Modeling Inherited Cardiac Disorders
– A Cell Is Worth a Thousand Genes –
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Advances in the understanding and treatment of cardiac disorders have been thwarted by the inability to study beating human cardiac cells in vitro. Induced pluripotent stem cells (iPSCs) bypass this hurdle by enabling the creation of patient-specific iPSC-derived cardiomyocytes (iPSC-CMs). These cells provide a unique platform to study cardiac diseases in vitro, especially hereditary cardiac conditions. To date, iPSC-CMs have been used to successfully model arrhythmic disorders, showing excellent recapitulation of cardiac channel function and electrophysiologic features of long QT syndrome types 1, 2, 3, and 8, and catecholaminergic polymorphic ventricular tachycardia (CPVT). Similarly, iPSC-CM models of dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) have shown robust correlation of predicted morphologic, contractile, and electrical phenotypes. In addition, iPSC-CMs have shown some features of the respective phenotypes for arrhythmogenic right ventricular dysplasia/cardioangiomyopathy (ARVD/C), LEOPARD syndrome, Pompe’s disease, and Friedreich’s ataxia. In this review, we examine the progress of utilizing iPSC-CMs as a model for cardiac conditions and analyze the potential for the platform in furthering the biology and treatment of cardiac disorders. ([Circ J 2014; 78: 784–794])

Key Words: Channelopathy; Dilated cardiomyopathy; Drug discovery; Human-induced pluripotent stem cells; Hypertrophic cardiomyopathy

Cardiac disease remains the most common cause of death in developed countries.1 Over the past 4 decades, there have been remarkable advances in our understanding and treatment of ischemic heart disease, while our understanding of cardiomyopathies and arrhythmic disorders has lagged behind. Indeed, the realization that many cardiomyopathies and sudden cardiac deaths (SCD) are in fact related to genetic disorders has heralded a remarkable era for hereditary cardiac disorders.2 With advances in genetic sequencing methods, more genetic targets are being identified as pathogenic variants of these disorders. Unfortunately, understanding the biology of how these putative genetic variants result in observed phenotypic changes has proven challenging.3

A big hurdle to understanding cardiac molecular and cellular physiology is the inability to maintain and study functional (beating) human cardiomyocytes in culture. This leaves scientists to rely on animal models or transfected cell models. Furthermore, the invasive nature of obtaining primary human cardiac tissue has hindered the field compared with other fields such as hematology or oncology. Induced pluripotent stem cells (iPSCs) have emerged as a reliable method of producing patient-specific somatic tissue lines via directed differentiation. By enabling the analysis and manipulation of human cardiac cells in culture, iPSC-derived cardiomyocytes (iPSC-CMs) have provided a robust platform for the study of genetic cardiac disorders (Table). The model has already proven complementary or superior when compared with transfected cell or animal models of cardiac genetic disorders. In this review, we aim to examine the growing body of work that has utilized iPSC-CMs to study cardiac disorders, offer some insights as to the strength of iPSC-CMs as a modeling tool, and how this could affect cardiovascular clinical practice and biomedical research (Figure 1).

History of iPSCs
iPSCs are made by reprogramming somatic cells into a more pluripotent phenotype that is capable of self renewal and differentiating into all 3 germ layers. This was first described by Takahashi and Yamanaka4 and later by Yu et al.5 Shortly thereafter, several groups showed that directed differentiation can produce somatic cell derivatives such as neurons, hematopoietic cells, and cardiomyocytes (Figure 1).6–8 The technology offers several advantages over the prior state of biomedical research. First, it bypasses the ethical concerns about the use of human embryonic stem cells, the only other pluripotent cell source. Second, because they are self-renewing, iPSCs can provide an unlimited supply of differentiated somatic tissues. Lastly, iPSCs as well as their derivatives recapitulate the genotype of their original somatic donor, which offers the unprecedented potential of obtaining patient-specific tissue types non-
invasively.

Since the first reports, there have been major advances in the methods of making iPSCs and lineage-specific differentiation protocols, which are outside the scope of this review. However, it is fair to say that obtaining patient-specific iPSCs and iPSC-CMs has become more reliable and more efficient. Given the difficulty in studying cardiac disorders in vitro, interest in utilizing iPSC-CMs as a model for cardiac disorders has grown very rapidly. Here we will highlight some of the studies that illustrate the advantages and limitations of the model.

**Inherited Channelopathy Models**

The most extensively studied iPSC-CM models are those of arrhythmic disorders. This is in part because the mechanisms by which gene variants lead to perturbations in ion flux are better defined than those of sarcomeric myopathies. Furthermore, patch clamp and multielectrode array (MEA) platforms reliably measure cellular action potential (AP) properties, ion channel currents, and arrhythmic potential. This detailed electrophysiologic assessment enables direct comparison and correlation with in vivo phenotypic changes such as ECG abnormalities or arrhythmia formation.

