STEM CELLS®

EMBRYONIC STEM CELLS/INDUCED PLURIPOTENT STEM CELLS

Concise Review: Cardiac Disease Modeling Using Induced Pluripotent Stem Cells

CHUNBO YANG,[a] JUMANA AL-AAMA,[b] MIODRAG STOJKOVIC,[c] BERNARD KEAVNEY,[d] ANDREW TRAFFORD,[d] MAJLINDA LAKO,[a] LYLE ARMSTRONG[a]

Key Words. Induced pluripotent stem cells • Embryonic stem cells • Differentiation • Cardiac

[a]Institute of Genetic Medicine, Newcastle University, The International Centre for Life, Central Parkway, Newcastle upon Tyne, United Kingdom; [b]Princess Al Jawhara Center of Excellence in Research, King Abdulaziz University, Jeddah, Saudi Arabia; [c]Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia; [d]Institute of Cardiovascular Sciences Core Technology, Manchester University, Manchester, United Kingdom

Correspondence: Lyle Armstrong, BSc, PhD, Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, U.K. Telephone: + 44-1912418695; Fax: 00+44 191-241-8666; e-mail: Lyle.Armstrong@ncl.ac.uk

Received November 3, 2014; accepted for publication May 12, 2015; first published online in STEM CELLS *EXPRESS* June 23, 2015.

1066-5099/2014/$30.00/0

http://dx.doi.org/10.1002/stem.2070

ABSTRACT

Genetic cardiac diseases are major causes of morbidity and mortality. Although animal models have been created to provide some useful insights into the pathogenesis of genetic cardiac diseases, the significant species differences and the lack of genetic information for complex genetic diseases markedly attenuate the application values of such data. Generation of induced pluripotent stem cells (iPSCs) from patient-specific specimens and subsequent derivation of cardiomyocytes offer novel avenues to study the mechanisms underlying cardiac diseases, to identify new causative genes, and to provide insights into the disease aetiology. In recent years, the list of human iPSC-based models for genetic cardiac diseases has been expanding rapidly, although there are still remaining concerns on the level of functionality of iPSC-derived cardiomyocytes and their ability to be used for modeling complex cardiac diseases in adults. This review focuses on the development of cardiomyocyte induction from pluripotent stem cells, the recent progress in heart disease modeling using iPSC-derived cardiomyocytes, and the challenges associated with understanding complex genetic diseases. To address these issues, we examine the similarity between iPSC-derived cardiomyocytes and their ex vivo counterparts and how this relates to the method used to differentiate the pluripotent stem cells into a cardiomyocyte phenotype. We progress to examine categories of congenital cardiac abnormalities that are suitable for iPSC-based disease modeling. STEM CELLS *2015;33:2643—2651*

SIGNIFICANCE STATEMENT

Induced pluripotent stem cells are increasingly important for creating in vitro models of genetic diseases. This approach is particularly valuable for modelling cardiac developmental abnormalities as long as the limitations of pluripotent stem cell derived cardiomyocytes are understood.

INTRODUCTION

Substantial numbers of congenital cardiac abnormalities result from mutations in several genes which underline the development of myocardial structures during embryonic ontogeny. The occurrence of such conditions is relatively common with approximately 1% of live births being affected by some form of cardiac abnormality [1] although the phenotype of these is often mild to produce overt symptoms and the disease may only be detected during autopsy indicated by death from causes other than heart disease. The creation of in vitro models for such congenital abnormalities is important not only for understanding the genetic mechanisms leading to disease but also for drug screening efforts aimed at the identification of potential treatments. Patient-specific cardiomyocytes are a valuable resource for studies of this nature.

CARDIOMYOCYTES CAN BE DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS

Single cardiomyocytes isolated from patients could be useful tools for disease modeling studies but they are rarely available and cannot be grown in culture prior to analysis. Studies have shown the possibility of in vitro differentiation of various types of somatic cells (such as endothelial progenitor cells from blood or bone marrow [2], mesenchymal stem cells from bone marrow or other tissues [3], and resident cardiac stem cells in the heart [4]) into cardiomyocytes, but it is not clear how well such cardiomyocytes replicate the functions of their ex vivo derived counterparts [5]. Embryonic stem cells (ESC) are a valuable cell source for regenerative medicine because they are capable of proliferating infinitely and differentiating into all cell types derived from the three embryonic germ layers. Cardiomyocytes are readily differentiated from

ESC. Appearance of spontaneously contracting cells in three-dimensional embryoid bodies (EBs) was reported 4 years after the derivation of mouse ESC, and in 2001 human cardiomyocytes were successfully generated following the establishment of human ESC lines 3 years earlier [6]. The problem with ESC is we are unlikely to obtain them from sufferers of congenital heart diseases and to mimic disease-specific mutations would have to be introduced. The advent of induced pluripotent stem cells (iPSCs) addresses this problem since these can be readily generated from the somatic tissues of patients. Moreover, iPSCs are similar to human ESC in morphology, proliferation, gene expression, epigenetic status of pluripotency genes, and differentiation potential both in vitro and in vivo [7–9]. Additionally, human iPSCs can differentiate ESC toward cardiomyocytes [10, 11] which can provide patient-matched lines for future cell therapy, but can also serve as in vitro models of congenital heart diseases for the study of underlying mechanisms, drug screening, and safety tests. Several strategies have been devised to reprogram somatic cells to iPSC most of which involve transduction of cells with the four original "Yamanaka" factors *OCT4, KLF4, SOX2*, and *c-MYC* and the majority of reprogramming protocols differ mainly in the type of vector used to transducer the cells. State of the art methods typically involve vectors that do not integrate into the target cell's genome such as Sendai viruses, plasmid vectors, polycistronic mini-circle vectors, and self-replicating selectable episomes [12–17]. Alternatively viral vector-free approaches including direct delivery of recombinant reprogramming proteins [18, 19] and repeated administration of synthetic modified messenger RNAs have been successfully applied, albeit with low efficiency [20].

