Cardiomyocytes from human induced pluripotent stem cells: capturing disease severity of LQT2 syndrome and the impact of chromosome aberrations

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Human Pluripotent Stem Cell Models of Cardiac Disease and their Therapeutic Implications

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ABSTRACT

It is now more than a decade since induced pluripotent stem cells (iPSCs) were first described. This has since become a robust technology that allows reprogramming of somatic cells from every individual to a pluripotent state. Importantly, it has revolutionised our ability to study human diseases as it captures all the genetic aspects of the patient from which they were derived. Combined with advances in generating the different cell types present in the human heart, this has opened new avenues to study cardiac disease in humans and investigate novel therapeutic approaches to treat these pathologies. Here we provide an overview of the current state of the field regarding the generation of cardiomyocytes from human pluripotent stem cells (PSCs) and methods to assess them functionally, an essential requirement when investigating disease and therapeutic outcomes. We critically evaluate whether treatments suggested by these *in vitro* models could actually be translated to clinical practice. Finally, we consider current shortcomings of this *in vitro* model and propose methods by which it can be further improved.

INTRODUCTION

Since the first reports describing the derivation of human embryonic stem cells (hESCs) in 1998, there has been the expectation this would usher in a new age of medicine, particularly for regenerative medicine. While this is becoming a reality for some diseases[1,2], for many others, including the replacement of heart tissue damaged by myocardial infarction, such therapies remain a long way off. However, the more recent ability to generate induced pluripotent stem cells (hiPSCs) from any individual has created new opportunities to study the mechanisms underlying human genetic diseases and as a corollary, develop new therapeutic strategies.

Indeed inherited cardiovascular disorders were among the first diseases for which hiPSC lines were derived[3]. Such pluripotent stem cell (hPSC) models are not only increasing insight into the pathogenesis of many inherited cardiac diseases but are also being used to develop novel ways to treat them. In this review, we summarise recent developments for differentiating hPSCs to cardiomyocytes efficiently and the ways they can be characterised functionally. We provide an overview of the many hPSC models of inherited cardiac diseases that have been described, focussing on examples that they have revealed novel disease mechanisms and therapeutic approaches to treat these pathologies. Finally, we comment on the current challenges faced by the field, and their possible solutions.

GENERATION OF HPSC-CMS

Initial strategies for generating cardiomyocytes from either hESCs or hiPSCs, including from patients, relied on either co-culture with endodermal stromal cells or by embryoid body (EB) differentiation[4]. However the yield of cardiomyocytes was low, protocols were time-consuming and poorly reproducible due to heterogeneity in the size of hPSC clusters in culture and the inclusion of undefined components

such as serum[5]. Later protocols therefore focused on generating cardiomyocytes from EBs containing defined numbers of cells, in serum-free medium with timed exposure to cytokines with a known role in gastrulation and mesoderm specification [6]. While these methods improved reproducibility and efficiency, cardiomyocyte yield is still low relative to the starting number of hPSCs. Also, some of these methods do not consistently work with hPSCs maintained in defined media such as mTeSR1 and E8 (RPD, CLM unpublished results).

To take advantage of these defined systems for maintaining hPSCs undifferentiated, it has been necessary to develop cardiomyocyte differentiation strategies suitable for use when the starting hPSCs are grown in monolayers. The same signalling cues are required in monolayer differentiation as in EB-based procedures[5,7–9], but because in this format the cells are more uniformly exposed to the differentiation signals, higher percentages and yields of cardiomyocytes can be obtained[10]. Furthermore, the growth factor supplements in monolayer differentiations can be completely replaced with small molecules which exhibit less lot-to-lot variation[11–15]. Despite these improvements, the resulting CMs are still mainly ventricular-like and foetal in phenotype, as evidenced by their relatively immature morphological, structural, metabolic, and electrophysiological state.

FUNCTIONAL ASSESSMENT OF HPSC-CMS

These phenotypic characteristics are also evaluated when using hPSC-CMs to investigate cardiac disease (Figure 1). Table 1 lists different methods widely employed to evaluate cardiomyocyte phenotypes, along with some examples of diseases that have been examined using these techniques. Electrophysiology and analysis of ion channel properties are among the most used methods. The cardiac action potential (AP) provides insight not only into whether hPSC-CMs are

ventricular-, atrial-, or nodal-like, but also into ion channel dysfunction, reflecting the abnormal electrocardiogram (ECG) profiles in some patients with congenital heart disorders. For example long QT syndrome (LQTS), characterised by prolonged AP duration (APD), can lead to an extended QT interval on an ECG[16]. This is also detected in disease hPSC-CM lines as the majority of ion channels involved in generating the AP are expressed in hPSC-CMs17.

The gold standard for performing these measurements is manual patch clamp electrophysiology, as the AP of individual cardiomyocytes as well as individual ion channels can be recorded. Nevertheless, this approach is technically demanding, requiring a skilled operator and has very low throughput. Automated patch clamp platforms can increase throughput with up to 384 cells being measured simultaneously[18], though are still more suitable for analysing cell lines expressing individual ion channels ectopically. With differentiation protocols generating highly enriched cardiomyocyte populations and improved dissociation procedures, automated patch clamp is becoming more consistent for measuring individual currents in hPSC-CMs, though presently not APs[19]. A compromise is the multielectrode array (MEA)[20]. This determines the extracellular field potential, a surrogate measure of the QT interval, in clusters of hPSC-CMs. This medium throughput method can detect disease phenotypes in LQTS hPSC-CM models, as well as drug responses. The incorporation of sharp electrodes to penetrate the cell membrane also allows APs to be recorded[21], though poor seal formation means it currently cannot replace patch clamp[22].

Alternatives for measuring changes in membrane potential and calcium flux in hPSC-CMs are optical-based approaches using fluorescence-based voltage or calcium-sensitive indicators. Various voltage sensitive-dyes, which intercalate into the lipid bilayer of the plasma membrane, have been shown to report membrane potential

changes through variations in fluorescence intensity[23,24]. However, unlike patch clamp, these do not provide absolute values. These dyes can also be combined with Ca^{2+} indicators, permitting simultaneous imaging of electrical and calcium dynamics[25]. Ca^{2+} probes frequently have been used to determine changes in Ca^{2+} flux and detect Ca^{2+} dysfunction in hPSC-CM disease models, and have been proposed as surrogates to measure the AP[26]. Nevertheless, both Ca^{2+} and voltage-sensitive indicators suffer from some collective limitations, including inter-assay loading variability and an inability to target specific cardiomyocyte subtypes. Recent developments in genetically encoded voltage or calcium indicators (GEVIs or GECIs) suggest that these could be alternatives for hPSC-CMs. Several GEVIs and GECIs have been introduced into hPSC-CMs to image APs and Ca^{2+} transients, including in hPSC-CM disease models (Table 1). While such genetic-based indicators allow specific cardiac populations to be targeted and also provide a homogenous signal, the GEVIs have slower response times than dyes[27,28]. However both GEVIs and GECIs continue to be improved, with newer iterations offering better signal-to-noise ratios and faster response kinetics[29,30].

