Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disorder characterized by abnormal Ca\textsuperscript{2+}-handling leading to catecholamine-induced Ca\textsuperscript{2+} leak, ventricular arrhythmias, and sudden cardiac death in young and otherwise healthy individuals. Most CPVT cases (CPVT1) are associated with dominant mutations in the cardiac ryanodine receptor gene (RyR2). The minority of cases results from autosomal recessive mutations in the cardiac calsequestrin gene (CASQ2), termed collectively CPVT2. The study of CPVT-related mutations provided invaluable insights on normal Ca\textsuperscript{2+}-handling in cardiomyocytes and the arrhythmogenic mechanisms of this disorder. Nevertheless, the study of this disorder has been hampered by the lack of appropriate human cardiomyocyte models and by the inability to study this disease in a patient-specific manner.

The introduction of the human induced pluripotent stem cells (hiPSCs) technology offers a unique opportunity to overcome these obstacles. This approach allows reprogramming of patient-specific somatic cells into pluripotent stem cells that can be coaxed to differentiate into several cell types including cardiomyocytes. In the cardiac field, hiPSCs were established from healthy individuals and from patients inflicted with acquired and several types of inherited cardiac disorders. Patient-specific hiPSC-derived cardiomyocyte (hiPSC-CM) models of different inherited arrhythmogenic syndromes (including the long-QT, Brugada, and sodium channel overlap syndromes; arrhythmogenic right ventricular cardiomyopathy; and both types of CPVT) were established and used to recapitulate the disease phenotype and drug response in the culture dish, to provide novel insights into disease and drug therapy mechanisms, and potentially to tailor patient-specific drug therapy.

**Background**—Catecholaminergic polymorphic ventricular tachycardia type 2 (CPVT2) results from autosomal recessive CASQ2 mutations, causing abnormal Ca\textsuperscript{2+}-handling and malignant ventricular arrhythmias. We aimed to establish a patient-specific human induced pluripotent stem cell (hiPSC) model of CPVT2 and to use the generated hiPSC-derived cardiomyocytes to gain insights into patient-specific disease mechanism and pharmacotherapy.

**Methods and Results**—hiPSC cardiomyocytes were derived from a CPVT2 patient (D307H-CASQ2 mutation) and from healthy controls. Laser-confocal Ca\textsuperscript{2+} and voltage imaging showed significant Ca\textsuperscript{2+}-transient irregularities, marked arrhythmogenicity manifested by early afterdepolarizations and triggered arrhythmias, and reduced threshold for store overload–induced Ca\textsuperscript{2+}-release events in the CPVT2-hiPSC cardiomyocytes when compared with healthy control cells. Pharmacological studies revealed the prevention of adrenergic-induced arrhythmias by \(\beta\)-blockers (propranolol and carvedilol), flecainide, and the neuronal sodium-channel blocker riluzole; a direct antiarrhythmic action of carvedilol (independent of its \(\alpha/\beta\)-adrenergic blocking activity), flecainide, and riluzole; and suppression of abnormal Ca\textsuperscript{2+} cycling by the ryanodine stabilizer JTV-519 and carvedilol. Mechanistic insights were gained on the different antiarrhythmic actions of the aforementioned drugs, with carvedilol and JTV-519 (but not flecainide or riluzole) acting primarily through sarcoplasmic reticulum stabilization. Finally, comparable outcomes were found between flecainide and labetalol antiarrhythmic effects in vitro and the clinical results in the same patient.

**Conclusions**—These results demonstrate the ability of hiPSCs cardiomyocytes to recapitulate CPVT2 disease phenotype and drug response in the culture dish, to provide novel insights into disease and drug therapy mechanisms, and potentially to tailor patient-specific drug therapy.

**Key Words:** drug therapy ■ induced pluripotent stem cells ■ myocytes, cardiac ■ stem cell ■ tachycardia, ventricular
WHAT IS KNOWN

- Catecholaminergic polymorphic ventricular tachycardia type 2 (CPVT2) results from autosomal-recessive CASQ2 mutations, causing abnormal Ca\(^{2+}\) handling and malignant ventricular arrhythmias.
- Patient/disease-specific human induced pluripotent stem cell derived cardiomyocytes hiPSC-CMs can recapitulate the abnormal clinical phenotype of a variety of inherited cardiac disorders in the culture-dish including of CPVT2.

WHAT THE STUDY ADDS

- The CASQ2 mutation reduced the threshold for development of SOICR in the CPVT2-hiPSC-CMs supporting a physiological role of CASQ2 in luminal Ca\(^{2+}\) sensing and in RyR2 stabilization.
- The patient/disease-specific hiPSC-CMs model allowed screening of potential therapies and provided novel insights into mechanism of action of several pharmacological agents (propranolol, labetalol, JTV519, carvedilol, flecainide and riluzole) in CPVT2.
- A positive correlation was noted between the in vitro effects of some of the drugs studied in the patient-specific hiPSC-CMs model and the clinical exercise-test results in the same patient, supporting the role of this technology in the field of precision medicine.