Differentiation of human iPSC toward the cardiac lineage can be achieved using a number of different methods [5, 6, 10, 11, 21–32]. It is not our intention in this review to focus upon the variety of differentiation methods available but rather the application of patient-specific iPSC to disease modeling. The development of protocols for generating cardiomyocytes ranges from simple spontaneous differentiation of pluripotent stem cells, either as EBs or adherent monolayers of cells coculture with other somatic cell types thought to induce cardiac mesoderm differentiation, to chemically defined media. The purpose of many such methods is to recapitulate the embryonic development process by manipulating molecular pathways that lead to specification and terminal differentiation of cardiomyocytes from embryonic mesoderm. It has been suggested that signals from the endoderm, such as bone morphogenic proteins, Activin/Nodal/TGFβ, fibroblast growth factors, and repressors of Wnt signaling, all play pivotal roles in establishment of the cardiovascular system [33].

How Similar are the iPSC-Derived Cardiomyocytes to their Adult Counterparts?

Differentiation of both ESC and iPSC using the methods described above yields areas of spontaneously contracting tissue containing large numbers of cardiomyocytes [34] that are capable of generating action potential (AP) specific for atrial, ventricular, and pacemaker cardiomyocytes which are detectable using patch clamping techniques [11, 35]. Early attempts to differentiate iPSC generated cardiomyocytes that were noticeably different to both their neonatal and adult ex vivo counterparts.

Expression of the subunits of the sodium channel current (I_{Na}) is consistent with native human ventricular cardiomyocytes [36, 37] despite reduced upstroke velocity of the ventricular-like AP in cardiomyocytes derived using earlier protocols [38–41]. Again, newer methods are able to generate iPSC-derived cardiomyocytes with ventricular APs that are more similar to those of adult cardiomyocytes [42]. Several solutions rely upon restoring the inward rectifier potassium current in iPSC-derived cardiomyocytes. Repolarization of the AP of native cardiomyocytes is controlled by four voltage-dependent potassium channel currents [43]. The activities of the delayed rectifier potassium channel currents (I_{Kr} and I_{Ks}) and the calcium-independent transient outward current (I_{to}) are similar in native and iPSC-derived cardiomyocytes [44, 45]; however, the activity of the inward rectifier potassium current (I_{K1}) is much lower in iPSC-derived cardiomyocytes when compared with native counterparts. The small I_{K1} current may explain the differences in electrophysiological characteristics of iPSC-derived cardiomyocytes. Several attempts have addressed this deficiency and restoration of I_{K1} either by ectopic expression of Kir 2.1 which encodes the channel protein that mediates I_{K1} [46], or by use of an in silico interface to provide an external I_{K1} current [47] restores the AP parameters to expected values.

Immaturity of the sarcoplasmic reticulum was observed in early studies of cardiomyocyte differentiation from iPSC. In fully differentiated cardiac muscle, the sarcoplasmic reticulum is the major source of Ca^{2+} required for contraction [48, 49] but an early publication suggested that both ESC and iPSC-derived cardiomyocytes show a significantly reduced response to β-adrenergic stimuli (such as isoproterenol) and a negative force-frequency relationship compared to a positive relationship for adult ex vivo cardiomyocytes [50]. More recent improvement of differentiation protocols, principally those which control the size and density of EBs generated in the early phase of differentiation, gives rise to cardiomyocytes with improved β-adrenergic responses [51, 52]. Transient calcium release (Ca^{2+} sparks) from the sarcoplasmic reticulum mediated by apparently normal ryanodine receptor function has been demonstrated [53, 54] and the smaller peak amplitude of the caffeine-induced calcium transient observed in some iPSC-derived cardiomyocytes compared to equivalent cells from hESC (suggestive of impaired ryanodine receptor function), can be improved by growth of iPSC-derived cardiomyocytes on microgrooved culture substrates [55].

Early differentiation protocols also produced cardiomyocytes with noticeably immature ultrastructure; electron microscopy studies showed abundant myofibrillar bundles and transverse Z-bands similar to those observed in fetal or neonatal cardiomyocytes [56] but with lower abundance of mitochondria than was normally observed for adult cardiomyocytes. Furthermore, mitochondria in the iPSC-derived cells showed lower density cristae. Again, improved differentiation protocols can give rise to iPSC-derived cardiomyocytes that show sarcomere and sarcoplasmic reticulum structures with a greater degree of similarity to adult cardiomyocytes. Similar effects can be achieved by long term maintenance of iPSC-derived cardiomyocytes in low density cultures [57]; however, it is not yet clear that such treatments significantly increase the structural maturity of mitochondria beyond a modest increase in respiratory activity [58]. In view of such data, manipulation of culture conditions, in particular the physical characteristics of the supporting matrix,

has significant influence on structural maturity of iPSC-derived cardiomyocytes [59] but some structural parameters, such as the absence of transverse tubules, remain to be addressed. Moreover, mature expression of some protein components of the sarcomere remains to be shown. In particular, expression of the adult isoform of cardiac troponin, cTnI is not yet achievable with human iPSC by any current cardiomyocyte differentiation protocol except stable transduction with adenoviral vectors encoding the adult *TNNI3* gene [60].

The levels of cytoskeletal proteins present in iPSC-derived cardiomyocytes also change in response to modification of differentiation protocols. Myosin heavy chain (MHC), myosin light chain (MLC) 2 atrial isoform, and MLC2 ventricular isoform are present but in particular, α-MHC is present in early stage iPSC-derived cardiomyocytes at higher levels than the β-isoform although expression of the latter increases with prolonged maintenance in culture, an occurrence suggested to reflect changes in contraction kinetics [57]. Differentiated iPSCs exhibited cardiomyocyte-specific markers, such as GATA4, NKX2.5, cTnT, MLC2a, sarcomeric α-actinin, connexin 43, α-sarcoglycan, and N-cadherin. The presence of α-sarcoglycan is interesting since it is prerequisite to the formation of functional connections between individual cardiomyocytes in the whole myocardium suggesting that iPSC-derived cardiomyocytes may be competent to integrate upon myocardial transplantation. This possibility has been underlined by animal model studies showing successful integration and apparent maturation of the iPSC-derived cardiomyocyte after transplant into the hearts of experimental animals [61, 62].