Cardiomyocyte excitation results in cyclic contraction and relaxation, thereby generating force. There are numerous techniques to measure contractility in hPSC-CMs, although each quantifies force differently so cross-comparison of measurements is not possible. Measurements are performed either on individual hPSC-CMs or two- or three-dimensional cell clusters and have been used to assess contractile dysfunction in hPSC models of cardiomyopathies (Table 1). Not surprisingly, three-dimensional engineered heart constructs mimic native cardiac tissue best[31]. Indeed the disease phenotype from a patient hiPSC line with a mutation in the sarcomeric protein titin could only be detected when the cardiomyocytes were cultured in three-dimensional aggregates[32]. However the

forces generated by hPSC-CMs even in these multicellular constructs remain much smaller than that of adult cardiomyocytes[33]. An additional limitation of many of these contractility assays is their low throughput.

Imaging systems have also been used to determine cardiomyocyte morphology as a measure of hypertrophy, assess metabolic activity within the cardiomyocyte or determine the content and organisation of organelles such as mitochondria. Mitochondrial (dys)function in hPSC-CM disease models can also be evaluated by measuring glycolysis and oxidative phosphorylation (Table 1).

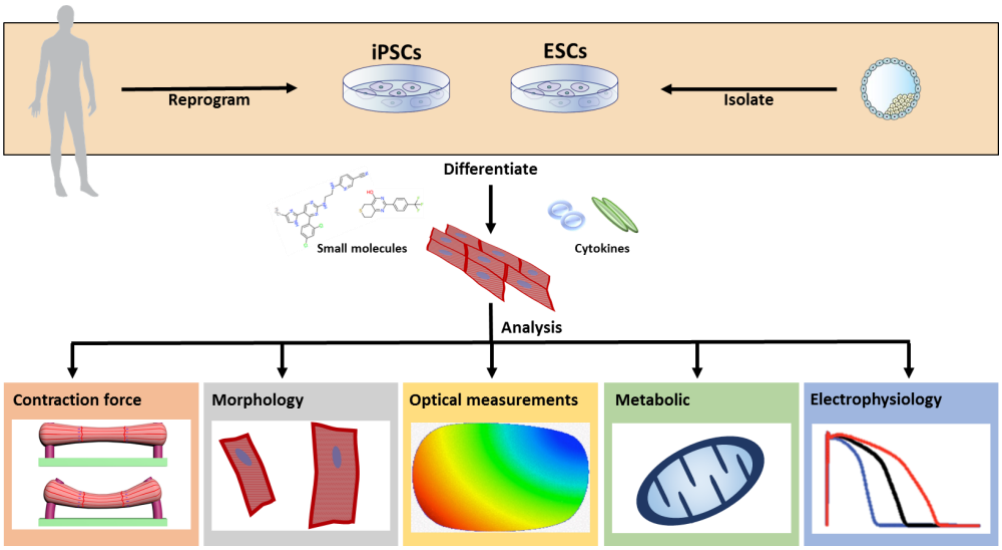

Figure 1. Examples of phenotypic properties that can be quantitatively assessed in hPSC-derived cardiomyocytes. Human iPSCs, generated by reprogramming adult somatic cells, or ESCs, isolated from human blastocysts, can be differentiated into cardiomyocytes using small molecules, cytokines or a combination of both. The resulting cardiomyocytes can then be used in downstream assays to measure for example their contractility, electrophysiology, Ca2+ flux, mitochondrial function or morphology. If the hPSC-CMs contain genetic mutations associated with cardiac disease, this can provide insight into the underlying disease mechanisms and also enable new therapeutic strategies to be evaluated.

hPSC Models of (Inherited) Cardiac Diseases

Despite improved understanding of the genetics underlying cardiac diseases, treatments (e.g., drug therapies) are still quite limited or else act primarily to delay disease progression. Furthermore, tailoring treatments to patients based on their genetic mutation and risk – one goal of precision medicine – is yet to become a reality. However, hPSC-CMs are now being used to model a wide range of cardiac disorders (Figure 2), not only to investigate the disease mechanism but also to evaluate therapeutic options in a patient-specific manner. While arrhythmias and cardiomyopathies continue to be the most investigated, cardiometabolic disorders and more complex diseases without clear genetic causes are also being modelled. Table 2 summarises the published hPSC models of these three groups of disorders as well as the strategies used to ameliorate the disease phenotype and their applicability for treating patients. In the following sections we review some of the key novel findings from hPSC-CM disease models, both from a mechanistic and clinical perspective.

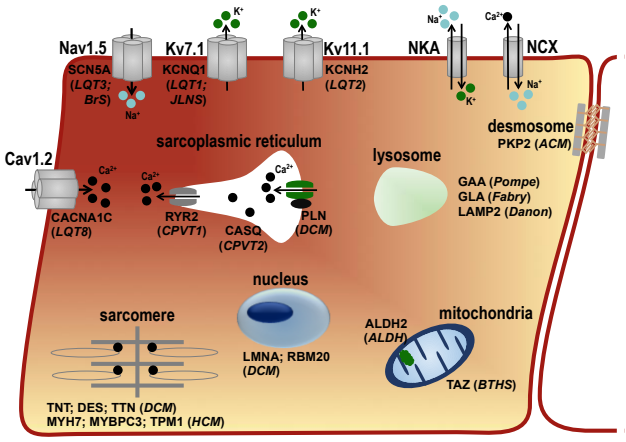

Figure 2. Overview of congenital cardiac diseases that have been modelled using human PSC-derived cardiomyocytes. The main cellular sub-localisation for the affected protein is indicated. A more extensive list of the mutations that have been examined is provided in Table 2.

PRIMARY ARRHYTHMIC DISEASES

Inherited channelopathies caused by mutations in cardiac ion channels are a group of diseases that has been extensively modelled using hPSC-CMs. These arrhythmic disorders include LQTS, Jervell and Lange-Nielsen syndrome (JLNS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT). Their hallmark is an abnormal ECG, either at baseline or for example during exercise[34]. This can predispose patients to an increased risk of cardiac arrhythmias, syncope, and even sudden cardiac death (SCD).

LQT1

One of the first cardiac disease hiPSCs were generated from LQT1 patients with a missense mutation (R190Q) in the potassium channel gene, *KCNQ1*[35]. The hiPSC-CMs showed a 70-80% reduction in the slow component of the delayed rectifier potassium current (I_{ks}) compared to healthy controls, a corresponding prolongation in the APD and the development of early afterdepolarisation (EAD) events in the presence of the β-adrenergic agonist, isoproterenol, an arrhythmic trigger in LQT1 patients. A novel frameshift mutation in *KCNQ1* was later shown to cause a similar electrophysiological phenotype and response to adrenergic stimulation in patient hiPSC-CMs[36]. In both cases, EADs were blunted in hiPSC-CMs by pre-treatment with the β-blocker, propranolol. This correlated well with clinical observations where β-blocker treatment is the first line of therapy in supressing arrhythmias in LQT1 patients[37], and indicated that hiPSC-CMs may be valuable for developing novel treatments.