Methods

Detailed information on various methods used in this study is available in the Data Supplement.

Generation and Cardiomyocyte Differentiation of the CPVT2-hiPSC-CMs

We used previously well-established healthy control and CPVT2 hiPSC lines,\(^2\) the latter derived from a 19-year-old male patient with CPVT2 because of the D307H-CASQ2 homozygous mutation) can recapitulate the CPVT phenotype in vitro, to provide novel mechanistic insights into the disease process, and offer a unique experimental platform for patient-specific drug testing. To test the latter hypothesis, we evaluated the potential antiarrhythmic effects of several pharmacological agents using the generated CPVT2-hiPSC-CMs model, investigated, and provided novel insights into the mechanism of action of these drugs and correlated the in vitro pharmacological responses of several agents with their in vivo clinical response in the same patient.

Pharmacological Studies

hiPSC-CMs were treated with isoproterenol (10 μmol/L), forskolin (10 μmol/L), carvedilol (0.3–1 μmol/L), propranolol (1 μmol/L), flecainide (1–6 μmol/L), riluzole (10 μmol/L), JTV519 (1 μmol/L), and labetalol (2 μmol/L) all from Sigma. Preparations were incubated with the tested drugs for >10 minutes except for carvedilol (0.3 μmol/L, 2 hours). Forskolin, carvedilol, riluzole, and JTV-519 were dissolved in dimethyl sulfoxide.

In all experiments quantifying arrhythmogenicity, a single ectopic Ca\(^{2+}\) release (Figure 1C) was regarded as the minimal finding defining Ca\(^{2+}\) cycling abnormality, similarly to previous reports.\(^3,22\) Early and delayed afterdepolarization (EADs, DADs, respectively) were defined as low-amplitude depolarizations occurring during phase 3 or phase 4 of the action potential, respectively. Arrhythmogenicity suppression was regarded as the complete resolution of any ectopic Ca\(^{2+}\) release events after drug application, whereas proarrhythmic effects were documented when new Ca\(^{2+}\)-cycling abnormalities occurred in previously normal cells. Similarly, SOICR suppression was regarded as the complete cessation of Ca\(^{2+}\) oscillations after drug application.

Clinical Correlation

Because of suboptimal medical response to maximal β-blocker therapy additional pharmacological solutions were sought and the patient’s response to flecainide and labetalol was evaluated. An exercise test was performed after 2 days of treatment with labetalol alone or flecainide in addition to β-blockers. The patient gave his written informed consent for the study, and the trial was approved by the institution’s ethics committees. Both investigators performing the
Figure 1. CPVT2-hiPSC-CMs are arrhythmogenic. A, Identifying the D307H-CASQ2 homozygous mutation (G/C substitution) in CPVT2-hiPSC-CMs (top). Positive immunostaining of the CPVT2-hiPSC-CMs for cTnI and α-actinin (bottom). B–F, Line-scan tracings depicting changes in intracellular Ca\(^{2+}\) in fluo4-loaded CPVT2-hiPSC-CMs. The CPVT2-hiPSC-CMs demonstrated a variety of Ca\(^{2+}\) abnormalities including local Ca\(^{2+}\)-release events (black arrows, B), broad double-humped transients (red arrows) appearing consistently (top) or intermittently (bottom). C, Multiple consecutive Ca\(^{2+}\) release events (red arrows) occurring while diastolic Ca\(^{2+}\) levels remain elevated (D), and sustained high-frequency oscillating Ca\(^{2+}\)-release events (red arrows; E and F). G and H, Line scan Ca\(^{2+}\) (Continued)
exercise test and the in vitro drug testing were blinded to results of the other study.

Statistical Analysis
Categorical variables were expressed as frequencies. The χ2 test was performed to compare such variables for unpaired groups. The McNemar test was used when paired nominal data were analyzed. P<0.05 was considered statistically significant.

Results
Characterization of Ca2+ Handling in the CPVT2-hiPSC-CMs

CPVT2-hiPSC-CMs Are Arrhythmogenic

The CPVT2-hiPSCs, displaying the D307H-CASQ2 mutation (Figure 1A), were coaxed to differentiate into cardiomyocytes (Figure 1A) using a modification of a directed monolayer differentiation system.31 Laser-confocal Ca2+ imaging of dispersed fluo4-loaded CPVT2-hiPSC-CMs revealed marked calcium-handling abnormalities in 69% of the cells (Figure 1B through 1F) and J. Such abnormalities in the CPVT2-hiPSC-CMs were manifested as multiple diastolic Ca2+ release events (Figure 1B) or as broad transients with ectopic Ca2+ releases ranging in complexity from single (Figure 1C) to multiple high-frequency consecutive Ca2+ release events occurring while diastolic Ca2+ levels remain elevated (Figure 1D through 1F). In contrast, the majority (85%) of healthy control hiPSC-CMs displayed normal Ca2+ cycling (Figure 1G and 1J). The remaining 15% (Figure 1H and 1J) displayed some Ca2+ cycling abnormalities, which were not as complex as those observed in the CPVT2-hiPSC-CMs.