In view of the studies described, human iPSC-derived cardiomyocytes appear to be useful for analyzing the molecular outcomes of heart disease and as screening tools to determine efficacy and toxicity of potential drugs to treat such conditions. Naturally, the utility of iPSC-based in vitro models will depend upon the nature of the disease we wish to study but overall, if genetic mutation is a causative or contributing factor to the disease then it is possible that cardiomyocytes differentiated from patient-specific iPSC may reflect dysfunctional characteristics comparable to those present in cardiomyocytes in vivo. If the mutations contribute to progressive deterioration of cardiac function then iPSC-derived cardiomyocytes may be less valuable; similarly, if the dysfunction present in the diseased cardiomyocytes contributes to the degeneration of other components of the heart or the ingress of fibrotic tissue, the disease may be difficult to model using cardiomyocytes alone. Similarly, if the causes of the disease are unknown, iPSC-based modeling will rely on the observation of similar functional defects in iPSC-derived and ex vivo cardiomyocytes. If we accept these limitations then iPSC-based disease models may be useful in some circumstances.

Modeling of Genetic Cardiac Diseases

Diseases Caused by Ion Channel Dysfunction

Genetic mutations that affect the function of ion channels are responsible for a group of diseases in which the APs of individual cardiomyocytes are suboptimal. Patients with such mutations often present with cardiac arrhythmia. These mutations, affecting 13 genes, are present in approximately 1:7,000 individuals and affect the transfer of ions across the plasma membrane of cardiomyocytes [63] with varying degrees of severity so the desirability of in vitro models of these conditions that may be used to test prospective therapeutic agents is considerable particularly since animal models are often poor representations of human cardiac electrophysiology.

Long QT Syndromes Arising from Sodium/Potassium Channel Dysfunctions

These are a group of heritable diseases associated with abnormal ion channel function characterized by prolongation of the QT interval on an electrocardiogram and a high risk of sudden cardiac death due to ventricular tachyarrhythmia. Patients with long QT syndromes (LQTS) can develop life-threatening Torsades de Pointes arrhythmias, which are polymorphic ventricular tachycardia characterized by QRS (which comprises the Q, R and S waves measured on an electrocardiogram) complexes twisting around the baseline in the ECG. Because of the degeneration into ventricular fibrillation and the massive drop in systemic blood pressure, Torsades de Pointes can result in syncope and sudden cardiac death with a mortality of up to 50% [64]. The two most common forms of the disease are LQTS1 and LQTS2 which account for 40%–55% and 30%–35% of all patients [65]. These forms of the disease result from loss of function mutations in the α-subunits of the repolarizing potassium channels. LQTS1 and LQTS2 result, respectively, from mutations in the *KCNQ1* and *KCNH2* genes which encode α-subunits of two distinct potassium currents mediating the delayed rectifier potassium current [66]. iPSC-based models of both these mutations have been developed. Moretti et al. [39] first reported iPSC-cardiac myocyte model for long-QT syndrome type 1 (LQT1) from patient carrying an R190Q mutation in the *KCNQ1* gene. The duration of the AP was markedly prolonged in ventricular- and atrial-like cells derived from patients with LQT1. Immunocytochemical tests in LQT1-iPSC-cardiomyocytes revealed the abnormal ER colocalization of KCNQ1, suggesting trafficking defect of the mutated protein. Compared with those from control subjects, LQT1-iPSC-cardiomyocytes showed substantially reduced I_{Ks} current densities and activation and deactivation properties of the tail I_{Ks} current were significantly altered, with activation being shifted toward more positive voltages and deactivation being decelerated. Due to abnormal I_{Ks} current, six out of nine LQT1-iPSC-cardiomyocytes developed early after depolarisation (EAD) following adrenergic stimulation, which could be attenuated by nonselective β-blockade. The LQT1 patient-derived cardiomyocytes recapitulated the electrophysiological features of the disorder, and appeared consistent response to arrhythmia suppressor of β-blockers. Several LQT2 models have been established from patients with mutations A422T, A614V, G1681A, and R176W in KCNH2 [40, 67, 68]. The LQT2-iPSC-cardiomyocytes had significantly prolonged duration of APs and smaller peak amplitudes of I_{Kr}. The I_{Kr} blocker E4031 provoked prolonged AP duration, resulting in pronounced EAD in LQT2-iPSC-cardiomyocytes. The effects of arrhythmia preventing agents on LQT2-iPSC-cardiomyocytes have been studied, including $I_{Ca,L}$ inhibitor nifedipine, $I_{K,ATP}$ channel opener pinacidil, and nicorandil, I_{Kr} channel enhancer PD-118057, and so forth. Application of these reagents resulted in AP shortening and abolished propensity to EADs, mimicking the clinical reactions of LQT2 to therapeutic medicines.

A third variant of LQTS has been modeled using iPSC. LQT3 (also known as Brugada Syndrome) is related to mutations in *SCN5A*, the gene encoding the α-subunit of the Na^+

channel. The mutations lead to persistent inward Na^+ current (I_{Na}) during the AP plateau phase and prolonged QT interval. From a patient with the *SCN5A*$^{1795insD/+}$ mutation resulting in the insertion of aspartic acid after tyrosine 1795, iPSCs were generated and differentiated into cardiomyocytes [69, 70]. Patch-clamp measurements on the derivative cardiomyocytes revealed significantly decreased I_{Na+} density, with peak I_{Na} density at −30 mV being 46% of that observed in control iPSC-cardiomyocytes. The persistent I_{Na+} was also larger compared with wild-type counterparts. AP measurements showed that LQT3-iPSC-cardioymyocytes had significantly smaller V_{max} and longer APD90 persisting at faster pacing frequencies. This iPSC-cardiomyocytes model emulated both gain-of-function and loss-of-function disorders with Na^+ channel mutations and will benefit the researches of genetic heart diseases with mutations in *SCN5A*, such as Brugada syndrome.

LQTS Arising from Calcium Channel Dysfunction: Timothy Syndrome

This is a rather more complicated illness than the LQTS problems caused by Na+/K + channel dysfunctions. Timothy syndrome (sometimes referred to as LQT8) is a multisystem disorder arising from a gain of function mutation in the *CACNA1C* gene which encodes the L-type calcium channel. This causes QT elongation that may result in lethal arrhythmia but since voltage gated calcium channels such as those encoded by *CACNA1C* are also expressed in the plasma membranes of other excitable cells such as skeletal myocytes and neurons, aberrant calcium transfer generates other dysfunctions in Timothy patients such as autism and intermittent hypoglycaemia [71]. Masayuki Yazawa et al. reported the modeling of Timothy syndrome from two patients [39, 72]. The Timothy syndrome cardiomyocytes contracted slowly (at half beating rate of the control) and irregularly compared with contraction of control cardiomyocytes, consistent with bradycardia in many Timothy syndrome patients. By whole-cell patch clamping, it was found that the L-type channel current in Timothy syndrome cardiomyocytes had significantly reduced voltage-dependent inactivation compared to control cells. Using current-clamp recording, it was revealed that ventricular-like Timothy-iPSC-cardiomyocytes had APs that were three times as long as those of control iPSC-cardiomyocytes. Indicated by Fluo-4, the spontaneously contracting Timothy syndrome cardiomyocytes showed more irregular, significantly larger and more prolonged Ca^{2+} elevations than those of control cardiomyocytes. The application of low doses of Roscovitine, a compound that increases the voltage-dependent inactivation of CaV1.2, significantly reduced the duration of APs, amplitude of Ca^{2+} transients, and decreased the frequency of abnormal depolarizing events in Timothy-iPSC-cardiomyocytes. This model provides a novel platform for the screening of new treatments to Timothy syndrome.

