 8 available baseline ECGs, a prominent U wave was observed in few precordial leads, with a repolarization pattern similar to what observed in Andersen-Tawil Syndrome26 (Supplemental Figure 4).

Other phenotypes

The other phenotypes observed in association to CALM mutations were IVF (n=7), SUD (n=4), overlap CPVT/LQTS (n=3) and an atypical cardio-neurological phenotype (n=1). Only 2 subjects, part of an IVF family, remain completely asymptomatic at age 14 and 60, and with a normal ECG. SUD, IVF (Supplemental Figure 5), and overlapping phenotypes frequently show adrenergically-induced ventricular arrhythmias and cardiac events. One patient, with a borderline QTc and peaked T waves, had recurrent neurologically-mediated seizures since age 3; cardiac events concomitant with seizures were excluded by an implantable loop recorder.

Neurological features in CALM-positive subjects

For 13 subjects, most with an LQTS phenotype (n=10) and a CALM1 mutation (n=9), a mild-to-severe neurological impairment was reported, including seizures, development delay, motor and/or cognitive disability. In 7 young patients, the neurologic deficits were observed following ACA and therefore these deficits are likely post-anoxic sequelae; six of them improved gradually during follow-up. In the 6 remaining patients, neurological features were unrelated to cardiac arrests and ranged from mental retardation and developmental delay to recurrent seizures and autism.

Treatment and outcome

Data on therapy were known for 69 of the 74 patients (Table 1); for 5 patients that originated from published literature, precise information on therapy was unavailable and could not be retrieved. Thirteen were never treated, 54 (78%) were treated with β-blockers, 21 (30%) with a
sodium channel blocker (mexiletine, flecainide, or ranolazine), 3 patients received verapamil, 1 nicoandil, and 4 (6%) underwent left cardiac sympathetic denervation (LCSD). In addition to 2 implantable loop recorders (ILR), 32 patients (46%) received a therapeutic device [(pacemaker, PM, n=7), ICD (n=25)] with a median age at first implant of 2 year (IQR 0-9.5).

In the two larger phenotypic groups (CALM-LQTS and CALM-CPVT), therapeutic attempts largely met with failure, as suggested by a global 56% prevalence of patients with cardiac events recurring despite β-blockers, sodium channel blockers, other antiarrhythmics, and LCSD. All these patients (n=45) with available data on treatment received β-blockers, either alone (n=15) or in combination with other therapies (n=30), either as concomitant or subsequent therapeutic attempts (Table 2). Over a median 5-year follow-up on therapy, at least one breakthrough event occurred in 25 (20 CALM-LQTS, 5 CALM-CPVT) of these 45 patients (56%), including 8 SCD (7 in CALM-LQTS.) Four subjects underwent LCSD, followed once by right cardiac sympathetic denervation; arrhythmias recurred in all 3 CALM-LQTS patients undergoing denervation surgery and with an adequate follow-up; however, they are all still alive. Twelve of the 26 patients with a device (7 PM, 19 ICD) were implanted in the first year of life (Table 2). During a median post-ICD observation time of 5 (IQR 3-9) years, 10 CALM-LQTS and 3 CALM-CPVT patients received at least one appropriate ICD shock (range 1-14 shocks). The effect of mexiletine could be assessed in 11 CALM-LQTS patients, and among them arrhythmia suppression was reported in 4 (36%). The only CALM-CPVT patient who received mexiletine together with β-blockers became asymptomatic. Verapamil was used in 3 patients but was discontinued in 2 because of VF recurrences (n=1) or intolerance (n=1).

DISCUSSION

The present report provides the first comprehensive assessment of what is currently known regarding the clinical manifestations of pathogenic and likely pathogenic variants in the genes encoding calmodulin. Only very recently has it become evident that genetic perturbations in
CALM1, CALM2, and CALM3 are associated with the occurrence of life-threatening cardiac arrhythmias during infancy or early in childhood. The availability of a mere handful of anecdotal reports of what are now referred to as calmodulinopathies has limited the understanding of the natural history, clinical diagnostic features, and clues for effective management of this rare and quite severe syndrome.

As it was done 40 years ago for LQTS, the creation of an International Calmodulinopathy Registry including most cases of this rare but potentially lethal syndrome appeared the best way to address these pressing clinical questions and to provide the critical mass of data needed to draw meaningful conclusions. This was accomplished by establishing the International Calmodulinopathy Registry. Here, we report the first findings and discuss their implications.

