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Topical Collection on Regenerative Medicine and Stem Cell Therapy:

Pluripotent Stem Cells as a Platform for Cardiac Arrhythmia Drug Screening

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Opinion statement

Since the first demonstrations of the differentiation of pluripotent stem cells to produce functional human cellular models such as cardiomyocytes, the scientific community has been captivated [1, 2•, 3]. In the time since that seminal work, the field has been catapulted forward by the demonstration that adult somatic cells can be reprogrammed to an induced state of pluripotency [4•], and more recently by the development of efficient and sophisticated genome editing tools [5•, 6•, 7], which together afford a theoretically unlimited supply of relevant genetic disease models. In particular, many of the early successes with induced pluripotent stem cell technology have been realized with cardiac arrhythmia syndromes [8•, 9–15]. There is interest in applying stem cell models in large-scale screens to discover novel therapeutics or drug toxicities. This manuscript aims to discuss the potential role of hPSC-derived cardiomyocyte models in therapeutic arrhythmia screens and review recent advances in the field that bring us closer to this reality.

Keywords

Pluripotent; Stem cell; Cardiomyocytes; Arrhythmia; Screening; Electrophysiology; Cardiac electrophysiology

Introduction

A major advance in disease modeling has been the demonstration that human induced pluripotent stem cell (hiPSC) technology can faithfully recapitulate many human diseases, including cardiac arrhythmia syndromes [10, 16, 14]. Most of the initial cardiac disorders to be modeled with hiPSC-derived cardiomyocytes (hiPSC-CM) have been Mendelian arrhythmia syndromes [8•, 9–15], though increasingly, other cardiac diseases have been modeled, such as familial hypertrophic and dilated cardiomyopathy [17, 18]. Importantly, several of these diseases lack targeted therapeutics that directly address their respective

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Conflict of interest

Dr. Jordan S. Leyton-Mange and Dr. David J. Milan each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

physiological defects. In addition to these unmet needs, stem-cell-derived models may hold promise in evaluation of drug-induced QT prolongation, one of the most common causes of post-market drug withdrawal, which remains difficult to predict in the pre-clinical setting [19]. Enthusiasm has been significant for applying stem-cell models to large-scale screens for both novel therapeutics and cardiotoxicity evaluations [20•, 21, 22]. However, most of the early studies in the stem cell field were limited in scope, despite being conceptually innovative. In recent years, the techniques for human pluripotent stem cell culture and cardiac differentiation have dramatically improved [23•, 24, 25]. This review will focus on the role that stem cell models can play in cardiac arrhythmia-related drug screens, and discuss the necessary steps to realize their potential.

Characteristics and relevance of stem-cell-derived cardiomyocytes

The contribution of any particular model is critically dependent on how faithfully it represents the native in vivo condition—in this case, a mature adult human cardiomyocyte (CM). As adult ventricular CMs are obtained only invasively and thereby in short supply, most studies characterizing the properties of human pluripotent stem cell derived cardiomyocytes (hPSC-CM) to date have compared parameters to previously published values [26].

Morphologically, most studies have reported that hPSC-CMs, both hiPSC-CMs and human embryonic stem cell-derived cardiomyocytes (hESC-CMs), are markedly smaller than adult CMs and lack organized sarcomeres and T-tubules, with a gene expression profile more closely resembling that of fetal CMs [27•]. These features of immaturity are similar to the immature electrical parameters recorded by patch clamp electrophysiology [27•, 28, 29•]. In contrast to adult human CMs, hPSC-CMs bear relatively depolarized diastolic potentials, slower action potential upstroke velocities, and spontaneous electrical activity [30].

In terms of action potential shape, most investigators have noted the appearance of three distinct hPSC-CM action potential subtypes, classified as ventricular-like, atrial-like, and nodal-like [28]. However, it has been acknowledged that there is a great deal of heterogeneity of AP characteristics reported between cell lines [31] and different laboratories [27•], and the relative cellular subtype proportions critically depend upon the criteria utilized to distinguish them [32•]. Despite this variability, the most frequently reported subtype population is ventricular-like [8•, 9, 29•, 33], characterized by a prominent plateau phase and longer action potential duration (APD), the length of which, while variable between studies, is comparable to reported values for native ventricular CMs [27•].