For example ML277, a compound identified as a potent activator of KCNQ1 channels[38], was shown to partially shorten APDs in LQT1- and healthy hiPSC-CM[39]. However it is important to note that KCNQ1 forms channel complexes with KCNE subunits and the precise stoichiometry in adult hearts is not clear[40]. Validating

effects of ML277 in more mature wildtype and LQT1 hiPSC-CMs will assist in determining whether it could become a targeted drug for LQT1. Similarly, a recent study investigated whether a novel hERG allosteric modulator (LUF7346) could be used to treat congenital and/or drug-induced forms of LQTS[41]. LUF7346 acts as a type-1 hERG activator by increasing the rapidly activating delayed rectifier K^+ current (I_{Kr}) window and slowing I_{Kr} deactivation in a voltage-dependent manner[42]. By correcting hiPSCs harbouring the KCNQ1 mutation R190Q, a pair of isogenic lines ($LQT1^{corr}$/ $LQT1^{R190Q}$) were created, thereby eliminating the influence of genetic background on the drug response[41]. Treatment with 3-5μM LUF7346 significantly shortened the APD in the LQT1 hiPSC-CMs, highlighting the potential of hERG allosteric modulation for treating congenital LQTS. Higher doses, however, stopped spontaneous beating and increased the risk of excessive QT interval shortening. This requires further investigation to determine whether this is an obstacle for clinical translation.

JLNS

While LQT1 is an autosomal dominant or sporadic disease, JLNS is a recessive disorder that is also due to mutations in *KCNQ1*. JLNS patients suffer from particularly severe cardiac symptoms and cannot be sufficiently protected by β-blocker therapy[43]. By combining hiPSC derivation from JLNS and LQT1 patient biopsies with genetic engineering, a collection of heterozygous and homozygous hiPSCs for two different classes of JLNS-causing mutations (a missense and a putative splice donor mutation), were generated[44]. Cardiomyocytes from both JLNS hiPSC lines showed similar repolarisation defects, despite the molecular consequence of each mutation being different. Treatment with another hERG activator, NS1643, shortened FPDs and protected both JLNS hiPSC lines from cisapride-induced arrhythmia[44]. Comparable reductions in QT interval were also observed with

LUF7346[41], suggesting a single drug might be able to protect against multiple forms of JLNS. However, both compounds have steep dose-response curves, so it may be difficult to find a dose that does not cause short QT.

LQT2

LQT2 is caused by mutations in the potassium channel gene, *KCNH2,* leading to a reduction in I_{Kr}. As for LQT1, LQT2 hiPSC-CMs also exhibit prolonged APD, arrhythmogenic events and irregular beating, thus reflecting typical aspects of the patient phenotype[45,46]. Moreover, as in LQT1, treatment of LQT2-hiPSC-CMs with β-blockers can correct EADs caused by adrenergic stimulation or pharmacological blockade of cardiac repolarisation currents (e.g. E4031)[45]. However not all β-blockers are equally as effective at preventing breakout cardiac events in LQTS patients[47]. Being able to test alternative drugs on a range of LQT2 hPSC lines could improve treatment strategies and also create opportunities to develop tailored therapies for patients depending on their mutation and genetic background. For example, Itzhaki et al. demonstrated that the clinically-approved compounds nifedipine (a Ca^{2+} channel blocker), and pinacidil (a K_{ATP} channel activator), shortened the APD and FPD and abolished EADs in hiPSC-CMs from a LQT2 patient[46]. However both compounds have a risk of causing hypotension which could limit their clinical applicability for this disease[48].

Treating LQT2 patients with I_{kr} channel activators is also of particular interest as several compounds have been identified which have a similar effect on I_{kr} but act through different mechanisms[42]. Matsa *et al.* examined the response of hiPSC-CMs to two experimental K^+ channel enhancers - nicorandil and PD-118057[45]. Both drugs shortened the prolonged APD of LQT2 hiPSC-CMs. However, as with LQT1 hiPSC-CMs, dosage needs to be carefully monitored to avoid excessive shortening of the

AP. More novel treatments have also been proposed such as the chaperone modulator, *N*-[*N*-(*N*-acetyl-L-leucyl)-L-leucyl]-L-norleucine (ALLN). This small molecule leads to re-trafficking of hERG and rescue of the LQT2 phenotype in a hiPSC model[49]. Also mRNA knockdown by mutated-allele-specific RNA interference could rescue the disease phenotype[50]. While neither of these approaches is directly translatable to the clinic, these findings underline the importance of understanding the complexity of different genetic defects at the molecular and cellular levels to develop alternative treatment strategies.

LQT3 & BrS

LQT3 is caused by gain-of-function mutations in *SNC5A*, which encodes the alpha subunit of the Na^+ channel ($Na_v1.5$). The result is persistent I_{Na} that increases APD and prolongs CM repolarisation[51]. β-blocker therapy in LQT3 patients is less effective than in other LQTS types, and in some instances can be harmful due to other underlying disorders[52]. This is because *SCN5A* mutations are also associated with loss-of-function arrhythmic disorders including BrS and conduction disease. These loss-of-function diseases are due to decreased peak I_{Na}, leading to slower AP upstrokes. Some mutations even result in the combination of several clinical manifestations and are commonly referred to as "Overlap Syndromes"[52]. However, associating different *SCN5A* mutations with particular phenotypes has been slow due to difficulties accurately modelling some of these mutations using heterologous cell culture systems[53,54]. We demonstrated the potential of hiPSC-CMs as an alternative model by establishing that despite their immaturity, these cells could display features of both BrS and LQT3[54]. More recently Liang et al. showed hiPSC-CMs could model the single phenotype of BrS, and by genome editing, corrected the *SCN5A* variant implicated and validated its pathogenicity[55].

Terrenoire *et al*. further demonstrated the possibility to use hiPSCs to develop personalised treatment regimens by deriving a hiPSC line from a LQT3 patient with a *de novo* mutation (F1473C) in *SCN5A* and a polymorphism (K891T) in *KCNH2*. An ICD and high doses of the Na^+ channel blocker mexiletine and propranolol helped reduce the numbers of arrhythmias experienced by the patient, however multiple episodes were still detected daily. The authors first used hiPSC-CMs to demonstrate that the disease was primarily due to the *SCN5A* mutation and not the *KCNH2* polymorphism. Treating the hiPSC-CMs with high doses of mexiletine led to both an anti-arrhythmic drug block of the late I_{Na} plus a pro-arrhythmic block of I_{Kr}, thus explaining the clinical symptoms of recurrent cardiac episodes. While Na^+ channel blockers can be beneficial in treating LQT3, this depends on how the mutation affects the biophysical properties of $Na_v1.5$. Indeed hiPSC models of different *SCN5A* mutations also reflect their different degrees of effectiveness[56,57].

LQT8

Timothy syndrome (TS), also known as LQT8, is a very rare, multisystem disorder caused by a single amino acid substitution in exon 8a of *CACNA1C*[58]. At the cellular level, the mutation causes impaired inactivation of the L-type Ca^{2+} channel, resulting in a persistent inward current that prolongs the AP[59]. While treatment with the Ca^{2+}-channel blocker verapamil, β-blockers or the Na^+-channel blocker ranolazine show some beneficial effects, the majority of TS patients die before puberty from cardiac arrhythmias[60]. Ca^{2+} imaging of TS hiPSC-CMs revealed excess Ca^{2+} influx and abnormal Ca^{2+} transients[59]. Additionally irregular contraction, prolonged APD and an increased incidence of delayed afterdepolarisations (DADs) were recorded. Roscovitine, a cyclin-dependent kinase inhibitor, was able to correct most of the alterations caused by channel dysfunction, validating earlier cellular studies. However due to its inhibition of multiple proteins involved in the cell cycle[61], this is

more likely to serve as a lead compound for developing new antiarrhythmics rather than as a potential treatment for TS patients.

