Mutant ryanodine receptors in catecholaminergic polymorphic ventricular tachycardia generate delayed afterdepolarizations due to increased propensity to Ca\(^{2+}\) waves

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- Arrhythmia;
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- Heart failure;
- Sudden death

**Aims** Mutations in cardiac ryanodine receptors (RyR2s) are linked to catecholaminergic polymorphic ventricular tachycardia (CPVT), characterized by risk of polymorphic ventricular tachyarrhythmias and sudden death during exercise. Arrhythmias are caused by gain-of-function defects in RyR2, but cellular arrhythmogenesis remains elusive.

**Methods and results** We recorded endocardial monophasic action potentials (MAPs) at right ventricular septum in 15 CPVT patients with a RyR2 mutation (P2328S, Q4201R, and V4653F) and in 12 control subjects both at baseline and during epinephrine infusion (0.05 μg/kg/min). At baseline 3 and during epinephrine infusion, four CPVT patients, but none of the control subjects, showed delayed afterdepolarizations (DADs) occasionally coinciding with ventricular premature complexes. In order to study the underlying mechanisms, we expressed two types of mutant RyR2 (P2328S and V4653F) causing CPVT as well as wild-type RyR2 in HEK 293 cells. Confocal microscopy of Fluo-3 loaded cells transfected with any of the three RyR2s showed no spontaneous subcellular Ca\(^{2+}\) release events at baseline. Membrane permeable cAMP analogue (Dioctanoyl-cAMP) triggered subcellular Ca\(^{2+}\) release events as Ca\(^{2+}\) sparks and waves. Cells expressing mutant RyR2s showed spontaneous Ca\(^{2+}\) release events at lower concentrations of cAMP than cells transfected with wild-type RyR2.

**Conclusion** CPVT patients show DADs coinciding with premature action potentials in MAP recordings. Expression studies suggest that DADs are caused by increased propensity of abnormal RyR2s to generate spontaneous Ca\(^{2+}\) waves in response to cAMP stimulation. Increased sensitivity of mutant RyR2s to cAMP may explain the occurrence of arrhythmias during exercise or emotional stress in CPVT.

**Introduction**

Mutations in the cardiac ryanodine receptor (RyR2) gene have been reported to underlie an autosomally dominantly inherited tachycardia syndrome called catecholaminergic polymorphic ventricular tachycardia (CPVT).\(^1,2\) CPVT is characterized by exercise- or stress-related ventricular tachycardia episodes in the absence of structural heart disease.\(^3\) Patients have syncopal events and a high probability of sudden cardiac death, with a mortality of ~30% by the age of 30 years. Arrhythmias include multifocal ventricular premature beats, bidirectional ventricular tachycardias, and polymorphic VTs.\(^3\) Although the molecular events underlying CPVT have been studied earlier in detail,\(^4,5\) the exact electrophysiological and cellular mechanisms of ventricular arrhythmias remain elusive.

RyR2s are expressed on the sarcoplasmic reticulum (SR) of cardiomyocytes, where they form intracellular Ca\(^{2+}\) release channels that play a central role in excitation-contraction (EC) coupling.\(^6\) We have earlier reported three unrelated families with missense mutations in RyR2 (RyR2-P2328S, RyR2-Q4201R, and RyR2-V4653F). All the identified mutations co-segregated with the clinical phenotype of a family history of sudden death, frequent ventricular premature depolarizations, or polymorphic ventricular arrhythmias.

In single-channel recordings, all three mutant RyR2s showed a significant gain-of-function abnormality due to the decreased binding affinity of the stabilizing FKBP 12.6 protein.\(^5\) However, the stabilizing role of FKBP 12.6 in CPVT has not been universally accepted.\(^7,8\) As an alternative
explanation for arrhythmias, Jiang et al.⁹ have suggested that RyR2 mutations have increased sensitivity to luminal Ca²⁺ activation and increased propensity for spontaneous Ca²⁺ release from the SR. Thus, during exercise sympathetic nervous system activation increases cAMP dependent protein kinase A (PKA) activity, resulting in increased SR Ca²⁺ load and arrhythmia triggering.

