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Optimizing catecholaminergic polymorphic ventricular tachycardia therapy in calsequestrin-mutant mice

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Abstract

BACKGROUND—Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal arrhythmia provoked by physical or emotional stress and mediated by spontaneous Ca²⁺ release and delayed after-depolarizations. Beta-adrenergic blockers are the therapy of choice but fail to control arrhythmia in up to 50% of patients.

OBJECTIVE—To optimize antiarrhythmic therapy in recessively inherited CPVT caused by calsequestrin (CASQ2) mutations.

METHODS—Murine heart rhythm telemetry was obtained at rest, during treadmill exercise, and after injection of epinephrine. The protocol was repeated after injection of different antiarrhythmic drugs. Results were then validated in human patients.

RESULTS—Adult CASQ2 mutant mice had complex ventricular arrhythmia at rest and developed bidirectional and polymorphic ventricular tachycardia on exertion. Class I antiarrhythmic agents (procainamide, lidocaine, flecainide) were ineffective in controlling arrhythmia. Propranolol and sotalol attenuated arrhythmia at rest but failed to prevent VT during sympathetic stimulation. The calcium channel blocker verapamil showed a dose-dependent protection against CPVT. Verapamil was more effective than the dihydropyridine L-type Ca²⁺ channel blocker nifedipine, and its activity was markedly enhanced when combined with propranolol. Human patients homozygous for CASQ2^{D307H} mutation, remaining symptomatic despite chronic β -blocker therapy, underwent exercise testing according to the Bruce protocol with continuous electrocardiogram recording. Verapamil was combined with propranolol at maximum

tolerated doses. Adding verapamil attenuated ventricular arrhythmia and prolonged exercise duration in five of 11 patients.

CONCLUSION—Verapamil is highly effective against catecholamine-induced arrhythmia in mice with CASQ2 mutations and may potentiate the antiarrhythmic activity of β -blockers in humans with CPVT2.

Keywords

Ventricular arrhythmia; Sympathetic; Murine model; Human; Calcium channel blocker

Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal hereditary disease characterized by exercise or emotional stress-induced ventricular tachycardia (VT), which can deteriorate to ventricular fibrillation.^{1–3} Patients experience episodes of seizures, syncope, and sudden death after stress. This arrhythmic disorder mainly affects children and young adults having a normal resting electrocardiogram (ECG), a tendency for sinus bradycardia, and no structural heart disease.^{4–7} Diagnosis of CPVT requires reproducing polymorphic or bidirectional VT during exercise testing or epinephrine infusion. Efficacy of pharmacological treatment is also assessed during repeat exercise testing. The treatment of choice, β -adrenergic blockers, fails to achieve complete arrhythmia control in up to 50% of cases,⁷ range 2%–62% according to Hayashi et al,⁸ and hence many patients receive an implantable cardioverter-defibrillator (ICD).

To date, two genetic modes of inheritance were identified. Autosomal dominant CPVT1 is caused by mutations in the cardiac ryanodine receptor (*RyR2*) gene located on chromosome 1q42.1-q43.⁷ CPVT2 is caused by recessively inherited mutations in the cardiac calsequestrin (*CASQ2*) on chromosome 1p13.3-p11.^{5,6} Both genes encode proteins essential for normal Ca^{2+} homeostasis and Ca^{2+} -induced Ca^{2+} release in the cardiomyocyte.^{4,9} The recessive mutations cause a more severe disease course and are manifested at a younger age. *CASQ2*^{D307H} was the first human CPVT2 mutation that was described in Bedouin Arabs from Galilee, Israel.^{5,10} The onset of symptoms occurred at a very early age (7 ± 4 years), and family history was remarkable for syncope, seizures, and mortality due to sudden death. *CASQ2* is a sarcoplasmic reticulum (SR) Ca^{2+} storage protein, which plays an important role in excitation-contraction coupling in the heart. *CASQ2* associates with triadin and junctin to form a complex called the SR Ca^{2+} release channel (the cardiac ryanodine receptor complex). Gene-targeted mice homozygous for either the D307H mutation (*CASQ2*^{D307H/D307H}) or calsequestrin knockout (KO; *CASQ2*^{−/−}) have been generated. These mouse models imitate with high fidelity the human recessively inherited CPVT.^{9,11} The aim of current experiments was to characterize the effects of different antiarrhythmic drugs on the prevalence and severity of arrhythmias in the mouse model of CPVT2 seeking to optimize the current available therapy for human CPVT patients homozygous for *CASQ2*^{D307H} mutation.