CPVT

CPVT is an arrhythmogenic disorder also characterised by abnormal intracellular Ca^{2+} handling and signalling in the cardiomyocytes. It causes DADs through the activation of the membrane Na^{+}/Ca^{2+} exchanger (NCX)[62]. Clinically, CPVT is triggered by situations that increase the level of catecholamines, such as physical exertion and emotional stress. CPVT1 is the most common type and is caused by autosomal dominant mutations in the cardiac ryanodine receptor type 2 gene (*RYR2*), a mediator of calcium release in the sarcoplasmic reticulum (SR)[63]. CPVT2 is a rarer, autosomal recessive form caused by mutations in the calsequestrin-2 gene (*CASQ2*), a calcium-binding protein also located in the SR[64]. Treatment of CPVT generally consists of β-blocker therapy, though 30% of patients still experience life-threatening arrhythmias[65]. It is therefore important to understand the functional consequences of a particular mutation to develop individualised treatments, particularly as both CPVT1 and CPVT2 have a similar clinical presentation yet different disease mechanism.

CPVT hiPSC-CMs exhibited similar phenotypes to those observed in the patients, with all mutations appearing to cause aberrant Ca^{2+} transients and the development of DADs, which in some cases was exacerbated with adrenergic stimulation[66–71]. As observed clinically, the Na^{+}-channel blocker flecainide restored intracellular ion concentration to normal levels in the hiPSC-CMs[66]. These models have also provided insight into the disease mechanism. It is proposed that *RYR2* mutations render the ryanodine receptors “leaky” following PKA-mediated phosphorylation, producing local depolarisations that trigger DADs via activation of NCX[72]. An alternative is that

RYR2 mutations can result in SR Ca^{2+} overload following β-adrenergic exposure, resulting in abnormal release of Ca^{2+} independent of FKBP modulation and leading to a similar electrophysiological phenotype[73]. Both of these mechanisms have been reported in hiPSC CPVT1 models[66,74], suggesting that the position of the mutation in *RYR2* plays a key role in the underlying cause of the abnormal Ca^{2+} handling and the different drug responses observed in patients. For example, dantrolene, a drug for treating malignant hyperthermia, abolished or reduced arrhythmias in patients where the *RYR2* mutation was in the N-terminal or central region, whereas no effect was seen when the mutation was in the transmembrane region[75]. These responses were also observed in hiPSC-CMs generated from each of these patients. Several other novel treatments of CPVT1 have also been reported. Both thapsigargin (a SERCA-inhibitor) and KN-93 (an antiarrhythmic drug that inhibits CaMKII), rescued the arrhythmic phenotype induced by catecholaminergic stress[66,70]. Neither of these compounds is likely to be clinically suitable as such due to their lack of target and tissue-specificity but are potentially useful lead compounds.

CARDIOMYOPATHIES

Inherited cardiomyopathies are a second group of cardiac disorders that have been widely studied using hiPSCs. Mutations in more than 50 genes have been linked to dilated (DCM), hypertrophic (HCM), and arrhythmogenic cardiomyopathies (ACM)[47]. Most of these disorders are characterised by sarcomere disorganisation, which can lead to reduced myocardial function and potentially heart failure. These diseases are also marked by large variability in clinical phenotype, with some patients remaining asymptomatic through life to SCD occurring in others during adolescence. Currently, treatments are typically initiated once the patient becomes symptomatic. Understanding the pathological mechanisms underlying these diseases, and in

particular the remodelling of the heart that often occurs before clinical symptoms are apparent, will help develop earlier treatments to prevent disease progression. In this regard, it is anticipated that hiPSC-CM cardiomyopathy models will prove useful.

DCM

DCM is one of the most common cardiomyopathy subtypes, with familial DCM having an estimated prevalence between 1 in 250 to 1 in 2500 individuals. It is clinically characterised by ventricular dilation and impaired contraction. More than 30 genes involved in various genetic pathways, including sarcomere and cytoskeleton formation and contraction, nuclear envelope stability, gene processing and transcription, and calcium handling have been identified in DCM[76]. DCM inheritance is usually autosomal dominant, with mutations in Titin (TTN) the most frequently identified[77]. Patients with familial DCM are treated with angiotensin converting enzyme (ACE) inhibitors, β-blockers, and diuretics similar to those used for other systolic heart failure conditions[78]. There are currently no etiology-specific cardioprotective treatments for asymptomatic familial DCM patients.

To date, mutations in six genes have been studied using DCM-hiPSC models (Table 2). A heterozygous missense mutation (R173W) in the sarcomere protein troponin T (TNNT2) was intensively studied with hiPSCs generated from 7 family members[79]. Key features of the disease were observed in the mutated hiPSC-CMs, including impaired Ca^{2+} handling, reduced contractility, and downregulation of SERCA2a. Metoprolol (a β-adrenergic blocker used to treat DCM patients) decreased the number of cardiomyocytes with abnormal sarcomere α-actinin staining, while transgenic overexpression of SERCA2a, a gene therapy treatment for heart failure undergoing clinical trials[80], improved their contractile function. A follow-up mechanistic study indicated that the R173W mutation increased the nuclear

translocation of TNNT2 and enhanced the epigenetic activation of the phosphodiesterase genes, *PDE2A* and *PDE3A*[81]. This upregulation led to compromised β-adrenergic regulation in DCM iPSC-CMs, resulting in contractile dysfunction. The use of the PDE2 and PDE3 pharmacological inhibitors, Bay-60-7550 and milrinone, improved the calcium handling and contractile force in the DCM iPSC-CMs. While milrinone has been prescribed to heart failure patients for many years, recent studies have questioned its safety and efficacy[82,83]. It will be interesting to see whether Bay-60-7550 or related PDE2 inhibitors are a better option, though currently there are no FDA-approved PDE2 inhibitors. In one severely afflicted family member, the myofibrillar architecture was also affected in the hiPSC-CMs[84]. Whether the observed sarcomeric shortening and slow actin assembly dynamics is due to the TNNT2 mutation, or the presence of other genetic variants warrants further investigation. Omecamtiv mecarbil, a myosin activator previously reported to improve cardiac function in acquired heart failure[85], reversed the phenotype by increasing contractility and improving sarcomere assembly[84]. Currently, only transplantation satisfactorily addresses depressed contractility in familial DCM. The possibility that omecamtiv mecarbil could treat this without adversely altering Ca^{2+} flux is an exciting prospect.