Although the molecular mechanism in CPVT is known to be the gain-of-function mutation in RyR2, the exact mechanism of relationship linking increased RyR2 activity and arrhythmias is not clear. Based on earlier results from experimental animal studies,¹⁰ we and others have hypothesized that CPVT patients have delayed afterdepolarizations (DADs) due to uncontrolled release of Ca²⁺ during diastole.⁷,⁸

In the present study, this hypothesis was tested by measurement of monophasic ventricular action potentials (MAPs) in the patients carrying RyR2 mutations. In addition, two of the mutant (RyR2-P2328S and RyR2-V4653F) as well as wild-type RyR2 (RyR2-wt) were expressed in human embryonic kidney (HEK 293) cells to verify the defect in Ca²⁺ release. Ca²⁺ signals were recorded with confocal imaging to clarify how mutant RyR2s respond to PKA stimulation in comparison with the wild-type channel.

Methods

Study subjects

We studied 15 CPVT patients and 12 age-matched controls. For this study, we reviewed all three Finnish families with a known RyR2 mutation. There were 22 affected live family members in these families who showed evidence of the disease and were 10 years or older. One patient had another serious disease and six patients refused the electrophysiology study. MAP recording was performed at the end of the routine electrophysiological examination.

We recruited control subjects from patients undergoing an electrophysiology study to assess the efficacy of previous catheter ablation of concealed atrioventricular accessory pathways during the same time period as patients carrying RyR2 mutations were studied. We included 18- to 50-year-old patients with normal QT interval and no structural heart disease and who did not have β-blockers or any other medication known to influence cardiac repolarization. Using these criteria, 12 consecutive patients were included. The MAP recording protocol was performed at the end of the routine electrophysiological examination.

The research protocol had been reviewed and approved by the Ethics Review Committee of the Department of Medicine, University of Helsinki, and written informed consent had been obtained from all study subjects. The procedures followed were in accordance with institutional guidelines.

Recording of monophasic action potentials and measuring of DADs

A bipolar silver–silver chloride catheter (model 006248, Bard Inc., Lowell, MA, USA) was introduced through a femoral vein and advanced into the right ventricle against the right ventricular septum under fluoroscopic guidance to record MAPs.¹¹ Three quadriripolar electrophysiological catheters (Bard Electrophysiology, Lowell, MA, USA) were placed in the right atrium, atrioventricular junction, and the right ventricular apex. Blood pressure was measured using a fluid manometer from the femoral artery. MAP signals amplified and filtered at a frequency from 0.05 to 250 Hz, standard 12-lead ECG and femoral arterial pressure were stored digitally at a sample rate of 1 kHz (Cardiolab, Prucka Engineering, Sugar Land, TX, USA).

MAPs were obtained during both sinus rhythm and during atrial pacing at a constant cycle length of 600 ms after placement of the catheter electrode in a position providing continuous recordings with a stable amplitude, smooth configuration, and isopotential diastolic baseline. Once the catheter was stabilized, stable MAPs were obtained in all patients. Study data were obtained at baseline and during infusions of epinephrine. For safety reasons, we limited epinephrine infusion to a target rate of 0.05 μg/kg of body weight per minute, which was reached stepwise while arrhythmias were carefully monitored.

DAD was defined as an abnormal, low-amplitude afterdepolarization occurring after repolarization is completed.¹² The amplitude of a DAD was required to be >0.2 mV and >3% of the action potential amplitude in five consecutive beats to be included in the measurements. The coupling interval of the DAD was defined as the interval from the upstroke of the preceding action potential to the peak of the DAD.¹³ We also recorded the diastolic upslope of the DAD by measuring the mean rate of rise (dV/dt) of the ascending limb of the DAD as a mean of three consecutive beats.