Catecholaminergic Polymorphic Ventricular Tachycardia Type 1

Catecholaminergic polymorphic ventricular tachycardia type 1 (CPVT1) is an inherited cardiac disorder characterized by emotional and physical stress-induced ventricular arrhythmia which can lead to sudden cardiac death in children and young individuals. 30%–40% of CPVT1 cases are linked to mutations in the cardiac ryanodine receptor type 2 gene (*RYR2*) encoding a Ca^{2+} channel on the membrane of sarcoplasmic reticulum, and studies based on RYR2 mutations suggested that arrhythmias in CPVT1 are related to the diastolic Ca^{2+} leakage from the sarcoplasmic reticulum (SR).

IPSCs were generated from a patient of CPVT1 with mutation p.F2483I in the *RYR2* gene and in CPVT-iPSC-derived cardiomyocytes abnormal cross-striation organization of the sarcomeric Z-disc protein, D-actinin was observed which is typical of immature cardiomyocytes [73]. Based on field potential recording of beating clusters (BCs), a higher percentage of CPVT1-iPSC-cardiomyocytes showed tendency of arrhythmia at basal conditions and upon treatment with the β-adrenergic agonist isoproterenol. APs in single cardiomyocytes measured by whole-cell patch clamp showed that isoproterenol caused positive chronotropic response in all the 32 control cardiomyocytes without arrhythmia. Whereas, upon isoproterenol application, 57.9% of CPVT1-iPSC-cardiomyocytes exhibited negative response and 34.2% developed arrhythmia and delayed after depolarizations, the typical CPVT1 phenotype. Confocal fluorescence imaging revealed higher amplitudes and longer durations of spontaneous local Ca^{2+} release events, indicating aberrant SR Ca^{2+} release in CPVT1-iPSC-cardiomyocytes due to mutant RYR2. With sarcolemmal Ca^{2+} currents (I_{Ca}) activated by depolarizing pulses, the Ca^{2+} release from internal Ca^{2+} stores continued long after repolarization of the membrane. When cells were exposed to forskolin or cAMP, a steep and sustained rise in cytosolic Ca^{2+} was observed. These findings from the in vitro CPVT1 model suggested that RYR2 mutation may increase the open probability of RYR2 especially upon adrenergic stimulation, leading to higher susceptibility of patients to ventricular tachycardia.

Diseases Caused by Dysfunctions in Intercellular Communication

Arrhythmogenic Right Ventricular Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) has a prevalence of 2–10/10,000 [74, 75]. The mean age of presentation is approximately 35 ± 15 years, typically with symptomatic ventricular tachyarrhythmias and may progress to heart failure [76, 77]. ARVC is familial in 25%–30% of cases and typically follows an autosomal dominant pattern of inheritance [78]. Pathogenic mutations of one or more genes coding for cardiac desmosomal proteins, namely plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*), and plakoglobin (*JUP*) are associated with ARVC [79]. Mutations in the nondesmosomal genes have been found in a minority of patients fulfilling the diagnostic criteria for ARVC.

Estimates of the prevalence of desmosomal gene mutations in unrelated probands range from 30% to 50% [80, 81]. The penetrance of these mutations is highly variable. Compound heterozygosity, modifier genes, and environmental influences have been suggested as explanations for this variability [82, 83]. Nuclear translocation of plakoglobin is a consistent finding in cardiac biopsy specimens from patients with ARVC and animal models of the disease and has been identified in probands with and without desmosomal gene mutations [84]. Nuclear translocation of plakoglobin suppresses canonical Wnt signaling which is a regulator of adipogenesis and apoptosis [85]. These processed have been identified as important in the pathophysiology of ARVC from histological studies [86]. Wnt signaling also regulates embryological development of the right ventricle. It has been

proposed that the nuclear translocation of plakoglobin leading to changes in Wnt signaling is a common pathway by which a range of processes produce the ARVC phenotype [87].

Generation of in vitro cellular models of ARVC using patient-specific iPSC-derived cardiomyocytes has been achieved [55, 56, 88, 89] in which a heterozygous mutations in the *PKP2* gene (c.1841T.C) were present. The expression of PKP2 and plakoglobin in cardiomyocytes derived from ARVC-iPSCs was significantly reduced compared to control iPSC-cardiomyocytes. Ultrastructural analysis by transmission electron microscopy (TEM) revealed notably greater maximum cell width of ARVC-iPSC-cardiomyocytes. The Z-bands appeared thicker, less organized, and more pleomorphic in ARVC-iPSC-cardiomyocytes. In addition, more lipid droplets were found accumulated in ARVC-iPSC-cardiomyocytes, in close agreement with in vitro cultured cardiomyocytes derived from ARVC patient showing increased potential for adipocytic changes. One further study [90] confirms these findings. The generated ARVC cell models recapitulated key features of the disease phenotype and provided a platform for further understanding of the underlying mechanisms as well as to evaluate novel clinical applications in diagnosis and treatments.

LEOPARD Syndrome

LEOPARD syndrome (LS) is an autosomal dominant developmental disorder belonging to a relatively prevalent class of inherited RAS/MAPK pathway syndromes, involving the skin, skeletal, and cardiovascular systems. Hypertrophic cardiomyopathy is the major phenotype in patients of LS. Approximately 90% of LS cases are caused by missense mutations in the *PTPN11* gene that encodes the protein tyrosine phosphatase SHP2. T468M and Y279C are major mutations in most LS cases. With sample cells from two patients with heterozygous T468M substitution mutation in *PTPN11*, iPSC lines were derived and induced into cardiomyocytes [91]. LS-iPSC-cardiomyocytes showed significantly larger surface area and increased sarcomere assembly compared to wild-type controls, indicating that cardiac hypertrophy in LS patients may occur through a cell autonomous mechanism due to the *PTPN11* mutation.