Long QT syndrome (LQT) is a disorder that is characterized clinically by prolongation of the QT interval on surface ECG and increased burden of ventricular arrhythmias and SCD. Advances in genetics and functional classification were able to further classify the syndrome into multiple subtypes based on common genetic loci and functional disturbances in ion channel flux. Although 13 loci associated with LQT have been identified, variants in 3 loci account for 60–70% of clinical cases. LQT is probably the most extensively studied arrhythmic disorder, given its prevalence and the fact that it has been described for over 50 years, compared with more recently described arrhythmic disorders such as short QT syndrome or catecholaminergic polymorphic ventricular tachycardia (CPVT). Because of the relatively advanced understanding of LQT compared with other arrhythmic disorder, LQT was an attractive first option for investigating the utility of iPSC-CMs as a functional model.

**Long QT1**

LQT1 is most commonly caused by variants in the KCNQ1 gene resulting in disruption in the slow delayed-rectifier potassium current (I\(_{Ks}\)). Classically, patients are described as developing exercise induced arrhythmia, which presumably results from inability of the cell to shorten the QT interval with exercise.

### Table. Summary of Major Studies Utilizing iPSC-CM to Model Hereditary Cardiac Disorders

| Disorder | Study | Gene (variant/s) | Histological or cellular abnormalities | Electrophysiological abnormalities | Response to standard pharmacologic agents | Force generation abnormalities | Novel drug testing |
|----------|-------|------------------|---------------------------------------|-----------------------------------|-----------------------------------------|-----------------------------|------------------|
| LQT1     | Moretti et al (2010) | KCNQ1 (p.R190Q) | –                                    | +                                  | +                                      | –                           | –                |
|          | Egashira et al (2012) | KCNQ1 (p.C1893del) | –                                    | +                                  | +                                      | –                           | –                |
| LQT1     | Itzhaki et al (2011) | KCNQ2 (p.A614V) | –                                    | +                                  | –                                      | –                           | –                |
|          | Matsa et al (2011)    | KCNQ2 (p.G6181A) | –                                    | +                                  | –                                      | –                           | –                |
|          | Lahti et al (2012)    | KCNQ2 (p.R176W)  | –                                    | +                                  | +                                      | –                           | –                |
| LQT3     | Terrenoire et al (2013) | SCNA5 (p.F1473C) | –                                    | +                                  | –                                      | –                           | –                |
|          | Ma et al (2013)       | SCNA5 (p.V1763M) | –                                    | +                                  | +                                      | –                           | –                |
| LQT8     | Yawata et al (2011)   | CACNA1C (p.G406R) | –                                    | +                                  | –                                      | –                           | –                |
| CPVT     | Itzhaki et al (2012)  | RYR2 (p.M4109R) | –                                    | +                                  | –                                      | –                           | –                |
|          | Fatima et al (2011)   | RYR2 (p.T7447A) | –                                    | +                                  | +                                      | –                           | –                |
|          | Novak et al (2012)    | CASQ2 (p.D307H) | –                                    | +                                  | –                                      | –                           | –                |
|          | Jung et al (2012)     | RYR2 (p.S406L)  | –                                    | +                                  | +                                      | –                           | –                |
| DCM      | Sun et al (2012)      | TNNT2 (p.R173W) | +                                    | –                                  | +                                      | +                           | –                |
|          | Siu et al (2012)      | LMNA (p.R225x, p.S18fs) | +                               | –                                  | +                                      | +                           | –                |
|          | Tse et al (2013)      | DES (p.A285V)   | –                                    | –                                  | +                                      | –                           | +                |
| HCM      | Lan et al (2013)      | MYH7 (p.R663H) | +                                    | +                                  | +                                      | +                           | –                |
| ARVD/C   | Kim et al (2013)      | PKP2 (c.2484C>T, p.G8286C, r.[2483_2489del]) | –                               | +                                  | +                                      | +                           | +                |
|          | Ma et al (2013)       | PKP2 (p.L614P) | –                                    | +                                  | +                                      | +                           | –                |
| LEOPARD  | Carvajal-Vergara et al (2010) | PTPN11 (p.T468M) | +                                  | –                                  | +                                      | +                           | +                |
| Pompe's disease | Huang et al (2011) | p.D645E/p.D645E, c.1935C>A/c.2040+1G>T | +                                     | –                                  | +                                      | –                           | –                |
| Friedreich's ataxia | Hick et al (2011) | Expanded GAA repeats in 1st intron of FXN | +                                     | –                                  | +                                      | –                           | +                |
| Other    | Liang et al (2013)    | KCNQ1 (p.G269S), MYH7 (p.R663H), TNNT2 (p.R173W) | +                                  | –                                  | +                                      | –                           | +                |

* Using electron microscopy.