Calmodulin is a ubiquitously expressed protein. In the heart, CaM is a critical modulator of several ion channels such as the L-type calcium channel, the sodium channel, different potassium channels, and the ryanodine receptor. Unique to biology, and underscoring its importance, the entire amino acid identity of the CaM protein is derived from the transcription/translation of three distinct CALM genes residing on three different chromosomes. In addition, the CaM protein sequence is extraordinarily conserved across vertebrates and highly across all eukaryotes. Only recently pathogenic variants involving these three calmodulin genes (CALM1, CALM2, CALM3), have been identified and associated to severe forms of LQTS, CPVT, IVF, and SUD.

Distinct clinical features of calmodulinopathy

LQTS is the most common and most malignant arrhythmogenic phenotype associated to disease-causative variants in the CALM genes. In 58% of the cases, a perinatal presentation with a mean QTc >600 ms, 2:1 functional AV block, T-wave alternans, and /or MAEs was observed. These were all heterozygous missense variants, mainly de novo, which is not surprising given their malignancy; much less frequently they were inherited from an unaffected parent with germline
mosaicism. LQTS-associated mutations were most often located in calcium binding sites at EF-hands III and IV, with a few recurrent variants (p. Asn98Ser, p.Asp130Gly and p. Phe142Leu).

The clinical manifestations were strikingly similar independently of the ethnicities represented in our cohort. The functional effect of these CaM perturbations is probably so strong to override any influence on the phenotype by the genetic background. In addition to the severe phenotype and the early occurrence of clinical manifestations, CALM-LQTS had some specific features. The trigger of cardiac events was mainly adrenergic stimulation, and when the onset of the arrhythmias was recorded, a rapid ventricular tachycardia, not preceded by a pause and quickly degenerating into ventricular fibrillation, was observed.

A distinctive feature of CALM-LQTS was an ECG pattern characterized by a late onset peaked T wave, resembling the ECG features observed in Timothy Syndrome and in LQT3. However, at variance with LQT324, mexiletine did not shorten the QTc in most calmodulin cases despite reducing arrhythmic events in almost 40% of the patients. There were some similarities between CALM-LQTS and Timothy Syndrome (TS), the other calcium-related variant of LQTS29, such as the presence of cardiac structural abnormalities and/or neurological features and hypoglycaemia as a possible cause of death30.

CPVT is the second most represented calmodulinopathy phenotype. Here too, the disease-causative variants were heterozygous missense variants, occurring in any of the three CALM genes. At variance with CALM-LQTS, there were true familial cases of CALM-CPVT, although the majority of the probands (7/9, 78%) had de novo mutations. Symptoms were present in all affected subjects; however, compared to the LQTS phenotype, the clinical manifestations were somewhat less severe, with rare perinatal presentation and with fewer structural cardiac and neurological abnormalities. The baseline ECG is either completely normal or shows prominent U waves. In most cases the clinical manifestations were not completely typical of CPVT. Indeed, exercise and adrenergic stimulation induced ventricular ectopic beats, also polymorphic, but sometimes only isolated; bidirectional VTs was seldom reported.
Eighteen patients (27%) showed co-existing cardiac structural abnormalities, mostly represented by atrial and ventricular septal defects. As these are the most common congenital cardiac malformations, it is likely that early detection occurred as a result of the extensive diagnostic evaluation performed in these very young patients with a severe clinical phenotype. However, the prevalence of these cardiac malformations in calmodulinopathy is apparently not trivial, compared with their general prevalence of approximately 1% of live births\(^3\). These observations, along with the reported neurological and neurodevelopmental deficits, either subsequent or independent of prior cardiac arrest, raise the possibility that calmodulinopathy could be syndromic in a number of cases.

**From genotype to phenotype**

Among the 25 distinct missense variants identified, 11 have been functionally characterized in different cellular settings (heterologous expression systems, mammalian ventricular myocytes, cardiomyocytes differentiated from human induced pluripotent stem cells, hiPSC-CMs) and are summarized in **Supplemental Table 4**. In our original description of CALM-LQTS\(^1\), we provided evidence that CaM mutations exhibited reduced Ca\(^{2+}\)-binding affinity and impaired Ca\(^{2+}\)-mediated signal transduction. Reduced Ca\(^{2+}\)-binding capacity was demonstrated for other LQTS-related CaM mutations\(^4,6\). The mechanism by which this leads to prolongation of action potential duration is an impairment of Ca\(^{2+}\)-dependent inactivation (CDI) of the L-type Ca\(^{2+}\) channel Cav1.2\(^6,32\), while the other ion channels regulated by CaM are less consistently affected.