Individual currents have also been extensively studied in hPSC-CMs using voltage clamp electrophysiology, demonstrating the presence of the major currents I_{Na} , I_{Kr} , I_{Ks} , $I_{Ca,L}$, and I_{to} [2•, 34•, 35, 36]. Unlike mature atrial and ventricular adult CMs, hPSC-CMs also universally feature a prominent funny current, I_f , and an absent or minimal inward rectifier, I_{K1} [37]. Furthermore, a rather large proportion of the Ca^{2+} release during an hPSC-CM action potential is IP_3 -sensitive [38]. A comprehensive analysis of the cellular electrophysiology of CMs derived from a single induced pluripotent stem cell (iPSC) line was recently reported [34•].

The electrophysiological responses of hPSC-CMs to various drug compounds have been explored by several investigators. hPSC-CM sensitivity has been demonstrated to adrenergic and cholinergic compounds [39], cardiac glycosides [40], I_{K_r} inhibitors [40, 34, 8••, 9], $I_{Ca,L}$ inhibitors [9, 38], $I_{K,ATP}$ activators [9], I_{K_s} inhibitors [28, 36], and various inhibitors of Na currents [35, 41]. Despite many subtle and some more substantive differences between hPSC-CMs and adult CMs, the action potentials and component currents do bear remarkable similarity, prompting hope that hPSC-CMs can recapitulate the adult CM intricacies more accurately than common animal models or heterologous expression systems.

Arrhythmia syndromes modeled in hPSC-CMs

Screens utilizing hPSC-CM models may aim to discover novel therapeutics, but could also be used to detect cardiotoxicity in either healthy or diseased states. In considering the value of therapeutic and toxicological screens using arrhythmic disease models, it is helpful to briefly review progress thus far (Table 1).

LQTS

The Long-QT Syndrome is characterized by a prolongation in the QT interval, the cellular correlate of which is the AP duration. In 2010, seminal work featured action potential recordings from iPSC-CMs derived from two patients harboring mutations in KCNQ1 (LQT1), encoding the alpha subunit of the channel mediating the I_{K_s} current [8••]. Other iPSC-derived LQT models have since been reported for LQT1 [42], LQT2 (I_{K_r} deficiency) [9, 10, 43], LQT3 (persistence of late I_{Na}) and LQT8/Timothy syndrome [11]. The quintessential feature of these has been demonstration of prolonged AP duration, as well as arrhythmogenic elements such as EADs. Importantly, the responses to drugs such as isoproterenol-induced EADs in LQT1 [8••], faithfully recapitulate expected clinical features of the respective disorders. Models of LQT3 syndrome, caused by a gain of function in $I_{Na,late}$ that prolongs inward current over the plateau phase have had mixed results. Ma et al. reported significant APD prolongation in iPSC-CMs derived from a patient with a V1763M mutation in SCN5A, which was reversible by mexilitine treatment [44], and Davis et al. reported similar results in cells derived from a patient with a clinical overlap syndrome with Brugada [12]. Another study of LQT3 hiPSC-CMs demonstrated variable APD prolongation not reaching statistical significance [45]. Finally, other investigators have chosen not to report AP comparisons from LQT3 hiPSC-CMs, reasoning that the relatively depolarized membrane potential in hPSC-CMs, compared to their adult counterparts, inactivates a large proportion of the Na^+ channel, which is thereby unable to contribute to the AP shape [46]. Supporting this notion, the $I_{Na,late}$ modulator ATX has been reported to have no apparent effect on AP duration [47].

