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Prevention of Ventricular Arrhythmia and Calcium Dysregulation in a Catecholaminergic Polymorphic Ventricular Tachycardia Mouse Model Carrying Calsequestrin-2 Mutation

Ronny Alcalai, MD^{1,2,*}, Hiroko Wakimoto, MD, PhD^{1,5,*}, Michael Arad, MD³, David Planer, MD², Tetsuo Konno, MD¹, Libin Wang, MD, PhD¹, Jon G. Seidman, PhD^{1,#}, Christine E. Seidman, MD^{1,4,#}, and Charles I Berul, MD^{5,6,#}

¹ Department of Genetics, Harvard Medical School, Boston, MA 02115

² Heart Institute, Hadassah Hebrew University Medical Centre, Jerusalem, Israel

³ Heart Institute, Sheba Medical Centre and Tel Aviv University, Israel

⁴ Howard Hughes Medical Institute and Cardiovascular Division, Brigham and Women's Hospital, Boston, MA 02115

⁵ Department of Cardiology, Children's Hospital Boston, Harvard Medical School, Boston, MA 02115

⁶ Children's National Medical Center, Washington, DC 20010

Abstract

Background—Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a familial arrhythmic syndrome caused by mutations in genes encoding the calcium-regulation proteins cardiac ryanodine receptor (RyR2) or calsequestrin-2 (CASQ2). Mechanistic studies indicate that CPVT is mediated by diastolic Ca²⁺ overload and increased Ca²⁺ leak through the RyR2 channel, implying that treatment targeting these defects might be efficacious in CPVT.

Method and results—CPVT mouse models that lack CASQ2 were treated with Ca²⁺-channel inhibitors, β -adrenergic inhibitors, or Mg²⁺. Treatment effects on ventricular arrhythmia, sarcoplasmic reticulum (SR) protein expression and Ca²⁺ transients of isolated myocytes were assessed. Each study agent reduced the frequency of stress-induced ventricular arrhythmia in mutant mice. The Ca²⁺ channel blocker verapamil was most efficacious and completely prevented arrhythmia in 85% of mice. Verapamil significantly increased the SR Ca²⁺ content in mutant myocytes, diminished diastolic Ca²⁺ overload, increased systolic Ca²⁺ amplitude, and prevented Ca²⁺ oscillations in stressed mutant myocytes.

Conclusions—Ca²⁺ channel inhibition by verapamil rectified abnormal calcium handling in CPVT myocytes and prevented ventricular arrhythmias. Verapamil-induced partial normalization of SR Ca²⁺ content in mutant myocytes implicates CASQ2 as modulator of RyR2 activity, rather than or in addition to, Ca²⁺ buffer protein. Agents such as verapamil that attenuate cardiomyocyte calcium overload are appropriate for assessing clinical efficacy in human CPVT.

Correspondence author: Charles I Berul, Current Address: Division of Cardiology, Children's National Medical Center, 111 Michigan Ave NW, Washington, DC 20010 USA, cberul@cnmc.org, Phone: (202) 476-5710, FAX: (202) 476-5700.

*These authors contributed equally to this work.

#These authors contributed equally to this work.

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Keywords

CPVT; Calsequestrin2; mouse model; calcium; antiarrhythmia agents; sudden death

Background

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic disorder caused by mutations in calcium-regulation genes, including dominant mutations in the cardiac ryanodine receptor gene (RyR2) or recessive mutations in the calsequestrin-2 (CASQ2) gene¹⁻⁴. Affected individuals experience polymorphic ventricular arrhythmias and sudden cardiac death, typically beginning in childhood⁵⁻⁷. Because CPVT arrhythmias are provoked by catecholamine discharge in response to exertion or emotional stresses, β -adrenergic inhibitors have long been considered as the treatment of choice⁸. While β -adrenergic inhibitors reduce the frequency of CPVT arrhythmias, they fail to prevent sudden cardiac death in all cases and are limited by dosing and side effects⁸⁻¹⁰. Recently, Hayashi et al reported significantly lower cardiac fatal event rates in patients receiving β -blockers, nonetheless β -blockers did not provide sufficient prevention of fatal arrhythmias during 8-year follow-up⁹. Verapamil and flecainide were recently reported to have beneficial effect on CPVT patients^{11, 12}, nevertheless these data are limited to small numbers of patients and short follow-up. Interventional approaches such as implantable cardiac defibrillators⁹ and sympathetic denervation^{13,14} are also partially efficacious in some CPVT patients, but these therapies are invasive and have disadvantages particularly in young children with the disorder.