Methods

Animal studies

The animal experiments conform to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication no. 85-23, revised 1996) and were approved by the institutional animal care and use committee of Tel Aviv University (no. 11-04-093). Our murine model for the recessively inherited CPVT was described elsewhere.¹¹ ECG and pharmacological studies were performed on adult male *SvEv* mice (8–10 months old) homozygous for CASQ2^{D307H} mutation (hence D307H) or CASQ2^{-/-} (KO), in comparison with age-matched wild-type (WT) mice. Mice were maintained and bred in a pathogen-free facility on regular rodent chow with free access to water and 12-hour light and dark cycles.

A murine ECG telemetry transducer weighing 3.8 g (DSI, St. Paul, MN) was surgically implanted under skin on the back of the animal as described elsewhere.¹¹ Mice were anesthetized with ketamin (75–90 mg/kg) and xylazine (5–8 mg/kg) intraperitoneally (IP; Kepro, Holland). Animals were allowed to recover after surgery for at least 48 hours before participating in any kind of experiments. Afterward, with ECG being continuously monitored, they were subjected to exercise stress and epinephrine injection.^{12,13} Baseline electrogram was obtained at rest while the animal was in the cage with no stressful stimuli. Mice were then forced to exercise on a rodent treadmill (Exer-6M; Columbus Instruments, Columbus, OH) with an adjustable belt speed and a slope of 15 degrees. The treadmill speed was gradually increased from 5 to 8 m/min, and the mouse was left to run 30 seconds at 8 m/min before obtaining a steady state heart rhythm recording. Then the animal was forced to run at 15 m/min, and ECG was recorded during the last 10 seconds of this sprint and the subsequent 60 seconds of recovery. After 5 minutes, mice were injected with epinephrine (0.5 mg/kg IP) and monitored for additional 5 minutes.

The same protocol was used to study the efficacy of the antiarrhythmic agents. Drugs were freshly diluted in saline (0.9%) and injected IP in 0.2 mL. Recordings at rest were obtained before injection and 15 minutes after drug delivery. Afterward mice underwent exercise and epinephrine testing as described elsewhere. Antiarrhythmic agents were selected to represent different classes and were used in the maximum doses that are reported to be effective in rodents/mice.^{14–20}

Human studies

The human studies conform to the principles outlined in the Declaration of Helsinki and were approved by the Institutional Review Board, Rambam Medical Center. CPVT patients homozygous for the CASQ2^{D307H} mutation⁵ who remained symptomatic despite chronic β -blocker therapy with oral propranolol (maximum tolerated dosage of a slow-release preparation) underwent a treadmill exercise test according to the Bruce protocol with continuous ECG recording. Oral verapamil was added to propranolol in three daily doses and uptitrated to the maximum tolerated dose. Exercise testing was repeated after 1 week of a combination therapy at stable drug doses, under the same experimental conditions. Exercise was terminated for prolonged VT (>10 beats), complex ventricular arrhythmia,

multifocal premature ventricular beats (PVCs), or inability to continue because of fatigue. Arrhythmia threshold was defined as sinus rate at the onset of complex ventricular arrhythmia. Clinical response to verapamil was defined as attenuation of exercise-induced arrhythmia and/or increased exercise performance by at least 3 minutes.

Data analysis

Human arrhythmia was defined according to established conventions.⁷ Murine heart rate and ventricular arrhythmia were analyzed manually from computer recordings (Figure 1) and defined as follows: nonsustained VT (NSVT)—four or more consecutive ventricular complexes; sustained VT (SVT)—a VT lasting >15 seconds; monomorphic VT (mVT)—a continuous ventricular rhythm with one predominant morphology; bidirectional VT (biVT)—ventricular rhythm with two predominant alternating morphologies; polymorphic VT (pVT)—a ventricular rhythm having at least three alternating morphologies.^{11,16} For purposes of drug efficacy analysis, the endpoint of VT was defined as NSVT or SVT recorded in an animal under any condition. All other ventricular arrhythmias (premature beats, bigeminy, salvos, couplets, and triplets) were classified as PVCs.

