An optogenetic arrhythmia model to study catecholaminergic polymorphic ventricular tachycardia mutations

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a condition of abnormal heart rhythm (arrhythmia), induced by physical activity or stress. Mutations in ryanodine receptor 2 (RyR2), a Ca^{2+} release channel located in the sarcoplasmic reticulum (SR), or calsequestrin 2 (CASQ2), a SR Ca^{2+} binding protein, are linked to CPVT. For specific drug development and to study distinct arrhythmias, simple models are required to implement and analyze such mutations. Here, we introduced CPVT inducing mutations into the pharynx of *Caenorhabditis elegans*, which we previously established as an optogenetically paced heart model. By electrophysiology and video-microscopy, we characterized mutations in *csq-1* (CASQ2 homologue) and *unc-68* (RyR2 homologue). *csq-1* deletion impaired pharynx function and caused missed pumps during 3.7 Hz pacing. Deletion mutants of *unc-68*, and in particular the point mutant UNC-68(R4743C), analogous to the established human CPVT mutant RyR2(R4497C), were unable to follow 3.7 Hz pacing, with progressive defects during long stimulus trains. The pharynx either locked in pumping at half the pacing frequency or stopped pumping altogether, possibly due to UNC-68 leakiness and/or malfunctional SR Ca^{2+} homeostasis. Last, we could reverse this ‘worm arrhythmia’ by the benzothiazepine S107, establishing the nematode pharynx for studying specific CPVT mutations and for drug screening.

CPVT is an inherited disturbance of the heart rhythm (arrhythmia) induced by adrenergic stress. It derives from an alteration of intracellular Ca^{2+} handling, involving the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism of myocytes. If untreated, CPVT is highly lethal. The exact prevalence of CPVT is not known, but it has been estimated to be around 1:10,000. The mean age of symptom onset is between 7 and 8 years, but a wide range of ages has been observed. Most mutations that have been linked to CPVT are found in two genes, ryanodine receptor 2 (RyR2; 50–55%) and calsequestrin 2 (CASQ2; 3–5%), two proteins fundamentally involved in regulation of intracellular Ca^{2+} in cardiac myocytes. The cardiac RyR2 is a Ca^{2+} release channel located in the SR. During CICR, Ca^{2+} is provided by the activated L-type voltage-gated Ca^{2+} channel (VGCC; Cav1.2) and binds to RyR2 thus triggering the opening of the channel, allowing fast Ca^{2+} efflux from the SR and subsequent muscle contraction. After reuptake into the SR lumen by the SERCA complex (Sarcoplasmic/Endoplasmic Reticulum Calcium transporting ATPase), Ca^{2+} is stored there at high concentrations. Calsequestrin is the major Ca^{2+} sequestering protein in the SR. It forms higher-order dynamic structures of monomers, dimers or long chains, depending on the Ca^{2+} concentration. Polymerization of calsequestrin occurs by back-to-back (C-terminal) and front-to-front (N-terminal) interactions. In mammals calsequestrin is linked to the RyR through an interaction with triadin and junctin.

For calsequestrin there is only one isoform present in *C. elegans*, CSQ-1, which shows ~40% sequence identity and 50–60% similarity to the human CASQ2. Cho et al. reported that UNC-68 appeared to affect the correct localization of CSQ-1 in vivo. Based on the genome sequence, there are no obvious homologs of junctin and...
triadin in *C. elegans*, therefore a direct interaction of the positively charged CSQ-1 C-terminus with the negatively charged luminal loops of UNC-68 is postulated

In vertebrates three RyR isoforms are present, whereas *C. elegans* has a single RyR gene encoded by the unc-68 locus. The UNC-68 protein of *C. elegans* shares 45% sequence identity and 63% homology with the human cardiac RyR2. A low-affinity Ca\(^{2+}\) binding site specific for RyR1 (aa 1872-1923) is not present in UNC-68, arguing that it is more related to RyR2. UNC-68 is expressed in the terminal bulb and posterior isthmus muscle cells of the pharynx, but also in body wall muscles and neurons. *unc-68* mutants are viable, but were identified as being defective in locomotion and to exhibit defects in pharyngeal pumping. In addition to visible defects in muscle function, *unc-68* mutants grow more slowly and have fewer offspring than the wild type. In contrast, mutant mice lacking RyR2 die as embryos with morphological abnormalities in the heart tube.

Up to now, many different (>170) CPVT mutations and polymorphisms are known (http://triad.fsm.it/cardmoc/). These result in a Ca\(^{2+}\) leakage from the SR, which leads to cytosolic Ca\(^{2+}\) overload generating delayed afterdepolarizations (DADs) (by driving the electrogenic sodium–calcium exchanger, NCX), triggered activity, and ventricular arrhythmias, in particular under adrenergic conditions. Disease-causing mutations have also been identified in RyR1, and are linked to malignant hyperthermia or central core disease. Recently, the significance of RyR function for aging was shown by single amino acid modifications, which conferred a reduction in lifespan and an accelerated decline in muscle integrity with age in *C. elegans*.

In this study we aimed to model CPVT arrhythmias in the *C. elegans* pharynx, a rhythmically active muscular pump that we previously established as an optogenetically controlled arrhythmia test system. We thus examined two available mutations of *C. elegans* calsequestrin. First, we analyzed possible defects of a deletion mutant. In addition, we tested a mutation at position P319 (mutated to S), which is conserved in vertebrate RyR2, and mutation of which is an established cause of CPVT. This mutation (however, to L, not to S) caused decreased Ca\(^{2+}\)-selectivity during polymerization of purified CASQ2-P308L protein as a structural disruptor for \(\alpha\)-helices and as a turning point in \(\beta\)-sheets, thus it was suggested that a substitution of P308 causes a profoundly altered conformation of the CASQ2 protein, and that this could lead to reduced Ca\(^{2+}\) binding. Therefore, we expected that any alteration of this proline in *C. elegans* CSQ-1 would also cause problems in folding or conformation, and consequently in Ca\(^{2+}\) binding capacity. Even though the worm allele introduces a polar residue instead of an aliphatic one, the structural consequences could be equally strong.

In the case of RyR2, we inserted two mutations (R2474S and R4497C) in *C. elegans* unc-68, identified in human patients with CPVT, and demonstrated in mouse models to be causative to CPVT. They represent two of the most investigated CPVT mutations in animal models or cell systems. Nevertheless, the mechanisms by which they induce arrhythmia are still not completely resolved. R2474S resides in the helical domain 1 segment 6b. One study suggested that the CPVT-associated RyR2 mutation results in leaky RyR2 channels due to decreased binding of calstabin-2 (FKBP12.6), which stabilizes the closed state of the channel. However, more recently it was found that the RyR2-FKBP12.6 interaction was not disrupted in R2474S/+ knock-in mice. These authors suggested that the R2474S mutation in the central domain of RyR2 induced a defective interaction between the central and the N-terminal domains, thus causing channel dysfunction. Jiang et al. could also not observe effects of CPVT mutations on FKBP12.6 binding. The mutation R4497C is located in the S0 segment of the RyR channel domain affecting the channel gating and creating a SR Ca\(^{2+}\) leak. A decreased binding of FKBP12.6 was also detected for this mutation. In other studies a normal RyR2-FKBP12.6 interaction was shown in RyR2\(^{R4496C}\) knock-in mice or in coimmuno-precipitation studies from resting HL-1 cardiomyocytes (CM). The reasons for these discrepancies are unclear.