The mechanisms by which mutations in CASQ2 and RyR2 cause CPVT have been recently explored using several engineered mouse models that carry human mutations in either the RyR2 or the CASQ2 genes¹⁵⁻¹⁷. These studies show that mutation in either gene increases Ca^{2+} leak through the RyR2 channel¹⁸. This defect is exacerbated by stress, inducing electrical instability in myocytes, leading to delayed afterdepolarizations and potentially fatal arrhythmias^{15, 19-21}. The central role of RyR2 dysfunction in CASQ2-mutation unifies the pathophysiologic mechanism for CPVT and suggests that pharmacologically targeting the intracellular Ca^{2+} overload may attenuate CPVT phenotype.

We assessed the efficacy and mechanism of several drugs including the L-type calcium channel inhibitors verapamil and diltiazem, the nonspecific calcium antagonist Mg^{2+} and the β -adrenergic inhibitor propranolol in preventing ventricular arrhythmias and Ca^{2+} mishandling in two engineered CPVT mouse models that lack CASQ2.

Methods

Mice

The knock-in mouse model that carry the homozygous human missense mutation D307H (CASQ^{307/307}) and the CASQ2 knockout mouse model that carry homozygous deletion of exon 9 (CASQ ^{$\Delta\text{E9}/\Delta\text{E9}$}) were generated and phenotyped as previously described²¹. CASQ^{307/307} and the CASQ ^{$\Delta\text{E9}/\Delta\text{E9}$} mice have complete or near complete deficiency of CASQ2 protein and both produce indistinguishable arrhythmic profiles. (herein denoted as CASQ^{-/-}). All mice were treated and maintained according to protocols approved by the Institutional Animal Care and Use Committee of Harvard Medical School.

Protein analysis

Enriched SR protein extractions from 10-20 week old mice were obtained according to SR isolation protocols²². Protein concentration was determined by the BCA method²³. Lysates (30µg) were separated by sodium dodecyl sulfate-polyacryl amide gel electrophoresis on a 4 to 20% gel (Pierce) and transferred onto PVDF membranes (Pierce). The following antibodies were used: rabbit polyclonal anti-calsequestrin (1:2500, Affinity BioReagents Inc.), goat polyclonal anti-SERCA2 ATPase (1:1000, Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-FKBP12 (1:500, Affinity BioReagents Inc.), mouse monoclonal anti-phospholamban (1:1000, Affinity BioReagents, Inc.), rabbit polyclonal anti-dephospho-RYR2(Ser-2809) (1:1000, Badriilla), rabbit polyclonal anti-phospho-RYR2(Ser-2809) (1:1000, Badriilla), rabbit polyclonal anti-calreticulin (1:1000, Upstate). The ECL blotting system (Pierce) was used to visualize proteins.

Electrocardiography, Telemetry and in vivo drug treatment

Electrocardiography (ECG) recordings and continuous telemetry were performed as previously described^{24, 25}. All electrophysiological data analysis was performed by two experienced observers blinded to the genotypes and pharmacological treatments. Surface 8-lead electrocardiograms (ECG) were obtained in anesthetized mice (pentobarbital, 0.033 mg/kg intra-peritoneally) using subcutaneous electrodes at the four limbs and 2 chest-leads (V1, V6). The ECG channels were filtered between 5 and 400 Hz and the analog signal was digitized with 12-bit precision at a sampling rate of 2 kHz. Mean sinus-cycle length (SCL), heart rate (HR), PR, QRS, and QT intervals were measured for each animal. Ambulatory single lead electrocardiograms were obtained by subcutaneously implanted telemetric radiofrequency transmitters (Data Sciences International, St. Paul, Minnesota, USA). After a 48-hours recovery period, resting ECGs were recorded for one hour periods during daytime.