It has been reported that active calcineurin dephosphorylation promotes the nuclear translocation of transcription factors NFAT [92]. Localization analysis of NFATc4 revealed a significantly higher proportion of LS cardiomyocytes with NFATc4 in the nucleus, suggesting that calcineurin-NFAT pathway plays important roles in the ontology of LS. To shed light on signaling pathways affected in LS, phosphoproteomic microarray chip containing ~600 pan and phospho-specific antibodies was applied to screen targets of *PTPN11* mutation. It was found that the phosphorylation of EGFR and MEK1 proteins was considerably increased in the LS-iPSC samples. However, bFGF stimulation did not elicit further activation of ERK, as observed in HES2 and wild-type iPSC. This LS model for the first time demonstrated that RAS-MAPK signal transduction is perturbed in LS as early as the pluripotent stem cell stage.

Diseases that may be Caused by Intrinsic Cardiomyocyte Dysfunction

Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is the most common form of nonischemic cardiomyopathy characterized by ventricular dilatation, systolic dysfunction, and progressive heart failure, and is the most common diagnosis leading to heart transplantation. Cardiac troponin T is one of the key components regulating sarcomeric thin filament activity and contraction of cardiomyocytes. Mutations in the cardiac troponin T encoding gene *TNNT2* are one of the main causes of DCM [93]. Due to the difficulty to obtain specimens from the hearts of DCM patients and the short surviving period of cultured cardiomyocytes, the study on DCM was challenging. Sun et al. reported the generation of iPSC-derived cardiomyocytes from DCM patients carrying a point mutation (R173W) in exon 12 of the *TNNT2* gene [94]. Compared to control cardiomyocytes, a much higher percentage of DCM-iPSC-cardiomyocytes showed punctate distribution of sarcomeric α-actinin. Highly variable sarcomeric organization, less well aligned Z lines, and scattered patterns of condensed Z bodies were detected by TEM as well, indicating increased heterogeneous sarcomeric pattern of DCM iPSC-derived cardiomyocytes. Treatment with the positive inotropic reagent norepinephrine markedly increased the proportion of DCM-iPSC-cardiomyocytes with disorganized sarcomeric pattern, and more severe scattered distribution of Z bodies. 10 mM norepinephrine treatment induced an initial positive chronotropic effect (increased rate of beating) that later became negative, eventually leading to failure of spontaneous contraction in DCM iPSC-derived beating EBs. When subjected to cycles of prolonged stretching, increased heterogeneity in sarcomeric pattern was observed in DCM-iPSC-cardiomyocytes. Thus, DCM iPSC-derived cardiomyocytes are more susceptible to the stress induced by β-adrenergic stimulation and biomechanical force. With excitation-contraction coupling examination, DCM-iPSC-cardiomyocytes exhibited significantly smaller intracellular calcium concentration ($[Ca^{2+}]i$) transient, indicating greatly reduced $[Ca^{2+}]i$ available for each contraction of DCM-iPSC-cardiomyocytes which is consistent with their weaker contraction force. With treatment of caffeine, DCM-iPSC-cardiomyocytes exhibited smaller amplitudes, prolonged time to peak, and delayed decay time compared to controls, suggesting lower Ca^{2+} storage in their sarcoplasmic reticulum and altered function of Ca^{2+} channels and Ca^{2+} pumps in the plasma and sarcoplasmic reticulum membranes.

Clinical studies have shown that blockade of the β-adrenergic pathway by metoprolol and Serca2a overexpression effectively alleviated the disease phenotype of DCM patients [95, 96]. Consistently, in vitro application of metoprolol helped DCM iPSC-derived cardiomyocytes to resist mechanical deterioration and improved their myofilament organization, overexpression of Serca2a increased the $[Ca^{2+}]i$ transients and contraction force of DCM-iPSC-cardiomyocytes and partially improved their function. Moreover, gene expression profiling further identified several new pathways related to DCM aetiology, which may lead to novel investigations for the specific disease mechanisms and drug screening.

Hypoplastic Left Heart Syndrome

Hypoplastic left heart syndrome (HLHS) is a serious congenital cardiovascular malformation characterized by underdevelopment of the left-sided cardiac structures, variably including hypoplasia or atresia of the left ventricle, ascending aorta, and aortic and mitral valves. HLHS is the commonest cause of heart transplantation in infancy and it is the cardiovascular malformation most frequently resulting in childhood death. The consensus view is that reduced flow through the left heart

during development is a key factor in the development of the condition. Also, aberrant expression or mutations of genes closely related to the development of left ventricular chamber have been reported presented in HLHS patients, such as *TBX5, LRX1*, and *HAND1*, which may contribute to the aetiology of HLHS [97–100]. However, any myocardial susceptibility component is as yet undefined. To study the molecular mechanisms underlying HLHS, iPSC lines were generated in our group from HLHS patients and differentiated into cardiomyocytes [101]. Alteration of gene expression profile was observed in HLHS-iPSC-cardiomyocytes, especially downregulation of key cardiac markers *MESP1* and *cTnT*. In addition, HLHS-iPSC-cardiomyocytes showed lower ability to give rise to BCs compared to control cardiomyocytes. HLHS-iPSC-cardiomyocytes showed more random arrangement of myofibrils and formed BCs with lower beating rate. Compared to control-iPSC-derived cardiomyocytes, HLHS-iPSC-cardiomyocytes showed an accelerated rate of Ca2 + transient decay and generated calcium transients in the presence of caffeine, implying the dysfunction of ryanodine receptor. Of note, *IP3R* expression was upregulated in HLHS derived, indicating that inositol triphosphate system may be an alternative source of calcium. When treated with adrenergic receptor agonist isoproterenol, the beating frequency of HLHS-iPSC-cardiomyocytes increases only by 9% compared to 66% for human ESC-derived cardiomyocytes. Therefore, it is possible that for HLHS, inositol triphosphate plays an important role in generating calcium transients. This is also an indicator of immature cardiac differentiation. For the first time, the HLHS-iPSC-cardiomyocytes model demonstrated that cardiomyocytes derived from HLHS-iPSC lines showed developmental and/or functional defects that could compromise their ability to contribute to cardiogenesis in vivo. Further studies are needed to characterize the molecular mechanisms involved in HLHS aetiology.

