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Modelling of Genetic Cardiac Diseases

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Abstract

Cardiac disease modeling is crucial to improve our understanding of the mechanism of various cardiac diseases and to discover new therapeutic approaches. Several modeling methods such as animal and computer simulations have been used to elucidate the cardiac diseases' mechanism and drug responses. However, each modeling technique has its own particular advantages and limitations. Human-based models would be particularly useful to investigate human cardiac diseases because humans and animals have differing cardiac physiologies and drug tolerability. In addition, the phenotype of cardiac diseases and response to therapeutic intervention differ not only between mutations but also among patients. Therefore, such diseases strongly demand the individualized/personalized strategies. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer the striking feature of retaining the same genetic information as donor, which guide us to investigate diseases and predict response to drug treatment individually. This feature of hiPSC-CMs is superior to the conventional in vitro modeling of cardiac diseases. Thus far, hiPSC-CMs have been successfully recapitulated many monogenic and also complex genetic cardiac diseases. hiPSC-CMs could be differentiated into different types of cardiomyocytes and non-cardiomyocyte cells, which empower us to understand cardiac chamber-specific arrhythmias such as atrial fibrillation and ventricular tachycardia.

Keywords: cardiac disease, modeling, hiPSC-CMs, drug

1. The importance of hiPSC-CMs

Cardiovascular diseases (CVDs) are the major causes of premature death and chronic disability worldwide [1]. Among CVD-related deaths, the occurrence of inherited lethal arrhythmias is the main reason for sudden cardiac death (SCD) in cardiac patients especially at young age [2]. Although many risk factors associated with SCD have been identified and understanding of pathogenesis of many cardiac diseases is progressing, the considerable number of cardiac patients still suffers SCD without warning, and we are still far from disease-specific treatment. Heterogeneous and multifactorial natures of genetic cardiac diseases are reasons for these complications. Furthermore, founder mutations causing cardiac disease have been reported in Finland [3], the Netherlands [4], and South Africa [5]. Not only disease phenotypes vary among different mutations, but also these vary among individuals carrying the same mutation. For example, long QT syndrome (LQTS) patients demonstrate a wide range of clinical phenotypes even among family members with the identical mutation [6]. Despite carrying the same gene variant resulting in cardiac disease, patients

often demonstrate the wide spectrum of clinical outcomes ranging from the absence of distinct electrocardiogram (ECG) abnormalities and being lifelong asymptomatic to clear abnormalities in ECG (e.g., prolonged QT interval and arrhythmias) and premature SCD. In addition, SCD could also be the first manifestation of cardiac disease. These suggest that the type of genetic mutation cannot always be the sole factor that dictates the prognosis of disease and clinical phenotype in all individuals who carry it [7]. Thus, genetic cardiac diseases exhibit the incomplete penetrance and differ among genetic cardiac diseases. For example, Brugada syndrome (BrS) has a penetration range from 12.5 to 50%; mean penetrance of LQTS is ~40%, while overall penetrance of catecholaminergic polymorphic ventricular tachycardia (CPVT) is 78% [7]. Another convoluting factor that hinders the genotype-phenotype correlation is variable expressivity within one phenotype because some mutation carriers display all the phenotypic symptoms, whereas some only display part of mutation-specific phenotypes [8]. The clinical heterogeneity of genetic cardiac diseases suggests that ultimate disease severity (i.e., penetrance and expressivity) does not solely depend on one particular gene causing cardiac disease, but instead results from the combination of many modifying factors such as age, gender, and environmental and lifestyle factors, which either exacerbate or protect against disease [9]. In addition, patients carrying more than one disease-causing mutations (i.e., not polymorphisms) either in the same gene or different genes yield to more severe clinical disease including earlier onset of disease, early heart failure, and premature SCD [10]. Besides these, some of the cardiac diseases overlap their phenotypes with other cardiac diseases (**Figure 1**). For example, mutations in cardiac sodium (Na^+) channel gene, *SCN5A*, are associated with type 3 long QT (LQT3), BrS, cardiac conduction diseases, and sinus node dysfunction [11]. These incomplete penetrance, variable expressivity, and phenotypic overlap impede the complete understanding of diseases' mechanism as well as disease-specific treatment. Furthermore, the treatment therapies are mainly targeted for symptomatic patients to prevent and counteract the symptoms, but treatments in asymptomatic individuals are still of concern with variable opinions. Nevertheless, pharmacological therapies have been resulted in poor outcomes in the