Pharmacological stress challenge was performed using intraperitoneal (IP) injection of epinephrine (0.4 mg/kg). On subsequent days, pharmacological stress was repeated 10 minutes after IP injection of antiarrhythmic agents as follow: magnesium (200mg/Kg), propranolol, (1mg/Kg), diltiazem (8mg/Kg) and verapamil (8mg/Kg). Dose-response studies were performed preliminarily, and these reported doses were the maximal tolerated by the mice according to heart rate (not less than 400bpm) and general condition (weakness and poor mobility occurring after drug injection). Each drug was tested after 24 hours washout period on the same mice. The order of treatments was changed in random order for each mouse. Telemetric monitoring was performed continuously for 10 minutes, 30 seconds after epinephrine injection and was observed for both provocation of ventricular arrhythmias and heart rate response. Ventricular arrhythmia time was defined when more than 50% of the QRS complexes were from ventricular origin, most commonly ventricular bigeminy or sustained bidirectional ventricular tachycardia (Fig 1).

Chronic drug treatment was performed for propranolol (0.5mg/ml) and verapamil (2mg/ml) given in drinking water for 14 days on 5 mice each group. The water was changed every 24 hours and the amount of water used by the mice was measured to ensure compliance and drug bioavailability. Telemetry recordings were performed at baseline and at days 7 and 14 of treatment before and after IP epinephrine injection; mice were monitored for ventricular arrhythmias and heart rate response similar to the acute drug treatment study. To assess the serum concentration of verapamil, blood was taken by direct heart puncture from anesthetized mice. Each test was performed on blood pooled from 3 different mice to obtain sufficient sample volumes.

Myocyte preparation and Ca²⁺ measurements

Ventricular cardiomyocytes of adult mice were isolated via a Langendorff-perfused heart preparation using enzymatic digestion as previously described²⁶. After isolation, the cells were suspended in Tyrode's buffers with gradually increasing Ca²⁺ concentrations (0.06, 0.24, 0.6 and 1.2 mM, pH 7.4 at room temperature) and were loaded with 1 μM fura-2AM calcium indicator (Molecular Probes, Eugene, OR, USA) as previously described²⁷. Myocytes were washed three times for 10-15 minutes with 1.2 mM Ca²⁺ Tyrode's solution containing 250 μM probenecid to retain the indicator in the cytosol. An additional 20 minutes were allowed for de-esterification of the indicator before imaging the cells. The experiments were then performed at room temperature in 1.2 mM Ca²⁺ Tyrode's solution containing (in mM): NaCl 140, KCl 4.5, MgCl₂ 0.5, glucose 5, and HEPES 10, pH adjusted to 7.4 with NaOH. All chemicals were obtained from Sigma (St. Louis, MO). Cardiomyocytes were electrically paced at 60 beats per minute via platinum wires. Sarcomere shortening/relengthening and fura-2 fluorescence ratios (which reflect the intracellular calcium transients) were simultaneously recorded and determined from discrete striation positions on the myocyte using a dual-excitation fluorescence imaging/contractility recording system (IonWizard® SarcLen detection and PMT Acquisition fluorescence system, IonOptix Inc., Milton, MA, USA). Sarcomere length and calcium transient recordings were performed in the baseline Tyrode's buffer and then after adding epinephrine 5.5 μM to the solution. Same recordings were performed also in a high Mg²⁺ (3 mmol/L) Tyrode's buffer and in Tyrode's buffer containing verapamil (1 μM). Sarcoplasmic reticulum (SR) Ca²⁺ load was assessed via a rapid puff of caffeine (20 mM) to fura-2-loaded myocytes without electric stimulation. Caffeine was superfused for the full duration of the transient until calcium levels returned to baseline. Caffeine application was performed in 0 mM Na⁺ and 0 mM Ca²⁺ Tyrode's solution containing 140 mM LiCl and 10 mM BDM²⁸. The caffeine application was also performed after cells were incubated for 3 minutes in 1 μM verapamil containing buffer.