All data were expressed as mean $\pm$ standard deviation or as percentages. Differences between groups of mutant and WT mice were determined using the Student's *t*-test for continuous variables and the χ^2 Fisher's exact test for probability assessment. Two-tailed *P* <.05 was considered statistically significant.

Results

Murine model of CPVT

WT mice had no arrhythmia at rest or during stress except for sporadic PVCs. D307H and KO mice had multiple arrhythmias at rest, and virtually all developed VT during exercise or after epinephrine injection (Table 1, Figure 1). The most common morphology was pVT followed by the biVT. Mice that had episodes of mVT also experienced pVT or biVT on other occasions. Because human CPVT patients do not suffer from arrhythmias at rest,¹⁰ we analyzed continuous overnight telemetry recordings from CASQ2 mutant mice that had VT on resting recording. Mutant mice had normal sinus rhythm most of the time (70% $\pm$ 9%), in particular when the room was empty, suggesting that an experimental setup evokes a sufficient stress to trigger arrhythmia in these very susceptible animals. It is noteworthy that arrhythmia at rest was very rare in younger mutant animals aged 3–4 months (data not shown). Therefore, we attributed CPVT at rest to the combination of environmental stimuli (noise, motion, etc.) with a low arrhythmia threshold in elderly mutant mice.

Finally, arrhythmia appeared to be more severe in KO compared with in D307H mice, as manifested by a higher prevalence of SVT (Table 1) and lower responsiveness to therapy.

Efficacy of antiarrhythmic drugs against CPVT in mice

Injecting vehicle (IP 0.2 mL saline) to a subset of mutant mice with documented VT (six KO and two D307H) had no effect on arrhythmia. The results of the antiarrhythmic drug screen against CPVT in CASQ2 mice are presented in Table 2. Medications were well tolerated.

Class I, II, and III antiarrhythmics partially attenuated arrhythmia at rest (Table 2) but provided no significant protection against CPVT evoked by exercise or epinephrine. The β -adrenergic blocker propranolol and the class III agent with β -blocking activity, sotalol, slightly decreased VT prevalence during exercise but not after epinephrine. The Ca^{2+} channel blocker verapamil significantly lowered VT prevalence in KO mice and completely abolished arrhythmia in D307H mice (Table 2, Figure 1).

Verapamil had a dose- and genotype-dependent effect on CPVT: 1 mg/kg significantly lowered arrhythmia prevalence in CASQ2^{D307H} but had no effect in KO mice; 5 mg/kg were needed to completely suppress arrhythmia in KO mice (Figure 2).

Since high-dose verapamil, apart from its L-type Ca^{2+} channel (LTCC) blockage, can block additional ion channels such as Na^{+} channels, K^{+} channels, ryanodine receptor, and possibly $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger,^{21–24} we tested whether its antiarrhythmic activity is related to blocking LTCC. Dihydropyridine LTCC blocker nifedipine reduced VT prevalence in CASQ2 mutant mice to 29% (two of seven; $P < .001$ compared with no therapy).

Synergism between β -blocker and Ca^{2+} channel blocker

Because β -blockade may potentiate the effect of LTCC blockade and vice versa, we investigated whether there was a synergistic antiarrhythmic action of β and Ca^{2+} channel blockers in CASQ2 mutant mice. For this experiment we used KO mice and exposed them to low-dose verapamil (1 mg/kg) and propranolol (10 mg/kg), doses that are ineffective when given alone. The drug combination completely abolished arrhythmia (Figure 3) and was associated with neither bradyarrhythmia (Table 3) nor conduction abnormalities.

Effects on heart rate

The resting heart rates were significantly lower in both CASQ2 D307H and KO mice compared with in WT mice (Table 3). Exercise caused only a slight increase of heart rate in WT mice. Sinus rate could not be determined in stressed mutant mice owing to severe ventricular arrhythmia. Propranolol and sotalol but not verapamil significantly decreased the sinus heart rate during rest and exercise.