Other DCM hiPSC models have examined variants in the genes encoding lamin A/C (LMNA) and TTN (Table 2). LMNA-related DCM is characterised by early onset of atrial fibrillation and conduction system disease leading to SCD and heart failure[86,87]. When hiPSC-CMs with two LMNA variants underwent electrical field stimulation, they exhibited increased nuclear senescence and cellular apoptosis compared to control hiPSC-CM, potentially explaining the development of premature cardiac aging seen in patients[88,89]. Pharmacological blocking of the ERK1/2 pathway with U0126 and selumetinib considerably reduced the pro-apoptotic effects of electric

field stimulation in the mutated lines, supporting earlier animal studies that suggested the MEK1 pathway might be a potential therapeutic target[90]. Similarly, hiPSC-CMs were used to investigate the pathogenicity of different TTN-truncating variants (TTNtvs)[32]. Using hiPSC-derived 3D cardiac microtissues, they found that truncating mutations located within the A band caused more contractile deficits compared to I-band TTNtvs due to alternative exon splicing mitigating their pathogenicity. This could explain why clinically more people with A-band TTNtvs exhibit a pathogenic phenotype[91], and illustrates the potential of hiPSC-CM models in prognostic evaluation. However, further improvement in cardiomyocyte maturation is needed for this to become a more reliable disease predictor.

HCM

HCM is also a common cardiomyopathy subtype with a prevalence of ~1 in 500 individuals. It is the most common cause of SCD in young people and athletes[78], and is clinically characterised by a thickened (≥15 mm) left ventricle, which can lead to heart failure due to diastolic dysfunction, LV outflow tract obstruction, or atrial fibrillation. Mutations in 23 genes encoding components of the sarcomere or sarcomeric-associated proteins have been linked to HCM, with the majority of mutations identified in β-myosin heavy chain (MYH7) and myosin-binding protein C (MYBPC3)[92]. However, mutations have only been identified in ~50% of cases, indicating that additional genes are likely involved. Moreover, phenotypic heterogeneity adds to the genetic complexity. Pharmacological treatment with β-blockers or verapamil can help manage the disease but does not reverse disease progression.

Despite their immaturity, hiPSC-CMs derived from HCM patients with mutations in MYH7 and MYBPC3 could reproduce in part many characteristics of the disease, such

as cellular enlargement, sarcomere disorganisation, disrupted contractility, as well as altered gene expression and calcium handling[93,94]. Using hiPSC-CMs with a missense mutation in MYH7 (R663H) to screen currently drugs used to treat HCM, Lan et al. confirmed that pharmaceutical inhibition of calcium entry with verapamil prevented the development of HCM[93]. This supports the hypothesis that dysregulation of Ca^{2+} cycling is a central pathogenic mechanism for the disease[92]. A second study modelling a different missense mutation (MYH7-R442G) observed similar phenotypes in the diseased hiPSC-CMs, and also were improved with verapamil[94]. Furthermore, whole transcriptome-sequencing indicated that genes implicated in cell proliferation, Notch and FGF signalling were involved in disease development, highlighting potential therapeutic targets. Indeed the histone deacetylase inhibitor Trichostatin A significantly ameliorated various hypertrophic phenotypes in the HCM iPSC-CMs, reflecting previous animal and cellular studies[94].

The majority of MYBPC3 mutations result in a truncated, unstable protein, suggesting that the ensuing HCM phenotype is caused by haploinsufficiency[95]. Using adenoviral gene transfer, it was demonstrated that expression of wild-type *MYBPC3* in a hESC line carrying a splice donor mutation in *MYBPC3* during early cardiomyocyte differentiation prevented HCM structural and functional phenotypes[96]. This is similar to observations in HCM mutant mice[97], and suggests gene therapy could be used to treat cardiomyopathies.

ACM

ACM is a primary cardiomyopathy characterised by ventricular arrhythmias and right ventricle dysfunction due to fibro-fatty infiltration of cardiomyocytes. It has an estimated prevalence of 1 in 5000, and like other cardiomyopathies displays highly variable penetrance and severity. The majority of mutations have been identified in

genes encoding components of the desmosome, with mutations in plakophilin-2 (*PKP2*) the most common[98]. Exactly how desmosomal protein mutations lead to the ACM phenotype is unclear, though alterations to Wnt–β-catenin signalling due to impaired desmosomal assembly is thought to induce a gene transcriptional switch from myogenesis to adipogenesis and fibrogenesis[99]. Modelling ACM using hPSCs could help in further elucidating the disease pathophysiology, though the late onset of the disease and suspected involvement of epicardial cells in mediating the fibrofatty myocardial phenotype poses a challenge. By inducing adult-like metabolism in hiPSC models of *PKP2* mutations, the resulting CMs not only displayed abnormalities in desmosome structure and gene expression, but also calcium-handling deficits and increased lipogenesis and apoptosis[100,101]. Furthermore lipid accumulation could be prevented by treating the diseased iPSC-CMs with either a GSK3β inhibitor or PPARγ antagonists[100,101]. The beneficial effect of inhibiting GSK3β has been observed in multiple model systems[102], supporting further research into the therapeutic potential of this strategy.

METABOLIC DISORDERS

Metabolic diseases are generally categorised as either inborn errors of metabolism (IEM) (i.e., inherited) or as acquired metabolic syndromes, due to their development in adulthood from the presence of additional risk factors. With both groups, the disease typically affects multiple organs, including the heart. The cardiac complications often present as either DCM, HCM or arrhythmias, and are frequently associated with IEM disorders that affect glycogen or lysosomal storage, fatty acid oxidation, and mitochondrial metabolism or function. Distinguishing IEM as the underlying cause of the disease rather than a primary cardiomyopathy is critical for developing disease management strategies. Therefore, hPSCs not only offer the

opportunity to develop new therapeutic approaches for these diseases but can also be used to understand how IEMs lead to cardiomyopathies. Similarly, the rise in cardiovascular disease through acquired metabolic syndromes also requires new models to better investigate these polygenic diseases.

Mitochondrial disorders

Barth Syndrome (BTHS) is a mitochondrial disorder caused by mutations in the gene encoding tafazzin (*TAZ*), which acetylates the mitochondrial phospholipid cardiolipin. Impaired cardiolipin acetylation results in impaired ATP production and mitochondrial dysfunction, with a clinical consequence being cardiomyopathy[103]. BTHS hiPSC-CMs were derived from two patients harbouring either a missense or frameshift TAZ mutation[104]. Additionally, introducing TAZ mutations into control hiPSCs via genome editing generated an isogenic pair of cell lines. Overall BTHS hiPSC-CMs exhibited impaired cardiolipin acetylation and mitochondrial dysfunction. The phenotypes could be reversed by gene replacement therapy whereby BTHS hiPSC-CMs were transfected with modified *TAZ* mRNA; however maximal respiratory capacity was not completely rescued. As myofilament disarray is a feature in BTHS patients, the authors examined sarcomeric organisation but only observed a decrease in the BTHS hiPSC-CMs with the frameshift and not missense mutation. However, this difference, as well as variation in contractile dysfunction, could also be due to clonal variation. A dramatic improvement in sarcomeric organisation and contractile dysfunction was observed when the BTHS hiPSC-CMs were treated with the antioxidant mitoTEMPO or linoleic acid, an essential unsaturated fatty acid precursor of mature cardiolipin. Whether these small molecule treatments can be easily translated into patient therapies remains to be seen.