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Several groups have published hiPSC-CM models derived from patients with CPVT, caused by gain of function mutations in RYR2 [13, 48, 49, 50] or loss of function in CASQ2 [51], and characterized by susceptibility of the sarcoplasmic reticulum to premature calcium release when stimulated by catecholamines. In the CPVT1 models, as in other studies of Ca^{2+} handling in hPSC-CMs [38, 52], transients were evident upon application of caffeine

and abolished by ryanodine, suggesting the presence of functional SR and ryanodine receptors (RyR). However, as hPSC-CM models are deficient in t-tubules, and Ca^{2+} wavefronts have been noted to be slow, suggesting that coupling between L-type Ca^{2+} channels and SR mediated Ca^{2+} release may be impaired. Thus, despite increased Ca^{2+} transients in CPVT hiPSC-CM models, the physiologic details of calcium handling may be less faithful to the native disease condition [27•]. Furthermore, the CASQ2 mutant hiPSC-CMs reported by Novak et al. feature action potential prolongation that differs substantially from the clinical features of the condition [51].

Arrhythmogenic right ventricular cardiomyopathy (ARVC)

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is marked by defects in desmosomal proteins leading to fibrofatty replacement of the right ventricular myocardium with age and increased incidence of ventricular arrhythmias. Three studies have produced hiPSC-CM models from patients with mutations in PKP2, the most frequently responsible gene for ARVC [53, 15, 14]. These reports revealed accumulation of intracellular lipid in response to lipogenic stimuli. Electrophysiological differences were subtle, with slightly higher mean diastolic potential and prolonged APD noted in one study [14], and prolonged field potential rise time by multi-electrode array in another [53], possibly reflecting slowed conduction and impaired coupling.

Other cardiac diseases

hiPSC-CM models of familial forms of hypertrophic cardiomyopathy [18], dilated cardiomyopathy [17], LEOPARD syndrome, Pompe's disease [54], and other disease have been reported elsewhere. While these clinical syndromes may be accompanied by arrhythmias, we considered them to be outside of the scope of this review.

Standardization is a necessary step

Original methods of cardiac differentiation from hPSCs relied on spontaneous differentiation of aggregates formed from hPSCs known as embryoid bodies, a process with low efficiency and highly variability between cell lines [55]. In contrast, current techniques aim to increase efficiency by recapitulating key developmental cues in embryonic cardiogenesis [56]. In general, there are three frequently reported methods: embryoid body differentiation combined with sequential addition of key growth factors [57], co-culture with visceral endoderm-like cells [29], and monolayer differentiation guided with recombinant growth factors [58, 23•, 59] or small molecules [24]. These techniques can be augmented to shift cell subtype lineage (atrial vs. ventricular) [33], or to improve differentiation, such as by inclusion of ascorbate [60], DMSO [61], or in the case of the monolayer technique, an extracellular matrix sandwich [23•], a method yielding highly efficient preparations of cardiomyocytes. However, efficiency varies even with these protocols, which in turn must be additionally optimized for different cell lines. In parallel, efforts have focused on enriching existing populations of differentiated mixtures of cells derived from hPSCs for cardiomyocytes [62], such as by Percoll gradient [3], genetic selection by fluorescence or antibiotic resistance [63, 64, 65•], selection by oxidative metabolic capacity [66], and sorting

by mitochondrial content [67] or cardiomyocyte cell surface markers such as SIRPA [68] or VCAM-1 [69].

Facilitating the maturation of hPSC-CMs has also been an important area of investigation, as prolongation of time in culture alone is insufficient. Each method of differentiation above involves culturing cells on a fixed protein substrate on a two-dimensional culture platform within an incubator controlling physiological temperature and CO₂ levels. While this embodiment aligns well with the infrastructure of most academic research laboratories, the conditions differ markedly from the in vivo environment, with the loss of three-dimensional ultrastructure and associated cellular contacts and mechanical stresses. One strategy to assist maturation encompasses growth on engineered substrates such as “biowires” to induce structural alignment and mechanical load [70]. Other strategies have involved genetic introduction of deficient rectifying currents accompanied by electrical field pacing [71], manipulation of bioenergetics [72•, 14], and other techniques. None of these has been adopted on a widespread level or has been shown to scale up for generation of the large supplies of cardiomyocytes necessary for high-throughput applications. An additional variable to consider is the time point after differentiation for cellular recordings, which may in turn depend on the differentiation protocols and cell line.