Human study

Verapamil was added to the chronic propranolol therapy in 11 CPVT patients aged 12–33 years. All had exercise-limiting ventricular arrhythmia and palpitations; nine also had syncope while on propranolol. Verapamil was generally well tolerated and did not affect the heart rate or blood pressure but had to be discontinued in one subject because of nausea and vomiting. On combination therapy, the maximum heart rate achieved increased by 9 ± 10 bpm (range -5 to 24 ; $P = .02$) and the exercise duration by 2.0 ± 2.4 minutes (range -0.5 to 6 ; $P = .03$). The drug was beneficial in five patients (50%, Table 4), leading to complete disappearance of stress-induced arrhythmia in four of them. In the other five patients, verapamil did not prevent arrhythmia but slightly increased the arrhythmia threshold (by 4 ± 3 bpm; $P = .03$). There were no significant differences between responders and nonresponders in the drug doses, the exercise duration, or the heart rate obtained (Table 4).

Responders were more often young males ($P = .06$), but the numbers are too small to draw a definitive conclusion.

Discussion

We studied mice and humans with *CASQ2* mutations to optimize CPVT therapy. The recessively inherited CPVT2, originally described in Bedouin Arabs from Galilee, is associated with early disease onset, severe arrhythmia, and adverse prognosis compared with dominantly inherited CPVT1 caused by *RyR2* mutations.^{6,8,10} In both cases the mechanism of arrhythmogenesis involves abnormal diastolic calcium leak through a dysregulated ryanodine channel.^{25,26} Extrusion of excess cytosolic Ca^{2+} and entrance of Na^{+} via the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX) is assumed to be responsible for delayed after-depolarizations. The principal therapies for CPVT are phenotype driven and include avoiding exercise, β adrenergic blockade, and ICD implantation.^{7,8} The response to β -blockers is incomplete and often declines with time because of an escape phenomenon.

We have previously described a murine model of recessive CPVT caused by either a null allele or a *CASQ2*^{D307H} mutation.¹¹ Normally, mice have a short action potential duration and a lower contribution of LTCC and NCX to Ca^{2+} transients. Despite these fundamental differences from humans, mutant mice successfully recapitulate the human arrhythmic phenotype. Physical or pharmacological stresses lead to development of VT, mostly of bidirectional or polymorphic morphology (Figure 1), which may be self-limited or sustained. Male and female, missense or null-allele mutant mice exhibit the same arrhythmic phenotype. Unlike human CPVT, murine arrhythmia does not deteriorate into ventricular fibrillation and does not cause death, possibly owing to a markedly smaller heart size in mice. On the other hand, arrhythmia is easily evoked and is prevalent even in resting mice, either owing to higher sensitivity of the animals to stressful environment or to a lower arrhythmia threshold in aged animals.

We therefore used both genotypes of *CASQ2* mutant mice to optimize the medical therapy of CPVT by studying the efficacy of different antiarrhythmic agents. We believe that the severe phenotype of these animals makes them very useful for pharmacological testing because the obtained results should be applicable to a broad range of CPVT by different *CASQ2* and probably *RyR2* mutations, which share a common electrophysiological mechanism.

Arrhythmia was more severe and resistant to therapy in *CASQ2*-deficient mice compared with in D307H animals that carry a residual amount of abnormal protein. These data suggest that the *CASQ2*^{D307H} protein has residual function and that decreased protein level (as opposed to abnormal protein structure) is the main cause of the phenotype.^{11,27}

Watanabe et al²⁶ recently demonstrated the efficacy of flecainide in *CASQ2*^{-/-} mice and two human CPVT patients, attributing the effect to the combination of reducing Ca^{2+} release through inhibiting ryanodine receptor and attenuating triggered activity through Na^{+} channel blockade. In our study, Na^{+} channel blockers and I_{Kr} blocker did not prevent CPVT (Table 2). The latter could be expected, given the small contribution of I_{Kr} and I_{Ks} to murine action

potential.²⁸ Flecainide failure was reproduced in four additional CASQ2^{-/-} and D307H mice, despite a similar administration protocol. A factor that could account for the disagreement is the older age of our animals (8–10 vs. 3–5 months), which is associated with a more severe arrhythmic phenotype. Results on flecainide for treating various CPVT patients, independent or in combination with β -blockers, are being awaited.