Site-directed mutagenesis

Point mutations P2328S and V4653F were generated in small fragments of RyR2 cDNA with Chameleon Double-Stranded, Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, California). The P2328S mutation was induced into a fragment comprising the nucleotides 2472-7678, and V4653F into a fragment consisting of the nucleotides 11756-15257. The following mutagenesis primers were used: P2328S, 5'-aga ttg ctc att cgg cca tcg gag tgt ttg gcct g-3' (includes conservative substitutions to generate a restriction site); V4653F, 5'-gg gac aata cta ggct ggt ttt ggt cct g-3' (includes conservative substitutions to generate a restriction site); V4653F, 5'-gg gac aata cta ggct ggt ttt ggt cct g-3' (includes conservative substitutions to generate a restriction site); V4653F, 5'-gg gac aata cta ggct ggt ttt ggt cct g-3' (includes conservative substitutions to generate a restriction site); V4653F, 5'-gg gac aata cta ggct ggt ttt ggt cct g-3' (includes conservative substitutions to generate a restriction site).
aaa tat gga gag ttc tac ggc cg-3'. The fragments with the desired mutations were subcloned back to their original positions in a full-length RyR2 cDNA in the pCMV5 expression vector with BstEII and KpnI (P2328S), or by two steps with Xhol and PpuMI, and Nhel and Sall (V4653F).

**Ryndarone receptor expression**

HEK 293 cells were grown in DMEM medium (Biological Industries, Israel) supplemented with 10% fetal calf serum, penicillin (50 IU/mL) + streptomycin (50 μg/mL) (ICN Biomedicals Inc., Aurora, Ohio, USA), HEPES (15 mM), sodium pyruvate (1 mM), L-glutamine (2 mM). Cells were maintained in 100 mm tissue culture dishes at +37°C under 5% carbon dioxide and they were split every second day. RyR2 cDNA was centrifuged and removed from the buffer solution. Transfection was carried out by a Ca²⁺ phosphate precipitation method using 2 μg of RyR2 cDNA for each 40 mm diameter round glass coverslip. The coverslips were treated with polylysine (1 mg/100 mL) for 1 h to allow attachment of the cells on the surface of the glass.

**Intracellular Ca²⁺ recordings**

Confocal Ca²⁺ measurements with HEK 293 cells were performed 24-30 h after the transfection. Experiments were carried out at room temperature (20-23°C). Cells were loaded on coverslips with 2 μM Fluo-3-AM (Molecular Probes, Leiden, The Netherlands) in buffer solution (150 mM NaCl, 5.4 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 5 mM HEPES) for 30 min in dark, followed by a 20 min de-esterification period in the same buffer solution. After the loading, the coverslip was mounted on the stage of a Nikon Eclipse TE300 inverted microscope attached to an Ultra View confocal imaging system (PerkinElmer Life Sciences, Cambridge, UK) with an 40x objective. Fluo-3 was excited using a 488 nM laser line and the emitted fluorescence was collected at wavelengths >505 nM. Confocal images were acquired using a CCD-camera at an image frequency of 10 frames/s. For the recordings a view of 10-20 cells was selected. After recording the baseline activity, the cells were stimulated with caffeine (5 mM) or cAMP analogue (DiocyanoylcAMP) by changing rapidly the external solution. The recordings were analysed off-line (NIH Image) and absolute values for Ca²⁺ were calculated as described earlier. A Kd value of 810 nM of fluo-3 was used for the calculations.

**Statistical analysis**

Continuous variables are presented as means ± SD or median (min-max). Differences in the normally distributed variables were assessed using the t-test and the paired t-test for dependent variables. Wilcoxon Signed Ranks test was used as a nonparametric test. All tests were two-sided and a probability value of P < 0.05 was considered statistically significant. No corrections were used to account for the potential inflation due to multiple testing. Categorical variables were assessed by the χ² test. The data were analysed using Systat statistical software version 9.

**Results**

**Patient characteristics**

The two study groups did not differ with regard to age or gender. The mean age of the CPVT patients was 34 ± 16 years and that of the control patients 38 ± 11 years; 6/15 of the CPVT patients and 6/12 of the control patients were male. Systolic blood pressure was also similar in the study groups (Table 2). There was a tendency to longer QT intervals in the CPVT patient group (434 ± 20 ms) in comparison with the control patients (418 ± 28 ms). All patients exhibited polymorphic ventricular extrasystoles or short runs of polymorphic ventricular tachycardia during the exercise test (Table 1).