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) deficiency is present in about 8% of the human population, predominantly in people of East Asian heritage[105]. The ALDH2*2 polymorphism (E487K) reduces ALDH2 enzyme activity, leading to a loss of its cardioprotective effects and increasing susceptibility for coronary artery and ischemic heart disease[106]. hiPSC-CMs from a cohort of East Asian individuals carrying the ALDH2*2 polymorphism demonstrated the expected accumulation of ROS and 4-hydroxynonenal (4HNE), a toxic aldehyde product, leading to cell cycle arrest and apoptosis signalling. Treating the ALDH2*2 hiPSC-CMs with Alda-1, a small molecule known to restore the enzymatic activity of the E487K mutant[107], rescued the apoptotic phenotype in the PSC-CMs[108]. While Alda-1 is not suitable for use in the clinic due to its relatively low potency and solubility, it does demonstrate the possibility of testing more clinically suitable analogues using this iPSC disease model.

Storage Disorders

Infantile-onset Pompe disease is an autosomal-recessive glycogen storage due to mutations in the *GAA* gene and typically manifests as severe hypotonia and cardiac hypertrophy between 3 and 5 months of age[109]. This is due to insufficient lysosomal α-glucosidase activity, leading to accumulation of glycogen in the heart[110]. Similarly Fabry disease (FD) results in the accumulation of globotriaosylceramide (GL-3) due to a deficiency in the lysosomal enzyme α-galactosidase A[111]. However Fabry disease usually develops in adulthood with clinical features including cardiac hypertrophy with diastolic dysfunction, arrhythmia, conduction defects, and myocardial fibrosis[112]. The current treatment for both disorders is based on enzyme replacement therapy (ERT) using either recombinant human α-glucosidase (rhGAA) or □-galactosidase A respectively. However these treatments are not curative as Pompe patients can develop immunogenic reactions as well as arrhythmias following repeated administration[113], while long term reduction of GL-3 deposits in

Fabry patients are not observed[110]. To develop improved therapeutic strategies, further understanding of the pathophysiology of these disorders is necessary.

The hiPSC-CMs from patients with the infantile form of Pompe disease exhibited many hallmarks of the disease including reduced lysosomal α-glucosidase activity, lysosomal glycogen accumulation, and lysosome enlargement[114,115]. Likewise treating with rhGAA, Pompe disease hiPSC-CMs showed a significant reduction in glycogen[114]. Moreover, treating with L-carnitine partially rescued some mitochondrial functions, resulting in an increase of oxygen consumption rate that was not observed with the standard treatment and suggesting this could be a valuable adjunct therapy.

Likewise FD hiPSC-CMs mirrored the patient phenotype with progressive lysosomal accumulation of GL-3, increased lysosomal storage inclusions and disorganised contractile fibres[116]. Substrate reduction therapy (SRT) has been proposed as an alternative to ERT to treat FD with the aim of reducing glycosphingolipid synthesis and therefore decreasing GL-3 levels[117]. Indeed SAR 402671, a glucosylceramide synthase inhibitor, is currently in clinical development for FD[118]. Treating FD hiPSC-CMs with SAR 402671 both prevented and reduced GL-3 deposits by more than 50%, corroborating results obtained using a FD mouse model[119] and highlighting the potential of SRT as an alternative approach for treating the cardiac phenotype of FD[116].

Endoplasmic reticulum disorders

By combining hiPSC disease modelling with next-generation sequencing to identify new genetic loci associated with SCD, Devalla et al. identified two new homozygous loss-of-function mutations in the novel gene encoding trans-2,3-enoyl-CoA reductase-like (TECRL)[120]. These mutations were present in patients from three

different families who exhibited characteristics of LQTS and CPVT. However, this new disorder is not thought to be a primary channelopathy as *TECRL* encodes an ER protein that may be involved in lipid metabolism. The clinical phenotype differed according to the mutation, with patients harbouring p.Arg196Gln diagnosed with LQTS, while patients with c.331+1G>A which causes incorrect protein splicing diagnosed as CPVT. The hiPSC-CMs derived from a patient with the c.331+1G>A mutation reflected this phenotype with abnormalities in calcium handling, including a smaller amplitude and slower decay of cytosolic Ca^{2+} transients. Additionally, prolongation of APD and increased propensity for DADs during catecholaminergic stimulation were observed. As shown with CPVT hPSC-CMs[66,71], flecainide reversed the phenotype in the TECRL hiPSC-CMs, though some DADs were still observed emphasising the need for additional or more effective drugs. Further understanding of the exact function of TECRL and how defects lead to altered calcium homeostasis would aid in this, as would additional hPSCs lines containing other TECRL mutations.

Diabetic cardiomyopathy

Diabetic cardiomyopathy is a long-term complication in type 2 diabetes. It is characterised by structural and functional abnormalities of the myocardium but without coronary artery disease or hypertension[121]. The underlying pathophysiologic mechanisms are not well understood due to its multifactorial etiology. Current clinical treatments include glycemic control, ACE inhibitors and β-blockers. In vitro modelling of complex diseases that include an "environmental" factor can be a challenge, but it was recently demonstrated that the cardiac phenotype of diabetic patients could be modelled using hiPSC-CM[122], supporting the view that a genetic component contributes to the disease[123]. Furthermore, when hiPSC-CMs from healthy donors were exposed to a *diabetic milieu* consisting of glucose, endothelin 1 and cortisol, they developed a cardiomyopathy phenotype that included cellular

hypertrophy, increased brain natriuretic peptide release, myofilament disarray, as well as lipid accumulation and peroxidation. To identify potential protective drugs, a 480-compound library was screened with 28 small molecules identified that prevented the diabetic cardiomyopathy phenotype developing. The most effective compounds across all the cellular models were thapsigargin and the voltage-gated Ca^{2+} channel inhibitor, fluspirilene[122]. Further studies incorporating both in vivo testing of this narrower list of effective compounds will provide a stronger base for subsequent clinical development, while a more diverse set of hiPSC-CMs derived from type 2 diabetic patients will assist in determining whether these hiPSC-CM models can define disease subtypes and potentially tailor drug treatments.

CHALLENGES IN CARDIAC DISEASE MODELLING

Maturity of hPSC-CMs

It is widely acknowledged that a key limitation of hPSC-CMs as disease models is their immaturity. The hPSC-CMs display characteristics typical of foetal cardiomyocytes with a small, round or polygonal morphology, disorganised sarcomeric structure, lack transverse tubules and are mainly mononuclear[124]. The gene expression profile of hPSC-CMs is also similar to first trimester gestational stage CMs[125,126], with low expression levels of several ion channel and contractile protein encoding genes[127]. Functionally, this contributes to the immature phenotype of spontaneous contraction, depolarised resting membrane potential (RMP) due to low or absent I_{K1} current and altered Ca^{2+} handling[128–130]. The conduction velocity in hPSC-CMs is also substantially slower than adult CMs[25]. Regarding metabolism, embryonic and hPSC-CMs predominantly produce energy through glycolysis, while adult CMs preferentially generate energy via fatty acid oxidation[101].