The β -adrenergic blocker propranolol had a very slight activity against CPVT in mice. Other drugs with β -adrenergic blocking activity, metoprolol (data not shown) and sotalol (Table 2), were comparable to propranolol. In light of its negative chronotropic effect and attenuation of heart rate response to exercise (Table 3), the discrepant effect of β -adrenergic blockers on mouse versus on human CPVT warrants attention. Mice are highly sympathetic animals and therefore should have a lower arrhythmia threshold. Beta-1 adrenergic receptor may become insensitive to propranolol but may still be amenable to catecholamine stimulation.²⁹ Other adrenergic receptors, which are not affected by propranolol, may mediate or contribute to arrhythmia in mice, for instance β_3 or α -adrenoceptor.³⁰

The Ca²⁺ channel blocker verapamil had a dose-dependent and genotype-dependent effect against murine CPVT. High doses of 2.5 and 5 mg/kg were needed to abolish arrhythmia in the CASQ2 D307H and KO mice, respectively. The recommended dose for blocking LTCC, 1 mg/kg,³¹ had only a partial antiarrhythmic effect in D307H mice and was ineffective in the KO. High concentrations of verapamil block other ion channels besides LTCC. Antagonism of sodium channel activity,²¹ the delayed rectifier (I_{Kr}) K⁺ channel,²² the ryanodine receptor,²³ and the NCX²⁴ were postulated as nonspecific effects of verapamil. To evaluate the contribution of LTCC blockade as opposed to other mechanisms, we repeated the experiment with a more specific LTCC blocker, dihydropyridine nifedipine. Nifedipine had a partial antiarrhythmic effect, implying that (1) LTCC does contribute to CPVT pathogenesis and (2) additional mechanisms participate in VT suppression by high-dose verapamil.

Propranolol markedly potentiated the antiarrhythmic action of verapamil, implying a synergistic activity (Figure 3). Sympathetic signaling increases the I_{Ca} as well as ryanodine channel open probability through phosphorylation of LTCC and RyR2, respectively.^{32,33} Blocking these pathways by propranolol is expected to cooperate with the inhibition of both currents by verapamil.³⁴ Decreasing the Ca²⁺ content in the SR²⁶ and of the RyR2 open probability should prevent diastolic Ca²⁺ waves and delayed after-depolarizations. Table 3 shows that the antiarrhythmic activity in mice was independent of the drug effect on heart rate at rest or exercise.

We further investigated whether the synergistic antiarrhythmic activity between β -receptor and Ca²⁺ channel blockers exists also in humans. Recent experience suggests that adding verapamil to β -blockers helps to prevent exercise-induced ventricular arrhythmia in nongenotyped patients.³⁴ The results of combining propranolol with verapamil in CPVT patients homozygous for the CASQ2^{D307H} mutation are reported in Table 4. Adding verapamil to symptomatic CPVT patients on maximally tolerated β -blockade helped five of 11 patients by attenuating arrhythmia and prolonging exercise duration. Severe bradycardia, a potential complication of this drug combination, was not seen in these young individuals but might be expected with prolonged therapy and in older subjects. Combining β -blockers

with dihydropyridine LTCC blockers may diminish this concern without compromising efficacy. Alternatively, propranolol combined with verapamil can be helpful and safe in those already protected by an ICD. We suggest that verapamil may be tried in severely symptomatic patients, in particular those with VT storms who are otherwise candidates for stellate ganglion ablation.³⁵

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ABBREVIATIONS

biVT	bidirectional ventricular tachycardia
CPVT	catecholaminergic polymorphic ventricular tachycardia
ECG	electrocardiogram
ICD	implantable cardioverter-defibrillator
KO	knockout
LTCC	L-type Ca^{2+} channel
mVT	monomorphic ventricular tachycardia
NCX$\text{Na}^+/\text{Ca}^{2+}$	exchanger
NSVT	nonsustained ventricular tachycardia
PVC	premature ventricular complex
pVT	polymorphic ventricular tachycardia
SR	sarcoplasmic reticulum
SVT	sustained ventricular tachycardia
VT	ventricular tachycardia
WT	wild type

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Figure 1.

