Mutation-specific differences in arrhythmias and drug responses in CPVT patients: simultaneous patch clamp and video imaging of iPSC derived cardiomyocytes

R. P. Pölönen1 · H. Swan2 · K. Aalto-Setälä1,3

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Abstract
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited cardiac disease characterized by arrhythmias under adrenergic stress. Mutations in the cardiac ryanodine receptor (RYR2) are the leading cause for CPVT. We characterized electrophysiological properties of CPVT patient-specific induced pluripotent stem cell-deprived cardiomyocytes (hiPSC-CMs) carrying different mutations in RYR2 and evaluated effects of carvedilol and flecainide on action potential (AP) and contractile properties of hiPSC-CMs. iPSC-CMs were generated from skin biopsies of CPVT patients carrying exon 3 deletion (E3D) and L4115F mutation in RYR2. APs and contractile movement were recorded simultaneously from the same hiPSC-CMs. Differences in AP properties of ventricular like CMs were seen in CPVT and control CMs: APD90 of both E3D (n = 20) and L4115F (n = 25) CPVT CMs was shorter than in control CMs (n = 15). E3D-CPVT CMs had shortest AP duration, lowest AP amplitude, upstroke velocity and more depolarized diastolic potential than controls. Adrenaline had positive and carvedilol and flecainide negative chronotropic effect in all hiPSC CMs. CPVT CMs had increased amount of delayed after depolarizations (DADs) and early after depolarizations (EADs) after adrenaline exposure. E3D CPVT CMs had the most DADs, EADs, and tachyarrhythmia. Discordant negatively coupled alternans was seen in L4115F CPVT CMs. Carvedilol cured almost all arrhythmias in L4115F CPVT CMs. Both drugs decreased contraction amplitude in all hiPSC CMs. E3D CPVT CMs have electrophysiological properties, which render them more prone to arrhythmias. iPSC-CMs provide a unique platform for disease modeling and drug screening for CPVT. Combining electrophysiological measurements, we can gain deeper insight into mechanisms of arrhythmias.

Keywords hiPSC · Cardiomyocyte · CPVT · Patch clamp · Contraction · Carvedilol · Flecainide

Introduction
Human induced pluripotent stem cells (hiPSCs) can be generated from patients’ somatic cells providing endless source for research in vitro [1]. The hiPSCs carry the same mutations as the patient who donated the primary cells and the differentiation of disease specific hiPSC-derived cardiomyocytes (CMs) allows disease modeling and drug development in human cells for personalized medicine.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a severe inherited cardiac disease in structurally normal heart associated with risk of sudden cardiac death [2]. CPVT patients have arrhythmias during mental or physical stress. CPVT is caused by mutations mainly in the RYR2 gene, coding for cardiac ryanodine receptor (RYR), but also mutations in other genes have been observed [3]. Therapy includes drug treatment, usually with beta-blockers, and, in
severe cases, implantable cardiac defibrillators or left cardiac sympathetic denervation. However, present therapeutic options do not provide complete protection against potentially lethal arrhythmias. A beta-blocker carvedilol and class Ic antiarrhythmic agent flecainide have been shown to have a direct effect on RYR and they, or their derivatives, are investigated as treatment options for CPVT [4–7].

CM contraction is driven by action potential (AP) induced release of intracellular Ca\(^{2+}\) from sarcoplasmic reticulum (SR) [8]. Inward current through sodium channels (I_{Na}) depolarizes the CM cell membrane which triggers inward flow of Ca\(^{2+}\) through \(\text{I}_{\text{Ca,L}}\) calcium channels, which leads to activation of RYRs and calcium induced calcium release (CICR) from SR. Ca\(^{2+}\) binds to sarcomeres and allows the contractile motion. Cell membrane is repolarized by several potassium currents and Ca\(^{2+}\) is reloaded into SR via SR Ca\(^{2+}\) ATPase (SERCA) [9]. The membrane potential (V_m) and intracellular Ca\(^{2+}\) concentration [Ca\(^{2+}\)]_i are the most crucial elements in cardiac excitation contraction coupling (ECC) and arrhythmogenesis. The [Ca\(^{2+}\)]_i influences the shape and duration of AP via Ca\(^{2+}\)-sensitive ionic transporters, such as I_{Ca,L}, Na\(^{+}\)-Ca\(^{2+}\) exchanger (NCX) and Ca\(^{2+}\)-activated nonselective cation channels, thereby modifying the electrophysiological properties for example refractoriness and membrane depolarization rate [9]. Conversely, membrane potential can influence the Ca\(^{2+}\) spark and waves [10].