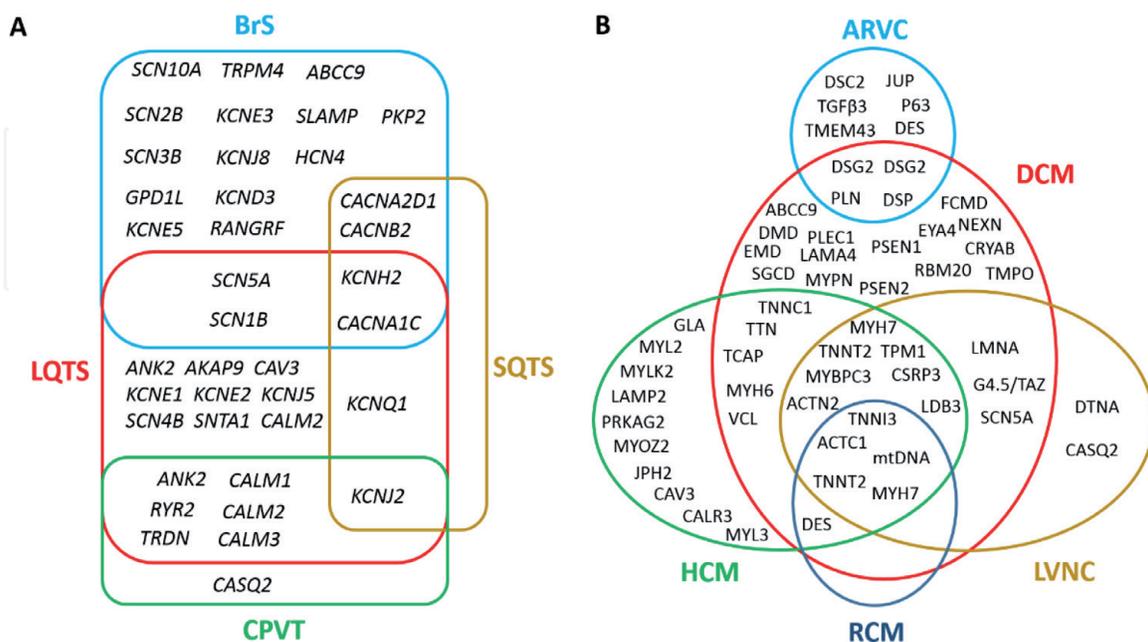


Figure 1. Heterogeneity of genetic cardiac diseases. (A) Overlapping genes causing channelopathies [27]. Brugada syndrome (BrS), long QT syndrome (LQTS), short QT syndrome (SQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT) (ref). (B) Overlapping genes causing cardiomyopathies [72]. Arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), left ventricular non-compaction cardiomyopathy (LVNC).

cardiac diseases [12]. So far, implantable cardioverter-defibrillator (ICD) is the only proven therapy for preventing detrimental consequences in cardiac patients with high risk of SCD [13]. However, ICD implantation is associated with its own complications and lower quality of life [14]. There are large groups of asymptomatic cardiac patients who do not have risk factors, which shift them into high-risk category as candidate for ICD implantation, but suffer SCD. Thus, the management for asymptomatic patients carrying pathogenic variant is the most challenging since SCD could be the first manifestation of disease [15, 16]. The clinical management of most cardiac diseases is suboptimal due to lack of comprehensive knowledge of mutations and possible mechanism involved. Thus, the mechanism of how mutation leads to modify the normal cardiac physiology and engender lethal arrhythmias should be deciphered so that the promising prevention and treatment could be established.

The prior cardiovascular research and drug screening have mostly been performed in animal models through knock-in/knock-out approaches. Although animal models have provided some fundamental information and led to many discoveries in genetic cardiac disease, physiological and pharmacological results cannot directly extrapolate from animals to humans because of some fundamental differences that exist between animal and human cardiac physiology [17]. For example, the resting heart rate of human is 75 bpm, while that of rat is 300 bpm, and the animal (mice and rats) can tolerate 6–400-fold higher concentration of some drugs compared to human [18]. The animal models become even worse when studying human cardiomyopathies due to mutations in contractile proteins, which are not highly expressed in mouse or rat. Therefore, it is more complicated to extrapolate physiological and pharmacological results from animal to human [17, 18]. Furthermore, most of cardiovascular drug screening and toxicology studies were performed in non-cardiac cell lines or animals, which do not accurately represent human CMs. Thus, considerable amount of cardiovascular drugs were withdrawn from market due to off-target effects [19]. Therefore, human tissues are required to study the human cardiac diseases and drug