To enable drug development for treatment of specific arrhythmias, but also to study distinct arrhythmia types, simple model systems are required, that allow implementing patient-specific mutations. Up to now, only a handful of mouse models for CPVT exist. Few RyR2 mutations have already been characterized in CM derived from induced pluripotent stem cells (iPSCs) and reflect basic aspects of CPVT that can be assessed well on the single-cell level. Novak et al. found in iPSC-CMs with the D307H CPVT mutation of calsequestrin that the \(\beta\)-adrenergic agonist isoproterenol causes DADs, oscillatory arrhythmic prepotentials, after-contractions and a diastolic (Ca\(^{2+}\)) rise. However, iPSCs are an expensive and complicated experimental system, precluding large scale screening approaches, and, more importantly, they cannot reflect the complex disease as it is present in an intact organ. For this reason we assessed the pharynx, the feeding organ of *C. elegans*, which is a rhythmically active muscular pump, as a heart model. Beside the easily accessible genetics and its high throughput potential, there are observations suggesting that the pharynx is orthologous to the vertebrate heart. Both the pharynx and the heart are tubes transporting material along their lumina. The pharynx utilizes homologues of most of the ion channels, pumps and transporters defining human CM physiology. Similar to the mammalian heart, the pharynx muscle cells are connected by gap junctions. The *C. elegans* EGL-19 L-type voltage dependent Ca\(^{2+}\) channel \(\alpha\)-subunit maintains depolarization during the plateau phase and sustains muscle contraction similar to the cardiac action potential (AP). In fact, with a plateau phase of ~200 ms, the pharynx AP resembles the shape of the human cardiac AP better than that of the mouse. EGL-19 slowly inactivates, and membrane repolarization is executed by a voltage-gated \(K^+\) channel, encoded by exp-2, which, though homology is low, is functionally similar to the human ether-a-go-go-related gene (hERG) channel.

Spontaneous pharynx pumping is too irregular to allow arrhythmia detection. To yield millisecond-precise and stable rhythmicity, we optically paced the pharynx using channelrhodospin-2 (ChR2) and Channelrhodopsin-2 (ChR2) gain-of-function variant, was directed to the plasma membrane of the pharyngeal muscle cells by the specific promoter pmyo-2 and its expression allows stimulation of pumping. We assessed pharynx pumping by extracellular recordings (electropharyngeograms, EPGs), similar to electrocardiograms, of dissected (cut-head preparation) or intact worms, which allows accurate measurements of pump rate and duration, and distinguishing muscle activity of different parts of the pharynx, as well as activity of pharyngeal neurons. Corpus and terminal bulb contraction/excitation, as well as their relaxation/repolarization, can be automatically analyzed. In addition, we used a video-microscopy based method, which allows recording of multiple intact animals simultaneously. Importantly, ChR2-paced pumping of the pharynx is not influenced by neuronal input.
Here, we extended our pharynx arrhythmia model to CPVT like arrhythmias. We characterized the role of RyR and calsequestrin in deletion mutants. Further we introduced different CPVT-related mutations of csq-1 and unc-68 and examined their effect on optogenetically paced pumping, exhibiting clear arrhythmia phenotypes and progressive loss of the ability to lock into the pacing. Finally, we demonstrate ameliorating effects of the benzothiazepine S107, emphasizing that our model can be used to identify and/or test allele-specific drugs.

Results

Deletion of calsequestrin causes arrhythmia in the *C. elegans* pharynx. To demonstrate its function in the pharynx, we expressed CSQ-1, fused to CFP at its C-terminus, under its own promoter in *csq-1* deletion mutants (Fig. 1a,b). Expression was observed in body wall muscle (BWM) cells and in the muscles of the pharyngeal terminal bulb. CSQ-1::CFP showed aggregated expression, that did not resemble the punctuate and mesh-like pattern as previously observed in antibody-staining for CSQ-1[10]. One reason could be overexpression of CSQ-1, or a disturbed back-to-back dimerization, due to an interference of the C-terminal CFP fusion. Nonetheless, we achieved complete rescue of *csq-1(ok2672)* deletion mutant phenotypes in a swimming locomotion assay depending on normal BWM function (Fig. 1c). Likewise, *csq-1(ok2672)* led to reduced pumping ability in the presence of bacterial food, and a complete rescue was observed by CSQ-1::CFP expression (Fig. 1d), while the point mutation P319S did not show significant differences to WT. The *csq-1(ok2672)* deletion mutant was unable to follow the 3.7 Hz optogenetic pacing (p ≤ 0.05; kymographic video analyses; Fig. 1e,f). In addition, the pump frequency of the deletion mutant showed an increased ‘jitter’ (here, defined as a deviation of more than 50 ms from the timing that would be achieved at precisely 3.7 Hz frequency, as dictated by the light pulses; Fig. 1g), resulting in periods with reduced pump frequency of about 2 Hz (Fig. 1h) and a reduced maximal pump rate in a stress test with a stepwise increase of the stimulation frequency (1 Hz steps, 5 s each step, 1–7 Hz) via EPG recordings of cut-head preparations (Fig. 1i,j). In humans, absence of CASQ2, induced by nonsense mutations like R33X, causes severe forms of CPVT[24]. The observed phenotypes in the *C. elegans* pharynx could thus be considered an analogous form of ‘worm arrhythmia’. Against our expectations, the point mutation CSQ-1::CFP (P319S) did not show significant defects. Probably the polar serine residue is not affecting the Ca2+ binding and polymerization properties as much as the aliphatic leucine found in the human P308L mutation[23] (Fig. 1d–j). There was no effect of the *csq-1* mutations on pump duration (Supplementary Fig. 1a,b), arguing that the phenotypes were not induced by prolonged AP duration, as for our earlier pharynx model of Timothy syndrome (long QT8; LQT8)[21].

Absence of the RyR/UNC-68 affects pharynx pumping. To modify the *C. elegans* RyR (UNC-68), we used fosmid recombineering. The fosmid we used contains a slightly truncated promoter, thus we first verified if this promoter fragment is expressed in pharyngeal muscles. This was the case, i.e. the *punc-68* promoter fragment included in the fosmid, transcriptionally fused to CFP, was expressed in pharyngeal muscles and BWMs (Fig. 2a). *unc-68* mutants were identified as showing uncoordinated locomotion[14], and to exhibit defects in pharyngeal pumping. In addition, *unc-68* mutants grow slowly and have fewer offspring than wild type (wt). The *unc-68(r1162)* mutant eliminates a large part of the channel pore domain, thus *r1162* can be considered a molecular ‘null’ allele[25].