Despite their immature phenotype it has been possible to detect clinically expected characteristics of genetic cardiac disorders using hPSC models. Naturally though, their sensitivity and accuracy as disease models would be further improved by generating cardiomyocytes that more closely resemble those in adults as many cardiovascular diseases, such as coronary artery disease and atrial fibrillation, are late onset[131]. Most attempts to mature hPSC-CMs adopt a similar approach – that is to mimic the cues that drive heart development in vivo. The most obvious is long-term culturing (80-100 days) of the hPSC-CMs which can induce morphological changes, as well as improve electrophysiological and Ca^{2+} handling[128], but is both impractical and costly. Other approaches include co-culture of hPSC-CMs with other cell types also present in the heart, such as endothelial, smooth muscle, and fibroblasts, to increase the resemblance to native myocardium[132].

Another tactic is to modify the culture medium. For example, thyroid hormones, such as triiodothyronine, have an important role in heart development[133], and have been shown to improve Ca^{2+} handling, bioenergetics, and contractile force in hPSC-CMs[134,135]. Indeed triiodothyronine in combination with IGF-1 and the glucocorticoid analogue dexamethasone, revealed a contractile force defect in the cardiomyocytes of an HCM hiPSC model that was not detected in medium without these components[136]. Similarly, the phenotypes of diabetic cardiomyopathy and ACM could be detected by metabolically maturing the hPSC-CMs through supplementing the medium with fatty acids and insulin or a lipogenic cocktail[101,122].

Altering the extracellular matrix surrounding hPSC-CMs can also increase maturity with improvements in contractility, electrophysiology, sarcomeric length and mitochondrial function reported[10,137,138]. Likewise modulating the stiffness of the substrate on which hPSC-CMs are plated can influence contractility[139], while forcing the hPSC-CMs to align and elongate using pre-patterned structures improved their

maturation based on faster Ca^{2+} kinetics[140]. By using these methods the impaired sarcomere assembly and contractility of BTHS iPSC-CMs could be detected[104]. Cyclic stretch and strain of hPSC-CMs, either mechanically or by electrical pacing, has also generated more mature CMs both structurally and functionally[132,141–144]. Pacing increased the expression of *KCNJ2*, which can lead to increased I_{K1} and lower RMP[145]. Similarly, adenoviral overexpression of *KCNJ2* in hPSC-CMs hyperpolarised the RMP and resulted in loss of automaticity[146]. This was recently used to generate more mature hiPSC-CMs to study the arrhythmia mechanism of a LQT9 Cav3 mutation[147]. Manipulation of the RMP can also be achieved *in silico* by dynamic patch clamp[148]. By artificially injecting I_{K1} into hPSC-CMs, the resulting RMP, upstroke velocity and amplitude are more similar to that of adult ventricular CMs. This approach improved the ability to model Na^+ channel mutations[149], and even to artificially model *KCNJ2* mutations responsible for Andersen-Tawil syndrome type 1 and short QT syndrome type 3[150].

It is apparent that a combination of different strategies will be required to generate hPSC-CMs with a more mature phenotype. Whether hPSC-CMs can reach the same of level of maturity as adult CMs in experimentally facile formats remains uncertain. Regardless, any advances made will likely improve the sensitivity of the readouts for hPSC-CM disease models.

Variability between PSC lines

Another aspect to consider when using patient hiPSC as disease models is the most suitable control. Comparing to an unrelated hiPSC line, genetic differences (i.e,. single nucleotide polymorphisms in the gene of interest or genetic mutations in genetic modifiers) could exacerbate or even mask the disease phenotype. Even between different control hPSC-CMs, the electrophysiological properties are

markedly variable[151]. A solution is to use gene targeting to produce isogenic cell lines differing only at the mutation or genetic loci of interest[152]. Recent developments in endonuclease-based gene editing systems, in particular that of CRISPR/Cas9[153,154], have made it significantly easier to correct genetic defects. It is likely that this approach will complement the more traditional method of recruiting patients to generate hiPSC disease lines, in particular when evaluating new therapeutic compounds[41]. However, the frequency of endonuclease-induced off-target mutations and the influence of clonal heterogeneity on the disease phenotype are issues that still require further investigation.

Directed differentiation to different cardiac cell types

To date most of the established differentiation protocols generate ventricular-like cardiomyocytes[4], and so most disease modelling studies have focussed on the cell-autonomous ventricular aspects of the disease. However many channelopathies can also affect other cardiomyocyte subtypes, such as nodal and Purkinje cardiomyocytes in cardiac conduction disorders and atrial CMs in atrial fibrillation[155]. Several methods have been reported to improve the generation of different cardiomyocyte subtypes using either directed differentiation protocols or through purification [156–161]. It will be interesting to determine whether subtype-specific disease-causing differences can be detected. Additionally, some diseases such as ACM and BrS are known to have ventricular-specific (right vs. left) features[162]. Developing technologies to generate and distinguish the type of ventricular hPSC-CMs will enable investigations into the chamber-specific characteristics of the disease.

As the heart also consists of vascular, smooth muscle and epicardial cells, it is essential that these cell types can be reliably generated from hPSCs to better mimic

its function *in vitro* and to study diseases caused by failing communication between these different cells[163]. Heterotypic cell models are the next step for investigating non-autonomous diseases, such as diabetic cardiomyopathy or myocardial infarction. Also familial cardiac diseases, such as BrS and ACM, can have a non-cardiomyocyte component with changes to the epicardium believed to contribute to the overall disease phenotype[162]. Methods to derive these cells from hPSCs have been developed[164,165] and it is anticipated that more complex multicellular culture systems will be developed. Indeed a three-dimensional engineered cardiac tissue model for HCM was recently reported, combining a fixed percentage of CMs with stromal cells[166]. While key aspects of the HCM phenotype were observed, it is unclear whether the stromal cells contributed to this.

SUMMARY

Despite these challenges, the generation of hiPSCs from patients and the ability to derive cardiomyocytes from these cells have resulted in a paradigm shift in the way we model cardiac diseases. While hPSCs are unlikely to completely replace animal or cell-based model systems, hPSC-CMs have demonstrated significant potential to model various cardiac disorders. This has led to novel mechanistic insights into the disease pathology and aided understanding of these disorders at the individual patient level. This means that new therapeutic compounds and strategies can be tested on human cardiomyocytes from a range of different hPSC lines and potentially lead to tailored treatment for each patient depending on their specific gene mutation – the ultimate goal of precision medicine.

COMPETING INTERESTS

CLM is a co-founder of Pluriomics B.V.