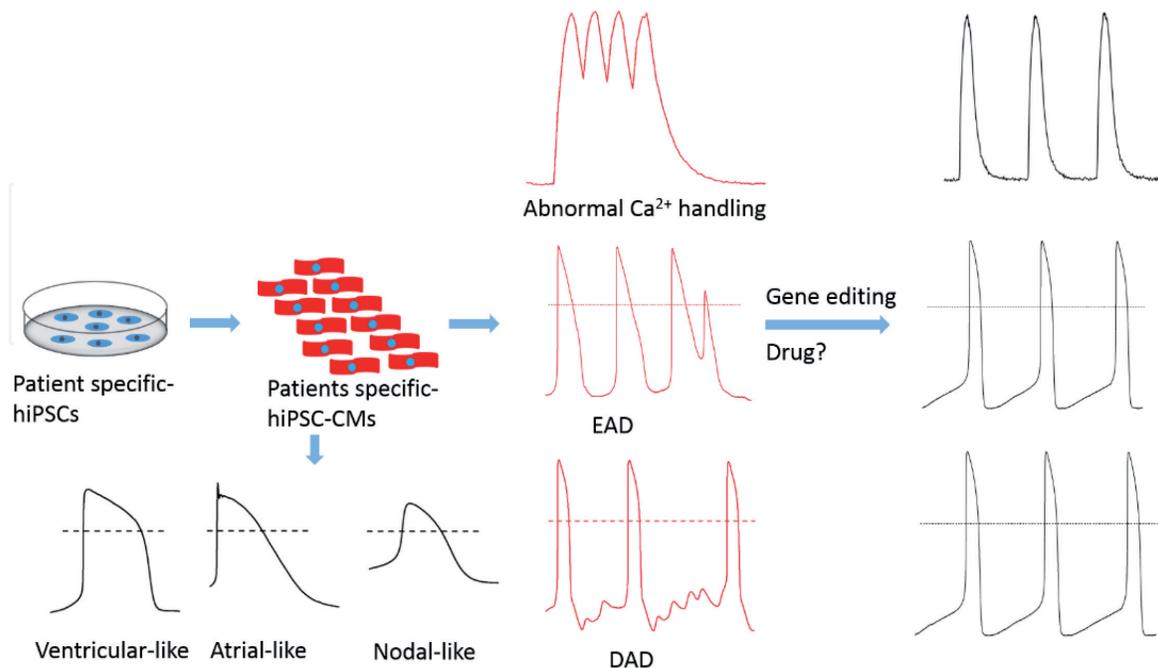


Figure 2. *hiPSC-CM-based modeling of human genetic cardiac diseases. Human-induced pluripotent stem cells (hiPSCs) can be differentiated into hiPSC-derived cardiomyocytes (hiPSC-CMs). There are at least three subtypes of hiPSC-CMs, namely, ventricular-like, atrial-like, and nodal-like hiPSC-CMs. hiPSC-CMs derived from cardiac patients carrying genetic mutation recapitulate calcium and electrical abnormalities (early afterdepolarization (EAD) and delayed afterdepolarization (DAD)). Newly emerging gene editing techniques were able to mitigate these abnormalities in hiPSC-CMs.*

testing. However, the human sample exhibits some of the major challenges: there is limited supply of human cardiac biopsies, and it involves complex procedures and ethical issues. In addition, these cardiac biopsies are typically obtained from the end stage of cardiac diseases; hence it is not possible to understand the mechanism of cardiac diseases [20, 21]. These obstacles are mostly overcome by the groundbreaking discovery of reprogramming adult somatic cells into induced pluripotent stem cells (iPSCs) [22, 23] which can be differentiated into cardiomyocytes (CMs) (hiPSC-CMs) [24–26]. The main advantages of hiPSC-CMs are iPSCs can be generated at any period of a patient's life, they have unlimited supply, and these retain the same genetic information as the donor, i.e., hiPSC-CMs are patient specific (**Figure 2**). These are superior features of hiPSC-CMs to the conventional in vitro modeling of cardiac diseases. In addition, hiPSC-CMs can be cultured for several months, which enable us to study acute and chronic effect of mutation and drugs on CMs. Thus, hiPSC-CMs not only provide the platform to investigate the mutation-specific mechanism but also assist to anticipate drug response on an individual basis and guide us to personalized medicine in future.

2. Channelopathy phenotypes in hiPSC-CMs

Channelopathy cardiac diseases are caused by mutations in cardiac ion channels located in the cellular membrane or organelles. Mutations in ion channels result in misbalance of fine-tuning ion exchange during excitation-contraction coupling (ECC), which could lead to cardiac arrhythmias and SCD in the worst case. The main cardiac channelopathies are CPVT, LQTS, BrS, and short QT syndromes (SQTS) [27]. These cardiac channelopathies have been extensively studied using hiPSC-CMs and described below.

2.1 Catecholaminergic polymorphic ventricular tachycardia (CPVT)

CPVT is an inherited cardiac disease with the prevalence of about 1:5000/10,000. This disease is characterized by premature ventricular contraction and/or polymorphic ventricular tachycardia (VT) induced by adrenergic stimulation in response to emotional stress or physical exercise in structurally normal heart. Over 150 mutations in ryanodine receptor type 2 (*RYR2* gene) are responsible for ~ 55% of CPVT type 1 cases (CPVT1), and mutation in calsequestrin 2 (*CASQ2* gene) CPVT accounts for 3–5% CPVT type 2 (CPVT2) cases [28, 29]. In addition, mutations in calmodulin (*CALM1*) genes and in triadin (*TRDN*) have been reported causing CPVT. *RYR2*, *CASQ2*, *CALM1*, and *TRDN* are involved in ECC, and mutation in any of these genes results in elevated intracellular Ca^{2+} , which leads to abnormal Ca^{2+} handling and arrhythmias [28, 29]. In consistency with clinical phenotype, many hiPSC-CM model had demonstrated the exacerbation of electrophysiological and Ca^{2+} handling abnormalities upon adrenergic stimulation [26, 30–32]. Furthermore, Zhang and colleagues had modeled hiPSC-CMs harboring CPVT1-associated F2483I mutation in *RYR2* gene and demonstrated that CPVT1 hiPSC-CMs had longer and wandering Ca^{2+} sparks and smaller sarcoplasmic reticulum Ca^{2+} content [32]. Later on, the same group corrected this mutation using clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) gene editing technique and showed that this mutation is causative rather than associative to the disease [33]. hiPSC-CM model for CPVT has also been used in studying the efficacy of various drugs. Previously we had directly compared the clinical results from CPVT1 patients with dantrolene medication, and the clinical response of dantrolene was similar as in hiPSC-CMs from the same patients; dantrolene

abolished or markedly reduced arrhythmias in patients and their hiPSC-CMs with certain mutation in *RYR2*, while it did not have any clinical effect with hiPSC-CMs or with other *RYR2* mutations [31]. Furthermore, an antiarrhythmic drug, flecainide, used to treat CPVT1 patients [34] was able to reduce the Ca^{2+} irregularities under adrenergic stimulation in CPVT1 hiPSC-CMs [30, 35]. CPVT2 patients harboring homozygous *CASQ2*-G112 + 5X mutation in *CASQ2* gene showed the rapid polymorphic VT under exercise stress test [36]. Adult rat ventricular myocytes were studied to understand the effect of *CASQ2* mutation in ECC, demonstrating that mutated CMs exhibited spontaneous extrasystolic Ca^{2+} elevations and delayed afterdepolarization (DADs) upon adrenergic stimulation [36]. Later, hiPSC-CM model harboring *CASQ2*-G112 + 5X mutation emulated these phenotypic features of disease, and AAV9-based gene delivery effectively prevents the development of adrenergic-induced DADs and triggered arrhythmias in CPVT2 hiPSC-CMs [37].