Once standardized protocols for cardiomyocytes differentiation are developed, in what state should hPSC-CMs be assayed? From a pure physiologist’s vantage point, it is attractive to study the singularized hPSC-CM. Isolated and electromechanically decoupled from neighboring cells, this state mimics traditional physiological experiments with isolated adult CMs. However, experiments with isolated CMs are often performed early after isolation, before the conditions of culture exert their confounding influences. Adult CMs de-differentiate when maintained in prolonged culture, exhibiting a higher diastolic potential, spontaneous activity, loss of T-tubular and sarcomeric organizational structure, and variable action potential phenotypes [73, 74]. Meanwhile, hPSC-CMs are kept in continual culture and are generally phenotyped following a recovery period after dissociation, and it is conceivable that dissociation to a single cell level may substantially alter cellular phenotype. Moreover, there is evidence that removal of the influence of non-myocytes from hPSC-CMs in embryoid bodies by genetic selection results in a functional stalling of maturation [75]. Protocols for cellular dissociation vary, with some resembling traditional cell culture techniques [23•], and others bearing resemblance to animal CM isolation [46]. Unlike freshly isolated adult CMs where the source is relatively homogeneous and the principle quality metric is Ca²⁺ tolerance, heterogeneous starting populations of hPSC-CM may be differentially affected by dissociation protocols, introducing survival biases. Finally, the appropriate cell plating density and optimal time window to assay hPSC-CMs following dissociation [76] requires exploration.

Streamlining phenotyping

Conventional electrophysiology

How should hPSC-CMs be phenotyped in high-throughput screens? Since its inception by Neher and Sakmann [77], conventional patch clamp electrophysiology has proven to be a rigorous method of evaluation and has previously been used to demonstrate the predictive

power of hPSC-CM models for cardiotoxicity [20••]. Perhaps its biggest downside is that manual patch clamping is also tedious and slow. In contrast, commercial 96-well and 384-well automatic patch clamp devices based on glass pipets or planar electrodes are available, the latter having been utilized on commercial hPSC-CMs with an average success rate of 46 % and mean seal resistance of 1.4 G Ω with mean stability of ~18 min [78, 79]. However, these automated systems have traditionally been employed for voltage-clamp recordings of individual currents in heterologous cell lines and may be less applicable for differentiated cells derived from hPSCs [80]. One possible advantage of patch clamp electrophysiology lies in the ability to directly augment the hPSC-CM physiology, such as by “electronically injecting” rectifier current I_{K1} using a dynamic clamp to model a more mature state [37]. The dynamic clamp may be an improvement over other studies that have employed negative current injection throughout the cardiac cycle to maintain diastolic potentials at more “adult” values between –80 and –100 mV [63, 35]. Finally, the successful throughput of all patch electrophysiology is critically dependent on membrane quality, which varies between cellular preparations. Particularly disadvantageous for drug studies, patch clamp electrophysiology tends to be limited in the capacity for prolonged or serial recordings.

Multi-electrode arrays

Another measure of the electrical activity which has grown in popularity is use of multi-electrode arrays (MEA) [81–83, 21]. MEAs consist of planar arrangements of electrodes that capture local extracellular field potentials from multicellular CM aggregates. As these systems permit simultaneous recordings from multiple electrodes, they are suited for high-throughput measurements, while their non-invasive nature facilitates serial or prolonged recordings. However, compared to traditional patch-clamp electrophysiology, MEAs do not yield as much direct information about the morphology of cellular action potentials, and recording is restricted to multicellular aggregates large enough to generate sufficient electrical signals.