**Monophasic action potential recordings**

The duration and the amplitude of MAPs did not differ between the study groups (Table 2). Epinephrine infusion increased heart rate in both study groups, but systolic blood pressure remained unchanged (Table 2). Epinephrine tended to shorten the duration of MAPs in the CPVT patient group (Table 2). The MAP amplitude tended to be lower during epinephrine infusion than during baseline, presumably because MAPs have a tendency to decrease their amplitude over time. During baseline MAP recordings, DADs were observed in 3 out of 15 (20%) CPVT patients. DADs were recorded in two patients carrying the RyR2-P2328S mutation and in one patient carrying the RyR2-Q4201R mutation. Figure 1 shows a distinct DAD in the MAP recording of a CPVT

| Table 2 Effects of epinephrine on systolic blood pressure, sinus cycle length, and monophasic action potential |
|---------------------------------------------------------------|------------------|------------------|
| Systolic blood pressure (mmHg)                                | CPVT patients (n = 15) | Control subjects (n = 12) | P-value |
| Baseline                                                      | 146 ± 29          | 139 ± 21          | 0.49    |
| Epinephrine                                                   | 144 ± 25          | 138 ± 21          | 0.58    |
| Sinus cycle length (ms)                                       |                  |                  |         |
| Baseline                                                      | 825 ± 104         | 918 ± 143         | 0.10    |
| Epinephrine                                                   | 692 ± 63          | 670 ± 130         | 0.64    |
| P-value                                                       | 4.9 x 10⁻⁵        | 5.3 x 10⁻⁷        |         |
| Duration of monophasic action potential during atrial pacing at a cycle length of 600 ms (ms) |                  |                  |         |
| Baseline                                                      | 306 ± 18          | 292 ± 17          | 0.12    |
| Epinephrine                                                   | 298 ± 18          | 293 ± 18          | 0.65    |
| P-value                                                       | 0.06              | 0.68              |         |
| Amplitude of monophasic action potential (mV)                 |                  |                  |         |
| Baseline                                                      | 11.8 ± 6          | 12.5 ± 4          | 0.75    |
| Epinephrine                                                   | 9.0 ± 4           | 8.7 ± 4           | 0.89    |
patient carrying the P2328S mutation. In the three CPVT patients, the DADs were continuously present showing minor beat-to-beat variation of the amplitude. One patient exhibited occasional double and triple oscillations of the DADs (Figure 2). We observed no DADs in any of the 12 control subjects. The three CPVT patients with DADs in MAP recordings at baseline had a significantly lower threshold heart rate for ventricular premature beats in exercise stress testing than the patients with no DADs (107 ± 21 vs. 131 ± 16 beats per minute, respectively, \( P < 0.05 \)).

During the low dose epinephrine infusion four out of the 15 (27%) CPVT patients showed DADs. DADs were observed in the same three patients who exhibited DADs during baseline and in one additional patient carrying the RyR2-P2328S mutation. We were not able to demonstrate DADs in patients carrying the RyR-V4653F mutation during baseline or after the low dose epinephrine infusion. Epinephrine tended to increase the relative amplitude and the slope of DADs (Table 3, Figure 3). We observed occasional premature ventricular beats in exercise stress testing at similar threshold heart rates, 130 ± 19 beats per minute and 130 ± 15 beats per minute, respectively. Epinephrine infusion did not induce DADs in any of the control subjects.