ARVD/C, arrhythmogenic right ventricular dysplasia/cardio myopathy; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQT, long QT syndrome.
Long QT2
Another defect in potassium channel flow results in LQT2, which is most commonly caused by variants in KCNH2 (also called human Ether-à-go-go-Related Gene: hERG), and creates an abnormal rapid cardiac delayed-rectifier potassium current (I_{Kr}). Patients who develop ventricular arrhythmia or SCD in response to auditory stimuli most commonly have LQT2.15

Another group published an iPSC-CM model of LQT2 caused by a known missense mutation in KCNH2 (p.A614V).17 Similar to the LQT1 model, the iPSC-CMs exhibited prolonged APD and field potential duration (FPD) at the single cell and tissue levels, respectively. In addition, the cells exhibited reduced IKr current, which also supports the proposed mechanism for LQT2, namely that the prolonged repolarization is caused by the loss of function of rapid-acting inward-rectifying potassium channels. Those authors found that at the multicellular level, 38% of LQT2 iPSC-CMs exhibited ectopic single and multiple triggered beats, which is the same mechanism hypothesized to trigger the premature beats that occur during the repolarization phase and to result in the ventricular arrhythmia of torsades de pointes (TdP) seen in LQT2 patients.

Timothy Syndrome (LQT8)
Timothy syndrome is a genetic disorder caused by mutation in the L-type calcium channel (Cav1.2) first described by...
Splawski et al in 2004. The disorder is characterized by syndactyly, immune deficiency, autism, and LQT (classified as LQT8). Yazawa et al created iPSC lines from 2 Timothy syndrome patients and 2 control patients, all of which differentiated into beating embryoid bodies (EBs). There were no gross differences among the lines on immunohistochemical staining of major cardiac proteins. As predicted by the genotypic variant, patch clamp analysis revealed reduced L-type calcium channel voltage-dependent inactivation in Timothy syndrome iPSC-CMs. This led to prolongation of the APD and increased incidence of delayed afterdepolarizations (DAD) in ventricular iPSC-CMs from Timothy syndrome patients compared with the controls. Roscovitine, which increased the voltage-dependent inactivation of the L-type calcium channels, reduced the timing and amplitude of calcium transients and APD in Timothy syndrome iPSC-CMs, bringing them closer to control cell levels.

**Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)**

CPVT is a hereditary arrhythmic disorder thought to be caused by abnormal calcium handling in cardiomyocytes. Clinically, the disorder is associated with states of catecholamine surge that precipitate bidirectional VT and SCD. The majority of patients harbor variants in the ryadone receptor gene (RYR2) and cardiac calsequestrin (CASQ2) that result in calcium leakage, potentiating DAD at the cellular level. The exact mechanism by which the variants lead to calcium-handling defects is not completely understood, which makes CPVT an especially interesting disorder to study using an iPSC-CM platform.

Itzhaki et al reprogrammed iPSCs from dermal fibroblasts of a CPVT patient with a heterozygous mutation in RYR2 (p.R663H) to make patient-specific iPSC-CMs. They noted no difference in baseline AP properties between CPVT and control iPSC-CMs. However, pacing at faster intervals resulted in development of DAD in 69% of CPVT iPSC-CMs compared with 11% of healthy control iPSC-CMs. Furthermore, they chemically simulated adrenergic surges by adding forskolin (adenylate cyclase activator) and isoproterenol, both of which increased the frequency of DAD and were associated with the development of triggered activity. Flecaïnide, which is thought to be clinically useful in CPVT patients, completely eliminated DAD. To confirm the proposed mechanism of CPVT as being increased intracellular levels of calcium, the authors applied a sarcoplasmic reticulum calcium ATPase inhibitor (thapsigargin) that depletes intracellular calcium stores. As predicted, depleting the cells of intracellular calcium decreased the incidence of DAD. Furthermore, the authors performed calcium imaging, demonstrating that abnormal calcium transients were exacerbated by faster pacing, adrenergic stimulation, and higher calcium concentration. Similar results were published by Fatima et al and Novak et al, who made iPSC-CMs from another RYR2 variant (p.F2483I) and a CASQ2 variant (p.D307H), respectively. The results were congruent, demonstrating the increased propensity to arrhythmia and diastolic calcium rise in response to isoproterenol.

**Inherited Cardiomyopathy Models**

All of the models described have relied on studying arrhythmic disorders by taking advantage of reliable tools available to measure cellular electrophysiology (EP). However, there was considerable doubt whether myopathies could be functionally modeled by iPSC-CM and if abnormalities in contractile function could be analyzed in vitro to validate the cellular phenotype of cardiomyopathy.

**Familial Dilated Cardiomyopathy (DCM)**

Dilated cardiomyopathy (DCM) is a disorder of progressive ventricular dilation and decreased systolic function that usually leads to the heart failure syndrome and possibly the development of lethal arrhythmias. Familial DCM refers to genetic cases of the disorder, but the term DCM is sometimes used interchangeably to refer to such cases. The pathophysiology of the progression of DCM is thought to be related to upregulation of catecholamines, the renin-angiotensin axis, and loading conditions. However, the pathophysiology by which individual causative variants lead to the observed phenotype is not well defined. The complexity in simulating systemic in vivo conditions, the limitations of measuring cellular phenotype, and incomplete understanding of the cellular pathophysiology have all been barriers to creating successful myopathy models.