Behavioral and morphological phenotypes of *unc-68* mutant animals suggest that intracellular Ca2+ release is not essential for excitation-contraction (EC) coupling in *C. elegans*, but may rather act to amplify a plasma membrane mediated calcium influx that itself is sufficient for contraction[11,25]. Thus, we observed a reduction of spontaneous pump rate on food of only 32% in the presence of bacterial food, and a complete rescue was observed by CSQ-1::CFP expression (Fig. 1d), while the point mutation P319S did not show significant differences to WT. The *csq-1(ok2672)* deletion mutant was unable to follow the 3.7 Hz optogenetic pacing (p ≤ 0.05; kymographic video analyses; Fig. 1e,f). In addition, the pump frequency of the deletion mutant showed an increased ‘jitter’ (here, defined as a deviation of more than 50 ms from the timing that would be achieved at precisely 3.7 Hz frequency, as dictated by the light pulses; Fig. 1g), resulting in periods with reduced pump frequency of about 2 Hz (Fig. 1h) and a reduced maximal pump rate in a stress test with a stepwise increase of the stimulation frequency (1 Hz steps, 5 s each step, 1–7 Hz) via EPG recordings of cut-head preparations (Fig. 1i,j). In humans, absence of CASQ2, induced by nonsense mutations like R33X, causes severe forms of CPVT[24]. The observed phenotypes in the *C. elegans* pharynx could thus be considered an analogous form of ‘worm arrhythmia’. Against our expectations, the point mutation CSQ-1::CFP (P319S) did not show significant defects. Probably the polar serine residue is not affecting the Ca2+ binding and polymerization properties as much as the aliphatic leucine found in the human P308L mutation[23] (Fig. 1d–j). There was no effect of the *csq-1* mutations on pump duration (Supplementary Fig. 1a,b), arguing that the phenotypes were not induced by prolonged AP duration, as for our earlier pharynx model of Timothy syndrome (long QT8; LQT8)[21].

unc-68 transgenes bearing homologous CPVT-mutations rescue unc-68 deletion mutant phenotypes. Since UNC-68 affects pharynx function, we wanted to assess if point mutations known to affect RyR2 and cardiac function in humans would also affect pumping. Because *unc-68* is a very large gene (the genomic sequence is 27 kb, the cDNA is 15 kb), we introduced mutations into a rescuing fosmid containing *unc-68* by recombineering. We chose two mutations (described above) from human CPVT patients that were demonstrated to cause arrhythmia also in mice: The R4743C mutation, analogous to the CPVT mutation R4497C in humans (R4496C in mice; resulting in a leaky channel[24,30], and R2729S analogous to the CPVT mutation R2474S in humans and mice (resulting in weak calstabin-2 binding[24,25] and/or defective interdomain interactions[28]). The engineered fosmids were used to generate transgenic lines containing extrachromosomal arrays, in the *unc-68(r1162)* mutant background. As a control, we also generated a rescuing transgene from the unmodified fosmid. Expression of mutant UNC-68 proteins, in the absence of the endogenous protein, resulted in complete (R4743C, Fig. 2d; R2729S, Fig. 2e) rescue of pumping behavior. The wt rescue line exhibited a complete rescue as well (Fig. 2d). The ryanodine receptor point...
Figure 1. CSQ-1 deletion and the point mutation P319S affect pharyngeal pumping. (a) Expression of pcesq-1::csq-1::CFP in BWM cells and (b) pharynx muscle of the deletion mutant csq-1(ok2672). Scale bars: 50 μm and 20 μm, as indicated. Left panel: Fluorescence micrograph, right panel: DIC image; dashed lines indicate pharynx position. Structural features of the pharynx are labeled: TB (terminal bulb), I (isthmus), C (corpus), X (grinder). (c) Swimming cycles/min and (d) pump rate on food, of csq-1(ok2672) deletion mutants, as well as full length pcesq-1::csq-1::CFP rescue in pmyo-2::ChR2::mCherry background. Also analyzed (d) is the point mutation CSQ-1(P319S), compared to wt (number of animals tested is indicated at the base of each bar). (e) Number of pumps evoked at 3.7 Hz pulse frequency was counted for 100 light stimuli and averaged in 10 stimuli bins for the indicated number of animals. Original kymographs are depicted for wt (black), csq-1(ok2672) (red) and csq-1(gk876502) (blue; P319S). (f) Percentage of light stimuli inducing a pump across 100 light stimuli (data in e). (g) Percentage of pumps that were induced with a deviation of >50 ms following a light stimulus (here defined as ‘jitter’). Shown (f and g) are wt (white), csq-1(ok2672) deletion mutant (grey) and P319S mutant (black). (h) Pump rate distribution (%) at 3.7 Hz pacing (white: >4.5 Hz, blue: 3.3–4.5 Hz, green: 2.5–3.3 Hz, red: 1.6–2.5 Hz, grey: 0.5–1.6 Hz, black: 0 Hz) in the indicated csq-1 mutants, compared to wild type (wt) (n = 11–15). (i) Original EPG recordings of cut-head preparations and (j) mean of maximal pump rate of deletion and P319S mutant achieved in a stress test (470 nm, 10 ms pulses at stepwise increasing pulse rate (1 Hz steps, 5 s each step, 1–7 Hz), as indicated by blue tick marks) compared to wt (n = 10–23). Statistically significant differences, 1-way ANOVA and Bonferroni post-hoc test: ***p < 0.001; **p < 0.01; *p < 0.05.
mutations, which could be expected to cause a basal increase of cytosolic Ca$^{2+}$, did not increase the spontaneous pump rate. This may be due to adaptation of the pharynx to this situation, or a potentially increased pump rate due to higher basal Ca$^{2+}$ might be counteracted by stronger muscle contraction that is less readily released in time for the next pump. Swimming assays, probing UNC-68 function in BWMs, exhibited a partial rescue for both transgenes (Fig. 2f,g). The same was found for the wt fosmid. Thus, incomplete rescue of locomotion may be due to mosaicisms of the array expression, or an incomplete expression pattern of the fosmid.