A few studies have been focused on simultaneous recording of V_m, calcium transients or contractile function from the same hiPSC CMs [11–19]. It is important to understand the highly inter-related nature between these key elements in ECC. The ability to combine ionic and biomechanic analyses can offer valuable details considering disease mechanisms, drug effects and arrhythmogenesis. In our previous studies, we have measured intracellular calcium, action potentials and field potentials from CPVT hiPSC CMs [20–23]. In this study, we combined current clamp recording with video imaging. We characterized electrophysiological and contractile properties of CPVT patient-specific hiPSC CMs carrying different mutations in RYR2. In addition, we evaluated effects of carvedilol and flecainide, agents that are shown to affect RYR2, on APs and contractility of hiPSC-CMs.

Materials and methods

Patient-specific human iPSC lines

In this study, two CPVT hiPSC lines were used carrying following RYR2 mutations: one with exon 3 deletion c.168-301c.273+t22del1128 (E3D) and one with point mutation p.L4115F (c.12343C>T) (L4115F). Mutation nomenclature was based on RYR2 reference sequence NM_001035.2. Wild type hiPSC line from one healthy individual was used as a control cell line. Several clones were established from the individuals but one per each was used in this study for characterization. The ethical committee of Pirkanmaa Hospital District approved collection of biopsies for generating patient-specific iPSC lines and written informed consent was obtained from all the donors (Aalto-Setälä R08070). Human iPSC-lines were established by sendai viral (CytoTune® IPS reprogramming kit, Thermo Fisher Scientific, Waltham, MA, USA) or retroviral transfection of OCT3/4, Sox2, KLF4 and c-MYC [1]. Characterization of hiPSC lines has been described previously [22].

Cardiac differentiation and dissociation

Differentiation into CMs was carried out by co-culturing hiPSCs with murine visceral endoderm-like (END-2) cells (prof. Mummery, Humbrecht Institute, Utrecht, The Netherlands) (supplementary data) [24]. Human iPSCs formed spontaneously beating clusters after 15 days of co-culturing. Beating clusters were cut and isolated with scalpel and dissociated with collagenase A (Roche Diagnostics) [24]. Not all beating hiPSC CM clusters dissociate into single cells, therefore resulting in suspension of mixed single cell CMs and small CM aggregates. These CMs were plated on glass coverslips coated with 0.1% gelatin.

Chemicals

All the drugs and chemicals for the experiments were purchased from the Sigma-Aldrich unless otherwise specified. Potassium methanesulfonate (KMeSO4) was ordered from MP biomedicals (USA). Stock solutions for extracellular solution were prepared in 1 M concentration and refrigerated until use. Extracellular solution was made freshly by diluting stock solution (in mM): 143 NaCl, 4.8 KCl, 1.8 CaCl2, 1.2 MgCl2, 5 glucose and 10 HEPES (pH adjusted to 7.4 with NaOH). Intracellular solution was made and refrigerated (in mM): 132 KMeSO4, 4 EGTA, 20 KCl, 1 MgCl2 and 1 CaCl2 (pH adjusted to 7.2 with KOH). Adrenaline, carvedilol and flecainide were dissolved in Milli-Q™ water and stored frozen until diluted to the final concentration (1 μM, 0.25 μM and 1.5 μM respectively) in extracellular solution at the day of the experiments. Amphotericin-B was dissolved in DMSO and diluted to intracellular solution to make a final concentration of 0.24 mg/mL. One drug (either carvedilol or flecainide) was used per each cardiomyocyte. Same cardiomyocytes were not exposed to both drugs.