2.2 LQT type 1 (LQT1)

LQT type 1 (LQT1) is caused by loss-of-function mutation in *KCNQ1* gene encoding α subunit of potassium (K^+) channel mediating slow delayed rectifier K^+ current (I_{Ks}). LQT1 is responsible for 30–35% of all LQTS cases [38]. LQT1 is characterized by prolongation of QT interval in ECG, which could lead to SCD due to VT, typically torsades de pointes [39]. hiPSC-CMs derived from LQT1 patients faithfully recapitulated the clinical hallmark by showing prolonged action potential duration (APD) which is analogous to QT duration in ECG, and reduced I_{Ks} current densities are held responsible for abnormal repolarization [40–42]. ML277, an I_{Ks} activator, increased the I_{Ks} amplitude by enhancing the activation of I_{Ks} , thus resulting in shortening of APD in LQT1 hiPSC-CMs [40]. In addition, adrenergic stimulation in LQT1 hiPSC-CMs induced the early afterdepolarization (EAD) [42], which is similar to arrhythmias triggered in LQT1 patients by exercise or emotional stress [39]. Clinically, β -blockers were effective in minimizing the risk of cardiac events in LQT1 patients [43]. Similar antiarrhythmic effect of β -blockers has been observed in LQT1 hiPSC-CMs [42]. Furthermore, hypokalemia is the electrolyte disturbance caused by lower K^+ level in blood serum, which aggravates the QT prolongation and facilitates the development of hypokalemia-induced torsades de pointes in LQT1 patients [39, 44]. We successfully developed and mimicked these disease phenotypes in LQT1 hiPSC-CMs carrying G589D or IVS7-2A > G mutation in *KCNQ1* gene. Additionally, lowering the extracellular K^+ concentration prolonged APDs and induced the formation of EADs in LQT1 hiPSC-CMs [45]. Both G589D- and IVS7-2A > G-specific LQT1 hiPSC-CMs displayed longer APD and higher Ca^{2+} abnormalities in baseline; G589D hiPSC-CMs demonstrated prolonged contraction, while IVS7-2A > G hiPSC-CMs showed impaired relaxation [46] observed in our video image-based software analysis [47].

2.3 LQT type 2 (LQT2)

LQT type 2 (LQT2) is an LQTS subtype, which is caused by loss-of-function mutations in *KCNH2* gene also known as human ether-a-go-go-related gene (*hERG*) encoding K^+ channel mediating rapid delayed rectifier K^+ current (I_{Kr}). LQT2 is responsible for approximately 25–30% of all LQTS cases [38]. Similar to LQT1, LQT2 patients also exhibit the prolongation of QT interval and torsades de pointes. As in LQT1 hiPSC-CM model, LQT2 hiPSC-CMs also recapitulated clinical phenotypes by displaying longer APD resulted from reduced I_{Kr} current densities and enhanced EAD following the adrenergic stimulation [48–50]. Our early study of LQT2 hiPSC-CMs carrying R176W mutation in *KCNH2* gene demonstrated the reduced I_{Kr} current densities, prolonged repolarization, and increased arrhythmogenicity although the donor is an

asymptomatic carrier [50]. These results are in parallel with clinical findings that LQT2 patients usually display symptoms when heart rate is slow. In addition, this report illustrated that electrophysiological abnormalities can be detected in hiPSC-CMs, although iPSCs are derived from asymptomatic carriers of *KCNH2* mutations. The application of I_{Kr} blockers (E4031 and sotalol) further prolonged the APD resulting in EADs, whereas Ca^{2+} channel blocker (nifedipine), $I_{K,ATP}$ channel opener (pinacidil and nicorandil), and I_{Kr} channel enhancer (PD-118057) reduced the APD and thus mitigated the formation of EAD in LQT2 hiPSC-CMs [48, 49]. Several novel pharmacological strategies including ICA-105574 (potent I_{Kr} activator) [51], chaperone modulator N-[N-(N-acetyl-L-leucyl)-L-leucyl]-L-norleucine (ALLN) [52], LUF7346 (hERG allosteric modulators) [53], as well as application of allele-specific RNA interference approach [54] have been attempts to rescue the LQT phenotype in LQT2 hiPSC-CMs. Correcting the mutation associated with LQT2 not only confirmed that mutation caused I_{Kr} reduction and APD prolongation but also suggested that trafficking defect as the pathological mechanism is responsible for the electrophysiological phenotype in LQT2 [51, 55].