The R4743C mutation of RyR/UNC-68 causes arrhythmic phenotypes in the pharynx. Next, we probed rhythmicity of pumping in the engineered CPVT pharynx models by optogenetic pacing. We presented 100 light pulses at a rate of 3.7 Hz and recorded pharynx activity by EPG-recordings of intact animals. Wild type animals and unc-68(r1162) mutants with the wt fosmid rescue could follow the pace rhythm faithfully until the end of the stimulus train (Fig. 3a). However, the unc-68(r1162) deletion mutant, as well as the mutant rescued with UNC-68(R4743C) exhibited pumping defects. To analyze these in a quantitative manner, we counted the actual pumps for each 10 light pulses. Both the deletion mutant as well as the point mutation R4743C could briefly follow the 3.7 Hz pumping period in the beginning of the stimulation protocol, but then exhibited a decrease of the pump rate, that was particularly pronounced for the R4743C CPVT mutant (Fig. 3b,c). This was also observed in kymographic video-analysis, in intact animals. The second CPVT-related mutation R2729S did not exhibit an inability to follow fast pacing (Fig. 3a–e). None of the unc-68 mutations had an effect on pump duration (Supplementary Fig. 1c–e), thus, as for csq-1, excluding prolonged AP duration as a cause for the observed inability to lock into the pacing stimulus.
Figure 3. The R4743C mutation, analogous to human CPVT mutation R4497C, induces arrhythmia in the pharynx. (a) Original EPG recordings of intact, pmyo-2::ChR2::mCherry-expressing animals containing unc-68 deletion mutant r1162, its rescue with wt unc-68 fosmid, the R4743C or R2729S mutant fosmids, and wt. (b) Mean and SEM number of pumps achieved over 100 consecutive light stimuli (3.7 Hz, 35 ms), binned per 10 light pulses, as in Fig. 1e, but obtained by EPG recordings. Genotypes of the animals tested are indicated. (c) Percentage of successful light pulses followed by a pump, across all 100 light stimuli, as recorded in (b). (d,e) Analyses analogous to (b) and (c), but obtained by kymographic analysis of video recordings in intact pmyo-2::ChR2::mCherry-expressing animals. Statistically significant differences, 1-way ANOVA and Bonferroni post-hoc test: ***p < 0.001; **p < 0.01; *p < 0.05.
The 1,4-benzothiazepine derivative S107 reverses the pump inability evoked by the CPVT mutation R4743C. Up to now β-blockers are the first therapeutic option for patients with CPVT. Although β-blockers reduce the occurrence of ventricular tachycardia, 30% of patients still experience cardiac arrhythmias and eventually require cardioverter defibrillator implantation to prevent cardiac arrest. Thus, there is a strong motivation to identify more specific drugs to treat CPVT arrhythmias, particularly to enable treatment with respect to the various mutations affecting the different domains of RyR2 or CASQ2, and their interactions with other proteins. Ideally, mutation-specific drugs may be identified that could prevent cardiac arrhythmias. The 1,4-benzothiazepine derivative K201 (a.k.a. JTV519) is an anti-arrhythmic drug currently under clinical investigation. However, K201 is a non-specific blocker of sodium, potassium and calcium channels. Thus, there are mixed results for K201, with side-effects outweighing benefits. Treatment with K201 enhanced the affinity of FKBP12.6 to the mutant RyR2 (R4743C) channel, inhibited the channel leak and prevented cardiac arrhythmias. Sasaki et al. could recently show in iPSCs for another C-terminal CPVT mutation (I4387V) that the development of DADs in the presence of isoproterenol was significantly suppressed by S107. But, to our knowledge, S107 was never tested on R4497C (R4743C in C. elegans). In fact, we found after a 30 min incubation in S107 (50 μM) a complete rescue of the pump ability of R4743C mutation bearing nematodes (Fig. 4a,b). Whereas kymographs showed that the R4743C mutants solely incubated in the vehicle DMSO (0.1%) quickly switched to pumping at half the pacing frequency, or sometimes stopped pumping at all, incubation in S107 enabled faithful 3.7 Hz pacing. In addition, there was no rescue of pump ability in the deletion mutant (Fig. 4b,d) indicating an unc-68 (RyR) specific effect of S107, that was not elicited via other, unknown channels.

**Discussion**

In a former study we established the C. elegans pharynx as a model for analyzing LQT8-like arrhythmogenic mutations affecting the Ca1.2 VGCC. Optogenetic stimulation of pharynx muscle via ChR2 enables pacing at about 4 Hz (and up to 6 Hz for > 1 minute). Here, we extended this model for CPVT-related mutations and characterized the role of RyR2- and CASQ2-mutations in C. elegans pumping, including homologous mutations causing CPVT in humans. The UNC-68(R4743C) mutation, homologous to human RyR2 (R4497C), causing channel leakiness, provoked a progressive drop in the ability to follow pacing. The experimental drug S107, a derivative of 1,4-benzothiazepine, caused a complete reversion of the phenotype, emphasizing that the C. elegans pharynx can indeed be used as a simple, genetically amenable model for cardiac arrhythmias, and possibly also for drug screening.

Eliminating the Ca2+ sequestering protein CSQ-1 (allele ok2672) weakly affected swimming locomotion (16% reduced swimming cycles), but had a more pronounced effect on spontaneous pharynx pump rate on food (38% reduction), which could be reversed by over-expression of CSQ-1::CFP. Inconsistently, in previous work, no significant effect on C. elegans locomotion speed could be observed, neither by RNAi against csq-1 nor in another deletion mutant csq-1(fh109) [10]. Interestingly, CASQ2-null mice are viable and display normal SR Ca2+ release and contractile function under basal conditions, although they exhibit striking increases in SR volume [29]. Nevertheless, these mice exhibit severe forms of CPVT, analogous to the phenotypes observed in humans lacking CASQ2 [30].

Here, we observed an arrhythmic phenotype for the deletion mutant of csq-1 in the paced pharynx model, which was unable to follow the 3.7 Hz stimulation (Fig. 1e) and in addition reached only reduced pump rates in the stress test (Fig. 1i). The point mutation P319S, similar to the human CPVT mutation P308L, showed no significant deviation from pulse frequency. Either, exchange of proline for a polar serine residue in the C-terminal domain of RyR2 or CASQ2 may be different than in vertebrates; this will have to be assessed in future work. A clear FKBP12.6 homolog is yet to be identified in C. elegans, which contains 8 members of the fkh gene class (we tested the presumably best homologue, FKB-2; Supplementary Fig. 2). Alternatively, the analogous C. elegans mutation R2729S has no effect on skeletal muscle, and might be a different functional allele in a different species. A knock-out mutation in R2729S was confirmed to affect skeletal muscle in C. elegans, and might thus be a different functional allele in a different species. A knock-out mutation in R2729S was confirmed to affect skeletal muscle in C. elegans, and might thus be a different functional allele in a different species.

Homozygous deletions of the RyR2 gene are embryonically lethal in mice [13,62]. The C. elegans RyR is required for normal locomotion and pumping. However, the unc-68 deletion mutant phenotype shows that UNC-68 is not essential for EC coupling or survival in the nematode. RyR could cooperate with the IP3 receptor for pumping at high rates, since a mild reduction of function mutant of itr-1(sa73) shows a similarly reduced pump rate on food as unc-68 deletions. Animals with strong itr-1 loss-of-function alleles are sterile [64].

We introduced the CPVT-related mutation R2729S into UNC-68 (R4743S in humans), but observed no effect on paced pump ability, only swimming locomotion was affected. Possibly, an interaction of UNC-68 with the C. elegans FKBP12.6 may be different than in vertebrates; this will have to be assessed in future work. A clear FKBP12.6 homolog is yet to be identified in C. elegans, which contains 8 members of the fkh gene class (we tested the presumably best homologue, FKB-2; Supplementary Fig. 2). Alternatively, the analogous C. elegans mutation R2729S does not impair the interaction between the two structural domains in UNC-68 (N-terminal and central domains) to the same extent as in human RyR2. The C. elegans UNC-68 protein could have a slightly altered structure, and R2729S may thus not exhibit an arrhythmia phenotype. In the mouse, it was proposed that the mutation alters RyR2 structure such that the whole complex becomes more susceptible to protein kinase A (PKA)-mediated phosphorylation modulation, thus causing increased arrhythmia. This could occur either by increased propensity to ‘unzipping’ of the N-terminal and central domains, and thus result in a significant increase in the frequency of spontaneous Ca2+ transients as shown in R2474S/ S + knock-in CM. Alternatively, it could be effected by dissociation of FKBP12.6 from human RyR2 following PKA phosphorylation. In C. elegans, no adrenergic signaling is present; however,
serotonin and other neuromodulators affect pharynx function as a response to the presence or absence of food. Yet, food-stimulated pumping was unaffected in the R2729S mutant animal (Fig. 2e). Last, we analyzed a homozygous mutant, while in mouse studies, heterozygotes were used, which may underlie discrepancies in the two systems.