Simultaneous patch clamp and video imaging

Experiments were conducted on 30+ days old (from start of differentiation) hiPSC CMs which were plated on cover
slips no more than 7 days before the experiments. Extracellular solution was heated to 36 ± 1 °C with an inline heater SH-27B controlled with a TC-324B controller unit (Warner instruments Inc., CT, USA). The perfusion was controlled by a gravity driven VC38 application system (ALA Scientific Instruments Inc., NY, USA). Patch pipettes (Harvard Apparatus Ltd., Holliston, MA, USA) were freshly prepared using PC-10 micropipette puller and flame polished with MF-830 microforge (both from Narishige Int., Tokyo, Japan). The patch electrodes had tip resistance of 1.8–2.2 MΩ with intracellular solution. AP recordings and video imaging were performed simultaneously. For patch clamp experiment, perforated patch using Amphotericin B was performed to record AP in Axon Series 200B patch-clamp amplifier connected to Digidata 1440a AD/DA converter driven by pCLAMP 10.2 software (all from Molecular devices LLC). APs were recorded in the gap-free mode in the current-clamp from the spontaneously beating hiPSC-CMs. Current-clamp recordings were digitally sampled at 20 kHz and filtered at 2 kHz using a low pass Bessel filter on the recording amplifier. Videos were captured with ANDOR iXon 885 EM-CCD camera (Andor Technology, Belfast, Northern Ireland) and a TH4-200 light source (Olympus, Tokyo, Japan). Video imaging was performed using an inverted Olympus IX70 microscope using LC Ach N PhC 0.40NA 20 × air objective (Olympus) and recorded with Live Acquisition software (TILL Photonics, Munich, Germany). The videos were recorded as 30 s-long sweeps with 49-105FPS before and after drug administration. Without binning of the camera and relatively large active pixel size, the videos were not the highest FPS possible. After establishing the seal, minimum of 2 min was allowed for the cardiomyocytes to stabilize before recording the baseline condition. The pCLAMP software was configured to perform the synchronization between patch clamp and video imaging system by receiving the synchronization pulse (SP) from the camera: SPs were recorded as 30 s-long sweeps with 49-105FPS before and after drug administration. Without binning of the camera and relatively large active pixel size, the videos were not the highest FPS possible. After establishing the seal, minimum of 2 min was allowed for the cardiomyocytes to stabilize before recording the baseline condition. The pCLAMP software was configured to perform the synchronization between patch clamp and video imaging system by receiving the synchronization pulse (SP) from the camera: SPs were recorded alongside with APs, thus when SP stopped, the video imaging was stopped.

**Action potential and contraction analysis**

Action potentials were analyzed with Origin™ 9.1 (OriginLab Corp., Northampton, USA) to extract the action potential parameters. The hiPSC-CMs were categorized as ventricular-like and atrial-like CMs when they showed APD90/APD50 < 1.30 and APA > 80 mV, and APD90/APD50 > 1.35, respectively. The different arrhythmias shown in the Fig. 3 were extracted (Fig. 3i). Most of the CMs were ventricular-like with APD90/50 ratio lower than 1.30. Properties of atrial like hiPSC CMs are shown in Table S3. Nodal like CMs were very few in number (data not shown). Only ventricular like CMs were studied further with adrenaline and then with either carvedilol or flecainide along with adrenaline. Representable traces from compound effects are in Fig. 3a–f.

**Statistical analysis**

Statistical analysis was carried out with SPSS software version 23 (SPSS, Chicago, IL, USA). Comparison within cell lines (before and after drug administration) were performed with non-parametric Wilcoxon and between cell lines (cell line to cell line comparison) with non-parametric Mann–Whitney U tests with Dunn-Bonferroni post hoc. p < 0.05 was considered statistically significant. Correlation of different parameters was done with Pearson correlation test.

**Results**

**Differences between CM aggregates and single cells**

There were no significant differences between AP or contraction parameters between CM aggregates and single cells (Tables S1 and S2). In this dataset, 68% were CM aggregates and 32% were single CMs. An aggregate consisted usually of two or few cells connected to each other (Fig. 3a–d, i, j). Area of CM aggregate was larger than the average cell area of single CM, approximately 8280 μm² and 5370 μm² respectively (Fig. S1). Single CMs were more spread out than the ones inside an aggregate (Fig. 3e–h). In addition, there was no significant correlation between cell area and any AP or contraction parameter. Therefore, results from aggregates and single cells were pooled.