The first successful model of a cardiomyopathy that tested the functional phenotype of iPSC-CMs was created by Sun et al, who recruited a 3-generation family with a point mutation TNNT2 mutation (p.R173W) to make patient-specific iPSC-CMs. They observed clear morphological differences between DCM iPSC-CMs and controls (Figure 2). Functionally, this translated into decreased contractility compared with the control iPSC-CMs as measured by atomic force microscopy. In addition, the authors mirrored the clinical effects of various drugs in DCM by demonstrating that treatment with norepinephrine resulted in exacerbation of the DCM phenotype, whereas treatment with metoprolol was protective.

Another effort to model DCM involved a lamin A/C (LMNA) variant, which causes a characteristic pattern of progressive conduction system disease and DCM. Those authors created iPSCs from a patient with LMNA mutation (p.R225X). Affected iPSC-CMs demonstrated nuclear senescence compared with control iPSC-CM. Electrical stimulation increased the prevalence of nuclear senescence and induced apoptosis in LMNA iPSC-CMs. The finding of tachycardia resulting in cell death is consistent with the prevailing hypothesis of the disorder that increased stimulation induces damage to myocytes with fragile nuclear properties.

**Familial Hypertrophic Cardiomyopathy (HCM)**

Hypertrophic cardiomyopathy (HCM) is a disorder of abnormal thickening of primarily the left ventricular wall. The condition is predominantly genetic in nature, and although it usually has a more benign course than DCM, it can lead to the heart failure syndrome and lethal arrhythmias. There are no approved life-prolonging drug therapies for HCM, and predicting any given patient’s course in terms of development of symptoms or lethal arrhythmias remains a challenge. Lan et al sought to evaluate the mechanism by which a well-reported genetic variant in MYH7 (p.R663H) results in cardiac hypertrophy. HCM iPSC-CMs showed a clear hypertrophic phenotype at the single cell level (Figure 3). In addition, EP assessment showed frequent DAD in HCM iPSC-CMs compared with control iPSC-CMs. The authors showed that impaired calcium handling and increased diastolic intracellular calcium concentration were implicated in cellular hypertrophy and arrhythmogenic burden. Interestingly, the administration of the L-type calcium-channel blocker, verapamil, improved calcium handling and ameliorated the hypertrophic and arrhythmogenic profile. This finding suggests that impaired calcium regulation is actually causative of the arrhythmia and hypertrophy rather than a secondary effect, contrary to the notion that the proarrhythmic effect was solely a function of tissue-level structural hypertrophy or intracavitary obstruction.
Apoptosis compared with EBs with low level Isl1+ cells. This supports the hypothesis that the Isl1+-derived RV-like cardiomyocytes confer the dominant pathology seen in ARVD/C.

Another model by Ma et al. created iPSC-CMs from a patient heterozygous for a different mutation in PKP2 (p.L614P). At baseline, iPSC-CMs expressed lower levels of PKP2 RNA and proteins but similar levels of other desmosomal proteins. But much like the study by Kim et al, no significant differences in lipid content were found at baseline. Also consistent with the findings of Kim et al, growing the cells in a lipogenic medium resulted in increased lipid content as detected by Oil Red O staining.

Although these studies were commendable first steps in attempting to model ARVD/C, they also revealed some of the significant challenges and limitations to iPSC-CM modeling. First, ARVD/C is a primarily adult-onset disease, usually manifesting by the 3rd or 4th decade, and observing any changes at a fetal stage of cardiomyocytes is more challenging. Second, ARVD/C is a tissue-level disorder characterized by multicellular abnormalities of RV morphologic changes, fatty infiltration, and arrhythmias that localize to the RV outlet tract. Furthermore, understanding the systemic and cellular conditions that contribute to the disorder is important for the creation of successful models. Although the manipulations involving activation of lipid pathways are conceptually useful and may help answer specific questions, they do not represent well-described mechanisms of the disease’s pathogenesis and do not necessarily mimic the physiologic process in vivo. This is not to say that there are not useful insights that could be gained from studying multicellular diseases at the single cell level, only that...

Figure 2. iPSC-CMs from familial dilated cardiomyopathy (DCM) patients show an abnormal sarcomeric α-actinin distribution. (A) Immunostaining of sarcomeric α-actinin and cardiac troponin T (cTnT) at day 30 after differentiation. (B) Compared with control iPSC-CMs, a higher percentage of DCM iPSC-CMs showed a punctate sarcomeric α-actinin staining pattern in greater than one-quarter of the total cellular area (**P=0.008). (C) No significant difference in cell size between control and DCM iPSC-CMs can be seen. (D) Transmission electron microscopy demonstrates that compared with controls, DCM iPSC-CMs show increased variability in the degree of organization. iPSC-CMs, induced pluripotent stem cell-derived cardiomyocytes; ZL, Z line; ZB, Z bodies; A, A band; I, I band; Mt, mitochondria. (Reproduced with permission from Sun et al. 24)
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40,000 individuals and can present in an infantile or late-onset form. The infantile form is characterized by cardiomyopathy and severe hypotonia, whereas the adult-onset form presents more as a skeletal myopathy that eventually leads to respiratory failure.