2.4 LQT type 3 (LQT3)

LQT type 3 (LQT3) is caused by gain-of-function mutations in *SCN5A* encoding α subunit of cardiac Na^+ channels [56]. The gain-of-function *SCN5A* mutation results in augmented late or persistent Na^+ current ($I_{Na,L}$), which leads to prolongation of QT interval in ECG and proarrhythmia. LQT3 is the third most common LQTS accounting for 5–10% of all LQTS cases [56]. LQT3 patients exhibit longer QT duration at slower heart rate, thus LQT3 patients are at higher risk for cardiac events during rest or sleep [57]. LQT3 patients harboring V1763 M mutation in *SCN5A* [58] R1644H mutation in *SCN5A* [59] or F1473C mutation in *SCN5A* and a polymorphism (K897 T) in *KCNH2* [60] had prolonged QT interval, and in vitro models using hiPSC-CMs derived from all those LQT3 patients demonstrated prolonged APD resulting in the larger $I_{Na,L}$ and altered biophysical properties of Na^+ channels [58–60]. Mexiletine, a Na^+ channel inhibitor commonly used in LQT3 therapy, lowered the $I_{Na,L}$ and thereby rescued the APD prolongation phenotype [58, 59] and suppressed the occurrence of EAD [59] and also corrected the altered Na^+ channel inactivation [60]. Incorporating the biophysics of Na^+ channel and pharmacological analysis illustrated that the improper functioning of Na^+ channel was responsible for LQT3 phenotypes rather than *KCNH2* polymorphism [60]. In addition to LQT3, mutation in *SCN5A* gene can cause BrS, and mixed phenotypes are often seen, which is also known as the “overlap syndrome.” Loss in function of Na^+ channel is often seen in BrS. Liang and co-workers had generated hiPSCs from two BrS patients, one with double missense mutation (R620H and R811H) in *SCN5A* gene (BrS(p1)) and another with one-base pair deletion mutation in the *SCN5A* gene (BrS(p2)), and showed that BrS hiPSC-CMs derived from both patients had reduced Na^+ current and increased triggered activity and abnormal Ca^{2+} handling [61]. These phenotypes were alleviated by correcting the mutation by CRISPR/Cas9 in hiPSCs derived from BrS (p2) [61]. Importantly, only BrS hiPSC-CMs harboring BrS-associated *SCN5A*-1795insD mutation displayed reduced Na^+ current and upstroke velocity, but not with three sets of hiPSC-CMs derived from BrS patients who tested negative for mutations in the known BrS-associated genes suggesting the Na^+ channel dysfunction may not be prerequisite for BrS [62]. In another study, Na^+ current and upstroke velocity were reduced, but not the voltage-dependent inactivation in BrS hiPSC-CMs carrying the mutations R1638X and W156X [63].

2.5 LQT type 7 (LQT7) or Andersen-Tawil syndrome (ATS)

LQT type 7 (LQT7) or Andersen-Tawil syndrome (ATS) is a rare inherited cardiac disease associated with mutation in *KCNJ2* gene (ATS type 1) encoding inward

rectifying K⁺ channel (Kir2.1) and accounts for ~70% of all ATS cases. However, the genetic cause of the remaining 30% of ATS (ATS type 2) remains unknown. In ATS patients, QT interval prolongation is not common, but prominent U wave and QU interval in ECG could be hallmarks of ATS, and they experienced cardiac arrhythmias including non-sustained VT and torsade de pointes [64]. Kuroda and co-workers generated hiPSCs from ATS patients carrying R218W, R67W, and R218Q mutations in *KCNJ2* gene and showed strong arrhythmic events and higher incidence of irregular Ca²⁺ handling in ATS hiPSC-CMs, but flecainide and KB-R7943 (a reverse-mode Na⁺/Ca²⁺ exchanger inhibitor) were able to suppress those events [65].

2.6 LQT type 8 (LQT8) or Timothy syndrome (TS)

2.6 LQT type 8 (LQT8) or Timothy syndrome (TS) is a very rare genetic cardiac disease which results from mutation in *CACNA1C* gene encoding Ca²⁺ channel (Ca_v1.2). LQT8 is the most severe type of LQTS, which is characterized by markedly prolonged QT interval, severe ventricular arrhythmia, and multiorgan dysfunction [66]. hiPSC-CMs derived from TS patients recapitulated the disease phenotypes, but roscovitine rescued those abnormalities such as altered Ca²⁺ channel inactivation, prolonged APD, higher incidences of arrhythmias, and abnormal Ca²⁺ handling [67].

2.7 Short QT (SQT)

SQT is a rare inherited cardiac disease characterized by QT interval shortening, which is in contrast to QT prolongation observed in LQTS. SQT is associated with mutations in genes associated with K⁺ channel or Ca²⁺ channels [68]. The prevalence of SQT is between 0.02–0.1% and 0.05% in adults and children, respectively [69]. Recently El-Battrawy and co-workers had generated hiPSCs from SQT type 1 patients carrying a mutation (N588K) in *KCNH2*, and hiPSC-CMs mimicked the clinical phenotype of SQT by showing a shortened APD as a result of increased I_{Kr} current densities [70]. In addition, SQT hiPSC-CMs exhibited abnormal Ca²⁺ transients and rhythmic activities, which are enhanced by carbachol, but quinidine alleviated those carbachol-induced arrhythmias and prolonged the APD [70].

3. Cardiomyopathy phenotypes in hiPSC-CMs

Cardiomyopathies are diseases of cardiac muscle and associated with structural and/or functional abnormalities. The most common genetic cardiomyopathies are hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). These genetic cardiomyopathies have been also extensively studied using hiPSC-CMs [71, 72].