**Action potential parameters and compound effects**

CMs were recorded in current clamp and AP parameters were extracted (Fig. 1). Most of the CMs were ventricular like with APD90/50 ratio lower than 1.30. Properties of atrial like hiPSC CMs are shown in Table S3. Nodal like CMs were very few in number (data not shown). Only ventricular like CMs were studied further with adrenaline and then with either carvedilol or flecainide along with adrenaline. Representable traces from compound effects are in Fig. 3a–f.
At baseline, hiPSC CMs differed from one to another: L4115F-CPVT CMs had the highest beating rate, E3D-CPVT CMs had the lowest APA and MDP (p < 0.05). Overall, APD90 and APA was lower and MDP more depolarized in CPVT compared to control CMs. Adrenaline had positive chronotropic effect on all hiPSC CMs: Beating rate was increased and APD10, 50 and 90 were decreased (p < 0.05). In addition, adrenaline decreased APA and depolarized MDP the most in E3D-CPVT CMs (p < 0.05). Furthermore, adrenaline increased upstroke velocity (dVdT) and triangularity (APD ratio) in L4115F and WT CMs (p < 0.05). Carvedilol and flecainide both had negative chronotropic effect on all hiPSC CMs: Decreased beating rate and increased APD90 (p < 0.05). In addition, both drugs decreased upstroke velocity and increased triangularity in CPVT CMs (p < 0.05). Carvedilol decreased beating rate the least but upstroke velocity the most in L4115F-CPVT CMs. Carvedilol had the least effect on APDs in control CMs. Flecainide decreased upstroke velocity the least but increased APD50 and 90 the most in L4115F CPVT CMs.

**Contraction parameters and drug responses**

Contraction properties of hiPSC CMs were studied with video imaging simultaneously along current clamp recording. Not all the videos could be analyzed in such a precision because of low signal to noise ratio, therefore the n amount in contraction parameters in Fig. 2 is less than in AP parameters in Fig. 1. In addition, the comparison of contraction amplitude (CTA) was not plausible between recordings because of variation in the lighting conditions affected by the liquid flow during each recording. The preheated perfusate created a turbulent flow and small bubbles in the recording chamber resulting in diffusion of light and different lighting and baseline conditions for the intensity based peak detection. Therefore, only the compound effects as the change of CTA can be compared within one recorded cell. At baseline, CPVT CMs had shorter contraction duration at 90% of relaxation (CTD90) and relaxation time (Fig. 2). Adrenaline decreased CTDs, time to peak and relaxation time more in controls than CPVT CMs. Flecainide prolonged CTDs and relaxation time the most in control CMs. Both drugs decreased contraction amplitude the most in E3D CPVT CMs. Interestingly, flecainide decreased the relaxation time and CTDs only in E3D CPVT CMs. Both drugs decreased contraction amplitude the most in E3D CPVT CMs. Flecainide decreased the CTA the most in E3D CPVT CMs. Carvedilol decreased the contraction amplitude more than flecainide in L4115F CPVT and control CMs but not in E3D CPVT CMs. Furthermore, carvedilol decreased CTDs the most in control CMs.
Correlation between AP and contraction

There was significant (p < 0.01) positive correlation between all respective APD and CTD parameters (Fig. S2). Figure 3 merged sections show the interrelation between $V_m$ and contraction. Contraction follows $V_m$ in various arrhythmic conditions. Weak but significant negative correlation between BPM and time to peak was found (p < 0.01) at baseline and adrenaline: time to peak decreases when beating rate increases. In addition, APD ratio and relaxation time also had weak and significant negative correlations (p < 0.01) at baseline and adrenaline: the smaller the ratio, the longer the relaxation time. After drug administration, the correlations were not as strong or significant anymore. Carvedilol and flecainide therefore had an impact on coupling of membrane $V_m$ and contraction.