Huang et al demonstrated that both the iPSC lines and the iPSC-CMs derived from 2 different patients had lower GAA activity, lower markers of metabolism (oxygen consumption rate and extracellular acidification), and higher glycogen content. The iPSC-CMs contained large glycogen vacuoles, deteriorating mitochondria, and autophagosome-like structures, all of which are histological characteristics of tissue from patients with Pompe's disease. The authors also showed that supplementation of recombinant human GAA, an existing therapy, rescued the phenotype.

LEOPARD Syndrome

The first study to explore cardiac modeling of iPSC derivatives tackled LEOPARD syndrome. LEOPARD syndrome is a genetic disorder marked by multiorgan abnormalities resulting from mutations in PTPN11. Up to 80% of patients with the disorder have HCM. LEOPARD iPSC-CMs were found to have a significantly higher median surface area compared with wild-type counterparts. In addition, the authors noticed increased nuclear expression of the hypertrophy-associated transcription factor NFATC4. The authors acknowledged their iPSC-CM population was not pure enough to carry out more detailed molecular analysis for hypertrophy. Despite these limitations, this landmark study was a proof-of-concept that iPSC-CMs recapitulate the genetic profile of their somatic derivatives and possibly some morphological features.

Pompe's Disease

Pompe's disease is an autosomal recessive metabolic disorder resulting from a mutation in the gene encoding α-glucosidase (GAA). The disorder has an incidence of approximately 1 in 40,000 individuals and can present in an infantile or late-onset form. The infantile form is characterized by cardiomyopathy and severe hypotonia, whereas the adult-onset form presents more as a skeletal myopathy that eventually leads to respiratory failure. Huang et al demonstrated that both the iPSC lines and the iPSC-CMs derived from 2 different patients had lower GAA activity, lower markers of metabolism (oxygen consumption rate and extracellular acidification), and higher glycogen content. The iPSC-CMs contained large glycogen vacuoles, deteriorating mitochondria, and autophagosome-like structures, all of which are histological characteristics of tissue from patients with Pompe’s disease. The authors also showed that supplementation of recombinant human GAA, an existing therapy, rescued the phenotype.

Friedreich's Ataxia (FA)

FA is a genetic disorder caused by mutations in the frataxin gene, which encodes the frataxin mitochondrial protein, resulting in cardiac and neurologic abnormalities. Clinically the disorder is most notable for severe ataxia and dysarthria caused by neurodegeneration of cerebellar pathways. However, over 50% of patients with the disorder die from congestive heart failure or arrhythmias, which are products of cardiac manifestation of the mitochondrial disorder. Hick et al created iPSC-CM models from patients with FA and showed that the cells contained a
higher frequency of structurally abnormal nuclei. Interestingly, the prevalence of abnormal nuclei increased over time, though this did not result in worsening electrical properties of the cell. However, the study was limited to image recordings and did not perform patch clamp analysis as they did for neurons. Thus, iPSC-CMs are not only useful for studying mechanical and electrical disorders of the heart, but also metabolic and mitochondrial disorders.

**Potential and Future Directions of Utilizing iPSC-CMs**

**Functional Classification of Genotypes**

An important adjuvant in treating heritable conditions, including cardiac disorders, is identification of the causative genetic variants. This is necessary to enable screening of family members and may also play a role in the diagnosis. However, the results of genetic testing are not binary positive or negative, but are subject to interpretation of the clinician evaluating the candidate variant and the existing evidence for the disease-causing mechanism. Clinicians who care for these patients often struggle with variants of uncertain significance.2 iPSC-CMs can play a key role in improving future medical evaluation by functionally characterizing these genetic variants.

Tse et al46 investigated a novel variant in the desmin gene identified in a patient with DCM (p.A285V). They created iPSC-CM models that demonstrated failure of colocalization of desmin with other sarcomeric proteins. The authors then used a viral transfection method to transfet control iPSC-CMs with A285-DES, which confirmed the phenotype. Although the confirmation of the DCM phenotype was molecular and not functional, it demonstrated an early potential for studying novel variants or variants of unclear significance identified in individuals.

Subsequently, Egashira et al47 created iPSC-CMs from a young patient who presented with sudden cardiac arrest and exhibited features of LQT1. The patient was found to have a novel variant of KCNQ1 (p.C1893del). Similar to the study by Moretti et al, the patient’s cells exhibited prolonged FPD and exhibited a trafficking defect of KCNQ1. One critique of the study is that the authors used a transfection model in HEK293 cells that demonstrated reduced IKs current to confirm variant pathogenicity. However, iPSC-CMs may offer an improved standard of testing pathogenicity, especially by applying genetic editing techniques, such as TALEN or CRISPR, and observing phenotypic changes. This has already been done in an iPSC model of Parkinson’s disease, but has yet to be shown in cardiac disorders.48

**Personalized Medicine**

Over the past 2 decades, our understanding of LQT syndrome has changed, the key realization being that different subtypes are caused by variants in different loci. The subtypes have different natural histories and treatment approaches. We now recognize that even within subtypes, different variants can predict a different disease course.49 Furthermore, it is now recognized that arrhythmic disorders sometimes do not have well-demarcated borders, but there is a spectrum of overlap syndromes.40 The ability to define and use the functional effect of de novo mutations for making therapeutic decisions is a promising feature of iPSC-CMs.