3.1 Hypertrophic cardiomyopathy (HCM)

HCM is one of the most common genetic cardiac diseases with an estimate prevalence of 1 in 500. HCM is characterized by unexplained symmetrical or asymmetrical left ventricular hypertrophy. Mutations in sarcomeric proteins account for ~60% of all HCM cases including mutation in β-myosin heavy chain (MYH7), cardiac myosin-binding protein C (MYBPC3), cardiac troponin I (cTnI), cardiac troponin T (cTnT), and tropomyosin (TPM1) [73]. Hypertrophy of myocytes and disarray of sarcomere are the histological hallmarks of HCM seen in cardiac biopsies from HCM patients [74], and these histological phenotypes are also observed in hiPSC-CM model of HCM [25, 75–77]. In addition, HCM hiPSC-CMs also demonstrated other hallmarks of HCM such as nuclear translocation of nuclear factor of activated T cells (NFAT) [75–77],

elevation of β -myosin/ α -myosin ratio, and calcineurin activation [75]. Furthermore, isolated CMs from HCM patients displayed the prolonged APDs, increased Ca^{2+} current densities, reduced transient outward K^+ current densities, abnormal Ca^{2+} handling, and increased frequency of arrhythmias [21]. These electrophysiological and Ca^{2+} transient irregularity phenotypes have been faithfully recapitulated in HCM hiPSC-CMs [25, 75, 76, 78]. When HCM tissues carrying a mutation in *MYBPC3* gene were compared with donor heart sample, no specific truncated MyBP-C peptides were detected, but the overall level of MyBP-C in myofibrils was significantly reduced [79]. Similar haploinsufficiency results were also shown in HCM hiPSC-CMs with mutation in *MYBPC3* gene [25, 80], and gene replacement in HCM hiPSC-CMs partially improves the haploinsufficiency and reduces cellular hypertrophy [80]. Similar to higher myofilament Ca^{2+} sensitivity observed in isolated cardiac biopsies from HCM with E99K mutation in cardiac actin [81], in vitro model of HCM hiPSC-CMs carrying E99K mutation in cardiac actin demonstrated significantly stronger contraction and increased arrhythmogenic events [82]. Furthermore, a study in HCM mice harboring I79N mutation in cTnT resulted in increased cardiac contractility, altered Ca^{2+} transients, and remodeling of action potential [83]. These phenotypes were faithfully recapitulated by HCM hiPSC-CMs carrying the same I79N mutation in cTnT [84]. These hypercontractility and increased arrhythmogenicity phenotypes were reversed in HCM hiPSC-CMs when the E99K mutation in cardiac actin [82] and I79N mutation in cTnT [84] were corrected using CRISPR/Cas9 gene editing technique. Recently, we have shown that HCM hiPSC-CMs carrying *TPM1-Asp175Asn* mutation exhibited VT type of arrhythmias [78], and this observation is in line with earlier clinical observation of HCM patients with *TPM1-Asp175Asn* mutation being at increased risk of fatal arrhythmias [85]. Currently, there is no specific pharmacological therapy for HCM patients, and drugs are prescribed mainly based on symptoms and personal history. However, drug therapy has also resulted in poor outcomes in HCM patients [12]. We reported the similar poor antiarrhythmic efficiency of β -blocker in preventing lethal arrhythmias in HCM hiPSC-CMs [78]. In another HCM report, several environmental factors were investigated with hiPSC-CMs to study their effect on disease progression [77]. They found that endothelin (ET)-1 was able to induce HCM phenotypes such as cellular hypertrophy and myofibrillar disarray in hiPSC-CMs, which are inhibited by ET receptor type A blocker [77]. HCM patients exhibited defects in mitochondrial functions and ultrastructure and abnormal energy metabolism [74]. These structural and functional phenotypes were recapitulated in hiPSC-CMs carrying m.2336 T > C mutation in mitochondrial genome causing HCM [86]. They reported that HCM hiPSC-CMs expressed reduced levels of mitochondrial proteins, ATP/ADP ratio, and mitochondrial membrane potential [86].

3.2 Dilated cardiomyopathy (DCM)

DCM is a myocardial disease characterized by ventricular chamber enlargement and systolic dysfunction and progressive heart failure without significant change in ventricular wall thickness. Mutations in >30 genes encoding proteins of cytoskeleton, sarcomere, and nuclear lamina are found in 30–35% of DCM patients [87]. DCM patients with mutations in *RBM20*, encoding RNA binding motif protein 20 (RBM20), have an early onset of disease phenotype [88]. Isolated CMs from DCM patients carrying mutation in *RBM20* displayed elongated and thinner sarcomere structure [88], and such disorganized sarcomeric structure phenotypes were recapitulated in DCM hiPSC-CMs carrying mutation in *RBM20* [89, 90]. *RBM20* is the main regulator of the heart-specific titin splicing, and N2BA isoform is predominantly expressed in CMs from DCM patient carrying mutation in the *RBM20* gene [91]. In vitro model of *RBM20* hiPSC-CMs successfully mirrored the altered titin