Familial Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy is associated with abnormal thickening of the left ventricular myocardium and estimated to be the most common hereditary cardiac disease being present in 0.17% of the population [102]. Mutations in a number of genes encoding proteins that contribute to sarcomere structure (such as the myosin heavy chain) are thought to cause this disease [103, 104] but the mechanisms by which these lead to myocyte hypertrophy and the resulting ventricular arrhythmia are unclear. Cardiomyocytes derived from patient-specific iPSC carrying an Arg663His mutation in the *MYH7* gene recapitulate many of the phenotypic consequences of this disease [105] such as greater cardiomyocyte volume compared to nondiseased controls, higher expression of β-myosin compared to α-myosin, calcineurin activation, and increased expression of several hypertrophy related genes such as *GATA4, TNNT2, MYL2*, and *MYH7*. Importantly, the electrophysiological characteristics of many of the iPSC-derived cardiomyocytes show arrhythmic waveforms and frequently failure of AP triggering. Subsequent analysis of Ca^{2+} handling properties of the iPSC-derived cardiomyocytes revealed Ca^{2+} transient irregularities and significantly reduced ability of the sarcoplasmic reticulum to release Ca^{2+}.

Summary

In conclusion, we have attempted to present the current state of the art regarding the use of iPSC to create in vitro models of cardiac diseases. To date, such investigations have been restricted to conditions arising from gene mutations that compromise the function of ion channels or signal transduction mechanisms for which a wealth of functional data are available. This simplifies the analysis of similar function in cardiomyocytes generated from patient-specific iPSC for although such cardiomyocytes are immature in comparison with adult cells obtained ex vivo, it is easy to show if they recapitulate the functional defects observed in the patient.

The utility of iPSC is less apparent for diseases in which the contribution of gene mutation is unknown. If there are discernible functional defects in the patients cardiomyocytes then it is possible that these will be present in cardiomyocytes derived from patient-specific iPSC and in this respect it is interesting to note the publication of studies such as those of HLHS where disease mechanisms are largely unknown but without a clear functional readout in a specific cell type, creation of iPSC-based in vitro disease models will be complicated. Moreover, the contribution made by the apparent immaturity of iPSC-derived cardiomyocytes is unclear and thus the development of robust protocols capable of producing cells with greater similarity to adult cardiomyocytes is a key challenge for iPSC models of cardiac disease.

Acknowledgments

We are grateful for financial support from BHF UK (PG/12/60/29799) and King Abdulaziz University (Deanship Scientific research Grant Number 3-287-1434-HiCi).

Author Contributions

C.Y.: writing of manuscript and final approval; J.A.-A., B.K., A.T., and M.L.: preparation and critical reading of manuscript and final approval and fund raising; M.S.: preparation and critical reading of manuscript and final approval; L.A.: manuscript writing, critical reading, final approval, and fund raising.

Disclosure of Potential Conflicts of Interest

There are no conflicts of interest.

References

1 van der Linde D, Konings EE, Slager MA et al. Birth prevalence of congenital heart disease worldwide: A systematic review and meta-analysis. J Am Coll Cardiol 2011;58:2241–2247.

2 Badorff C, Brandes RP, Popp R et al. Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. Circulation 2003;107:1024–1032.

3 Gonzales C, Pedrazzini T. Progenitor cell therapy for heart disease. Exp Cell Res 2009; 315:3077–3085.

4 Beltrami AP, Barlucchi L, Torella D et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114:763–776.

5 Perino MG, Yamanaka S, Li J et al. Cardiomyogenic stem and progenitor cell plasticity and the dissection of cardiopoiesis. J Mol Cell Cardiol 2008;45:475–494.