Examples of murine CPVT and the principal effects of drugs. Representative heart rhythm traces in mice undergoing the stress protocol: **A:** adult WT and **(B)** *CASQ2*^{-/-} (KO). WT mice did not have arrhythmia. KO mouse had severe arrhythmia: ventricular premature beats, bigeminy, and couplets (*stars*) at rest, polymorphic VT during exercise (*middle panel*), and monomorphic VT with epinephrine (*lower panel*). Arrows indicate atrioventricular dissociation. **C:** Representative traces from a KO mouse treated by verapamil (2.5 mg/kg) and **(D)** propranolol (10 mg/kg). Verapamil completely abolished the arrhythmia, while propranolol was ineffective. Note the bidirectional VT in the stressed propranolol-treated mouse.

Figure 2.

Dose-dependent effect of verapamil on VT prevalence in CASQ2 mutant mice. Control (no drug); WT: wild type mice; D307H: CASQ2^{D307H}; KO: CASQ2^{-/-}. #*P* < .01 and **P* < .001 compared with untreated mice. Numbers of mice tested and mice having VT are provided next to each column.

Figure 3.

Combining propranolol and verapamil for CPVT prevention. *CASQ2*^{-/-} (KO) mice were treated IP with propranolol (10 mg/kg) and/or verapamil (1 mg/kg) and were subjected to the stress protocol. While treatment by any of these agents had no effect on arrhythmia prevalence, their combination completely prevented VT. $\chi^2 \sim P < .0001$ comparing VT prevalence on combined therapy versus no therapy. Numbers of mice tested and mice having VT are provided next to each column.

Table 1

Arrhythmia recorded in CASQ2 mutant mice at rest, exercise and pharmacological stress

Genotype	No. of mice	PVC, %	Bigeminy, %	Complex PVC, %	NSVT, %	SVT, %	MVT, %	PVT, %	BiVT, %
Rest:									
WT	7	14	0	0	0	0	0	0	0
D307H	12	83	25	42	58	50	0	42	25
KO	14	86	43	36	79	50	0	36	57
Stress:									
WT	7	43	0	0	0	0	0	0	0
D307H	12	100	33	42	83	50	8	67	42
KO	14	100	7	36	100	100 ^a	29	93	64
Epinephrine:									
WT	7	14	0	0	0	0	0	0	0
D307H	12	92	0	50	83	33	17	67	8
KO	14	100	0	21	100	86 ^a	14	64	43

Note: KO mice had more SVT. Complex PVCs: couplets, triplets or salvos.

^a $P < .01$ compared with D307H mice after exercise stress or epinephrine.

Table 2

Effect of antiarrhythmic agents on VT prevalence

Genotype	No. of mice	Rest PVC	Rest VT	Stress PVC	Stress VT	Epi PVC	Epi VT	Total VT (%)
Control:								
D307H	12	11	7	12	10	11	10	10 (83)
KO	14	14	14	14	14	14	14	14 (100)
Mutant	26	25	21	26	24	25	24	24 (92)
Procainamide 300 mg/kg, KO	4	2	1	4	2	4	3	3 (75)
Lidocaine 50 mg/kg:								
D307H	2	1	1	2	2	2	2	2 (100)
KO	3	0	0	3	3	3	3	3 (100)
Flecainide 20 mg/kg:								
D307H	2	2	2	2	2	2	2	2 (100)
KO	4	1	1	3	2	3	3	3 (75)
Total class I antiarrhythmic:								
D307H	4	3	3	4	4	4	4	4 (100)
KO	11	3	2	10	7	10	9	9 (82)
Mutant	15	6	5 ^a	14	11	14	3	13 (87)
Class II propranolol 10 mg/kg:								
D307H	5	2	2	4	4	5	5	5 (100)
KO	6	2	0	6	3	6	5	5 (84)
Mutant	11	4	2 ^b	10	7 ^c	11	10	10 (91)
Class III sotalol 10 mg/kg:								
D307H	5	1	1	3	3	5	5	5 (100)
KO	5	3	0	5	3	4	4	4 (80)
Mutant	10	4	1 ^b	8	6 ^c	9	9	9 (90)
Class IV verapamil 2.5 mg/kg:								
D307H	8	0	0	4	0	0	0	0 (0)
KO	5	2	1	3	1	3	2	2 (40)
Mutant	13	2	1 ^d	7	1 ^d	3	2 ^d	2 (15) ^d

Note: Effects of different antiarrhythmic agents on CASQ2 mutant mice undergoing the stress protocol. Antiarrhythmic agents were grouped according to the Vaughan Williams classification. No arrhythmia was provoked in WT mice. Since in general D307H and KO mice had a similar response to drugs, they were pooled into "Mutant." All class I (Ia–c) drugs were pooled as "Total class I." Data are presented in absolute number of mice or percent mice having VT. Statistical comparisons refer to VT prevalence in treated mice versus no treatment. Epi: epinephrine.