isoform expression (titin isoform switch) [89, 90]. Furthermore, RBM20 hiPSC-CMs showed delayed Ca^{2+} extrusion and reuptake and more Ca^{2+} being released during each ECC, which resulted into deficient muscle contraction, the hallmark of cardiac dysfunction of DCM patients [89, 90]. In addition, a three-dimensional engineered heart muscle generated from RBM20 hiPSC-CMs showed an impaired force of contraction, and passive stress was decreased in response to stepwise increase in strain, suggesting higher viscoelasticity caused by mutation in *RBM20* [89]. Besides HCM, mutation in cTnT also caused DCM and resulted in shifts in Ca^{2+} sensitivity and force of contraction [92]. Sun and co-workers generated iPSCs from DCM patients carrying R173W mutation in cTnT and reported that DCM hiPSC-CMs exhibited altered Ca^{2+} handling, decreased contractility, and abnormal sarcomeric α -actinin distribution [93]. DCM patients with lamin A/C (LMNA) mutations show a highly variable phenotype. Cardiac biopsies from DCM patients harboring LMNA mutations exhibit reduced LMNA in nuclei with nuclear membrane damage such as focal disruption and nuclear pore clustering [94]. Nonsense mutation (R225X) in exon 4 of the LMNA gene causing DCM was associated with accelerated nuclear senescence and apoptosis of DCM hiPSC-CMs under electrical stimulation [95]. In another in vitro modeling of DCM, harboring A285V mutation in desmin (*DES*) using hiPSC-CMs displayed the pathogenic phenotypes of DCM such as diffuse abnormal DES aggregation, poor co-localization of DES with cTnT, and Z-disk streaming with accumulation of granulo-filamentous materials or pleomorphic dense structures adjacent to the Z-disk or between the myofibrils [96]. DCM patients harboring R14del mutation in phospholamban (PLN) result in ventricular dilation, contractile dysfunction, and episodic ventricular arrhythmias [97]. Similarly, hiPSC-CMs carrying R14del mutation in PLN induced the Ca^{2+} handling abnormalities, irregular electrical activity, and abnormal intracellular distribution of PLN in DCM hiPSC-CMs [98]. These PLN R14del-associated disease phenotypes were mitigated upon correction of PLN R14del mutation by transcription activator-like effector nuclease (TALENs) gene editing technique [98]. Furthermore, genetic correction of PLN R14del mutation by TALENs improved the force development and restored the contractile function in three-dimensional human engineered cardiac tissue derived from R14del-iPSCs [99].

3.3 Arrhythmogenic right ventricular cardiomyopathy (ARVC)

ARVC is rare genetic cardiac disease with the prevalence ranging from 1:000 to 1:5000 worldwide. The histopathological hallmark of ARVC is the substitution of the cardiac myocytes with fibro-fatty deposits, particularly within the free wall of the right ventricle. The consequent results from the disruption of normal myocardial architecture can lead to right ventricular dysfunction, life-threatening arrhythmias, and SCD [100]. ARVC is caused by mutations in genes encoding desmosomal proteins such as plakoglobin (JUP), desmoplakin (DSP), plakophilin-2 (PKP2), desmoglein-2 (DSG2), and desmocollin-2 (DSC2) [100]. Similar to immunohistological results from the biopsy sample from ARVC patients [101], ARVC hiPSC-CMs harboring a plakophilin 2 (PKP2) gene mutation mimicked the reduced PKP2 immunosignal [102, 103]. In addition, clusters of lipid droplets accumulating within the cytoplasm were identified in ARVC-hiPSC-CMs associated with structural distortion of desmosomes [103]. Another study showed that induction of adult-like metabolic energetics from an embryonic/glycolytic state and abnormal peroxisome proliferator-activated receptor gamma (PPAR γ) activation underlie the pathogenesis of ARVC [104]. It has been observed that male ARVC patients develop earlier and more severe phenotype than female ARVC patients [105]. To understand whether sex hormones in serum may contribute to the major arrhythmic cardiovascular events in ARVC, Akdis and co-workers combined a clinical study and in vitro

hiPSC-CM model and showed that increased levels of testosterone accelerate ARVC pathologies, while premenopausal female estradiol levels slow down exaggerated apoptosis and lipid accumulation in ARVC hiPSC-CMs [106].

4. Limitations and future prospective

The reprogramming of somatic cells into pluripotent stem cells and subsequent differentiation into specific cell types is a newly emerging technique and is certainly not free from limitation.

One of the most questionable issues of hiPSC-CMs is their maturity. Despite expressing relevant ion channels [107] and structural genes [25, 26, 75, 76, 89, 108], hiPSC-CMs lack t-tubules and exhibit lower expression of Kir2.1 and weaker contractility; thus they do not fully resemble adult CMs. In order to improve the maturity of hiPSC-CMs and consequently upgrade the functionality of hiPSC-CMs, various techniques have been investigated in different groups. Three-dimensional construction of engineered heart tissue is a rapidly growing technique for structural and functional maturation of hiPSC-CMs [109], which resulted in higher Na^+ current density and upstroke velocity [110], and enhances the metabolic maturation [111] comparable to adult CMs. Furthermore, Shadrin and co-workers introduced the “Cardiopatch” platform for three-dimensional culture and maturation of hiPSC-CMs; this platform produces robust electromechanical coupling, consistent H-zone and I bands, and evidence of t-tubules and M-bands [112].