6 Kehat I, Kenyagin-Karsenti D, Snir M et al. Human embryonic stem cells can differ-

entiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 2001;108:407–414.
7 Mallon BS, Chenoweth JG, Johnson KR et al. StemCellDB: The human pluripotent stem cell database at the National Institutes of Health. Stem Cell Res 2013;10:57–66.
8 Guenther MG, Frampton GM, Soldner F et al. Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. Cell Stem Cell 2010;7:249–257.
9 Mallon BS, Hamilton RS, Kozhich OA et al. Comparison of the molecular profiles of human embryonic and induced pluripotent stem cells of isogenic origin. Stem Cell Res 2014;12:376–386.
10 Narazaki G, Uosaki H, Teranishi M et al. Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. Circulation 2008;118: 498–506.
11 Zhang J, Wilson GF, Soerens AG et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 2009;104:e30–41.
12 Stadtfeld M, Nagaya M, Utikal J et al. Induced pluripotent stem cells generated without viral integration. Science 2008;322: 945–949.
13 Okita K, Nakagawa M, Hyenjong H et al. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322:949–953.
14 Kaji K, Norrby K, Paca A et al. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 2009;458:771–775.
15 Fusaki N, Ban H, Nishiyama A et al. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 2009;85:348–362.
16 Jia F, Wilson KD, Sun N et al. A nonviral minicircle vector for deriving human iPSC cells. Nat Methods 2010;7:197–199.
17 Yu J, Hu K, Smuga-Otto K et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324: 797–801.
18 Zhou H, Wu S, Joo JY et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4: 381–384.
19 Kim D, Kim CH, Moon JI et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 2009;4:472–476.
20 Warren L, Manos PD, Ahfeldt T et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 2010;7:618–630.
21 Laflamme MA, Gold J, Xu C et al. Formation of human myocardium in the rat heart from human embryonic stem cells. Am J Pathol 2005;167:663–671.
22 Kehat I, Gepstein A, Spira A et al. High-resolution electrophysiological assessment of human embryonic stem cell-derived cardiomyocytes: A novel in vitro model for the study of conduction. Circ Res 2002;91:659–661.
23 Xue T, Cho HC, Akar FG et al. Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: Insights into the development of cell-based pacemakers. Circulation 2005;111:11–20.
24 Passier R, Oostwaard DW, Snapper J et al. Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures. Stem Cells 2005;23:772–780.
25 Xu XQ, Graichen R, Soo SY et al. Chemically defined medium supporting cardiomyocyte differentiation of human embryonic stem cells. Differentiation 2008;76:958–970.
26 Freund C, Ward-van Oostwaard D, Monshouwer-Kloots J et al. Insulin redirects differentiation from cardiogenic mesoderm and endoderm to neuroectoderm in differentiating human embryonic stem cells. Stem Cells 2008;26:724–733.
27 Graichen R, Xu X, Braam SR et al. Enhanced cardiomyogenesis of human embryonic stem cells by a small molecular inhibitor of p38 MAPK. Differentiation 2008; 76:357–370.
28 Mummery C, Ward-van Oostwaard D, Doevendans P et al. Differentiation of human embryonic stem cells to cardiomyocytes: Role of coculture with visceral endoderm-like cells. Circulation 2003;107:2733–2740.
29 Kattman SJ, Witty AD, Gagliardi M et al. Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. Cell Stem Cell 2011;8:228–240.
30 Laflamme MA, Chen KY, Naumova AV et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol 2007;25:1015–1024.
31 Yang L, Soonpaa MH, Adler ED et al. Human cardiovascular progenitor cells develop from a KDR + embryonic-stem-cell-derived population. Nature 2008;453:524–528.
32 Pesl M, Acimovic I, Pribyl J et al. Forced aggregation and defined factors allow highly uniform-sized embryoid bodies and functional cardiomyocytes from human embryonic and induced pluripotent stem cells. Heart Vessels 2014;29:834–846.
33 Olson EN. Development. The path to the heart and the road not taken. Science 2001; 291:2327–2328.
34 Mummery CL, Zhang J, Ng ES et al. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: A methods overview. Circ Res 2012;111:344–358.
35 David R, Stieber J, Fischer E et al. Forward programming of pluripotent stem cells towards distinct cardiovascular cell types. Cardiovasc Res 2009;84:263–272.
36 Ma J, Guo L, Fiene SJ et al. High purity human-induced pluripotent stem cell-derived cardiomyocytes: Electrophysiological properties of action potentials and ionic currents. Am J Physiol Heart Circ Physiol 2011;301: H2006–2017.
37 Davis RP, Casini S, van den Berg CW et al. Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. Circulation 2012;125:3079–3091.
38 Zhang J, Wilson GF, Soerens AG et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 2009;27:e30–41.
39 Moretti A, Bellin M, Welling A et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med 2010;363:1397–1409.
40 Lahti AL, Kujala VJ, Chapman H et al. Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. Dis Model Mech 2012;5:220–230.
41 Itzhaki I, Maizels L, Huber I et al. Modelling the long QT syndrome with induced pluripotent stem cells. Nature 2011;471:225–229.
42 Meijer van Putten RM, Mengarelli I, Guan K et al. Ion channelopathies in human induced pluripotent stem cell derived cardiomyocytes: A dynamic clamp study with virtual IK1. Front Physiol 2015;6:7.
43 Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. Physiol Rev 2005;85:1205–1253.
44 Hoekstra M, Mummery CL, Wilde AA et al. Induced pluripotent stem cell derived cardiomyocytes as models for cardiac arrhythmias. Front Physiol 2012;3:346.
45 Cordeiro JM, Nesterenko VV, Sicouri S et al. Identification and characterization of a transient outward K + current in human induced pluripotent stem cell-derived cardiomyocytes. J Mol Cell Cardiol 2013;60:36–46.
46 Lieu DK, Fu JD, Chiamvimonvat N et al. Mechanism-based facilitated maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Arrhythm Electrophysiol 2013; 6:191–201.
47 Bett GC, Kaplan AD, Lis A et al. Electronic "expression" of the inward rectifier in cardiocytes derived from human-induced pluripotent stem cells. Heart Rhythm 2013;10: 1903–1910.
48 Trafford AW, Diaz ME, Negretti N et al. Enhanced Ca2 + current and decreased Ca2 + efflux restore sarcoplasmic reticulum Ca2 + content after depletion. Circ Res 1997; 81:477–484.
49 Walden AP, Dibb KM, Trafford AW. Differences in intracellular calcium homeostasis between atrial and ventricular myocytes. J Mol Cell Cardiol 2009;46:463–473.
50 Xi J, Khalil M, Shishechian N et al. Comparison of contractile behavior of native murine ventricular tissue and cardiomyocytes derived from embryonic or induced pluripotent stem cells. FASEB J 2010;24:2739–2751.
51 Pesl M, Acimovic I, Pribyl J et al. Forced aggregation and defined factors allow highly uniform-sized embryoid bodies and functional cardiomyocytes from human embryonic and induced pluripotent stem cells. Heart Vessels 2014;29:834–846.
52 Weng Z, Kong CW, Ren L et al. A simple, cost-effective but highly efficient system for deriving ventricular cardiomyocytes from human pluripotent stem cells. Stem Cells Dev 2014;23:1704–1716.
53 Zhang GQ, Wei H, Lu J et al. Identification and characterization of calcium sparks in