Percent sarcomere shortening (%SL) was expressed as the ratio of the difference between resting and twitch length normalized to resting cell length. Calcium transient analysis include: baseline levels, transient amplitude (peak calcium change from baseline), departure time, time constant for calcium transient decay- τ (represent the reuptake time), duration of transient (time from departure to return to baseline level) and the area under the Ca²⁺ transient curve. Sarcomere length and Ca²⁺ transients were analyzed using the IonOptix transient analysis software (IonWizard®, www.ionoptix.com).

Myocytes included in the study were rod-shaped with a clear striation pattern, quiescent in the absence of electrical stimulation and resting sarcomere length of more than 1.6 μm. All drug treatments were done on myocytes with normal contraction and Ca²⁺ transient at baseline.

Statistical analysis

EP studies—Mann-Whitney test was used to compare response to treatment and heart rate between genotypes (wildtype and CASQ^{-/-}) and between treatment groups, using Bonferroni correction for multiple comparisons. In addition, data were categorized into two categories: *complete response* – less than 5% of the time in ventricular arrhythmia versus partial and *no response*. McNemar test was used to compare categorized data between baseline and each drug separately. Fisher's exact test was used to compare categorized response rate between verapamil and propranolol treatment groups in the chronic phase.

Myocyte studies—Differences between groups of wild-type and CASQ^{-/-} cardiomyocytes in different solutions were determined using the unpaired Student T-test for continuous variables and the Fisher's exact test for categorized variables.

Data were analyzed using SPSS version 14.0 (SPSS Inc, Chicago, Ill). A two-sided *P* value of less than 0.05 was considered significant. All values are presented as mean $\pm$ SE.

Results

In vivo studies

Stress was imposed on CASQ^{-/-} mice with epinephrine given at baseline and after IP injection of propranolol (n=11), Mg²⁺ (n=11), verapamil (n=8), or diltiazem (n=6). The heart rate and rhythms were continuously recorded for 10 minutes. Since both CASQ^{307/307} and the CASQ^{ΔE9/ΔE9} mice had the same response to drug treatments, we present the analysis for both mice collectively (denoted as CASQ^{-/-}). All mice had ventricular arrhythmias (Fig. 1) after epinephrine injection and each drug significantly decreased the average duration of arrhythmias compared to baseline (Fig. 2A). Arrhythmia durations were reduced by approximately 50% after propranolol, Mg²⁺ or diltiazem, while verapamil produced a 90% reduction in ventricular arrhythmia time. In comparison to beta-adrenergic inhibition with propranolol, only verapamil had significantly more reduction (p=0.006) of the arrhythmia burden (Fig 2A).

Epinephrine-induced ventricular arrhythmias were reduced to less than 5% of the recording time in only 1 of 11 mice after propranolol (p=1.0 vs. baseline), in 4 of 11 after Mg²⁺ (p=0.12 vs. baseline), in 3 of 6 mice after diltiazem (p=0.25 vs. baseline) and in 7 of 8 mice after verapamil (p=0.02 vs. baseline). Only verapamil was able to completely prevent arrhythmia in the vast majority of mice.

CASQ^{-/-} mice, like humans with CASQ2 mutations, have lower basal heart rates^{6, 21}. Resting heart rates in CASQ^{-/-} mice treated with propranolol or Mg²⁺ were further reduced, whereas the heart rates after treatment with either verapamil or diltiazem were higher than baseline and significantly higher than after propranolol treatment (Fig. 2B).

Based upon the suppression of epinephrine-induced arrhythmias by verapamil, we assessed the efficacy of chronic verapamil administration in arrhythmia prevention. CASQ^{-/-} mice received verapamil or propranolol (n=5 per treatment group) in drinking water for 2 weeks. Telemetry recordings at baseline and at days 7 and 14 (after drug administration) were assessed. Propranolol reduced arrhythmias duration to an average of 24.6% (range 10-48%) compared to 89.9% at baseline but did not suppress the arrhythmias below 5% in any of the mice (Fig. 3a). On the other hand, verapamil completely suppressed arrhythmia (to 0% of the recording time) in 4 of 5 mice (p=0.048 vs. propranolol; Fig.3B), but similar to the short-term study, was ineffective in one mouse.