Contractile motion/impedance analysis

Contractile motion and beat rate variability [84] can be measured non-invasively using impedance recordings, and systems for high-throughput have been commercially developed. Guo and colleagues [40] developed an assay utilizing confluent monolayers of hPSC-CMs. These authors assayed a panel of 83 drugs for concentrations that elicited arrhythmic beating and/or slowing of beat rate, noting that the resulting concentration correlated with clinical arrhythmia and QT-prolongation levels. Similar to MEA-based recording, impedance-based assays are non-invasive and amenable to high-throughput. However, while the contractile properties of cardiomyocytes correlate with their electrophysiology, the electromechanical coupling of hPSC-CMs is not as mature as their adult counterparts. Furthermore, the effects of some compounds that harbor independent or differential contractile vs. electrical toxicity may produce readouts that confound measurements of “true” arrhythmogenicity.

Fluorescent optical mapping

Fluorescent Optical “mapping” with voltage-sensitive and calcium-sensitive indicators is a well-established method of imaging electrical activity [85•]. AP morphology and wavefront propagation in whole hearts has been performed with the zwitterionic membrane dyes, di-4-

ANEPPS and di-8-ANEPPS for years [85•]. These probes were recently combined with Ca^{2+} imaging dyes and used to simultaneously image electrical and calcium dynamics in hPSC-CM monolayers [72••]. However, the ANEPPS dyes feature prominent phototoxicity and photobleaching that limit cumulative illumination and degrades signal quality [86]. Furthermore, these dyes have had limited application to the study of isolated cells [87]. While the newer red-shifted probe di-4-ANBDQBS features a higher signal quality, its adequacy has yet to be evaluated for use with hPSC-CMs. In contrast, Ca^{2+} -based dyes such as fluo-4 and rhod-2 have much lower toxicity and higher fluorescence [88]. Some hPSC-CM screens have used high-throughput imaging systems with fluo4-based Ca^{2+} transients as a surrogate reporter for electrical activity [89, 90]. In addition to small-molecule based membrane dyes, the field of genetically encoded voltage [91–94] and calcium sensors [95] has been evolving rapidly. One of these voltage indicators, A242-ArcLight, was recently demonstrated to robustly report electrical activity in hPSC-CMs, with a signal to noise ratio of ~22 dB when measured in single isolated cells with standard fluorescence microscopy [96]. As genetically encoded probes continue to offer improved signal-to-noise quality, photostability, and reduced toxicity, they may find a place in the non-invasive characterization of electrophysiology in hPSC-CM models.

Screening in practice

Once a suitable method of phenotypic evaluation is chosen, how would a high-throughput drug screen be conceived in practice? Numerous chemical libraries are commercially available for the purpose of drug screening, from small collections of characterized compounds to larger libraries of up to ~1 million compounds with uncharacterized function. As hPSC-CM screening technology develops, initial efforts may be geared towards smaller libraries of characterized molecules where “hits” are more easily clinically translatable, such as from collections of US Food and Drug Administration (FDA) approved drugs. To minimize the confounding influence of cellular heterogeneity and experimental variation, multiple measurements could be taken for each condition or drug concentration. Data may also be recorded pre-drug and post-drug application, with each cell or cell aggregate being its own control. In the case of fluorescence evaluations and MEA recordings, cells may be arrayed multi-well configurations, with up to hundreds of cells or cell aggregates per well. For measures such as action potential duration having inherent rate dependence, rate control, such as by electric field stimulation, could be considered.

Furthermore, to ensure reproducibility, the relative purity of the cell type of interest should be considered. hPSC-CMs destined for screening purposes might be either freed of contaminating non-cardiomyocyte cell types prior to plating, or marked by fluorescent reporters that define and validate their identity as specific cell types. For example, reporters for the ventricular myosin light chain-2-isoform (MLC-2v), which has been demonstrated to be a marker for hPSCs committed to the ventricular lineage, could be utilized to obtain a ventricular lineage [63]. Similarly, as βMHC is the dominant isoform of the myosin heavy chain in the adult heart and detectable in EBs at around 90 days or later [65•], it may be suitable as a marker of relative maturity. In addition to purity, future screens should optimize and report information on cell quality. It is foreseeable that the field may advance beyond small panels of expression markers and instead rely on multiple parameters,

including incorporating functional ones towards an overall assessment of cell quality. Recently, Sheehy and colleagues [97] devised a 64-parameter quality assessment on commercially available murine pluripotent stem cell-derived cardiomyocytes based on structural (i.e., sarcomere alignment), electrophysiological (i.e., AP duration), and contractile performance, in comparison to freshly isolated neonatal ventricular myocytes.