### Verification of RyR2 expression in HEK 293 cells by caffeine

Expression vectors for RyR2-wt, RyR2-P2328S, and RyR2-V4653F were transfected into HEK 293 cells, followed by an examination of the cells by confocal Ca\(^{2+}\) imaging. Functional activity of expressed RyR2s was verified by intracellular Ca\(^{2+}\) release by caffeine, an activator of RyR2 Ca\(^{2+}\) release channels. HEK 293 cells do not have endogenous RyR expression, and therefore any observed Ca\(^{2+}\) release in response to caffeine exposure is supposed to originate from transfected RyR2s. We found no caffeine-induced Ca\(^{2+}\) release in nontransfected cells, which is in agreement with the lack of endogenous RyR expression in HEK 293 cells.\(^{14}\)

Sarcoplasmic reticulum Ca\(^{2+}\) loading was investigated by examining the characteristics of caffeine-induced Ca\(^{2+}\) release. HEK 293 cells transfected with RyR2-wt, RyR2-P2328S, and RyR2-V4653F responded to caffeine with a rapid global increase of cytoplasmic [Ca\(^{2+}\)]. The peak of the caffeine-induced Ca\(^{2+}\) release was not significantly different in the cells transfected with RyR2-wt, RyR2-P2328S, and RyR2-V4653F.
RyR2-P2328S, or RyR2-V4653F (363 ± 68, 299 ± 59, and 274 ± 33 nM, respectively) when stimulated with a 5 mM caffeine solution at the end of each experiment. Both wt- and mutant RyR2 plasmids had transfection efficiencies of ~50%. These results suggest that the studied RyR2 mutations did not cause a significant change in SR Ca\(^{2+}\) content when compared with wild-type RyR2s.

At baseline recordings (1.8 mmol/L extracellular [Ca\(^{2+}\)]), confocal imaging of fluo-3-loaded cells transfected with any of the RyR2 plasmids displayed an extremely low number of spontaneous Ca\(^{2+}\) transients, and we found no difference in the occurrence of Ca\(^{2+}\) release events between the cells transfected with wild-type or mutant RyR2s. Only two isolated Ca\(^{2+}\) sparks were observed in the baseline recording of 43 cells. These events localized to a small region in the cell, similar to what has been reported earlier with RyR1 and RyR3 expression in HEK 293 cells. No spontaneous or caffeine stimulated subcellular Ca\(^{2+}\) release events were observed in non-transfected control cells.

Sensitivity of transfected HEK 293 cells to dioctanoyl-cAMP

The characteristic induction of arrhythmias in CPVT patients is thought to occur through stimulation of the adrenergic system. To simulate sympathetic stimulation, cells were stimulated with dioctanoyl-cAMP (di-cAMP). di-cAMP is a cell permeable cAMP analogue that mimics the action of endogenous cAMP with approximately 100 times higher activity.

Figure 5 shows representative Ca\(^{2+}\) imaging recordings of HEK 293 cells transfected with wild-type and mutant RyR2 plasmids.
Ca$^{2+}$ and V4653F, baseline Ca$^{2+}$ concentrations of di-cAMP (50 nM) triggered Ca$^{2+}$ sparks and waves at all of the di-cAMP concentrations used (1, 10, and 50 μM). The increase in the number of Ca$^{2+}$-transients with 1 and 10 μM di-cAMP was observed without an increase in the baseline Ca$^{2+}$-level in cells transfected with RyR2-P2328S (103 ± 12 vs. 103 ± 4 and 108 ± 20 nM) or RyR2-V4653F (102 ± 8 vs. 109 ± 20 and 107 ± 9 nM). The highest di-cAMP concentration increased the basal cytosolic Ca$^{2+}$ level by 50 ± 38 nM in RyR2-V4653F and 27 ± 14 nM in RyR2-P2328S transfected cells (P < 0.05). None of the untransfected cells showed any Ca$^{2+}$-events in response to stimulation with di-cAMP.

Quantitative analysis of n = 590 cells transfected with RyR2-wt or mutant RyR2 is illustrated in Figure 6. A significantly larger amount of cells transfected with mutant RyR2s responded with Ca$^{2+}$ waves when compared with cells transfected with wild-type RyR2s when stimulated with 10 or 50 μM di-cAMP (P < 0.001).