Another issue of hiPSC-CMs is the purity of differentiated CMs. The CMs differentiated from hiPSCs yield in heterogeneous population of CMs. There are at least three subtypes of CMs such as ventricular, atrial, and nodal CMs; among them the majority (~70%) of CMs are ventricular-like, and only a minority of CMs are atrial-like (~20%) and nodal-like (~10%) [40, 58, 93, 107]. Although many molecular and functional characteristics are shared among these CMs subtypes, they also exhibit their own unique features. For example, ventricular CMs have prominent plateau phase (phase 2) in action potential profile, atrial CMs exclusively exhibit I_{Kur} channels, and nodal CMs lack strong upstroke velocity [113]. Most of the published methods of differentiation protocol yield in a lower amount of atrial-like and nodal-like CMs [40, 58, 93, 107], but sufficient numbers of subtype-specific CMs are needed to understand the subtype-related disease mechanism and development of specific therapeutic approaches. Atrial fibrillation (AF) is one of the most common cardiac arrhythmias; however, current antiarrhythmic drugs for treatment of AF are not atrial-specific and could cause unacceptable ventricular events [114]. Thus, sufficient supply of atrial CMs is crucial for investigating the AF cellular mechanism. hiPSCs have been differentiated into high-purity atrial-specific CMs by using retinoic acid signaling at the mesoderm stage of development [115]. These patient-specific atrial CMs allow us to investigate in detail mechanisms of AF and to develop atrial-specific therapeutic drugs. Furthermore, sinoatrial node (SAN) dysfunction can manifest bradycardia and asystolic pauses, but its pathophysiology is not completely understood [116]. SAN pacemaker cells from hiPSCs would facilitate the study of the disease mechanism and provide a cell source for developing a biological pacemaker. Protze and co-workers had reported the transgene-independent method for the generation of pacemaker cells (nodal-like CMs) from human pluripotent stem cells by stage-specific manipulation of developmental signaling pathways [117]. Besides CMs, the heart also consists of many other cell types such as fibroblast, endothelial and vascular smooth muscle cells, and also extracellular matrix. Importantly, the origin of cardiac diseases may not always exclusively originate from CMs, but might

involve non-CMs. Thus, incorporating the fibroblasts [118], endothelial cells [119], and vascular smooth muscle cells [120] into CMs from the same hiPSCs could offer new insight of disease mechanism.

The establishment of appropriate control is another challenge in disease modeling using hiPSC-CMs. It is generally argued/suggested that when comparing the results between control and mutated hiPSC-CMs, both should have the same genetic background. This objective is achieved in somehow by using healthy family members as control [58, 93]. However, only ~50% of genome is shared between siblings, and phenotypic difference could result from DNA variants in the rest of genome besides disease-associated mutation [121]. Mutated genes can be corrected with the help of newly growing gene editing technology such as TALENs [98] and CRISPR/Cas9 [33, 51, 84], thus establishing the so-called isogenic lines. This isogenic line would be the most appropriate control for comparison as it differs only in the presence and absence of mutation. Therefore, advance genome engineering will not only provide more reliable control lines but also guide us to understand how mutation modifies the normal functioning of cells. However, for diseased CMs without known mutation, healthy family members or otherwise controls are still the best.

5. Conclusion

While animal models fail to recapitulate human cardiac disease phenotype properly, hiPSC-CMs have been successful in recapitulating crucial phenotypes of many genetic cardiac diseases in terms of morphology, contractility, Ca^{2+} handling, ion channel biophysics, cell signaling, and metabolism. Most strikingly, hiPSC-CMs provide the patient-specific platform to study the disease mechanism and drug response individually, which the traditional disease modeling technique would never offer. In addition, cardiac subtype-specific arrhythmias and drug screening could be performed with the help of unlimited supply of hiPSC-CMs; thus chamber-specific treatment modalities could be identified. Certainly, by improving the current weaknesses of hiPSC-CMs and incorporating with new gene editing techniques, complex cardiac disease mechanism could be deciphered, and novel effective treatment therapies could be identified to improve the life of cardiac patients.

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Conflict of interest

No conflict of interest.

Abbreviations

AF	atrial fibrillation
ARVC/D	arrhythmogenic right ventricular cardiomyopathy/dysplasia
APD	action potential duration
ATS	Andersen-Tawil syndrome

BrS	Brugada syndrome
Ca ²⁺	calcium ion
CPVT	catecholaminergic polymorphic ventricular tachycardia
CRISPR	clustered regularly interspaced short palindromic repeats
cTnT	cardiac troponin T
CVDs	cardiovascular diseases
DADs	delayed afterdepolarization
DCM	dilated cardiomyopathy
DSC2	desmocollin-2
DSG2	desmoglein-2
DSP	desmoplakin
EAD	early afterdepolarization
ECC	excitation-contraction coupling
ECG	electrocardiogram
ET	endothelin
hiPSC-CMs	human-induced pluripotent stem cell-derived cardiomyocytes
ICD	implantable cardioverter-defibrillator
iPSCs	induced pluripotent stem cells
K ⁺	potassium ion
LMNA	lamin A/C
LQTS	long QT syndromes
MYBPC3	cardiac myosin-binding protein C
MYH7	myosin heavy chain
Na ⁺	sodium ion
PKP2	plakophilin-2
PLN	phospholamban
SAN	sinoatrial node
SCD	sudden cardiac death
SQTS	short QT syndromes
TALNs	transcription activator-like effector nucleases
TS	Timothy syndrome
VT	ventricular tachycardia

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