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Table 1: Evaluation of methods used to measure disease phenotypes in hPSC-derived cardiomyocytes

Measurement type	Advantages	Limitations	Examples		
			Disease model	Method/Reagent	Ref.
Electrophysiology					
Patch Clamp (manual)	• Gold-standard with high resolution & accuracy • Precise measurements of currents & membrane potentials • Can record kinetics & gating properties of individual ion channels • Identify cardiac subtypes & drug responses • High degree of flexibility in recording configurations	• Labour-intensive • Requires trained specialist • Low-throughput • Follow-up measurements of same cell not possible	LQT1 LQT2 LQT3/Overlap	Whole-cell patch clamp Whole-cell patch clamp Perforated patch clamp	35 46 54
Patch clamp (automated)	• Permits high-throughput phenotyping • Requires less operator training • Reduces labour	• Reduced quality and lifespan of cells due to measurements performed in suspension • Better for measuring individual currents than APs • Requires large quantities of very pure hPSC-CM (>80%) preparations • Follow-up measurements of same cell not possible	CPVT	Planar patch clamp	19
Microelectrode arrays (MEAs)	• Permits medium-throughput phenotyping • Requires less operator training • Non-invasive, permitting long-term recordings • Measures cardiac-conduction velocities	• Measures multi-cellular clusters & requires pure (>60%) hPSC-CM preparations • High variability in measurements due to attachment of hPSC-CM to the electrode • Cannot measure individual currents or APs • Surrogate measure of QT interval	LQT2 JLNS DCM	Planar electrode array Planar electrode array Planar electrode array	45 44 71
Optical measurements					
Calcium dyes	• Can be combined with high-throughput imaging systems • High signal-to-noise ratio	• Prone to interassay variability in dye concentration • Affinity to Ca^{2+} can artificially prolong Ca^{2+} transient	CPVT1 HCM ACM	Fluo-4 AM Rhod-2 AM Fura-2 AM	67 96 101
Genetically-encoded voltage indicators (GEVIs)	• Non-invasive • Permits long-term recordings • Can target to specific hPSC-CM subtypes • Homogeneous signal intensities	• Do not obtain absolute values • Slow response kinetics • Generating cell lines is labour-intensive • Limited spectral range	LQT1 LQT2 LQT8	VSFP-CR ArcLight ArcLight	167 28 168

Genetically-encoded calcium indicators (GECIs)	• Non-invasive • More sensitive than Ca^{2+} dyes • Better biocompatibility compared to Ca^{2+} dyes	• Can result in unwanted intracellular interactions • Do not obtain absolute concentrations • Slow response kinetics for cardiac mapping • Generating cell lines is labour-intensive	LQT8 CPVT2	gCamp5G R-GECO1	168 28
Contraction force					
Bead displacement	• Permits long-term recordings • High resolution	• Sensitive to small errors in bead displacement measurements • Very small twitch forces • Force measured in 2-dimensions on single cells	HCM	polyacrylamide-embedded fluorescent beads	136
Engineered heart tissue	• Improved cellular alignment & sarcomeric organisation • Generates higher contraction forces • Can combine measurement of contractility with voltage and calcium measurements	• Low-throughput - requires 0.5 - $1x10^6$ hPSC-CMs • Challenging to image • Does not recapitulate in vivo structure	LQT3 DCM Pompe	filamentous matrix micro-fabricated tissue gauges force transducer	169 32 115
Atomic force	• Combines measurement of cellular elasticity, beat force, and rate • Very sensitive	• Low throughput (single cell) • Can damage the cell	DCM HCM	Cantilever tip Cantilever tip	79 170

Table 2: Summary of published PSC cardiac disease models and tested strategies for therapeutic rescue

Disease	Gene	Mutation	Therapeutic strategy	Prescribed to treat disease	Clinical potential	Ref.
Primary Arrhythmias						
LQT1	*KCNQ1*	R190Q	propranolol	yes		35
		P631fs	propranolol	yes		36
		R190Q; G269S; G345E	nifedipine	no	potential/lead compound	173
			pinacidil	no	potential/lead compound	
		V308_V344del	ML277	no	lead compound	39
		R594Q; R190Q	LUF7346	no	potential compound	41
LQT1/JLNS	*KCNQ1*	E160fs; R594Q	NS1643	no	lead compound	44
		R594Q	LUF7346	no	lead compound	41
LQT2	*KCNH2*	A561T	propranolol; nadolol; nicorandil	yes		45
			PD118057	no	lead compound	
		A561V	ALLN	no	lead compound	49
		N996I	LUF7346	no	lead compound	41
		A614V	nifedipine	no	potential/lead compound	46
			pinacidil	no	potential/lead compound	
		A422T	nifedipine	no	potential/lead compound	26
		R176W	n/a			174
		N996I	n/a			175
		A561P	n/a			176
LQT3/BrS	*SCN5A*	R1644H	mexiletine	yes		57
			ranolazine	no	clinical trial	
			phenytoin	no	potential/lead compound	
		F1473C	mexiletine	yes		177
		V1763M	mexiletine	yes		56
		N406K	TTX	no	no	26
			nifedipine	no	potential/lead compound	
			propranolol	yes		169
		V240M; R535Q	n/a			178
		1795insD	n/a			54
		R620H/R811H; K1397fs	n/a			55
LQT8	*CACNA1C*	G406R	roscovitine	no	lead compound	59

CPVT1	*RyR2*	S406L	dantrolene	no	clinical trial	[67]
		T2538R; L4115F; Q4201R; V4653F; N57_G91del	dantrolene	no	clinical trial	[75]
		L3741P	flecainide	yes		[71]
		M4109R	flecainide	yes		[66]
			thapsigargin	no	lead compound	
		E2311D	KN-93	no	lead compound	[70]
		R20Q	n/a			[69]
		F2483I	n/a			[179]
		P2328S	n/a			[68]
CPVT2	*CASQ2*	D307H	n/a			[180]
Primary Cardiomyopathies						
DCM	*LMNA/C*	S18fs, R225X	U0126; selumetinib	no	lead compound	[88;181]
	TNNT2	R173W	metoprolol	yes		[79]
			Serca2a gene therapy	no	clinical trial	
			Bay-60- 7550	no	lead compound	[81]
			milrinone	yes	potential/lead compound	
			omecamtiv mecarbil	no	clinical trial	[182]
	PLN	R14del	PLN gene therapy	no	potential	[183]
	DES	A285V	n/a			[184]
	TTN	W976R; A22352fs; P22582fs; N22577fs	n/a			[32]
	RBM2O	R636S	n/a			[185]
HCM	*MYH7*	R663H	cyclosporin A	no	no	[93]
			FK506	no	no	
			propranolol; verapamil; diltiazem; nifedipine	yes		
		R442G	metoprolol +/- verapamil	yes		[94]
			trichostatin A	no	lead compound	
	MYBPC3	c.2905+1G >A	MYBPC3 gene therapy	no	potential	[96]
		G999_Q1004del	n/a			[186]
		W792fs	n/a			[187]
		G1061X	n/a			[188]

	TPM1	D175N	n/a			[188]
ACM	*PKP2*	A324fs	BIO	no	lead compound	[100]
		G828fs; K672fs	GW9662; T0070907	no	lead compound	[101]
		L614P	n/a			[189]
Metabolic						
Pompe	*GAA*	D645E; Y354X/D645E	rhGAA	yes		[114]
		P266S; M439K	gene therapy	no	potential	[171]
		G828_N882 del W746X/W481fs	n/a			[115]
Fabry	*GLA*	W162X; R220X	rh α-galA	yes		[116]
			l-carnitine	no	potential compound	
			SAR 402671	no	clinical trial	
Danon	*LAMP-2*	A44fs; c.64+1 G>A	rapamycin	yes	potential compound	[172]
BTHS	*TAZ*	N173fs; S110P	gene therapy	no	potential	[104]
			mitoTEMPO	no	lead compound	
			linoleic acid	no	potential/lead compound	
ALDH	*ALDH2*	E487K	alda-1	no	lead compound	[108]
TECRL	*TECRL*	R196G; c.331+1G>A	flecainide	yes		[120]
Diabetic cardiomyopathy			thapsigargin	no	lead compound	[122]
			fluspirilene	yes	potential/lead compound	

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