Laser-confocal action-potential recordings, using the voltage-sensitive fluorescent indicator FluoVolt, confirmed that the aforementioned Ca2+ abnormalities led to the development of arrhythmias in the CPVT2-hiPSC-CMs. Thus, 63% of CPVT2-hiPSC-CMs displayed afterdepolarizations (mostly DADs but also EADs) and even triggered arrhythmias (Figure 1I and 1J) compared with only 13% of control hiPSC-CMs after isoproterenol administration. It was previously suggested that Ca2+ release from the sarcoplasmic reticulum (SR) may occur not only after Ca2+ entry via the cell membrane but also in the absence of membrane depolarization in a process termed SOICR.5,21,30,33 It was proposed that some RyR2 mutations might decrease SOICR event threshold.5,21,30,33 We, therefore, evaluated the effect of α-adrenergic activation in the CPVT2-hiPSC-CMs through the application of phenylephrine (10 µmol/L). This resulted in development of new Ca2+-cycling abnormalities in 41% of the phenylephrine-treated CPVT2-hiPSC-CMs (Figure 2C). Interestingly, both the incidence and complexity of such events (Figure 2C) were less severe than that after β-adrenergic activation (Figure 2A).

Store Overload–Induced Ca2+ Release

It was previously suggested that Ca2+ release from the sarcoplasmic reticulum (SR) may occur not only after Ca2+ entry via the cell membrane but also in the absence of membrane depolarization in a process termed SOICR.5,21,30,33 It was proposed that some RyR2 mutations might decrease SOICR event threshold.5,21,30,33 We have previously described the presence of normal SOICR activity in CPVT1-hiPSC-CMs.21 It is not clear, however, whether such a phenomenon could also be modeled in the CPVT2-hiPSC-CMs.

Adrenergic Stimulation

Because arrhythmias in CPVT are usually triggered by exercise or emotional stress, we evaluated the effect of adrenergic pathway activation in the hiPSC-CMs. Isoproterenol (10 µmol/L) application was highly arrhythmogenic in the CPVT2-hiPSC-CMs (Figure 2 A) but not in healthy control hiPSC-CMs (Figure 2B). Thus, focusing on normally beating cells at baseline, we observed the development of new Ca2+-cycling abnormalities in 77% of isoproterenol-treated CPVT2-hiPSC-CMs. In contrast, no new arrhythmias were observed in the healthy control hiPSC-CMs after isoproterenol administration.

It was recently reported that α-adrenergic receptor stimulation might cause ventricular arrhythmias in the CASQ2−/− mouse model.12 We, therefore, evaluated the effect of α-adrenergic activation in the CPVT2-hiPSC-CMs through the application of phenylephrine (10 µmol/L). This resulted in development of new Ca2+-cycling abnormalities in 41% of the phenylephrine-treated CPVT2-hiPSC-CMs (Figure 2C). Interestingly, both the incidence and complexity of such events (Figure 2C) were less severe than that after β-adrenergic activation (Figure 2A).

Drug Screening Using the CPVT2-hiPSC-CMs

We next aimed at using the CPVT2-hiPSC-CMs model to evaluate the potential therapeutic effects and mechanism of action of several pharmacological agents, which could...
theoretically be beneficial in treating CPVT2. Both the suppression of arrhythmias and SOICR were evaluated (Figures 3 and 4). Suppression of arrhythmias was regarded as the complete resolution of any ectopic Ca\textsuperscript{2+} release events after drug application (Figure 3A, 3B, 3D, and 3E, in contrast to Figure 3C and 3F). A proarrhythmic effect was defined when new Ca\textsuperscript{2+}-cycling abnormalities occurred after drug application in previously normal cells.

**Figure 2.** Adrenergic stimulation and store overload–induced Ca\textsuperscript{2+} release (SOICR) in CPVT2 and control human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs). A and B, Ca\textsuperscript{2+}-cycling abnormalities (red arrows) were induced in the CPVT2-hiPSC-CMs by isoproterenol (10 µmol/L; A) but not in control hiPSC-CMs (B). C, Induction of Ca\textsuperscript{2+}-cycling abnormalities (red arrows) in the CPVT2-hiPSC-CMs by phenylephrine (10 µmol/L). D, Line-scan images showing the development of SOICR in the CPVT2- (left) and healthy control (right) hiPSC-CMs when increasing extracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{o}) concentrations. All tracings were recorded from the same cell, respectively. The incidence and complexity of the SOICR events were significantly greater in the CPVT2-hiPSC-CMs at each [Ca\textsuperscript{2+}]\textsubscript{o} concentration. E, Summary of the percentage of oscillating CPVT2- (red) and control- (blue) hiPSC-CMs at increasing [Ca\textsuperscript{2+}]\textsubscript{o} concentrations. Note that the proportion of oscillating cells was significantly higher in the CPVT2-hiPSC-CMs at each [Ca\textsuperscript{2+}]\textsubscript{o} concentration (n=27, 27, 37, 27, 22, 27, 27; P=0.03, 0.01, 0.03, 0.03, 0.01, 0.002, 0.001, respectively) when compared with healthy control cells (n=24 in all [Ca\textsuperscript{2+}]\textsubscript{o} levels). *Statistically significant differences.