Arrhythmias seen in patch clamp recording reflected on contraction

None of the control CMs had arrhythmias at adrenaline whereas all the CPVT CMs were arrhythmic. Overall, hiPSC CMs had more DADs than EADs (Fig. 4). Representative traces of different arrhythmias are presented in Fig. 3. E3D CPVT CMs had the most arrhythmias at all conditions. Furthermore, CPVT CMs had increased amount of DADs and EADs after adrenaline exposure, however this result was not statistically significant (p > 0.05). DADs are seen as a swift depolarization immediately after repolarization (Fig. 3g). These minor changes in $V_m$ are not reflected in contraction profile. Phase 3 and burst EADs are present in both $V_m$ and contraction (Fig. 3g, h). The extra beats resulting from EADs have decreased contraction amplitude. Both drugs were able to decrease the amount of DADs in CPVT CMs whereas flecainide slightly increased EADs in E3D CPVT CMs (Fig. 4). Carvedilol abolished almost all arrhythmias in L4115F CPVT CMs while flecainide decreased DADs 67% and EADs 49% (Fig. 3c–f). In addition, flecainide decreased DADs 60% and carvedilol 51% in E3D CPVT CMs. APD alternans was seen in 2/25 (8%) L4115F CPVT CMs after adrenaline as alternating APD in $V_m$ and amplitude in contraction (Fig. 3I). Long AP results in lower amplitude and vice versa. Flecainide abolished both of the alternans cases. Tachyarrhythmia were seen in 4/20 (20%) E3D CPVT CMs and 1/25 (4%) L4115F CPVT CMs as a rapid major change in beat rate and depolarized MDP continuing for over several seconds (Fig. 3j). Flecainide abolished one of the tachyarrhythmia, whereas it caused two CMs undergo tachyarrhythmia. Carvedilol abolished three of the four tachyarrhythmia cases.

Discussion

Following the discovery of linking RYR2 mutations to CPVT, studies utilizing patch clamp in RYR2-R4496C mutant mouse model have been published [2, 26–28]. Increased open probability of mutated RYR2 leading to spontaneous calcium release and lowered threshold for triggered activity has been identified as a primary cause.
**Fig. 3** Representative traces of drug responses and arrhythmias in hiPSC CMs. Action potential and contraction measured simultaneously from the same cell.  

- **A** Normal baseline trace of WT control.  
- **B** Normal adrenaline trace from the same cell as in (a).  
- **C** Adrenaline trace from CPVT showing extra beat (arrow).  
- **D** Carvedilol trace from the same cell as in (c).  
- **E** Adrenaline trace from CPVT showing DADs (arrows).  
- **F** Flecainide trace from the same cell as in (e).  
- **G** DADs and phase 3 EADs (arrows) from CPVT CM.  
- **H** EAD burst and DADs (arrows) from CPVT CM.  
- **I** Alternans from CPVT CM where long AP (L) results in lower contraction amplitude (arrow) and short AP (S) in higher.  
- **J** Tachyarrhythmia from CPVT CM. Dashed lines at 0 mV.
for DADs and arrhythmia. Patch clamp recordings showing DADs under isoproterenol or other adrenergic stimulus have confirmed this. However, electrophysiological properties of mouse and human CMs are different, e.g. beating rate and presence of repolarizing ionic currents [29]. Therefore, hiPSC derived CMs have been introduced, and electrophysiology studies using CPVT patient derived hiPSC CMs have been published, and the disease phenotype has been confirmed in human-based cell model in vitro [23, 30–32].

In this study, we characterized electrophysiological properties of E3D and L4115F CPVT hiPSC-CMs and control CMs at baseline and after adrenaline provocation and evaluated antiarrhythmic effects of carvedilol and flecainide. CPVT CMs had shorter APD90 and more depolarized MDP compared to controls at baseline. The more depolarized MDP led to lower APA in CPVT CMs. In addition, the shorter APD90 probably was caused by the sarcolemmal calcium transient affected via the leaky RYR2 mediated higher intracellular calcium concentration. Furthermore, MDP change affects the availability of ion channels at the cardiomyocyte membrane laying effect on the AP profile. Adrenaline had positive and carvedilol and flecainide negative chronotropic effect in all hiPSC CMs. CPVT CMs had increased number of DADs and EADs after adrenaline exposure. Adrenaline provocation increases the SR calcium load and results to calcium leak via the mutated RYR2s. The results suggest that the depolarized MDP plays a role in the generation of arrhythmias in E3D CPVT CMs. Alternans was seen in L4115F CPVT CMs. Carvedilol and flecainide decreased the number of DADs but flecainide increased EADs in E3D CPVT and control CMs. In addition, both drugs decreased contraction amplitude in all hiPSC CMs.