Most functional studies thus far performed, attempted to assess CaM mutation effects in heterologous expression systems or mammalian cardiomyocytes. Although these experiments contributed to delineating the underlying calmodulinopathy mechanism, their results were significantly limited by not having employed a physiologically reliable experimental platform accounting for the native stoichiometric ratio of CaM. Since CaM is encoded by three different genes, i.e. six alleles, a single mutation of one allele implies that mutant CaM and wild-type CaMs co-exist in the cell in a 1:5 ratio.
To overcome this issue and to provide a deeper understanding of the effects conferred by CaM mutations in their native CM environment, we generated patient-specific induced pluripotent stem cells (iPSC) from skin fibroblasts of one of our patients with CALM-LQTS (p.Phe142Leu in CALM)\(^1\) and differentiated them into CMs\(^33\) (iPSC-CMs). By performing extracellular field potential, membrane action potential, and intracellular Ca\(^{2+}\) measurements, we demonstrated a strong dominant-negative reduction in the CDI of Cav1.2, resulting in increased inward I\(_{\text{Ca,L}}\) and repolarization delay as the variant’s predominant effect\(^33\). Similar results were obtained by two other independent studies using an iPSC-CM platform for calmodulinopathy modeling\(^34,35,35\).

CPVT-associated disease-causative \textit{CALM} variants have been studied less extensively and never in the context of patient-derived iPSC-CMs; however, the major \textit{in vitro} effect of these variants is a higher binding affinity for RyR2 producing a greater RyR2 channel open probability with spontaneous formation of Ca\(^{2+}\) waves\(^10,32,36,37\).

Overall, these studies have provided insights into the pathophysiological mechanisms underlying life-threatening arrhythmias in the context of calmodulinopathy.

At this time it is evident that no gene-specific phenotypic correlations can be made since mutations in all 3 \textit{CALM} genes may give rise to different phenotypes. On the other hand, a few mutation-specific phenotypic correlations seem to emerge, such as the p.Asp130Gly and p.Phe142Leu mutations always associated with an LQTS phenotype. Another emerging correlation is that of mutation topology and phenotype. In fact, CALM-LQTS mutations seem to mainly affect amino acids residing in the Ca\(^{2+}\) binding loops (EF-hands III and IV). Functional characterization of several LQTS-associated CaM mutations has indeed shown that they mostly affect CaM’s Ca\(^{2+}\) binding affinity, with a consequent impairment of the Ca\(^{2+}\)-dependent inactivation of the Ca\(^{2+}\) channel\(^32-35,37-39\). This is an established calmodulinopathy mechanism and is in accordance with the fact that the most frequently encountered phenotype across all mutations in all 3 genes is LQTS.

Conversely, CPVT-associated CaM mutations only occasionally involve amino acids directly responsible of Ca\(^{2+}\) binding. Their few functional studies suggest that they strengthen
CaM’s affinity for the RyR2 channel, promoting its open conformation and increasing the frequency of Ca\(^{2+}\) waves.

The p.Asn98Ser variant, associated with both a CPVT and LQTS phenotype, has shown multiple effects \textit{in vitro} as it leads to greater RyR2 single-channel open probability\textsuperscript{36} as well as to impaired CDI\textsuperscript{34,37}, which fits with the two phenotypes observed \textit{in vivo}. As with other arrhythmogenic diseases of genetic origin, only detailed functional studies on a case-by-case basis may explain how each specific mutation may give rise to more than one phenotype.

**Management**

Despite the strength of the adrenergic triggers observed in both CALM-LQTS and CALM-CPVT, it is disappointing and rather surprising that anti-adrenergic strategies effectively used in conventional LQTS and CPVT (β-blocker therapy and LCSD) are inadequately protective for patients with calmodulinopathy\textsuperscript{23}. Indeed, β-blocker therapy, the mainstay treatment for LQTS\textsuperscript{40}, seems to offer modest benefit in controlling the life-threatening arrhythmias of calmodulinopathy. Mixed results were observed with the sodium channel blocker mexiletine, possibly because of a mutation-specific effect\textsuperscript{41}. Ca\(^{2+}\) channel blockade may seem a rational therapeutic strategy in CALM-LQTS given that impaired CDI of Cav1.2 is a prominent underlying mechanism\textsuperscript{33–35}. Unfortunately, at this time, the data available with verapamil are too limited to draw any conclusion\textsuperscript{1,42}. As part of this largely negative picture, also LCSD, the other major pillar in LQTS\textsuperscript{40,43,44} and CPVT\textsuperscript{45} treatment, failed to prevent life-threatening arrhythmias in few CALM-LQTS patients.