**JTV-519**

We first evaluated the effect of JTV-519, a potent RyR2 stabilizer.\textsuperscript{34,35} Application of JTV-519 (1 µmol/L) resulted in significant suppression of the CPVT2-hiPSC-CMs arrhythmic behavior, even when Ca\textsuperscript{2+}-cycling abnormalities were complex at baseline (Figure 3A). Overall, JTV-519 eliminated Ca\textsuperscript{2+}-cycling abnormalities in 72% of arrhythmic CPVT2-hiPSC-CMs (Figure 4A).
Because β-blockers are the mainstay treatment for both CPVT types, we next evaluated their therapeutic potential in the CPVT2-hiPSC-CMs model. Administration of propranolol (1 µmol/L) or carvedilol (1 µmol/L) before adrenergic stimulation with isoproterenol (10 µmol/L) completely prevented the development of Ca²⁺-cycling abnormalities (Figure 3A and 3B). At lower (and more clinically relevant) concentrations of these β-blockers, some isoproterenol-induced Ca²⁺-cycling abnormalities were still noted, albeit at a much lower frequency than compared with isoproterenol alone (Figure 3B).

It was suggested recently that carvedilol, but not other β-blockers (such as propranolol), may possess direct antiarrhythmic properties in CPVT by stabilizing RyR2. 36,37

**β-Blockers and Combined α/β-Adrenergic Blockade**

Because β-blockers are the mainstay treatment for both CPVT types, we next evaluated their therapeutic potential in the CPVT2-hiPSC-CMs model. Administration of propranolol (1 µmol/L) or carvedilol (1 µmol/L) before adrenergic stimulation with isoproterenol (10 µmol/L) completely prevented the development of Ca²⁺-cycling abnormalities (Figure 5A and 5B). At lower (and more clinically relevant) concentrations of these β-blockers, some isoproterenol-induced Ca²⁺-cycling abnormalities were still noted, albeit at a much lower frequency than compared with isoproterenol alone (Figure 5B).

It was suggested recently that carvedilol, but not other β-blockers (such as propranolol), may possess direct antiarrhythmic properties in CPVT by stabilizing RyR2. 36,37

**Figure 3.** Antiarrhythmic drug screening. A–F, Line scan Ca²⁺ recordings from CPVT2-hiPSC-CMs at baseline (top tracings, red arrows indicate abnormal Ca²⁺ cycling) and after application of different pharmacological agents (bottom tracings). Note the antiarrhythmic actions of JTV-519 (A), carvedilol (B), flecainide (D), and riluzole (E). In contrast, propranolol and labetalol (C and F) did not suppress baseline Ca²⁺-cycling abnormalities in CPVT2-hiPSC-CMs.
We also noted a direct antiarrhythmic action of carvedilol, significantly reducing the incidence of Ca\(^{2+}\)-cycling abnormalities in the CPVT2-hiPSC-CMs even in the absence of isoproterenol (Figures 3B and 4A) and using clinically relevant doses (0.3 \(\mu\)mol/L). In contrast, although propranolol effectively prevented isoproterenol-induced arrhythmogenicity (Figure 5A and 5B), it had no effect on eliminating baseline Ca\(^{2+}\)-cycling abnormalities (Figures 3C and 4A). Overall, carvedilol (0.3 and 1 \(\mu\)mol/L) eliminated such abnormalities in \(\approx\)50% of previously abnormal CPVT2-hiPSC-CMs (Figure 4A). In agreement with previous studies,\(^{36}\) a longer incubation period was required to achieve a therapeutic effect with the lower dose. In contrast, propranolol demonstrated no significant reduction in baseline Ca\(^{2+}\)-cycling abnormalities (Figures 3C and 4A).

Labetalol (a mixed \(\alpha/\beta\)-adrenergic blocker) was recently reported to prevent arrhythmias in the CPVT2 mouse model.\(^{32}\) Because phenylephrine was somewhat arrhythmogenic in