The second CPVT-related mutation R4743C (R4497C in humans), as well as the deletion of \( \text{unc-68} \), caused a significant progressive decrease in the ability to follow paced pumping at high rate, both in EPG-recordings and video microscopy of intact animals, where the majority of animals were able to follow only every second light pulse or stopped pumping. This finding manifests a ‘worm arrhythmia’ phenotype of the CPVT-like mutation in our optogenetic model. The equivalent mutation R4496C in mice was suggested to play an essential role in RyR2 channel gating, and it was shown that the channel is more likely to open even at low \( \text{Ca}^{2+} \) concentration. Thus, a faster depletion of \( \text{Ca}^{2+} \) could be underlying the inability of UNC-68(R4743C) pharynxes to follow 3.7 Hz pacing.

The arrhythmic phenotype observed in the pharynx of the \( \text{C. elegans} \) mutant UNC-68(R4743C) argues for our system as a valuable arrhythmia model that enables to study CPVT mutations. To assess whether this also enables drug testing, we analyzed effects of the benzothiazepine S107 on restoring the pump ability of the arrhythmic pharynx. Indeed this was possible. It is interesting that S107 was considered to stabilize the FKBP12.6-RyR2 interaction in humans, yet, here it also showed beneficial effects for R4743C (R4497C in humans). This strengthens the assumption that the respective mutation influences the FKBP12.6-RyR2 interaction, as already shown by Lehnart et al. when using S107 treatment for the R2474S mutation. Our work demonstrates that potential anti-arrhythogenic drugs can be studied in the \( \text{C. elegans} \) system, which may be used as a rapid, initial model for introducing patient-specific CPVT mutations, and then to screen potential drugs in a relatively straightforward manner. Of course, this may not always be possible if the affected residue in humans is not conserved in UNC-68. However, possibly the human RyR2

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**Figure 4.** The benzothiazepine S107 reverses arrhythmia in the UNC-68(R4743C) mutant. (a) Original kymographs of video-microscopic recordings of the unc-68(r1162) rescue with wt and R4743C mutant fosmids, with or without incubation in 50 \( \mu \text{M} \) of the benzothiazepine S107 (inset) in 0.1% DMSO. (b) Mean and SEM number of pumps achieved over 100 consecutive light stimuli (3.7 Hz, 35 ms), binned per 10 light pulses, obtained by kymographic video analysis of intact, pmyo-2::ChR2::mCherry-expressing animals. As indicated, unc-68(r1162) deletion mutants, after 30 min incubation in 50 \( \mu \text{M} \) S107 (red; or without S107 (0.1% DMSO; black), were compared to wt with (green) or without (blue) S107 incubation (n = 4–9). (c) Analysis as in (b), but for r1162 deletion mutants expressing the R4743C fosmid, with S107 (green) or without (blue), or the rescue wt fosmid (red and black, respectively) (n = 20–29). (d) Percentage of successful light pulses followed by a pump, across all 100 light stimuli, as recorded in (b) and (c). Statistically significant differences, 1-way ANOVA and Bonferroni post-hoc test: ***p < 0.001; **p < 0.01; *p < 0.05.
can be introduced into *C. elegans* to replace UNC-68, thus generating a ‘humanized’ model. Worms can be treated with drugs and then assessed in a high-throughput manner by video microscopy.

In conclusion, we have established the pharynx of *C. elegans* as a model to characterize CPVT mutations, for two loci affected by this genetic condition, CASQ2 and RyR2 (csp-1 and unc-68 in *C. elegans*, respectively). The optogenetically paced pharynx model enables testing of drugs like S107 on specific mutations. The approach may enable characterizing new CPVT mutations found in patients, but possibly also mutations of RyR1, which are associated with malignant hyperthermia or central core disease.

**Methods**

**C. elegans strains.** *C. elegans* strains were cultivated at 20 °C on nematode growth medium (NGM) fed with *E. coli* strain OP-50-1. We used the following strains kindly provided by the Caenorhabditis Genetics Center (CGC): N2 (wild type), *RB2019: csq-1(ok2672)X, VC40907: csq-1(ok876502)X; altering P319 to S, TR2171: unc-68(r1162)V, CB540: unc-68(e540)V, JT73: itr-1(sa73)IV and RB2222: fkb-2(ok3007)I, unc-13(s691)I* was kindly provided by E. Jorgensen.

Furthermore, we generated the following transgenic strains: *ZX1534: csq-1(ok2672)X; zxE754[pcse-1(2.4kb):csp-1::CFP (5ng); pmyo-3::mCherry]; zxIs20[pmyo-2::ChR2(H134R)::mCherry, pges-1::nlsp::GFP], ZX1581: csq-1(ok2672)X; zxE754[pcse-1(2.4kb):csp-1::CFP (5ng); pmyo-3::mCherry], ZX1651: unc-68(r1162)V; zxE752[punc-68::unc-68 (1ng); pmyo-3::mCherry]; zxIs20[pmyo-2::ChR2(H134R)::mCherry, pges-1::nlsp::GFP], ZX1654: unc-68(r1162)V; zxE749[punc-68::unc-68(R2729S) (1ng); pelt-2::mCherry]; zxIs20[pmyo-2::ChR2(H134R)::mCherry, pges-1::nlsp::GFP], ZX1819: csq-1(ok876502)X; zxE752[pmyo-2::ChR2(H134R)::mCherry, pges-1::nlsp::GFP], ZX1833: unc-68(r1162)V; zxE748[punc-68::unc-68(R4743C) (1ng), pelt-2::mCherry]; zxIs20[pmyo-2::ChR2(H134R)::mCherry, pges-1::nlsp::GFP], ZX2121: unc-68(r1162)V; punc-68::unc-68 (exon1–4):CFP (10 ng).

**Plasmids.** Plasmid pmyo-2::ChR2(H134R)::mCherry was generated as described in Schuler et al.21. The *csq-1* gene was amplified by PCR from *C. elegans* genomic DNA (primer pair: oEF48, oEF49) and inserted into pmyo-3::CFP (based on pPD115.46) via restriction sites BamHI and KpnI. All primer sequences are listed in Supplementary Table S1. The *myo-3* promoter was exchanged to the endogenous *csq-1* promoter, by obtaining a 2.4 kb DNA fragment upstream of the *CSQ-1* encoding region by PCR from the genomic DNA (octs3, octs4) and insertion into the pmyo-3::csq-1::CFP plasmid via PsiI and BsaI restriction sites, resulting in pcsq-1::csq-1::CFP.

**Punc-68::unc-68 (exon1–4):CFP**, which was used for the verification of expression via *punc-68*, was generated using the In-Fusion cloning HD Kit (Clontec Takara Bio Europe, Saint-Germain-en-Laye, France). The *pmyo-2::CFP* plasmid was restricted by Acc651 and Sall and served as backbone. *punc-68::unc-68* was amplified from fosmid WRM063aG12 by primer pair oEF150 and oEF151 containing homology arms annealing to the restricted pmyo-2::CFP plasmid and ligated.