Whether or not to implant an ICD in these young patients is a difficult decision, as exemplified by the following two cases. The decision not to implant an ICD in an asymptomatic infant, with a \textit{CALM2} mutation and a QTc of 552 ms, resulted in his SCD due to documented VF at age of 20 months despite full dose β-blockade. By contrast, the decision to implant an ICD in a \textit{CALM3} neonate contributed to his death due to device-related complications. These tragic examples
highlight the urgent need to identify appropriate management strategies and therapies for life-threatening calmodulinopathies\(^\text{35}\).

Overall, it is difficult to provide more granular information on response to therapy because in most cases, given the lack of protection and the dramatic situations with these small children, one therapy was added or substituted to another in relatively rapid sequence which makes hazardous to provide an accurate and non-misleading picture. Not infrequently, therapies followed by early recurrences were associated subsequently with a stabilization of the condition. This is why we regard as more correct to provide our overall sense of the therapeutic outcome as we have gathered from the detailed series of events in the individual cases. Indeed, the Registry data do not as yet allow the identification of a promising approach to an effective clinical management. What appear as legitimate conclusions are that: 1) monotherapy with β-blockers is usually insufficient; 2) combination therapy with β–blockers, sodium channel blockers and LCSD may not be sufficient, particularly in CALM-LQTS with perinatal presentation; and 3) ICDs are probably necessary in most patients but, in the very young ones, they represent a double-edged sword and the appropriate time for implantation should be carefully balanced considering symptoms and age. Only for truly desperate cases, cardiac transplantation could be considered as a last resort treatment option\(^\text{46}\).

**LIMITATIONS**

All Registry data on rare and life-threatening conditions unavoidably suffer from the same limitation: namely, that despite careful and systematic collection of all possible information, the very nature of the individual management of these high risk children usually results in a series, even large, of anecdotal cases. This complicates the attempt to generalize the results, as it would be necessary and it is why we thought more correct to provide our gestalt view. More granular data, greater numbers, and longer follow-up will be necessary to define the efficacy of specific therapies or of their combination.
CONCLUSION

This first report from the International Calmodulinopathy Registry provides novel information on the natural history and clinical presentation of these life threatening disorders. The presence of distinct genotype-phenotype correlations is beginning to emerge. As to the response to the conventional therapies used for LQTS and CPVT, the current data are not encouraging but a longer follow-up is necessary. The future inclusion in the Registry of many more cases will provide more insights and better guidance for the best management of these patients.
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CONFLICT OF INTERESTS

M.J.A. is a consultant for Audentes Therapeutics, Boston Scientific, Gilead Sciences, Invitae, Medtronic, MyoKardia, and St. Jude Medical. M.J.A. and Mayo Clinic have an equity/royalty relationship (without remuneration so far) with AliveCor, Blue Ox Health Corporation, and StemoniX. M.V. receives salary/stock from Invitae Corporation. H.H.O. have received salary from Abbott and have research partnership with Medtronic. However, none of the disclosures pertain directly to this paper and none of the companies provided financial support for this study. The other authors report no conflicts.
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FIGURE LEGENDS

**Figure 1.** Schematic model of calmodulin with the 4 Ca$^{2+}$ binding loops (EF-hands I-IV) and amino acids principally involved in the binding of Ca$^{2+}$ ions denoted with light grey lines. Coloured amino acid residues (circles in EF-hands and squares in linkers and N-/C- terminal regions) represent positions affected by genetic variants, identified either in patients of the Registry or in subjects of the general population (gnomAD database$^{22}$). All amino acid changes so far identified are listed in circular boxes according to a colour code for the associated phenotype: red for LQTS, green for CPVT, yellow for IVF, SUD or atypical phenotype, grey for variants identified in the gnomAD database. The corresponding amino acid positions in the protein are highlighted with the same colour code. Shaded colours stand either for overlap phenotype or for an association with multiple distinct phenotypes.

**Figure 2.** Event-free survival (to any first event) in the entire study population (A) and according to gender (B); cumulative survival (to SCD) in the entire study population (C).

**Figure 3.** Representative ECGs of CALM-LQTS patients. A) CALM3-D130G, male, 4 days, 12-lead ECG showing markedly prolonged QTc and 2:1 atrioventricular block. B) CALM1-D130G, female, 2 years, Holter recording showing markedly prolonged QTc and phases of T wave alternans. C) Holter ECG showing the VF onset in a CALM-LQTS, *CALM1*-D130G, female, 9 months.