![Figure 4. Pharmacological effects on arrhythmogenicity and store overload–induced Ca\(^{2+}\) release (SOICR).](image-url)
Figure 5. Drug effects on adrenergic-induced arrhythmogenicity, postpacing triggered activity, and diastolic Ca^{2+} release (DCR). A, Line-scan recordings from normally beating CPVT2-hiPSC-CMs pretreated with different pharmacological agents, before (left) and after (right) isoproterenol (upper tracings) or phenylephrine (lower tracing) treatments. Note the protective action of carvedilol, propranolol, flecainide, and riluzole against isoproterenol-induced arrhythmias. Labetalol, in contrast, failed to completely prevent phenylephrine-induced arrhythmias (arrows). B, Summary of the isoproterenol- (upper) and phenylephrine- (lower) induced arrhythmogenicity in CPVT2-hiPSC-CMs. Isoproterenol application induced novel arrhythmias in 77% of the cells (10 µmol/L; n=13; P=0.002). Application of (Continued)
Figure 5 Continued. carvedilol and propranolol either completely prevented isoproterenol-induced arrhythmias at high doses (1 µmol/L; n=6; P=0.003 and n=7; P=0.003, respectively) or significantly reduced their incidence at lower concentrations (carvedilol [0.3 µmol/L] to 15%; propranolol [0.5 µmol/L] to 30%; n=13; P=0.005 and n=10; P=0.04, respectively). Similarly, pretreatment with flecainide (6 µmol/L; n=6; P=0.04) and riluzole (10 µmol/L; n=10; P=0.003) also significantly reduced isoproterenol-induced arrhythmia incidence to 17% and 10% respectively, whereas labetolol (2 µmol/L; n=8; P=0.63) was not protective. Phenytoamine application induced novel arrhythmias in 41% of the cells (10 µmol/L; n=17; P=0.01). Carvedilol (1 µmol/L; n=10; P=0.02) and riluzole (10 µmol/L; n=10; P=0.02) applications completely prevented phenylephrine-induced arrhythmia. Pretreatment with labetolol (2 µmol/L) resulted in only a modest (and statistically insignificant) decrease in the incidence of phenylephrine-induced Ca2+-cycling abnormalities (41% to 20%; n=10; P=0.4) whereas propranolol (1 µmol/L; n=10) had no protective effect at all (60% vs 41%; P=0.7). * Statistically significant differences as compared with isoproterenol or phenylephrine alone, respectively. C–E, Forskolin-treated CPVT2-hiPSC-CMs were paced at 1 Hz (vertical indicators). Note the appearance of triggered activity (red arrows) and DCR events (black arrows) in the postpacing period. Note that carvedilol suppressed both DCR and triggered activity events (D), whereas flecainide (C) and riluzole (E) suppressed triggered activity without significantly affecting DCR. F, Summary of flecainide (1 and 6 µmol/L), riluzole (10 µmol/L), and carvedilol (1 µmol/L) effects on triggered activity and DCR events in the postpacing period. Note the significant suppression of triggered activity with application of flecainide (1 µmol/L by 60%; n=10; P=0.03; 6 µmol/L by 94%; n=18; P=0.00001), riluzole (by 100%; n=13; P=0.00002), and carvedilol (by 100%; n=8; P=0.008). Importantly, in contrast to carvedilol that significantly prevented DCR events in 86% of the cells (n=7; P=0.03), flecainide (1 and 6 µmol/L) abolished DCR in only 27% and 30% of the cells (n=11; P=0.25 and n=10; P=0.25, respectively). Similarly, riluzole suppressed DCR events in only 14% of the cells (n=7; P=1). * Statistically significant differences.

Mechanisms of Antiarrhythmic Drug Activities in the CPVT2-hiPSC-CMs

Traditional therapeutic approaches in CPVT focused on either blocking the adrenergic signaling pathway (β-blockers) or on stabilizing RyR2. Newly suggested drugs presented new or unexpected mechanisms. For instance, carvedilol was suggested to stabilize RyR2 beyond its β-blocking action.66,67 Similarly, many recent in vivo and clinical studies suggested a potential role for flecainide in CPVT. Two main mechanisms were suggested (1) flecainide may increase triggered-activity threshold by directly blocking Na+ channels39,44,46 or (2) flecainide may stabilize SR thereby decreasing diastolic Ca2+ leak.74,80,42,44

To provide mechanistic insights into the antiarrhythmic action of the tested pharmacological agents, we initially evaluated their ability to alter SOICR incidence in the CPVT2-hiPSC-CMs as a surrogate for SR stabilization. To provide additional information on their capacity to alter excitability or stabilize the SR, we also evaluated their effects on triggered activity and diastolic Ca2+-release events after pacing.

SOICR Suppression

**JTV519 and Forskolin**

To validate our model, we first evaluated JTV519 and forskolin, based on the known RyR2-stabilizing property of the former and the direct adrenergic activation by the latter. As expected, application of JTV519 (1 µmol/L) resulted in suppression of SOICR events in 46% of oscillating CPVT2-hiPSC-CMs (at [Ca2+]o of 1.8 mmol/L; Figure 4B), whereas forskolin (10 µmol/L) induced Ca2+ oscillations in 55% of previously quiescent CPVT2-hiPSC-CMs (Figure 4B).

**Carvedilol, Propranolol, and Labetolol**

In agreement with recent reports suggesting RyR2-stabilizing properties of carvedilol,66 we found that carvedilol suppressed SOICR in the CPVT2-hiPSC-CMs model, decreasing the percentage of oscillating cells by 37% (0.3 µmol/L) and 44% (1 µmol/L; Figure 4B). Carvedilol was also hypothesized to
Figure 6. Clinical correlation of flecainide and labetalol effects. ECG tracings obtained during exercise testing of the CPVT2 index patient after pretreatment with either flecainide (A) or labetalol (B). Flecainide displayed a significant antiarrhythmic effect, eliminating almost all ventricular ectopy (with only a few remaining isolated premature ventricular complexes, red arrows, appearing at a heart rate of 115 beats per minute during Bruce protocol level 6 workload). Labetalol, in contrast, did not prevent arrhythmias, with development of multiple episodes of polymorphic ventricular tachycardia (red arrow) at a heart rate of 69 beats per minute soon after stress test initiation.
stabilize RyR2 independently of its β/α adrenergic blocking properties. In support of this hypothesis, we found that neither propranolol nor labetalol affected SOICR in the CPVT2-hiPSC-CMs (Figure 4B).