Propranolol decreased the mean heart rate from 454 $\pm$ 22 bpm at baseline to 416 $\pm$ 12 bpm after 2 weeks of treatment, while verapamil caused no significant change (533 $\pm$ 11 bpm to 524 $\pm$ 16.5 bpm). There was no difference in PR, QRS or QT intervals after treatment in both groups.

The response to verapamil was all or none. Thirteen mice were treated with the drug either orally or by injection. While in 11 mice there was complete response to treatment with elimination of the arrhythmia, in 2 mice there was no response at all. Pooled serum levels of verapamil after IP injection (8mg/kg) were 106ng/ml and after 14 days of oral treatment (2mg/ml of water) were 1158ng/ml, both either within or above therapeutic levels (100-600ng/mL).

Ca²⁺ transients and myocytes contractility

We tested whether the pathological handling of Ca²⁺ by CASQ^{-/-} myocytes was improved by verapamil, as we previously reported occurred following Mg²⁺ administration²¹. CASQ^{-/-} myocytes treated with verapamil had significantly reduced Ca²⁺ reuptake time in comparison to untreated cells and both agents normalized the decay constant (TAU) to values comparable to wild-type myocytes (Table 1). Ca²⁺ amplitudes were not changed in response to either agent, but both decreased fractional shortening, indicating that Mg²⁺ and verapamil significantly diminished myocyte contractility (Table 1).

Epinephrine-stimulated CASQ^{-/-} myocytes had elevated diastolic Ca²⁺ levels and diminished Ca²⁺ transient amplitudes (Table 1 and Fig. 4). Pre-treatment with verapamil or Mg²⁺ attenuated this elevation and maintained diastolic Ca²⁺ in CASQ^{-/-} myocytes at levels comparable to wild type-cells (Table 1 and Fig. 4). The pre-treatment also increased the Ca²⁺ transient amplitudes of CASQ^{-/-} myocytes treated with epinephrine (Table 1 and Fig. 4). In the presence of Mg²⁺, epinephrine restored contractile parameters more closely to values in wild-type cells (Table 1), while contractility remained significantly depressed in verapamil-treated myocytes after exposure to epinephrine, despite improved Ca²⁺ transients (Table 1).

Following epinephrine, CASQ^{-/-} myocytes had frequent spontaneous Ca²⁺ release (SCR) events and Ca²⁺ oscillations. Both Mg²⁺ and verapamil decreased SCR and oscillation frequency, but verapamil was significantly more effective. The percentage of cells with SCR after epinephrine was reduced from 100% to 15% with verapamil (p<0.05) but only to 85% with Mg²⁺ (p=ns, Table 1).

We investigated how verapamil might alter total SR Ca²⁺ content in CASQ^{-/-} myocytes. Previous studies^{15, 29} indicate that in comparison to wild-type myocytes SR Ca²⁺ content in CASQ^{-/-} myocytes is significantly lower. To investigate whether verapamil can restore SR content we treated CASQ2 deficient cells with verapamil for 3 minutes and then applied caffeine. Verapamil-treated CASQ^{-/-} myocytes had significantly higher caffeine induced Ca²⁺ release amplitudes and prolonged duration of Ca²⁺ transients (Fig. 5). These data indicate that verapamil increased the total SR Ca²⁺ content in CASQ^{-/-} myocytes.

Protein analysis

We examined the expression of key Ca²⁺ regulatory proteins in CASQ^{-/-} hearts at baseline and after 2 weeks of verapamil treatment. No significant changes were found in the levels of expression of sarcoplasmic reticulum Ca²⁺ATPase (SERCA2a), phospholamban, FKBP12.6, calsequestrin and calreticulin (Supp. Fig 1). Total levels of RyR2 and phosphorylated RyR2 were also unchanged by verapamil treatment. We concluded that verapamil effects on Ca²⁺ handling and arrhythmia was not directly mediated by modulation of these proteins.