This was elegantly demonstrated by Terrenoire et al,45 who created iPSC-CMs from a proband with LQT syndrome and severe arrhythmia phenotype (QTc of 825 ms) that was refractory to multiple treatments. Genetic testing identified a de novo mutation in SCN5A (p.F1473C), which normally leads to LQT3. In addition, the patient was heterozygous for a common polymorphism in KCNH2 (p.K897T). LQT3 is caused by a gain-of-function variation in SCN5A, resulting in decreased voltage-dependent inactivation of sodium channels. Prior work by those authors showed that transfection of this variant in HEK293 cells resulted in defects in inactivation (producing a gain of function) consistent with the LQT3 phenotype.42 However, the patient was particularly refractory to therapy, and the role of the KCNH2 polymorphism in the phenotype remained unclear. The effect of this polymorphism is controversial; that is, whether it exerts a protective or harmful effect.43,44 Using patch clamp and current clamp techniques, the authors clearly showed a defect in sodium channel inactivation kinetics, but did not find any abnormalities in the IKs current or the activation properties of the channel. Furthermore, high-dose mexiletine, a sodium-channel blocker, as well as faster pacing were able to significantly reduce the late sodium current. This is precisely how the patient was treated clinically, pacing at a higher rate and administering high-dose mexiletine therapy. This model of personalized medicine will likely emerge as a powerful clinical application for iPSC-CMs in cases of complex genetic disorders with poorly defined therapeutic pathways. A more classic model of a de novo SCN5A variant (p.V1763M) causing LQT3 was later published, showing similar findings.45

**Risk Stratification**

One of the challenges in treating many hereditary cardiac disorders is the variable expression of phenotype and severity of phenotype, even in carriers from the same family.3 Thus, even in cases of well-defined variants within the same family, a family member will exhibit severe disease, while others will be completely asymptomatic. This makes risk stratification based on genotype alone challenging, and the creation of a platform that can better risk stratify carriers would prove very helpful clinically. This then raises the question whether patient-specific iPSC-CMs can be that platform by recapitulating disease severity and not just gross features of the disease.

This issue was examined in study by Matsa et al40 using iPSC lines from an affected LQT2 patient and an asymptomatic gene carrier. The symptomatic patient had a more prolonged surface ECG and markedly symptomatic arrhythmias, whereas the asymptomatic gene carrier had a less prolonged surface ECG but otherwise no evidence of arrhythmias. Interestingly, the APD of the iPSC-CMs from the asymptomatic carrier patient was more prolonged than that of the control cells, but still significantly shorter compared with the symptomatic patient’s iPSC-CMs. Furthermore, the asymptomatic carrier’s iPSC-CMs did exhibit the large EAD burden with isoproterenol stimulation that the symptomatic LQT patient’s iPSC-CMs did. Similarly, Lahti et al47 derived iPSC-CMs from an asymptomatic carrier of a known LQT2 variant (p.R176W). The iPSC-CMs showed a prolonged APD, but interestingly did not exhibit more spontaneous EAD than the control cells, correlating with the patient’s clinical phenotype of prolonged surface ECG QT interval but lack of arrhythmia. This is in contrast to other LQT2 iPSC-CM models derived from more symptomatic LQT2 patients that showed higher incidence of spontaneous EADs.17,46

Taken together, these results suggest that there may be a correlation between the observed clinical phenotype and iPSC-CM phenotype. These observations are very preliminary and need to be further tested on a larger scale and across multiple disease models. However, if indeed iPSC-CMs could recapitu-
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of the FPD was observed. This iPSC-CM platform supported the hypothesis that there is a certain redundancy in the repolarization currents ($I_{Ks}$ and $I_{Kr}$), and that there is a compound effect to genetic and exogenous modifying factors that affect the FPD.

Toxicity Testing

Cardiac toxicity remains a leading cause of withdrawal of candidate drugs either during development or after market release. The current approach to cardiovascular safety assessment focuses on animal testing to test systemic cardiovascular effects and evaluation of hERG channel blockade, which most often involve drug testing in cell transfection models expressing the hERG channel. The transfected cellular models have several limitations. First, the cardiac action potential involves complex and sequential activation of multiple ion channels, and the evaluation of a single channel (even an influential and commonly affected one) fails to predict the effects on the entire AP. Of equal importance, there are non-EP mechanisms of cardiac drug toxicity that have become increasingly clinically significant. To date, iPSC-CMs are the closest surrogates for human in vivo cardiac cells that can be manipulated and studied in culture, and thus would appear to be a much more predictive model of human cardiac toxicity.

This direct comparison was addressed in a study in which hERG channel-transfected HEK293 cells (hERG-HEK293) were directly matched to iPSC-CMs in terms of predicting drug toxicity. Liang et al were able to demonstrate the superiority of iPSC-CMs to hERG-HEK293 cells in predicting late the severity of phenotype, this would redefine the clinical approach to risk-stratifying patients with LQT and possibly other hereditary cardiac disorders.