^a $p < .005$.

^b $p < .001$.

^c $p^* < .05$.

^d $p < .0001$.

Table 3

Sinus rate at rest and exercise stress in mice during therapy with β -adrenergic and Ca^{2+} channel blockers or their combination

Heart rate and genotype	Control	Propranolol, (10 mg/kg)	Sotalolol (10 mg/kg)	Verapamil (1 mg/kg)	Verapamil (2.5 mg/kg)	Verapamil (5 mg/kg)	Propranolol (10 μ g/g) + verapamil (1 mg/kg)
Rest:							
WT	636 $\pm$ 80	522 $\pm$ 28 ^a	553 $\pm$ 30 ^a	693 $\pm$ 17	667 $\pm$ 26	—	—
D307H	495 $\pm$ 144	503 $\pm$ 24	467 $\pm$ 76	613 $\pm$ 49	593 $\pm$ 70	—	—
KO	541 $\pm$ 68	445 $\pm$ 34 ^a	431 $\pm$ 96 ^a	548 $\pm$ 44	584 $\pm$ 39	544 $\pm$ 18	435 $\pm$ 29 ^a
Stress:							
WT	715 $\pm$ 27	536 $\pm$ 25 ^b	585 $\pm$ 22 ^b	738 $\pm$ 24	715 $\pm$ 25	—	—
D307H	NA	533 $\pm$ 50	494 $\pm$ 88	644 $\pm$ 37	614 $\pm$ 68	—	—
KO	NA	459 $\pm$ 10	498 $\pm$ 43	615 $\pm$ 28	627 $\pm$ 24	624 $\pm$ 41	433 $\pm$ 35

Note: All data are presented as mean $\pm$ standard deviation. NA: data not available because of sustained or frequent ventricular arrhythmia. All comparisons refer to sinus rate at baseline.

^a $P < .05$.

^b $P < .0001$.

Table 4

Effect of combined therapy by verapamil and propranolol on exercise performance and arrhythmia in CPVT2 patients with CASQ2^{D307H} mutation

	Responders	Nonresponders	P
n	5	5	
Age	13 ± 3	22 ± 9	.11
Sex (male/female)	4/1	1/4	.06
Propranolol dose, mg/kg/day	3.4 ± 0.9	4.5 ± 1.5	.21
Verapamil dose, mg/kg/day	2.8 ± 0.8	3.0 ± 0.5	.76
Exercise test I:			
Resting heart rate, bpm	63 ± 6	58 ± 4	.21
Maximum heart rate, bpm	97 ± 11	103 ± 18	.53
Cause of exercise termination: multifocal PVC/VT	2/3	5/0	
Exercise duration, minutes (Bruce protocol)	9.1 ± 2.6	9.1 ± 3.2	1
Exercise test II:			
Resting heart rate, bpm	61 ± 6	57 ± 4	.30
Maximum heart rate, bpm	111 ± 13	107 ± 16	.71
Cause of exercise termination: multifocal PVC/VT/fatigue	1/0/4	4/1/0	
Maximum heart rate, bpm	13 ± 12	4 ± 3	.14
Exercise duration, minutes (Bruce protocol)	13.1 ± 3.2	9.0 ± 2.9	.07
Exercise duration, minutes	4.0 ± 1.4	-0.1 ± 0.7	.001

Note: Responders: attenuation of exercise-induced arrhythmia and/or increased exercise performance by at least 3 minutes in the presence of verapamil; exercise test I: propranolol therapy; exercise test II: propranolol + verapamil; maximum heart rate: sinus rate at arrhythmia threshold or the maximum rate in the absence of arrhythmia; exercise duration/maximum heart rate: change between test I and test II.