Discussion

CPVT is a familial disorder with a high rate of life-threatening arrhythmias, typically presenting in childhood and adolescence⁵. Although this syndrome has been recognized for about 30 years, to date there is no effective proven approach for treatment of CPVT. β -blockers have empirically been considered the best therapeutic option in CPVT, but no study has directly compared its long-term efficacy with other antiarrhythmic medications or interventions. ICDs are used in some CPVT patients for prevention of sudden cardiac death; however, painful defibrillation shocks can trigger further adrenergic stress and arrhythmia storms that can potentially result in death despite appropriate ICD shocks. Sympathetic denervation has also been recently suggested as a therapeutic option for resistant cases¹³, but

this invasive approach is not always available and has only been demonstrated in very small numbers of young CPVT patients. Two studies^{11, 30}, suggested a beneficial effect of verapamil in combination with β -blockers for CPVT, although the combination may not be well tolerated due to potentially synergistic side-effects. Another study demonstrated that flecainide may be effective in CPVT¹², nevertheless flecainide can be proarrhythmogenic and routine use of this drug cannot yet be recommended until further studies define its risks and efficacy in a larger sample of humans with CPVT.

Two CPVT disease-related genes have been reported so far: RyR2, encoding the cardiac ryanodine receptor Ca^{2+} release channel, and CASQ2, encoding cardiac calsequestrin-2⁸. Studies of mouse models carrying mutations in these genes demonstrated that mutations cause destabilization of the RyR2 complex, stress induced diastolic calcium leak through the RyR2 channel, spontaneous SR Ca^{2+} release and Ca^{2+} oscillation which are the basis for development of ventricular arrhythmias *in vivo*^{15, 17, 18, 31}. In the present study, we utilized 2 CASQ2 engineered mouse models that have been shown previously to recapitulate the clinical CPVT phenotype²¹ in order to investigate several therapeutic options that directly target calcium regulation in cardiomyocytes. We found that intraperitoneal injection of verapamil before epinephrine completely prevented ventricular arrhythmias in most of these mice and was the only drug that was superior to propranolol in stress-induced arrhythmia prevention (Fig 2). The results remained the same when we evaluated long-term oral treatment of verapamil compared to propranolol. While propranolol reduced the frequency of the arrhythmias, it could not prevent it completely similar to the experience in humans with CPVT treated with β -blockers⁹. Verapamil, on the other hand, completely abolished the arrhythmia in most mice treated with the drug. This is a critical point in treatment of CPVT since a single drug-refractory arrhythmic event might be fatal, thus the partial response seen in the case of propranolol is not adequate.

Surprisingly, heart rates after treatment with verapamil or diltiazem were higher than at baseline. The explanation of this paradoxical response in heart rate to calcium channel blockers is not clear. The mechanism of relative bradycardia seen in both humans and mice with CASQ2 mutations^{3, 21} is not known and it is possible that calcium channel blocking can reverse some of the effect of CASQ2 deficiency on heart rate. Alternatively, it may be tachycardia reflex in response to hypotension caused by the drugs; nevertheless, we did not measure blood pressures of the mice so we can not rule in or rule out this possibility.

To assess the mechanism by which verapamil prevents arrhythmia, we have tested the various effects of verapamil on calcium transients of isolated cardiomyocytes. We found that after exposure to epinephrine the verapamil-treated mutant cells exhibited significantly less elevation in the diastolic Ca^{2+} levels, higher systolic amplitude of Ca^{2+} transients and significantly lower frequency of the spontaneous Ca^{2+} releases and oscillations. Similar effects to verapamil were seen when CASQ2 deficient cells were tested in high Mg^{2+} solution probably by reduction of the L-type Ca^{2+} current by Mg^{2+} and possibly by a decrease in the stress-induced magnesium efflux³² causing increased intracellular magnesium levels that directly or indirectly reduce RyR2 activity³³. By reduction of the L-type Ca^{2+} current and RyR2 activity, Mg^{2+} prevent diastolic Ca^{2+} overload and Ca^{2+} oscillations in mutant cells. Nevertheless, Mg^{2+} could not completely normalize the Ca^{2+} mishandling of mutant cells in response to catecholamines as was the case with verapamil. In vitro calcium studies with caffeine reveal additional effect of verapamil on CASQ2 deficient cardiomyocytes. Caffeine normally causes activation of the RyR2 release channel and SR Ca^{2+} depletion³⁴. Mutant cells previously shown to have small caffeine-induced transients due to decreased Ca^{2+} content within the SR^{15, 21}. Following verapamil treatment, these transients were significantly higher (Fig. 5) indicates that the SR Ca^{2+} content can be partially restored by pre-treatment with verapamil.