Going forward

Current cardiotoxicity evaluations with animal cells and I_{K_r} /hERG-expressing heterologous systems have limitations in their prediction of clinical toxicology. Compared to heterologous cell lines, hPSC-CM technology recapitulates at least some of the native milieu and intricacies of human cardiomyocytes, and thus holds promise to be a valuable tool drug discovery and disease modeling. Notably, the FDA and the Health and Environmental Sciences Institute (HESI) recently laid out a new paradigm for preclinical cardiotoxicity testing, which includes tests on stem-cell-derived cardiomyocytes [98]. However, efforts to date have been limited both by the variability, immaturity, and heterogeneity of cardiomyocytes produced by current protocols and by the labor-intensive nature of conventional phenotyping. In particular, the immature nature of the present iteration of hPSC-CM technology is a major barrier to clinical translatability. As techniques for the production of hPSC-CMs continue to advance and standardize, and we gain a clearer understanding of the reproducibility and predictive power of hPSC-CM assays, we can begin to use hPSC-CM screens in a widespread fashion. This will represent a significant advance, moving cardiovascular medicine closer to personalized therapies and targeted therapeutics.

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* Of importance

** Of major importance

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Table 1
Progress thus far of therapeutic and toxicological screens using arrhythmic disease models

Disease	Mutation	Derivation	Control comparison	Method of differentiation	Cardiomyocyte characterization	Novel Findings	Reference
LQT1	KCNQ1 R190Q	Retroviral (OSKM)	Healthy volunteer hiPSC	Embryoid body	Patch clamp, Immunostaining for cardiac markers	Prolonged APD in LQT1 subjects	Moretti A et al. [8•]
LQT1	KCNQ1 P631fs/33	Retroviral (OSKM)	Healthy volunteer hiPSC	Embryoid body	Patch clamp, MEA, Immunostaining	Prolonged MEA FPD, dominant negative physiology only evident in heterologous system	Egashira T et al. [42]
LQT2	KCNH2 A614V	Retroviral (OSK)	Healthy volunteer hiPSC	Embryoid body	Patch clamp, MEA, Immunostaining	Prolonged APD, pharmacologic shortening effect	Itzhaki et al. [9]
LQT2	KCNH2 G1681A	Lentiviral (OLSN)	Unaffected carrier mother, Healthy volunteer hiPSC and hESC H7 line	Forced aggregation of defined cell number into embryoid bodies in V-96 plates	Patch clamp, MEA, Immunostaining	Prolonged APD and FPD, patch clamp but not MEA revealed prolonged APD in unaffected carrier mother	Matsa et al. [10]
LQT2	KCNH2 R176W	Retroviral (OSKM)	Healthy volunteer hiPSC and hESC H7 line	END-2 visceral endoderm cell co-culture	Perforated patch clamp, MEA, Immunostaining	Prolonged APD, noted that patch clamp LQT2 recordings were prolonged by 66 %, whereas MEA FPD was only prolonged by 10–20 %	Lahdi A et al. [43]
LQT2	KCNH2 N996I	Retroviral (OSKM) Targeted genetic mutation by HR in NKX2.5 ^{eGFP/w} hESCs	Targeted genetic correction by HR in mutant hiPSC, Targeted hESCs compared to untargeted hESCs	END-2 visceral endoderm cell co-culture	Patch clamp, MEA, Immunostaining	KCNH2 N996I confers a prolonged APD and MEA FPD in both hiPSC and hESC lines studied Between line differences were great, as hiPSC corrected APD measurements were even longer than WT hESC	Bellin M et al. [31]