Flecainide
To test whether flecainide may harbor RyR2-stabilizing properties, we exposed oscillating CPVT2-hiPSC-CMs to flecainide at 1 and 6 µmol/L. As presented in Figure 4B, flecainide did not alter SOICR at 1 µmol/L, whereas at 6 µmol/L flecainide suppressed SOICR in 15% of the cells (not reaching, however, statistical significance).

Riluzole
Radwaniński et al.49 showed that riluzole’s antiarrhythmic action in the CPVT mouse model was not associated with RyR2 blocking. Supporting this observation, we found that riluzole (10 µmol/L) application did not alter SOICR events in the CPVT2-hiPSC-CMs (Figure 4B).

Postpacing Triggered Activity and Diastolic Ca2+ Release Events
It was shown that rapid pacing of CPVT cardiomyocytes causes SR Ca2+ overload, which may translate into diastolic Ca2+ release (DCR) events and triggered activity at the post-pacing interval, especially during adrenergic stimulation.21,39 To simulate this scenario, we studied forskolin-treated CPVT2-hiPSC-CMs after pacing. As demonstrated in Figure 5, the CPVT2-hiPSC-CMs developed marked DCR events and triggered activity during the postpacing period (Figure 5C through 5E). Carvedilol (1 µmol/L) completely suppressed triggered activity, concomitantly with marked suppression of DCR events (Figure 5D and 5F). Flecainide (1 µmol/L) also significantly reduced the incidence of triggered activity in the postspacing interval (Figure 5C and 5F). However, its effect on reducing DCR was only modest (not reaching statistical significance, Figure 5C and 5F). This differential effect was even more pronounced with flecainide 6 µmol/L, almost completely abolishing triggered activity, whereas only modestly reducing DCR (Figure 5F). Similarly, riluzole (10 µmol/L) completely suppressed triggered activity with minor (and statistically nonsignificant) reduction of DCR (Figure 5E and 5F).

Clinical Correlation
Finally, we aimed to evaluate the correlation between our in vitro CPVT2-hiPSC-CMs findings and the clinical setting. To this end, we compared the drug-testing results from the patient-specific CPVT2-hiPSC-CMs model with those from the exercise-test studies performed in exactly the same patient. The patient studied had a history of multiple exercise-induced arrhythmias, which were only partially relieved by high-dose β-blocker (320 mg/d propranolol) treatment.

Pretreatment with flecainide (200 mg/d for 2 days), as shown previously in CPVT2 patients,43 resulted in a significant antiarrhythmic effect in our patient. Thus, although a few premature ventricular complexes were still noted during the exercise test (at a heart rate of 115 beats per minute, Bruce protocol level 6), no ventricular tachycardia events were documented (Figure 6A).

In contrast, labetalol treatment (800 mg/d for 2 days) failed protecting against such ventricular arrhythmias, and short runs of polymorphic ventricular tachycardias were frequently recorded during the stress test, initiating already at a heart rate of 69 beats per minute shortly after stress-test initiation (Figure 6B).

Our in vitro results correlated with these clinical findings, with propranolol at clinically relevant concentrations protecting (but not completely preventing) isoproterenol-induced arrhythmia in the CPVT2-hiPSC-CMs. Importantly, flecainide application was also found to be protective against the development of arrhythmias in the CPVT2-hiPSC-CMs, whereas labetalol was ineffective (Figure 5A and 5B).

Discussion
We used a patient/disease-specific hiPSCs-CMs model of the autosomal recessive form of CPVT (because of the D307H-CASQ2 mutation) to provide mechanistic insights into disease pathogenesis and treatment. Our results show that (1) CPVT2-hiPSC-CMs recapitulate the clinical CPVT phenotype in vitro by displaying significant Ca2+-handling abnormalities, diastolic Ca2+-leak, and arrhythmic activity that was aggravated by adrenergic stimulation; (2) the CASQ2 mutation resulted in reduced SOICR threshold in the CPVT2-hiPSC-CMs when compared with healthy control cells; (3) using this patient-/disease-specific approach, we were able to screen several therapeutic agents (propranolol, labetalol, JTV519, carvedilol, flecainide, and riluzole) in clinically relevant concentrations and to provide mechanistic insights into their potential mode of action; and (4) the in vitro effects of some of these agents in the patient-specific hiPSC-CMs were concordant with the clinical exercise-test results in the same patient.