Taken together, our data suggest that L-type Ca^{2+} channel inhibition paradoxically normalizes Ca^{2+} release in stressed CASQ2 deficient myocytes, restores SR Ca^{2+} content and prevents VT in vivo. These effects are probably due to significant reduction of end-diastolic Ca^{2+} levels in the cytosol after exposure to verapamil. Since SR Ca^{2+} leak is very sensitive to changes in cytosolic Ca^{2+} in CASQ2 deficient myocytes³⁵, preventing the rise in diastolic Ca^{2+} will likely reduce leak and paradoxically increase SR content (Fig. 5). These effects are in opposition to the β -adrenergic effects on CASQ2 deficient myocytes that cause paradoxical reduction in systolic release and in the total SR content. Watanabe et al have showed that flecainide prevents arrhythmia in CASQ2 knock-out mouse model by RyR2 inhibition¹². Verapamil can bind the skeletal RyR and reduces its activity³⁹, hence it is plausible that some of the effects of verapamil seen in isolated myocytes studies are mediated by RyR2 inhibition explaining the remarkable effect of verapamil on Ca^{2+} dysregulation and on arrhythmia prevention in vivo, similar to flecainide. Further studies may be necessary in permeabilized myocytes and in isolated RyR2 channels in order to conclusively differentiate between the direct action of verapamil on the L-type calcium channels and RyR2 channels.

Previous studies have shown that calsequestrin is associated with the Ca^{2+} release channel protein complex of the SR through protein-protein interactions³⁶ and controls the SR Ca^{2+} release by effects on luminal Ca^{2+} dependent RyR2 gating^{37, 38}. Our studies further suggest that CASQ2 is primarily a regulatory protein of RyR2 release channel and is less essential as SR Ca^{2+} buffer at rest conditions. This can be an explanation for the normal heart structure and viability of humans and mice despite complete deficiency of CASQ2. Our studies point out that CASQ2 appears to be mostly needed to prevent uncontrolled catecholamine-induced activation of the RyR2 and thus to prevent constant SR Ca^{2+} leak. Accordingly, the phenotype of humans with CASQ2 mutations is limited to stress and not observed in the resting state.

The best clinical therapeutic approach for CPVT is still uncertain, and it seems that in order to achieve full response to verapamil, high dose treatment is needed. Verapamil also has strong negative inotropic effects as seen in the isolated myocyte studies, and therefore may not be appropriate to prevent arrhythmia in patients with significant heart failure as described in human cases⁴⁰. Nevertheless, in patients with normal heart structure and ventricular function like in CPVT, verapamil is a promising therapeutic option and should be considered as first line treatment in some cases of CPVT or in combination with β -blockers or flecainide. Larger human studies would be needed to assess which drug is the most effective for these patients, but these studies will be challenging due to the rarity of this inherited disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Typical ventricular arrhythmias recorded in CASQ2^{-/-} mice

(A) Representative traces of normal sinus rhythm at baseline, (B) ventricular bigeminy after epinephrine IP injection, (C) sustained polymorphic ventricular tachycardia with bidirectional pattern after epinephrine IP injection.

Figure 2. Effect of IP injection of several drugs on arrhythmia and heart rate in CASQ2^{-/-} mice (A) Percentage of time in sustained ventricular arrhythmia during 10 minutes of recording after IP injection of Epinephrine. Recording were made without treatment and 2 minutes after test drug injection as indicated. * - $p < 0.05$ compare to no treatment, # - $p < 0.05$ compare to propranolol treatment. (B) Mean heart rate at baseline and after test drug IP injection as indicated. * - $p < 0.05$ compare to baseline, # - $p < 0.05$ compare to propranolol treatment.