**E3D CPVT CMs: depolarized MDP and high DAD frequency**

In this study, E3D CPVT CMs had the most DADs and EADs. The more positive, depolarized MDP seems to be favoring arrhythmic events by setting lower threshold for triggered activity. SR calcium leak through RYR can lead to small changes in membrane voltage leading to activation of ICa,L or influx through NCX and eventually CICR, favoring arrhythmic events by setting lower threshold for triggered activity. In Fig. 3e, g, h, two kind of DADs appear: right after repolarization in the root of AP and later in the diastolic depolarization (Fig. 3h). The latter hold a remarkable resemblance to OPPs. Flecainide cured all of the first mentioned DADs, but only a portion of the latter. This could indicate distinctive ways these two types of DADs are produced. However, the APD prolongation caused by flecainide may mask the first type of DADs. Furthermore, some CMs exhibited long irregular periodical beating patterns (Fig. S3). There is similar voltage oscillation during OPPs and the gaps between these periods. This might be due to aberrant CM automaticity and different from the mechanism of DADs. Cells, which were beating inconsistently in this manner, were excluded from the further analysis.

**CPVT CMs had more DADs than EADs**

In this study, CPVT hiPSC CMs were more arrhythmic than control CMs (Fig. 4). In addition, there were more DADs than EADs in CPVT CMs. Carvedilol was more efficient abolishing EADs than flecainide (Fig. 4b). Carvedilol acts by blocking beta-adrenergic receptors, inhibiting phosphorylation of calcium handling proteins (RYR, SERCA, ICa,L) and altering calcium handling. Flecainide acts by blocking sodium channels leading to reduced excitability, but also IKr leading to risk for arrhythmia. In addition, both drugs have been shown to reduce RYR2 calcium leak associated with DADs [4–7]. In this study, both drugs decreased the number of DADs in CPVT CMs (Fig. 4).

Novak et al. reported oscillatory prepotentials (OPPs), distinct from DADs, described as voltage oscillations during late diastolic depolarization [32, 33]. This irregularity in the membrane potential is very similar to what have been earlier described as DADs [23, 27, 28, 30, 31]. In our current study, flecainide abolished majority of DADs but not all of them. In Fig. 3e, g, h, two kind of DADs appear: right after repolarization in the root of AP (Fig. 3e) and later in the diastolic depolarization (Fig. 3h). The latter hold a remarkable resemblance to OPPs. Flecainide cured all of the first mentioned DADs, but only a portion of the latter. This could indicate distinctive ways these two types of DADs are produced. However, the APD prolongation caused by flecainide may mask the first type of DADs. Furthermore, some CMs exhibited long irregular periodical beating patterns (Fig. S3). There is similar voltage oscillation during OPPs and the gaps between these periods. This might be due to aberrant CM automaticity and different from the mechanism of DADs. Cells, which were beating inconsistently in this manner, were excluded from the further analysis.
that study to APDs in this study. In addition, the beating rates of the CMs were different. Since the repolarization of AP is much more rapid than calcium decay, similar oscillation patterns were not detected in membrane voltage in this study. In addition, it could be that the CMs exhibiting periodical beating were the ones captured with the long calcium oscillations in the previous study, and in this study, they were not included in the ventricular like dataset. In addition, calcium imaging does not allow distinguishing between CM subtypes. Furthermore, the previous study showed that E3D CPVT CMs had lower calcium transient amplitude, which could be an indication of elevated diastolic calcium [22]. We returned to that calcium imaging dataset and analyzed the baseline fluorescence intensity values at diastole and indeed, in CPVT cells it was higher compared to the controls and the highest in E3D CPVT CMs (p < 0.001) (Fig. S4). These findings suggest that the RYR2 exon 3 deletion results in elevated diastolic calcium, which contributes to the depolarized diastolic potential therefore facilitating arrhythmic behavior in these cells.

Alternans

Similar to our previous study, L4115F CPVT CMs had alternans during adrenergic stress [22]. In this study, two alternans cases were recorded, both in L4115F CPVT CMs. Both of these were discordant, negatively coupled and electromechanically out of phase, in which the long AP results in low and short AP in high contraction amplitude (Fig. 3i). It has been shown with in silico modeling that in these cases the driving force of the alternans is unstable intracellular calcium [39]. In addition, the RYR2-L4115F point mutation is located in the central domain of the protein leading to gain of function of the channel and could therefore facilitate the formation of alternans via imbalanced calcium cycling [40]. In this study, we did not conduct calcium imaging but it is known from the previous studies that calcium and contraction go hand in hand [15]. Membrane voltage, calcium and contraction are tightly coupled in cardiac ECC. In addition to causing APD alternans, we showed that calcium alternans can cause very small scale MDP alternans leading to more negative MDP after a long AP and a bit more positive after a short one (Fig. S5). MDP changes between consecutive beats were only approximately 0.5 mV. This indicates that higher calcium content during longer calcium transient, associated with the short AP, contributes to the repolarization to a more positive MDP.