More than a decade has passed since RyR2 mutations were identified as responsible for CPVT12,23 and CASQ2 mutations for CPVT2.9 Studies in mouse models of different CASQ2 mutations (including the D307H-CASQ2 mutation) suggested several possible mechanisms explaining CPVT2 arrhythmogenicity. It was proposed that CASQ2 mutations decrease CASQ2 protein levels and SR Ca2+ buffering or interfere with CASQ2-mediated regulation of RyR2 and luminal Ca2+-sensing thereby causing catecholamine-induced diastolic Ca2+ leak.46 Nevertheless, the clinical correlation between such animal-based models and human patients was suboptimal at times.47

Using hiPSC-CMs to establish disease-in-a-dish models may allow the study of diseased human cardiomyocytes in a patient-specific manner. Subsequently, many hiPSC-CMs models of both CPVT types20–29 were shown to recapitulate the clinical phenotype in the culture-dish by displaying abnormal Ca2+ handling and arrhythmias. Here, we focused on CPVT2 and found, similar to previous reports,24,25,29 that most CPVT2-hiPSC-CMs display marked Ca2+-handling abnormalities including local Ca2+-release events, whole-cell Ca2+ irregularities, and arrhythmogenicity of varying complexity, which worsened after adrenergic stimulation. Using voltage-sensitive dyes to record optical action potentials, we noted that membrane potential manifestations of the aforementioned calcium abnormalities in the CPVT2-hiPSC-CMs were the development of DADs, EADs, and triggered arrhythmias. By the simultaneous recording of Ca2+ transients and
optical action potentials, we were able to document a complex interplay between Ca\(^{2+}\)-handling abnormalities and membrane potential changes, with ectopic Ca\(^{2+}\) releasestriggering membrane depolarizations and vice versa. Such Ca\(^{2+}\)-triggered EADs could be the result of increased Na\(^{+}\)/Ca\(^{2+}\)-exchanger activity because of elevated intracellular Ca\(^{2+}\) levels.

It is hypothesized that DAD formation is preceded by diastolic Ca\(^{2+}\) leak, occurring when SR Ca\(^{2+}\) content exceeds a certain threshold.\(^{5,30}\) Such SOICR events were implicated as a fundamental component associated with arrhythmia during Ca\(^{2+}\) overload. RyR2 mutations were shown to decrease SOICR threshold.\(^{21,30}\) We recently investigated this phenomenon in a CPVT1-hiPSC-CMs model demonstrating a reduced SOICR threshold in the affected cells.\(^{21}\) Here, we demonstrated the ability to model SOICR also in the CPVT2-hiPSC-CMs and revealed that the D307H-CASQ2 may also reduce SOICR threshold in the affected cardiomyocytes. These findings further elucidate the mechanistic nature of arrhythmogenicity in CPVT2, supporting the potential role of CASQ2 in luminal Ca\(^{2+}\) sensing and in RyR2 stabilization.\(^{33,47,48}\)

Subsequently, we performed drug-screening studies using the CPVT2-hiPSC-CMs model. Initially, we showed that JTV-519 suppressed Ca\(^{2+}\)-cycling abnormalities in CPVT2-hiPSC-CMs. This stresses the potential antiarrhythmic role of RyR2 stabilization not only in CPVT1 but also in CPVT2 and further suggests that CPVT2 arrhythmogenicity is mediated via RyR2 dysregulation. We next showed, similarly to the mainstay clinical treatment of CPVT2, that β-blockers were protective against isoproterenol-induced arrhythmogenicity in the CPVT2-hiPSC-CMs. Interestingly, carvedilol at clinically relevant doses, but not propranolol or labetalol, exhibited a direct antiarrhythmic action in CPVT2-hiPSC-CMs, suppressing Ca\(^{2+}\)-cycling-related abnormalities even in the absence of adrenergic stimulation. This effect could not be attributed to its β- or α-adrenergic blocking activity and supports the potential future use of carvedilol (or its recently described analogues with RyR2-stabilizing activity) for the treatment of CPVT2.

Flecainide was recently suggested as a potent antiarrhythmic agent in CPVT.\(^{21,38-42}\) Our results support this concept by showing that flecainide, at clinically relevant concentrations, could significantly suppress baseline Ca\(^{2+}\)-cycling abnormalities and protect against isoproterenol-induced arrhythmogenicity in the CPVT2-hiPSC-CMs. Finally, riluzole, a neuronal Na\(^{+}\) channel blocker used for treatment of amyotrophic lateral sclerosis, which was recently shown to be antiarrhythmic in a mouse model of CPVT,\(^{48}\) was also found beneficial in our CPVT2-hiPSC-CMs model.

We also documented a phenylephrine-induced (α-adrenergic) proarhythmic effect in CPVT2-hiPSC-CMs, consistent with recent findings in a mouse model of CPVT2. Importantly, carvedilol, flecainide, and riluzole, but not propranolol, were protective against such arrhythmia. Because propranolol presents no α-blocking activity, its ineffectiveness in this setting was not surprising. Based on our findings, carvedilol could presumably suppress such arrhythmogenicity by both an α-blocking activity and a direct antiarrhythmic effect, whereas flecainide and riluzole could do so merely via a direct antiarrhythmic action.