Figure 3. Effect of oral treatment of Propranolol and Verapamil on arrhythmia rate in CASQ2^{-/-} mice

Each line represents the percentage of time in sustained ventricular arrhythmia during 10 minutes of recording after IP injection of epinephrine in one mutant mouse at baseline and after two weeks of oral drug treatment as indicated.

Figure 4. SR Ca^{2+} transients of $CASQ2^{-/-}$ myocytes

Representative traces from $CASQ2^{-/-}$ myocytes during constant epinephrine infusion for 5 minutes with electrical pacing at 1 Hz (ticks in the upper panel). The recordings were made in Tyrode solution (upper panel), in High Mg^{2+} (3mM) solution (middle panel) and in solution containing 1μM Verapamil (lower panel). Note elevation of diastolic Ca^{2+} levels (arrow), reduction of transient peak height, and development of Ca^{2+} oscillation after 4 minutes of epinephrine infusion (right side of upper panel). These pathologic responses to epinephrine were prevented by Mg^{2+} and more effectively by verapamil.

Figure 5. Verapamil increased SR Ca^{2+} content of $\text{CASQ2}^{-/-}$ myocytes

(A) Representative traces of caffeine-induced (20 mM) Ca^{2+} transients in wild type (WT) and $\text{CASQ2}^{-/-}$ myocytes with and without pre-treatment with verapamil. (B) Bars denote pooled data from 10 untreated wild-type cells (white), 23 untreated $\text{CASQ2}^{-/-}$ cells (black) and 22 treated $\text{CASQ2}^{-/-}$ cells (gray) from at least 3 independent myocyte preparations. *- $p < 0.01$ compare to wildtype cells, #- $p < 0.05$ compare to $\text{CASQ2}^{-/-}$ cells.

Table 1
Isolated cardiomyocyte calcium transients and contractility studies - baseline and after epinephrine (5.5μM)

	Baseline				Epinephrine			
	WT (N=23)	CASQ ^{-/-} (N=31)	CASQ ^{-/-} +Mg ²⁺ (N=13)	CASQ ^{-/-} +Ver (N=17)	WT (N=14)	CASQ ^{-/-} (N=12)	CASQ ^{-/-} +Mg ²⁺ (N=13)	CASQ ^{-/-} +Ver (N=13)
Rest (F2 ratio)	0.53±0.14	0.58±0.11	0.52±0.10	0.55±0.10	0.49±0.09[*]	0.75±0.13	0.58±0.12[^]	0.53±0.09[#]
Ca ²⁺ amplitude (F2 ratio)	0.43±0.13	0.44±0.14	0.39±0.12	0.41±0.25	0.56±0.17[*]	0.27±0.17	0.44±0.19[^]	0.47±0.2[#]
Time to peak 50% (msec)	9±1.8[*]	11±3	14±6	10±3	8±2	12±7	10±7	9.5±3
Tau (msec)	122±22[*]	141±28	120±15[^]	103±26[#]	68±9	66±22	79±23	83±27
Rest Sarcomere length (μm)	1.75±0.06	1.75±0.06	1.74±0.05	1.8±0.04	1.72±0.06	1.74±0.06	1.73±0.06	1.73±0.06
% Sarcomere shortening	6.5±2.1	7.8±2.9	4.1±2.1[^]	3.7±2.2[#]	12.9±3.3	11.7±3.3	10.6±4.1	7.1±3[#]
Spontaneous Ca ²⁺ releases (No. of cells with more than 2 events)	n/r	n/r	n/r	n/r	8(57%)[*]	12(100%)	11(85%)	2(15%)[#]
Ca ²⁺ Oscillations (No. of cells with one event or more)	n/r	n/r	n/r	n/r	0[*]	9(75%)	6(46%)[^]	1(7.6%)[#]

Data presented as mean±SE

^{*} p<0.05 for comparison between CASQ^{-/-} and WT

[^] p<0.05 for comparison between CASQ^{-/-} and CASQ^{-/-} +Mg

[#] p<0.05 for comparison between CASQ^{-/-} and CASQ^{-/-} + Verapamil

WT-wild type, Ver – Verapamil, n/r – not relevant

N – represent number of cells from at least 3 independent myocyte preparations