Disease	Mutation	Derivation	Control comparison	Method of differentiation	Cardiomyocyte characterization	Novel Findings	Reference
LQT2	KCNH2 A561V	Episomal plasmid transfection	Healthy volunteer hiPSC	Embryoid body	Patch clamp, MEA, Immunostaining	LQT2 CMs exhibited prolonged APD and FPD, lower E4031-sensitive currents. Mutant hERG was located peri-nuclear. Treatment with ALLN restored membrane localization and shortened APD	Mehia A et al. [99]
LQT3	SCN5A V1763M	Synthetic mRNA (OSKML)	Unaffected sibling control hiPSC	Guided embryoid body differentiation with small molecules SB203580 and IWP-2	Patch clamp, Immunostaining	Increased late Na^+ current and significantly prolonged APD in mutant iPSC-CMs.	Ma D et al. [44]
LQT3	Two patients: SCN5A R535Q & SCN5A V240M	Retroviral (OSKM)	Unaffected unrelated iPSC control and hESC (H1) control lines	END-2 visceral endoderm cell co-culture	Patch clamp, Immunostaining	Non-significant trend towards APD prolongation. Significant variability in AP parameters between individual iPSC-CMs	Fatima A et al. [45]
LQT3	SCN5A F1473C With KCNH2 polymorphism K897T	Polycistronic lentiviral vector: hSTEMCCA-loxP lentiviral (OSKM)	Mother with KCNH2 polymorphism K897T and SCN5A WT, Father WT for each	Embryoid body formation, with growth factor and small molecule application: BMP4, Activin A, DKK1, SB-431542, BMP	Patch clamp	APD not compared, authors noting the minimal contribution of late I_{Na} with the depolarized resting membrane potential of hPSC-CM models KCNH2 polymorphism without influence on anti-arrhythmic efficacy Increased stimulation frequency reduced late I_{Na} late, correlating with clinical effect of	Terrenoire C et al. [46]

Disease	Mutation	Derivation	Control comparison	Method of differentiation	Cardiomyocyte characterization	Novel Findings	Reference
						increasing pacemaker rate	
LQT3/Brugada	SCN5A 1798insD	Retroviral or lentiviral (OSKM)	hiPSC WT for SCN5A	END-2 visceral endoderm cell co-culture	Patch clamp, Immunostaining	Increased I_{Na} late, in mutant hiPSC. Small degree of APD prolongation vs WT (217.2±14.9 vs 173.5±12.2 msec)	Davis RP et al. [12]
LQT8 “Timothy syndrome”	CACNA1C G406R	Retroviral (OSKM)	Five diseased and five healthy volunteer controls iPSC lines.	Embryoid body	Patch clamp, single cell RT-PCR for MLC _{2v} to isolate ventricular cells, Live calcium imaging with Fluo-4	Prolonged APD by ~three fold. Higher amplitude and irregular Ca^{2+} transients in mutants	Yazawa M et al. [11]
CPVT1	R YR2 M4109R	Retroviral (OSK) plus valproate	Healthy volunteer hiPSC	Embryoid body	Patch clamp, MEA, Live calcium imaging with Fluo-4	Increased frequency of delayed afterdepolarizations in CPVT iPSC-CMs, which increased with adrenergic stimulation and were abolished by flecainide. Increased Ca transients and increased store-overload-induced Ca release at high extracellular Ca^{2+}	Itzhaki I et al. [13]
CPVT1	R YR2 S406L	Retroviral (OSKM)	Healthy volunteer hiPSC	Embryoid body	Patch clamp, Immunostaining, Live calcium imaging with Fluo-4	CPVT iPSC-CMs exhibited increased Ca^{2+} cycling abnormalities including alternans, transient fusion, and irregular oscillations. Upon exposure to isoproterenol, diastolic Ca^{2+} levels elevated in CPVT cells. Dantrolene rescued	Jung CB et al. [48]