**Contraction of hiPSC CMs**

Contraction parameters were acquired by analyzing videos of beating CMs. Sampling interval at lower video frame rates can affect the accuracy of the analysis of rise time of the contraction peaks. We used larger active camera chip without binning to capture the whole cell area of the CMs and CM aggregates for the analyses. In addition, contraction parameters had relatively high deviation (Fig. 2). Consequently, there was a lack of statistical significance in these results. Figure 3 shows how different arrhythmias were recapitulated in contraction profile. EAD triggered extra beats were characterized with lower contraction amplitude (Fig. 3g, h). Discordant negatively coupled alternans led to electromechanically out of phase beating pattern (Fig. 3i). Tachyarrhythmia...
Flecainide decreased CTDs most in E3D CPVT CMs. At more positive diastolic potential, APs are driven more by calcium than at more negative diastolic potentials because I_{Ca,L} open at more positive membrane potential than sodium channels. Interestingly, E3D CPVT CMs had higher contraction amplitude at baseline. Flecainide blocking both voltage gated sodium channels and RYR2 results in decreased CTDs and more decreased contraction amplitude compared to CMs with more repolarized MDP. Carvedilol, however, affects the SR calcium content by inhibition of adrenergic pathway and RYR2 block. The different pathways of action of these two drugs might explain the differences.

Future perspectives

Detailed investigation of electrophysiological properties of hiPSC CMs gives valuable information about arrhythmic conditions and their triggers, even though they do not completely recapitulate the phenotype of adult human CMs. However, they can serve several different purposes, for example drug screening and disease modeling. In addition, more detailed and fine-tuned in silico models can be developed to simulate human cardiac/cardiomyocyte physiology [41]. These cell models and approaches can be utilized by pharmacological industry to fulfil the need of more cost efficient and human-based drug development already in preclinical phases. Human iPSC derived CMs can offer physiologically more authentic model to study cardiotoxic effects in vitro, decrease the amount of animal studies and cut costs in the drug development process. Combining in vitro and in silico modeling will hopefully accelerate the cardiovascular research in the future.

Conclusions

In this study, simultaneous patch clamp and video imaging was conducted with CPVT patient derived hiPSC CMs to study effect of carvedilol and flecainide on AP and contractile properties. Differences in baseline properties and drug effects were found. CPVT CMs were more arrhythmic than controls. In addition, there were more DADs than EADs in the hiPSC CMs. The E3D CPVT CMs had the most arrhythmias and electrophysiological properties distinct from others favoring arrhythmic behaviour. Different types of arrhythmias in membrane voltage were reflected in the contraction profile of the CMs. In this study, there were still substantial amount of DADs present after drug administration, indicating these treatments provide only partial protection from arrhythmias. Therefore, it is important to look for additional drug therapies for CPVT. Patient specific hiPSC CMs recapitulate disease hallmarks and drug responses therefore providing a powerful tool to study CPVT.

Limitations

In this study, only one clone per hiPSC line from a healthy donor was used to create control CMs. APD ratio was used to distinguish CM subtypes. However, controversy for the determination of the CM subtypes exists [42]. The immaturity of the hiPSC derived CMs, their calcium handling and electrophysiology; especially I_{K1} and I_{K1} is still a relevant problem [43]. Therefore, these results may not be translatable to patients. However, in our earlier studies, our hiPSC derived CMs have been characterized by various techniques showing their electrophysiological properties [18, 22, 23, 44].

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Compliance with ethical standards

Conflict of interest  The authors declare that they have no conflict of interest.
Ethical approval  Approval from the Ethics Committee of Pirkanmaa Hospital District was given to conduct the research on hiPSC lines (Aalto-Setälä R08070). Patients donating skin biopsies were provided with an informed consent in the Heart Hospital, Tampere University Hospital.

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