Studying Molecular Mechanisms of Disease

Beyond genotype-phenotype investigations, other groups have used iPSC-CMs to study longstanding questions that were previously impaired by the inability to culture and grow functional human cardiomyocytes in vitro. For example, clinicians often recognize that some patients will experience drastic prolongation of QT with certain hERG-blocking drugs, while others have minimal symptoms. This phenomenon led to the hypothesis of repolarization reserve, namely the ability of cells to compensate for reduced repolarization currents via other channels and a degree of built-in redundancy between repolarization currents. Given that iPSC-CMs express virtually all channels expressed by human myocytes, they offer a platform with some advantages over animal models and HEK293 transfection models to study the concept of repolarization reserve. Braam et al investigated the effects of 9 antiarrhythmic drugs on the FPD of iPSC-CMs as measured by MEA. Most compounds showed responses in terms of prolonging or shortening the field potential that were congruent with those of other models. The exceptions were HMR1556 and JNJ303, both of which were expected to prolong the field potential by blocking the $I_{Ks}$ current, but actually had a minor effect in wild-type cells. When the repolarization reserve was challenged by administering sotalol ($I_{Kr}$ blocker) or administering the compounds in LQT2 iPSC-CMs (reduced $I_{Kr}$), a clear prolongation of the FPD was observed. This iPSC-CM platform supported the hypothesis that there is a certain redundancy in the repolarization currents ($I_{Ks}$ and $I_{Kr}$), and that there is a compound effect to genetic and exogenous modifying factors that affect the FPD.

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Cardiac toxicity remains a leading cause of withdrawal of candidate drugs either during development or after market release. The current approach to cardiovascular safety assessment focuses on animal testing to test systemic cardiovascular effects and evaluation of hERG channel blockade, which most often involve drug testing in cell transfection models expressing the hERG channel. The transfected cellular models have several limitations. First, the cardiac action potential involves complex and sequential activation of multiple ion channels, and the evaluation of a single channel (even an influential and commonly affected one) fails to predict the effects on the entire AP. Of equal importance, there are non-EP mechanisms of cardiac drug toxicity that have become increasingly clinically significant. To date, iPSC-CMs are the closest surrogates for human in vivo cardiac cells that can be manipulated and studied in culture, and thus would appear to be a much more predictive model of human cardiac toxicity.

This direct comparison was addressed in a study in which hERG channel-transfected HEK293 cells (hERG-HEK293) were directly matched to iPSC-CMs in terms of predicting drug toxicity. Liang et al were able to demonstrate the superiority of iPSC-CMs to hERG-HEK293 cells in predicting.
toxicity of multiple drugs, including verapamil and alfuzosin. They also screened diseased iPSC-CMs derived from individuals with LQT, HCM, and DCM with a panel of drugs known to interact with different cardiac ion channels, including cisapride, which is a potent hERG-blocking drug removed from the market in 2000 (Figure 4). Another study showed that iPSC-CMs were equally effective to hERG-HEK293 cells in predicting zolpidem toxicity.\textsuperscript{52} Thus, the data suggest iPSC-CMs represent an advanced model for predicting cardiac toxicity compared with hERG-transfection models.

In addition to toxicity and efficacy testing, iPSC-CMs provide a platform to study the mechanism of efficacy and toxicity in human cells, which can be difficult to ascertain. Sunitinib is a chemotherapeutic agent used to treat renal cell carcinoma and gastrointestinal stromal tumors, and is known to be associated with left ventricular dysfunction. Cohen et al\textsuperscript{53} administered sunitinib to iPSC-CMs and demonstrated that it induced cell death by ATP depletion, increasing oxidative stress, and apoptosis. Because animal models have suggested aminomiodazole carboxamide ribonucleotide (AMPK) or ribosomal S6 kinase (RSK) as possible mediators of sunitinib-induced cardiotoxicity, the authors used iPSC-CMs as a platform to test these hypotheses and showed that neither AMPK nor RSK was the mediator of sunitinib cytotoxicity.

**Identifying New Drug Targets**

In most iPSC-CM disease modeling studies to date, researchers have utilized drugs with well-defined clinical responses to validate the respective iPSC-CM models. In other words, they were retrospective tests to validate predicted human in vivo clinical response. Jung et al\textsuperscript{54} studied whether iPSC-CMs can be used to prospectively test for drug efficacy. The investigators created iPSC lines from a patient with CPVT and documented the abnormal calcium handling and arrhythmic potential seen by other groups.\textsuperscript{21–23} The cells were then treated with dantrolene, a drug used to treat a skeletal muscle ryanodine receptor condition, which restored normal calcium handling and abated the DAD seen in CPVT iPSC-CMs. Similar work done by Siu et al showed that blockers of the MEK1 pathway (U0126 and selumetinib) could ameliorate cell death in LMNA iPSC-CMs.\textsuperscript{55} Care should be taken to avoid the inference that such data necessarily signify clinical efficacy, systemic safety, or an alternative to clinical testing. However, these studies do provide examples of the potential of iPSC-CMs for novel drug discovery based on improved understanding of mechanisms of action. When combined with a high-throughput platform, iPSC-CM can be especially useful in screening large chemical libraries for novel drug discovery.