**Generation of unc-68 point mutations.** Insertion of point mutations in the *unc-68* gene was performed by a modified protocol of the heat induced recombination strategy in fosmids previously published by Tursun et al.46. Electro-competent *E. coli* SW105 which contain an arabinose-activatable FLP recombinase (not used in these experiments) and a heat-activatable λ red recombinase, were transfected with fosmid WRM063aG12 by electroporation (MicroPulser ™ Electroporator, Biorad, California, USA) at 2000 V, 6 ms. Successful transfection was verified by isolation of fosmid DNA and test digestion with AgeI. For insertion of point mutations, a two-step recombination and selection strategy was used. For the first step, λ red recombinase was heat-activated and electro-competent SW105 containing fosmid WRM063aG12 were generated46 and transfected with a PCR product containing a galactokinase- K (GalK) encoding gene with corresponding em-7 promoter, serving as a selection marker and flanked by 50 bp *unc-68*-homology sequences including the respective point mutation (Supplementary Fig. 4, Step 1). The cassette was amplified from the pBALU-1 plasmid46, with primers containing 50 bp of *unc-68* homology (R2729S: oEF200, oEF201; R4743C: oEF202, oEF203). Recombined clones were selected on galactose minimal agar plates and analyzed by colony-polymerase chain reaction (PCR; R4743C: oEF15, oEF16; R2729S: oEF204, oEF205). Mixed clones containing both WT-*unc-68* and GalK-*unc-68* fosmids were observed and “onlyGalK” clones were separated by repeatedly streaking out on MacConkey agar plates46, containing the pH indicator neutral red for an indication of galactose degradation, and analyzed via colony PCR screening (Supplementary Fig. 4, Step 1). The GalK sequence was replaced by the respective point mutation in a second step as follows: Mutation-specific PCR products, containing ~350 bp of homology and the point mutation were used for the second λ red recombinase and selection step (Supplementary Fig. 4, Step 2). Using fosmid WRM063aG12 as template these PCR products were generated by overlap PCR. At first PCR fragments containing the point mutations and overlapping regions were amplified with oEF206, oEF207 and oEF208, oEF209 for the R2729S mutation as well as oEF210, oEF211 and oEF212, oEF213 for the R4743C mutation, verified by gel electrophoresis and purified by gel extraction. In a second PCR reaction, the fragments were combined by overlap PCR to a final recombining construct with the mutant corresponding primer pairs oEF206, oEF209 for R2729S and oEF210, oEF213 for R4743C. Electro competent, λ red recombinase heat-activated SW105 “onlyGalK” bacteria were generated, transfected with the corresponding PCR product and cells were selected on deoxy-galactose minimal agar plates (Supplementary Fig. 4, Step 2). Colony-selection occurred via colony PCR with primers and the final *unc-68* constructs with the desired point mutations were verified by sequencing of colony PCR products with primers (R4743C: oEF15, oEF16; R2729S: oEF204, oEF205) (Supplementary Fig. 4, Step 2).

**Microinjection and generation of transgenic animals.** Transgenic animals expressing endogenous UNC-68 or UNC-68 with point mutations, respectively, were generated by microinjection of 1 ng/µl of the particular fosmid and the co-injection marker pmyo-3::mCherry (10 ng/µl) or pelt-2::mCherry (20 ng/µl).
in the deletion mutant unc-68(r1162). Generation of a strain for verification of unc-68 expression in the pharynx occurred by microinjection of 10 ng/μl of punc-68::unc-68(exon1–4)::CFP in the deletion background unc-68(r1162)V. The CSQ-1 rescue construct was expressed in mutant csq-1(ok2672) after microinjection of 5ng/μl pcqs-1::pcqs-1::CFP with co-injection marker pmyo-3::mCherry (10 ng/μl).

Genotyping of mutants. unc-68(r1162)V mutants were selected by locomotion phenotype, and were confirmed by PCR genotyping (oEF59, oEF60, oEF61). Genotyping of csq-1(ok2672)V was likewise done by PCR (oNH1, oNH2, oNH3). The mutation csq-1(P319S) was genotyped by PCR amplification (oEF214, oEF215), digestion with HinfI and analysis by electrophoresis on a 5% acrylamide gel to visualize the resulting small DNA fragments. The positively genotyped mutants were verified by sequencing.

Fluorescence microscopy. Expression of pcqs-1::csq-1::CFP and punc-68::unc-68(exon1–4)::CFP was analyzed on a Zeiss Axio Observer microscope, with an 40x/0.25 Zeiss APlan oil objective and CFP filter set (Carl Zeiss, Göttingen, Germany). Animals were transferred on 2% agarose pads in M9 buffer (K2PO4, 20 mM; Na2HPO4, 40 mM; NaCl, 80 mM; MgSO4, 1 mM) and immobilized with 1 μl freshly prepared 50 mM sodium azide solution (Sigma-Aldrich, USA, St. Louis) in M9 buffer from a 1 M stock in water.

Determination of spontaneous pump rate on food. One day before experiments, L4 larvae were picked on NGM dishes (55 mm, 8 ml NGM) seeded with 320μl OP505 culture. Spontaneous pumping of animals on food was video recorded (Powershot G9, Canon, Tokio, Japan) and visually counted for 20 s. Mean values, standard error of the mean (SEM) and further statistics (1-way ANOVA with Bonferroni post-hoc test) were calculated with OriginPro (OriginLab, Northampton, MA, USA).

EPG-Recording and optical pacing. One day before experiments young adult hermaphrodites were placed on fresh NGM plates with or without all-trans retinal (ATR, Sigma Aldrich, St. Louis, MO, USA). ATR (0.65 μl of a 100 mM stock in ethanol) was added to 650 μl of OP505 culture and spread onto 94 mm culture dishes (vented, Greiner Bio-One, Kremsmuenster, Austria) containing 25.2 ml of NGM. We performed EPG recordings on intact animals and on cut head preparations. For cut head preparations, animals were transferred into a recording chamber containing a Sylgard-covered cover slip (25 mm diameter) and filled with 1.5 ml of EmD50 buffer (NaCl, 140 mM; KCl, 3 mM; CaCl2, 3 mM; MgCl2, 1 mM; Hepes, 10 mM; D-Mannitol, 50 mM; pH 7.3 adjusted with NaOH). The head of an animal was cut away from the body with a scalpel (Braun Aesculap, Tuttingen, Germany) directly posterior to the terminal bulb. Upon dissection, the body wall muscles contract and expose the posterior pharynx.

Electrophysiology was performed as described in Schuler et al.21. The tip of the worm head was sucked into an EPG-suction electrode (~20 μm inner diameter tip) using a 10x objective. For optical pacing with a 470 nm LED (KSL-70, Rapp Optoelectronics, Hamburg, Germany) the pharynx was positioned below a 60x water immersion objective (ULMplanFI/IR, 0.9 NA); an EGFP-ET filter set (AHF Analysentechnik AG, Tuebingen, Germany) was used. Recording of EPGs and triggering of light pulses was synchronized by PatchMaster software (Heka, Lambrecht, Germany).

Either spontaneous pumping (at least for 1 minute) was recorded or the pharynx was stimulated with 470 nm light pulses (1.5–2 mW/mm2, 35 ms) over a period of 30 s or in a stress test (stepwise increasing stimulation frequency: 1–7 Hz; pulse duration: 10 ms, over a period of 5 seconds each). Review software (Bruxton Corporation, Seattle, WA, USA) was used to translate PatchMaster files to ABF files. Pump rate and duration were analyzed by AutoEPG20 (kindly provided by Dr. Christopher James, Embody Biosignals Ltd., UK). Excel was used for calculation of means and SEM, 1-way ANOVA with Bonferroni post-hoc test was performed using OriginPro.