Discussion

Main findings

Our results show that CPVT patients carrying a RyR2 mutation display DADs in right ventricular endocardial MAP recordings whereas healthy control subjects do not exhibit corresponding DADs at similar conditions. The DADs coincide with separate late-appearing U waves in the surface ECG, and a premature ventricular beat occasionally occurred on a DAD. Cells transfected with two disease-causing missense RyR2 mutations showed spontaneous Ca$^{2+}$ release events at lower concentrations of di-cAMP than cells transfected with a wild-type RyR2 receptor.

DAD in arrhythmogenesis

DADs are caused by nonelectrically driven Ca$^{2+}$ events depolarizing the membrane potential during diastole by activating the Na/Ca exchanger and Cl$^{-}$ currents. When the membrane potential reaches the threshold, a premature action potential is triggered initiating the arrhythmia. DAD-mediated triggered activity is believed to play an important role in arrhythmias associated with strong sympathetic stimulation, heart failure, and RyR2 mutations. In the clinic, patients with a RyR2 mutation typically show multiformal ventricular premature complexes and relatively slow polymorphic ventricular tachycardia during exercise, as was also observed in our patients. The polymorphic ventricular tachycardia is expected to be caused by multiple spontaneous Ca$^{2+}$ release events causing DAD-induced firing of multiple sites in the ventricles, as recently reported by Katra and Laurita. However, the transition of the slow polymorphic ventricular tachycardia to ventricular fibrillation may be related to other mechanisms.

The fact that DADs measured at one endocardial site in our study was observed in only three of 15 CPVT patients at baseline is consistent with an experimental finding, indicating a regional distribution of DAD development. Our MAP recording may show a far field signal of DAD; therefore, the regional distribution of DAD development may also explain why no amplification of DAD was observed at the time of premature ventricular beat. On the other hand, abnormal Ca$^{2+}$ handling may not regularly manifest in all CPVT patients during resting conditions. Furthermore, patients having DADs at baseline showed premature ventricular complex at a lower heart rate than others. This finding advocates that observation of DADs in CPVT patients at resting conditions is associated with an increased likelihood of ventricular premature beats and polymorphic ventricular tachycardia.

Molecular background of DADs

Reported RyR2 mutations cluster in the following three areas of RyR2: N-terminal (amino acids 176–433), central (2246–2504), and C-terminal region (4104–4653). The three RyR2 mutations studied here are from three unrelated families and result in a structural and functional phenotype with an increased open probability and gating consistent with a gain-of-function of the RyR2 receptor phenotype. In the present study, DADs were recorded from three patients carrying a P2328S mutation and one patient carrying a Q4201R mutation, but we were not able to show DADs in V4653F mutation carriers.

The P2328S missense mutation is located near the binding site of the regulatory protein calstabin 2, whereas the V4653F mutation is in the pore region of the RyR2. It remains unclear whether this difference in the location of the mutation results in a different phenotype between P2328S and V4653F carriers. Both P2328S and V4653F mutation carriers have similar mortality rates of 30–33% by the age of 35 years. During exercise testing, V4653F mutation carriers had ventricular premature beats in ECG at the same threshold heart rate as P2328S carriers. When expressed in HEK 293 cells, both mutations had an increased propensity to Ca$^{2+}$ waves during PKA stimulation. It is probable that the low-dose adrenaline infusion in the present study was not sufficient to produce DADs in V4653F mutation carriers.

Figure 6  Quantitative analysis of spontaneous Ca$^{2+}$ release events in HEK 293 cells transfected with wild-type (n = 209) or mutant (P2328S, n = 170 and V4653F, n = 211). RyR2s. Bars represent percentage of cells showing Ca$^{2+}$ waves in response to the addition of di-cAMP (1, 10, and 50 μM).
carriers or that our approach to record MAP at one endocardial site is not sufficient to expose DADs occurring at different locations. An alternative explanation would be that arrhythmias in V4653F carriers are generated by another mechanism than DADs.