To further characterize the mechanistic nature of the antiarrhythmic actions exhibited by these agents, we evaluated their influence on SOICR in the CPVT2-hiPSC-CMs as a surrogate for potential SR stabilization.\(^{36}\) Interestingly, both JTV-519 and carvedilol displayed marked SOICR suppression. Other β-blockers (propranolol and labetalol) did not affect SOICR in the CPVT2-hiPSC-CMs, supporting the notion\(^{26,37}\) that the SR-stabilizing property may be unique to carvedilol. Moreover, flecainide (1 µmol/L) or riluzole, despite possessing important antiarrhythmic actions, failed to significantly suppress SOICR, suggesting that their main antiarrhythmic action may not be related to SR stabilization. Interestingly, at 6 µmol/L, flecainide demonstrated a trend for decreasing SOICR, not reaching, however, statistical significance. To complement these findings, we compared the effects of carvedilol, flecainide, and riluzole during the postpacing period of forskolin-treated CPVT2-hiPSC-CMs. Although carvedilol significantly suppressed both triggered activity and DCR events at the postpacing period, flecainide, and riluzole markedly suppressed triggered activity but had only a mild effect on DCR.

Taken together, our findings indicate that the antiarrhythmic action of carvedilol in CPVT2 also involves RyR2 stabilization. In contrast, although flecainide may possess some SR-stabilizing properties at higher concentrations (as evident from the trends in the SOICR and postpacing experiments), at lower concentrations, flecainide’s direct suppression of triggered activity in CPVT2 is probably the more important mechanism.

Finally, to explore the potential of hiPSC-based models in predicting pharmacological clinical responses, we compared our in vitro drug-testing findings in the CPVT2-hiPSC-CMs model with the exercise test results performed in exactly the same patient. Similar to our in vitro observations, the clinical data and stress test results advocated that propranolol may not be entirely protective against catecholamine-induced arrhythmia in our patient and that flecainide treatment may contribute an additional antiarrhythmic effect while preventing exercise-induced arrhythmias. In contrast, although labetalol was recently suggested to exhibit beneficial antiarrhythmic activity in a CPVT2 mouse model,\(^ {23}\) it failed to achieve similar effects in both the in vitro CPVT2-hiPSC-CMs model and in the corresponding clinical study. These findings may suggest that hiPSC-based models could potentially aid in predicting patient-specific clinical response, as was also evident from a recent report comparing the flecainide clinical response with in vitro findings using hiPSC-CMs from a CPVT1 patient.\(^ {28}\)

Although of significant potential, hiPSC-CM–based disease models of arrhythmogenic syndromes are not flawless. One limitation of such models, including our current work, is that cells are usually obtained from only a small number of individuals (because of the rare incidence of such syndromes and to the large amount of work associated with creation and detailed characterization of the generated cell lines), and hence, the results of these studies cannot necessarily be generalized to differences between patient populations. This is also an obvious limitation for correlating the in vitro results with the clinical setting, necessitating many more patients to prove significant trends. Indeed, extensive further research is warranted to test the prediction ability of drug screening studies using hiPSC-based models with the clinical setup.
Finally, a major limitation in the field is the degree of maturity of the hiPSC-CMs, which do not reach the full adult phenotype, therefore potentially affecting the clinical relevance of at least some hiPSC-based disease models. In this regard, the high frequency of baseline arrhythmia noted in the current study and in previous CPVT-hiPSC-CM-based models may be at least partially attributed to the early-stage phenotype exhibited by hiPSC-CMs and to the experimental setting using single cells rather than multicellular tissues. Such augmented arrhythmogenicity may in the one hand facilitate the screening for antiarrhythmic agents but on the other hand may not accurately reflect the actual clinical setup.

Nevertheless, despite the aforementioned limitations, our findings stress the unique potential of hiPSC-CMs for modeling arrhythmogenic syndromes in general and CPVT in particular. This approach allowed recapitulating the disease phenotype in the patient-specific CPVT2-hiPSC-CMs, provided important insights into the mechanistic nature of arrhythmias and drug response in this syndrome, and highlighted the potential of hiPSC-CMs to serve as a platform for drug screening and potentially for individualizing drug therapy.

Acknowledgments
We thank Dr Doron Aronson for his statistical advice, Dr Ilanit Itzhaki for the custom-written analysis software, and Dr Edith Suss-Toby for her help in imaging.

Sources of Funding
This study was funded in part by the European Research Council (ERC) Ideas-Program [ERC-2010-STG-260830-Cardio-iPS].

Disclosures
None.

References


Patient-Specific Drug Screening Using a Human Induced Pluripotent Stem Cell Model of Catecholaminergic Polymorphic Ventricular Tachycardia Type 2
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_Circ Arrhythm Electrophysiol._ 2017;10:
doi: 10.1161/CIRCEP.116.004725

_Circulation: Arrhythmia and Electrophysiology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3149. Online ISSN: 1941-3084

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