High-throughput kymograph recording and optical pacing. Transgenic L4 larvae cultivated with ATR were placed on fresh NGM dishes containing ATR one day prior to the assay. 1 μl of polystyrene microspheres (Polysciences 00876-15, 2.5% w/v suspension, Hirschberg an der Bergstrasse, Germany) were added on pads composed of 10% agarose. About 10 animals were transferred into the beads and gently overlaid with a coverslip.

Measurements were performed on a Zeiss Axios Observer, equipped with a 100 W HBO lamp, EGFP Filter (Ex. 472/30, beam splitter 570, Em. 520/50) and an 10x/0.25 Zeiss APlan objective. Animals were stimulated with blue light pulses (35 μs, 1.5 mW/mm2) with a computer-controlled shutter (Sutter Instruments, Novato, CA, USA) every 250 μs (4 Hz) over a period of 30 s. Recording was performed using an ORCA Flash 4.0 sCMOS camera (Hamamatsu, Japan; 20 fps, 10 μs exposure time, 2 × 2 binning, 1024 × 1024 Pixel) and μManager v1.4 software. For drug tests, animals were incubated 30 min in 50 μL of 50 μM S107 (Calbiochem-Order number: 500469; Merck, Darmstadt, Germany) in M9 buffer containing 0.1% DMSO before the measurements.

Multi-kymographs of grinder movements were generated and analyzed as described in Schuler et al.21. Excel was used for calculation of means and SEM, 1-way ANOVA with Bonferroni post-hoc test was performed using OriginPro.

Data availability. The data that support the findings of this study (video recordings, original records of electrophysiological data) are available from the corresponding authors upon reasonable request.