In order to directly compare the impact of RyR2-P2328S and V4653F mutations on Ca\(^{2+}\) handling, plasmids carrying mutant and wild-type receptors were transfected into HEK 293 cells. At baseline, both the wild-type and the RyR2 carrying CPVT mutations showed an extremely low frequency of spontaneous Ca\(^{2+}\) release events. This was in contrast to an earlier report of increased basal activity of RyR2s carrying a gain-of-function mutation.\(^{24}\) The lower baseline activity in our experiments may be explained by differences in the molecular characteristics of the studied mutations. Loading of the Ca\(^{2+}\) stores at baseline may also affect the results. Stimulation with a cAMP analogue unmasked the defect in RyR2s, and cells transfected with either RyR2-P2328S or V4653F plasmids showed an increased number of spontaneous Ca\(^{2+}\) release events such as Ca\(^{2+}\) sparks and waves when compared with cells transfected with wild-type RyR2.

An increase in baseline Ca\(^{2+}\) levels were detected only after the highest level of PKA stimulation, and a similar rise in cytosolic Ca\(^{2+}\) was measured in the cells transfected with native and mutant channels. These results suggest that Ca\(^{2+}\) oscillations are not caused by an increase in baseline Ca\(^{2+}\) due to a ‘leaky’ channel, but rather by an increased propensity to Ca\(^{2+}\) waves in response to cAMP stimulation. cAMP may increase RyR2 receptor opening probability either by dissociating the stabilizing protein FKBP 12.6\(^{25,26}\) or by increasing SR Ca\(^{2+}\) load. It was recently demonstrated that RyR2s carrying CPVT mutations demonstrate increased sensitivity to luminal Ca\(^{2+}\).\(^{9,25}\) cAMP stimulation increases Ca\(^{2+}\) load in the SR and may hence trigger arrhythmias in patients carrying a CPVT mutation.

Abnormal luminal Ca\(^{2+}\) activation might explain the observations of multiple repetitive Ca\(^{2+}\) waves with declining amplitude in HEK 293 cells transfected with mutant RyR2s causing CPVT. Reflecting this phenomenon, repetitive DADs were also displayed in MAP recordings (Figure 2). The multiple DADs occurred with decreasing amplitude similarly to what has been previously reported in single cell studies provoked by Ca\(^{2+}\) overload.\(^{26}\) In cardiac myocytes, repetitive Ca\(^{2+}\) release may occur when luminal Ca\(^{2+}\) reaches a certain threshold during Ca\(^{2+}\) removal from the cytosol.

Study limitations
Due to safety reasons, we used only a relatively low dose (0.05 μg/kg/min) of epinephrine infusion resulting in an average heart rate of 87 beats per minute in CPVT patients and 90 beats per minute in control patients. Thus, the heart rates achieved remained well below the clinical target rate of 127 beats per minute before premature ventricular complexes appeared during the exercise test. This might have explained why DADs and premature ventricular complexes were not observed in all patients carrying RyR2 mutation. We recorded DADs at one endocardial site only. The observed DADs were of low amplitude and the ventricular premature complexes recorded on DADs may thus also be coincidental. We are aware of the mechanical artifacts which may cause oscillations in MAP recordings resembling DADs. Our previous experience in long QT syndrome\(^ {27}\) and the fact that our control subjects did not exhibit DADs support the idea that the DADs observed in the CPVT patients are real events.

In cardiac myocytes, RyR2s are grouped in the SR membrane near the T-tubule system. HEK293 cells lack a T-tubule system, which may have an effect on the organization and function of the channels. Furthermore, Ca\(^{2+}\) events in HEK293 are much slower in comparison with cardiac myocytes. Additional exploration of RyR2 mutations in a cardiac myocyte model is therefore implicated in future studies.

Conclusions
In conclusion, our results show that patients carrying an inherited gain-of-function defect in RyR2s display DADs coinciding with premature action potentials. By expressing RyR2s in a cell model, we showed that mutations increase the probability of spontaneous Ca\(^{2+}\) waves during adrenergic stimulation. We suggest that arrhythmias are caused by Ca\(^{2+}\) oscillations generating DADs rather than by an increase in the basal cytosolic Ca\(^{2+}\) level. The increased sensitivity of RyR2s to cAMP may explain the occurrence of clinical arrhythmias in CPVT during exercise or emotional stress.

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References