Disease	Mutation	Derivation	Control comparison	Method of differentiation	Cardiomyocyte characterization	Novel Findings	Reference
CPVT1	R/YR2 P2328S	Retroviral (OSKM)	Healthy volunteer hiPSC	END-2 visceral endoderm cell co- culture	Patch clamp, Immunostaining, Live calcium imaging with Fura-2 AM	CPVT iPSC-CMs showed higher Ca ²⁺ cycling abnormalities (14 % vs 8 %), which augmented with adrenergic stimulation. CPVT cells had higher diastolic calcium levels and lower SR load.	Kujala KP et al. [49]
CPVT1	R/YR2 F2483I	Retroviral (OSKM)	Healthy fibroblast hiPSC clone hiPSC1, hESC lines H1, H9, HES- 2	END-2 visceral endoderm cell co- culture	Patch clamp, MEA recording, Live calcium imaging with Fluo-4, Caffeine releasable SR-Ca ²⁺ stores	Non-significant excess of arrhythmic activity on MEA in CPVT CMs, excess of DADs in CPVT CMs Slow decay of Ca transients in mutants, Increased Ca- induced Ca- release gain in mutants upon adrenergic stimulation, yet lower SR Ca ²⁺ - load	Zhang XH et al. [50] and Fatima et al. . [100]
CPVT2	CASQ2	Polycistronic lentiviral vector: hSTEMCCA -loxP lentiviral (OSKM)	Three healthy volunteer hiPSCs	Embryoid body	Patch clamp, Immunostaining, Live calcium imaging with Fura-2 AM, Transmission electron microscopy	Isoproterenol induced after- contractions and DADs in CPVT2- CMs and elevated diastolic [Ca ²⁺] _i levels. **APD was longer in CPVT2- CMs	Novak A et al. [51]
ARVC	<i>PKP2</i> 2484C>T causing cryptic splicing in exon 12	Retroviral (OSKM)	Healthy volunteer hiPSCs, and H9 hESCs	Embryoid body, Lipogenic conditions (5F protocol: PPAR γ and PPAR α agonists, with insulin, dexamethasone, and IBMX)	TUNEL staining for apoptosis, Patch clamp, Energetics, Immunostaining	ARVC-CMs had excess apoptosis upon lipogenic induction, depressed energy production and increased glycolytic reliance, mutant cells had	Kim et al. [14]

Disease	Mutation	Derivation	Control comparison	Method of differentiation	Cardiomyocyte characterization	Novel Findings	Reference
ARVC	PKP2 L614P	Retroviral (OSKM)	Healthy volunteer hiPSC, H9 hESCs	Embryoid body, In some renditions, Lipogenic conditions (indomethacin, insulin, dexamethasone, and IBMX)	Patch clamp, Immunostaining and Oil Red O staining, Live calcium imaging with Fluo-4, Transmission electron microscopy	prolonged relaxation time Slightly higher mean diastolic potential and prolonged APD ARVC-CMs feature reduced PKP2 and plakoglobin desmosomal proteins at cell periphery. Increased Oil Red O staining in mutant cells after exposure to lipogenic conditions	Ma D et al. [15]
ARVC	Two patients; PKP2 c972InsT/ N leading to premature stop codon PKP2 c148-151delAC AG/N causing premature stop codon	Retroviral (OSK) with valproate	Healthy volunteer hiPSC	Embryoid body, Lipogenic conditions (insulin, dexamethasone, IBMX, 20 % fetal bovine serum, and StemPro LipoMax)	Patch clamp, Immunostaining, MEA Transmission electron microscopy	Significant reduction in PKP2 immunosignal in ARVC-CMs, Prolonged field potential rise time by MEA 33 % of ARVC-CMs featured lipid droplets on EM compared to none in healthy hiPSC-CMs, Lipogenic conditions caused an extensive amount of lipid accumulation in ARVC-CMs ARVC-CMs featured hazy, dissymmetric desmosomes and widened desmosomal gaps ARVC-CMs featured higher percentage of apoptotic cells (3.8 % vs. 1.0 %)	Caspi O et al. [53]

OSKM: Oct-4, Sox-2, Klf-4, c-Myc

OSK: Oct-4, Sox-2, Klf-4

OLSN: Oct-4, Lin28, Sox-2, Nanog

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OSKML: Oct-4, Sox-2, Klf-4, c-Myc, Lin28

** criticized as non-representative of the clinical disorder

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