References
1. Liu, N., Ruan, Y. & Priori, S. G. Catecholaminergic polymorphic ventricular tachycardia. _Prog. Cardiovasc. Dis._ 51, 23–30 (2008).
2. Lehmann, H. & Priori, S. G. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. _Circulation_ 91, 1512–1519 (1995).
3. Priori, S. G. et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. _Circulation_ 106, 69–74 (2002).
4. Faggioni, M. & Knollmann, B. C. Calsequestrin 2 and arrhythmias. _Am. J. Physiol. Heart Circ. Physiol._ 302, H1250–1260 (2012).
5. Wang, S. et al. Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. _Nat. Struct. Biol._ 5, 476–483 (1998).
6. Park, H., Wu, S., Dunker, A. K. & Kang, C. Polymerization of calsequestrin. Implications for Ca2+-regulation. J. Biol. Chem. 278, 16176–16182 (2003).
7. Guo, W. & Campbell, K. P. Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. J. Biol. Chem. 270, 9027–9030 (1995).
8. Jones, L. R., Zhang, L., Sanborn, K., Jørgensen, A. O. & Kelley, J. Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum. J. Biol. Chem. 270, 30787–30796 (1995).
9. Cho, J. H. et al. Calsequestrin, a calcium sequestering protein located at the sarcoplasmic reticulum, is not essential for body-wall muscle function in Caenorhabditis elegans. J. Cell Sci. 113, 3947–3958 (2000).
10. Cho, J. H. et al. Functional importance of polymerization and localization of calsequestrin in C. elegans. J. Cell Sci. 120, 1551–1558 (2007).
11. Maryon, E. B., Saari, B. & Anderson, P. Muscle-specific functions of ryanodine receptor channels in Caenorhabditis elegans. J. Cell Sci. 111, 2885–2895 (1998).
12. Brenner, S. The genetics of Caenorhabditis elegans. Genetics 77, 71–94 (1974).
13. Takeshima, H. et al. Embryonic lethality and abnormal cardiac myocytes in mice lacking ryanodine receptor type 2. EMBO J. 17, 3309–3316 (1998).
14. Medeiros-Domingo, A. et al. The RYR2-encoded ryanodine receptor/calium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. J. Am. Coll. Cardiol. 54, 2065–2074 (2009).
15. Leenhardt, A., Denjoy, I. & Guichet, P. Catecholaminergic polymorphic ventricular tachycardia. Circ. Arrhythm. Electrophysiol. 5, 1044–1052 (2012).
16. Lanner, J. T., Georgiou, D. K., Joshi, A. D. & Hamilton, S. L. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. Cold Spring Harb. Perspect. Biol. 2, a003996, https://doi.org/10.1101/cshperspect.a003996 (2010).
17. Betzenhauser, M. J. & Marks, A. R. Ryanodine receptor channelopathies. Pflugers Arch. 460, 467–480 (2010).
18. MacLennan, D. H. et al. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature 343, 559–561 (1990).
19. Zhang, Y. et al. A mutation in the human ryanodine receptor gene associated with central core disease. Nat. Gen. 5, 46–50 (1993).
20. Baines, K. N., Ferreira, C., Hopkins, P. M., Shw, M. A. & Hope, I. A. Aging effects of Caenorhabditis elegans ryanodine receptor variants causing to human myopathic mutations. G37, 1451–1461 (2017).
21. Schuler, C., Fischer, E., Shaltiel, L., Steuer Costa, W. & Gottschalk, A. Arrhythmogenic effects of mutated L-type Ca2+-channels on an optogenetically paced cardiac pump in Caenorhabditis elegans. Sci. Rep. 5, 14427, https://doi.org/10.1038/srep14427 (2015).
22. de la Fuente, S., Van Langen, I. M., Postma, A. V. & Meijer, A. A. Specificity of catecholaminergic polymorphic ventricular tachycardia caused by two calsequestrin 2 mutations. Pacing Clin. Electrophysiol. 31, 916–919 (2008).
23. Bal, N. C. et al. Probing cationic selectivity of cardiac calsequestrin and its CPVT mutants. Biochem. J. 435, 391–399 (2011).
24. Priori, S. G. et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 103, 196–200 (2001).
25. Lehnhart, S. E. et al. Leaky Ca2+-release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. J. Clin. Invest. 118, 2230–2245 (2008).
26. Cerrone, M. et al. Bidirectional ventricular tachycardia and fibrillation elicited in a knock-in mouse model carrier of a mutation in the cardiac ryanodine receptor. Circ. Res. 96, e77–82 (2005).
27. Peng, W. et al. Structural basis for the gating mechanism of the type 2 ryanodine receptor RyR2. Science 354, aah5326 (2016).
28. Uchinoumi, H. et al. Catecholaminergic polymorphic ventricular tachycardia is caused by mutation-linked defective conformational regulation of the ryanodine receptor. Circ. Res. 106, 1413–1420 (2010).
29. Jiang, D. et al. Enhanced store overload-induced Ca2+ release and channel sensitivity to luminal Ca2+ activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. Circ. Res. 97, 1173–1181 (2005).
30. Jiang, D., Xiao, B., Zhang, L. & Chen, S. R. Enhanced basal activity of a cardiac Ca2+-release channel (ryanodine receptor) mutant associated with ventricular tachycardia and sudden death. Circ. Res. 91, 218–225 (2002).
31. Liu, N. et al. Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R4496C knock-in mouse model. Circ. Res. 99, 292–298 (2006).
32. George, C. H., Higgs, G. V. & Lai, F. A. Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. Circ. Res. 93, 531–540 (2003).
33. Knollmann, B. C. et al. Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca2+ release, and catecholaminergic polymorphic ventricular tachycardia. J. Clin. Invest. 116, 2530–2520 (2006).
34. Kannankeril, P. J. et al. Mice with the R176Q cardiac ryanodine receptor mutation exhibit catecholamine-induced ventricular tachycardia and cardiomyopathy. Proc. Natl. Acad. Sci. USA 103, 12179–12184 (2006).
35. Loaiza, R. et al. Heterogeneity of ryanodine receptor dysfunction in a mouse model of catecholaminergic polymorphic ventricular tachycardia. Circ. Res. 112, 298–308 (2013).
36. Zhao, Y. T. et al. Arrhythmogenesis in a catecholaminergic polymorphic ventricular tachycardia mutation that depresses ryanodine receptor function. Proc. Natl. Acad. Sci. USA 112, E1669–1677 (2015).
37. Wang, Y. Y. et al. RyR2R242Q mutant effect in the heart of mice. PLOS ONE 7, e44660, https://doi.10.1371/journal.pone.0044660 (2012).
38. Fatima, A. et al. In vitro modeling of ryanodine receptor 2 dysfunction using human induced pluripotent stem cells. Cell. Physiol. Biochem. 28, 579–592 (2011).
39. Novak, A. et al. Functional abnormalities in iPSC-derived cardiomyocytes generated from CPVT1 and CPVT2 patients carrying ryanodine or calsequestrin mutations. J. Cell Mol. Med. 19, 2006–2018 (2015).
40. Jung, C. B. et al. Dantrolene rescues arrhythmogenic RyR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. EMBO Mol. Med. 4, 180–191 (2012).
41. Di Pasquale, E. et al. CaMKII inhibition rectifies arrhythmic phenotype in a patient-specific model of catecholaminergic polymorphic ventricular tachycardia. Cell Death Dis. 4, e843, https://doi.org/10.1038/cddis.2013.369 (2013).
42. Kaszki, K. et al. Patient-Specific Human Induced Pluripotent Stem Cell Model Assessed with Electrical Pacing Validates S107 as a Potential Therapeutic Agent for Catecholaminergic Polymorphic Ventricular Tachycardia. PLOS ONE 11, e0164795, https://doi.org/10.1371/journal.pone.0164795 (2016).
43. Iltzaki, I. et al. Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. J. Am. Coll. Cardiol. 60, 990–1000 (2012).
44. Freiminger, M. M. et al. A human pluripotent stem cell model of catecholaminergic polymorphic ventricular tachycardia recapitulates patient-specific drug responses. Dis. Model. Mech. 9, 927–939 (2016).
45. Novak, A. et al. Cardiomyocytes generated from CPVTD307H patients are arrhythmogenic in response to beta-adrenergic stimulation. J. Cell. Mol. Med. 16, 468–482 (2012).
47. Avery, L. & You, Y. J. C. elegans feeding. WormBook: The online review of C. elegans biology, 1–23, https://doi.org/10.1895/wormbook.1.150.1 (2012).
48. Mango, S. E. The C. elegans pharynx: a model for organogenesis. WormBook: The online review of C. elegans biology, 1–26, https://doi.org/10.1895/wormbook.1.129.1 (2007).
49. Altun, Z. F., Chen, B., Wang, Z. W. & Hall, D. H. High resolution map of Caenorhabditis elegans gap junction proteins. Dev. Dyn. 238, 1936–1950 (2009).
50. Davis, M. W., Fleischhauer, R., Dent, J. A., Joho, R. H. & Avery, L. A mutation in the C. elegans EXP-2 potassium channel that alters feeding behavior. Science 286, 2501–2504 (1999).
51. Raizen, D. M. & Avery, L. Electrical activity and behavior in the pharynx of Caenorhabditis elegans. Neuron 12, 483–495 (1994).
52. Cook, A., Franks, C. I. & Holden-Dye, L. Electrophysiological recordings from the pharynx. WormBook: The online review of C. elegans biology, 1–7, https://doi.org/10.1895/wormbook.1.110.1 (2006).
53. Dillon, J. et al. AutoEPG: software for the analysis of electrical activity in the microcircuit underpinning feeding behaviour of Caenorhabditis elegans. PLOS ONE 4, e8482, https://doi.org/10.1371/journal.pone.0008482 (2009).
54. Postma, A. V. et al. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. Circ. Res. 91, e21–e26 (2002).
55. Maryon, E. B., Coronado, R. & Anderson, P. unc-68 encodes a ryanodine receptor involved in regulating C. elegans body-wall muscle contraction. J. Cell Biol. 134, 885–893 (1996).
56. Richmond, J. E., Davis, W. S. & Jorgensen, E. M. UNC-13 is required for synaptic vesicle fusion in C. elegans. Nat. Neurosci. 2, 959–964 (1999).
57. Kaneko, N., Matsuda, R., Hata, Y. & Shimamoto, K. Pharmacological characteristics and clinical applications of K201. Curr. Clin. Pharmacol. 4, 126–131 (2009).
58. Driessen, H. E., Bourgonje, V. J., van Veen, T. A. & Vos, M. A. New antiarrhythmic targets to control intracellular calcium handling. Neth. Heart J. 22, 198–213 (2014).
59. Houle, T. D., Ram, M. L. & Cala, S. E. Calsequestrin mutant D307H exhibits depressed binding to its protein targets and a depressed response to calcium. Cardiovasc. Res. 64, 227–233 (2004).
60. Postma, A. V. et al. Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. Science 304, 292–296 (2004).
61. Sacherer, M. et al. FIV359 (K201) reduces sarcoplasmic reticulum Ca2+ leak and improves diastolic function in vitro in murine and human non-failing myocardium. Br. J. Pharmacol. 167, 493–504 (2012).
62. Liu, Y. et al. Generation and characterization of a mouse model harboring the exon-3 deletion in the cardiac ryanodine receptor. PLOS ONE 9, e95615, https://doi.org/10.1371/journal.pone.0095615 (2014).
63. Walker, D. S., Gower, N. J., Ly, S., Bradley, G. L. & Baylis, H. A. Regulated disruption of inositol 1,4,5-trisphosphate signaling in Caenorhabditis elegans reveals new functions in feeding and embryogenesis. Mol. Biol. Cell 13, 1329–1337 (2002).
64. Salvador, D., Logan, M. A., Chisholm, A. D. & Jorgensen, E. M. The inositol trisphosphate receptor regulates a 50-second behavioral rhythm in C. elegans. Cell 98, 757–767 (1999).
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66. Macconkey, A. Lactose-Fermenting Bacteria in Faeces. J. Hyg. 3, 333–379 (1905).
67. Thompson, O. et al. The million mutation project: a new approach to genetics in Caenorhabditis elegans. Genome Res. 23, 1749–1762 (2013).

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Author Contributions

Designed research: C.S., A.G., with help of the other authors. Performed experiments: C.S., E.F. Performed data analysis: C.S., E.F. Wrote the paper: C.S., A.G., with help of the other authors.

Additional Information

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