**Limitations of the iPSC-CM Model**

**Maturity and Modeling Late-Onset Disorders**

One of the main criticisms of iPSC-CMs is that they are an immature phenotype and as such represent an inadequate model of pediatric and adult disorders. This statement is more deduction than empirical science, as some data suggest that the LQT syndrome phenotype is already expressed at the fetal stage, reaffirming some of the findings of the model.\textsuperscript{56} It is certainly true that iPSC-CMs are mostly still in the fetal stage of development, and ongoing efforts to induce maturation via prolonged culturing, 3-dimensional growth, and pacing have shown promise.\textsuperscript{56–60} Nonetheless, the immature phenotype may make it difficult to draw correlations with adult cells and to successfully model some late-onset disorders such as ARVD. However, it should be noted that iPSC-CMs are a model or surrogate for functional human cardiomyocytes, and not an equivalent or full replacement. Animal models have limitations in modeling human cardiac disorders as well, but they still play an important role in understanding the disorders. Ultimately, the success of a model should be based on how it performs against the actual disorder. Finally, to date the relevant data have shown that iPSC-CMs perform fairly well in modeling most genetic cardiac disorders. There could be a publication bias affecting some of these results, but it is worth noting that the iPSC-CM phenotype for LQT1, LQT2, LQT3, and CPVT has been reproduced by independent investigators.\textsuperscript{16,17,21–23,37,41,45–47,54}

**Recaptulating Cardiac-Systemic Interactions**

Another limitation of iPSC-CMs is that they are not analogous to a whole heart. The progression of cardiac disorders is a product of different loading conditions, complex hormonal milieu, and environmental insults faced by human cardiomyocytes. Furthermore, cardiac effects of drugs may depend on metabolic activation of the compounds by the liver, a feature not reproducible in cell culture models. Recaptulating all of these conditions and complex multiorgan interactions is impossible to do in vitro. This is not to say that simple interventions cannot unmask the cardiomyopathy or arrhythmia phenotype, but such simple interventions do not reflect the in vivo complexity of the whole body. Thus, iPSC-CMs are not a replacement for in vivo studies that aim to study the complex interactions of different systemic conditions.

**Modeling Multicellular Disorders**

The use of iPSC-CM modeling has largely been limited to hereditary disorders, especially those in which the pathology is reproducible at the single cell level. This limitation makes it difficult to model disorders that would appear to be more multicellular, particularly if the genetic component is less pronounced as in the case of atrial fibrillation. Furthermore, iPSC-CMs do not replicate complex cardiac embryogenesis processes, making it difficult to model congenital heart disorders. Lastly, the behavior of a monolayer or dissociated single cells of myocytes do not replicate the behavior of human cardiac tissue.

This was challenged by Kadota et al,\textsuperscript{61} who differentiated iPSC-CMs and plated them in cell sheet formations that were capable of electric coupling. They confirmed that the propagation of depolarization was not only lateral but to abutting ends of the cells. With careful titration of cell density and stimulation frequency, the authors were able to induce a spiral wave that resembled the development of reentrant arrhythmias in human myocardium. Furthermore, the authors were able to demonstrate that nifekalant, E-4031, sotalol, and quinidine terminated the spiral wave. Utilizing iPSC-derived tissues in 3-dimensional models has already yielded powerful models of other tissue types.\textsuperscript{57,62} The study by Kadota et al is pioneering on multiple levels and reveals more potential for iPSC-CMs by (1) modeling a non-hereditary disorder of cardiomyopathy; (2) incorporating novel engineering methods to allow for modeling of a multicellular disorder, including those that are dependent on 2- or 3-dimensional spatial organization; and (3) proving that drug testing in a multicellular cardiac model is feasible.

**Conclusions**

In less than 4 years, iPSC-CMs have been used to model more than 10 cardiac hereditary conditions. In most cases, the model has shown robust recapitulation of the phenotype. Although the cells are not an in vivo model and have a fetal phenotype,
most studies have offered novel insights or confirmed known pathology of the disorder in question. Some early work also shows an emerging role for iPSC-CMs in functional evaluation of genes, risk stratification of patients, drug testing, and personalized medicine. This promising work likely represents the scratching of the surface of the full potential of iPSC-CM. Large studies will be needed to confirm these applications, but iPSC-CMs hold great potential to advance cardiac biomedical research and clinical care.

Acknowledgments
We gratefully acknowledge Blake Wu and Joseph Gold for critical reading, Ms. Amy Thomas for her assistance with illustrations, and funding support from National Institute of Health (NIH) T32 (KS), the Uehara Memorial Foundation Research Fellowship (K.K.), Leducq Foundation, American Heart Association 13EIA14420025, NIH R01 HL113006, NIH U01 HL099776, California Institute for Regenerative Medicine (CIRM) TR3-05556, and CIRM DR2-05394 (JCW).

Conflict of Interest
JCW: scientific advisory board and cofounder of Stem Cell Theranostics.

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