cardiomyocytes derived from human induced pluripotent stem cells. PLoS One 2013;8: e55266.
54 Rao C, Prodromakis T, Kolker L, Chaudhry UA, Trantidou T, Sridhar A, Weekes C, Camelliti P, Harding SE, Darzi A, Yacoub MH, Athanasiou T, Terracciano CM. The effect of microgrooved culture substrates on calcium cycling of cardiac myocytes derived from human induced pluripotent stem cells. Biomaterials 2013;34:2399–2411.
55 Rao C, Prodromakis T, Kolker L et al. The effect of microgrooved culture substrates on calcium cycling of cardiac myocytes derived from human induced pluripotent stem cells. Biomaterials 2013;34:2399–2411.
56 Yu T, Miyagawa S, Miki K et al. In vivo differentiation of induced pluripotent stem cell-derived cardiomyocytes. Circ J 2013;77: 1297–1306.
57 Lundy SD, Zhu WZ, Regnier M et al. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cells Dev 2013;22:1991–2002.
58 Yang X, Rodriguez M, Pabon L et al. Triiodo-l-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells. J Mol Cell Cardiol 2014;72:296–304.
59 Arshi A, Nakashima Y, Nakano H et al. Rigid microenvironments promote cardiac differentiation of mouse and human embryonic stem cells. Sci Technol Adv Mater 2013;14: 025003.
60 Bedada FB, Chan SS, Metzger SK et al. Acquisition of a quantitative, stoichiometrically conserved ratiometric marker of maturation status in stem cell-derived cardiac myocytes. Stem Cell Reports 2014;3:594–605.
61 Ye L, Chang YH, Xiong Q, Zhang P, Zhang L, Somasundaram P, Lepley M, Swingen C, Su L, Wendel JS, Guo J, Jang A, Rosenbush D, Greder L, Dutton JR, Zhang J, Kamp TJ, Kaufman DS, Ge Y, Zhang J. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. Cell Stem Cell 2014;15:750–761.
62 Ye L, Chang YH, Xiong Q et al. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. Cell Stem Cell 2014;15:750–761.
63 Müller M, Seufferlein T, Illing A et al. Modelling human channelopathies using induced pluripotent stem cells: A comprehensive review. Stem Cells Int 2013;2013: 496501.
64 Moss AJ, Schwartz PJ, Crampton RS et al. The long QT syndrome. Prospective longitudinal study of 328 families. Circulation 1991;84:1136–1144.
65 Hedley PL, Jorgensen P, Schlamowitz S et al. The genetic basis of long QT and short QT syndromes: A mutation update. Hum Mutat 2009;30:1486–1511.
66 Wilde AA, Bezzina CR. Genetics of cardiac arrhythmias. Heart 2005;91:1352–1358.
67 Matsa E, Rajamohan D, Dick E et al. Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. Eur Heart J 2011;32:952–962.
68 Spencer CI, Baba S, Nakamura K et al. Calcium transients closely reflect prolonged action potentials in iPSC models of inherited cardiac arrhythmia. Stem Cell Reports 2014; 3:269–281.
69 Davis RP, Casini S, van den Berg CW et al. Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. Circulation 2012;125:3079–3091.
70 Fatima A, Kaifeng S, Dittmann S et al. The disease-specific phenotype in cardiomyocytes derived from induced pluripotent stem cells of two long QT syndrome type 3 patients. PLoS One 2013;8:e83005.
71 Splawski I, Timothy KW, Sharpe LM et al. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell 2004;119:19–31.
72 Yazawa M, Hsueh B, Jia X et al. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature 2011;471:230–234.
73 Fatima A, Xu G, Shao K et al. In vitro modeling of ryanodine receptor 2 dysfunction using human induced pluripotent stem cells. Cell Physiol Biochem 2011;28:579–592.
74 Peters S, Trummel M, Meyners W. Prevalence of right ventricular dysplasia-cardiomyopathy in a non-referral hospital. Int J Cardiol 2004;97:499–501.
75 Corrado D, Thiene G. Arrhythmogenic right ventricular cardiomyopathy/dysplasia: Clinical impact of molecular genetic studies. Circulation 2006;113:1634–1637.
76 Marcus FI, Zareba W, Calkins H et al. Arrhythmogenic right ventricular cardiomyopathy/dysplasia clinical presentation and diagnostic evaluation: Results from the North American Multidisciplinary Study. Heart Rhythm 2009;6:984–992.
77 Hulot JS, Jouven X, Empana JP et al. Natural history and risk stratification of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circulation 2004;110:1879–1884.
78 Dalal D, Nasir K, Bomma C et al. Arrhythmogenic right ventricular dysplasia: A United States experience. Circulation 2005; 112:3823–3832.
79 Ackerman MJ, Priori SG, Willems S et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: This document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Europace 2011;13:1077–1109.
80 Sen-Chowdhry S, Syrris P, Ward D et al. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. Circulation 2007;115:1710–1720.
81 den Haan AD, Tan BY, Zikusoka MN et al. Comprehensive desmosome mutation analysis in north americans with arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circ Cardiovasc Genet 2009;2: 428–435.
82 Sen-Chowdhry S, Syrris P, Pantazis A et al. Mutational heterogeneity, modifier genes, and environmental influences contribute to phenotypic diversity of arrhythmogenic cardiomyopathy. Circ Cardiovasc Genet 2010;3:323–330.
83 Xu T, Yang Z, Vatta M et al. Compound and digenic heterozygosity contributes to arrhythmogenic right ventricular cardiomyopathy. J Am Coll Cardiol 2010;55:587–597.
84 Asimaki A, Tandri H, Huang H et al. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. N Engl J Med 2009;360:1075–1084.
85 Garcia-Gras E, Lombardi R, Giocondo MJ et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J Clin Invest 2006;116:2012–2021.
86 Basso C, Ronco F, Marcus F et al. Quantitative assessment of endomyocardial biopsy in arrhythmogenic right ventricular cardiomyopathy/dysplasia: An in vitro validation of diagnostic criteria. Eur Heart J 2008;29:2760–2771.
87 Ai D, Fu X, Wang J et al. Canonical Wnt signaling functions in second heart field to promote right ventricular growth. Proc Natl Acad Sci USA 2007;104:9319–9324.
88 Ma D, Wei H, Lu J et al. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. Eur Heart J 2013;34:1122–1133.
89 Caspi O, Huber I, Gepstein A et al. Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. Circ Cardiovasc Genet 2013;6:557–568.
90 Kim C, Wong J, Wen J et al. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. Nature 2013;494: 105–110.
91 Carvajal-Vergara X, Sevilla A, D'Souza SL et al. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. Nature 2010;465:808–812.
92 Molkentin JD. Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. Cardiovasc Res 2004;63:467–475.
93 Willott RH, Gomes AV, Chang AN et al. Mutations in troponin that cause HCARDIOYMYOCYTE, DCARDIOYMYOCYTE AND RCARDIOYMYOCYTE: What can we learn about thin filament function? J Mol Cell Cardiol 2010;48:882–892.
94 Sun N, Yazawa M, Liu J et al. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. Sci Transl Med 2012;4:130ra147.
95 Hajjar RJ, Zsebo K, Deckelbaum L et al. Design of a phase 1/2 trial of intracoronary administration of AAV1/SERCA2a in patients with heart failure. J Card Fail 2008;14:355–367.
96 Engelmeier RS, O'Connell JB, Walsh R et al. Improvement in symptoms and exercise tolerance by metoprolol in patients with dilated cardiomyopathy: A double-blind, randomized, placebo-controlled trial. Circulation 1985;72:536–546.

97 Pellikka PA, McCully RB. Stress echocardiography: So much to do, so little time. J Am Coll Cardiol 2007;50:1990–1991.

98 Reamon-Buettner SM, Ciribilli Y, Inga A et al. A loss-of-function mutation in the binding domain of HAND1 predicts hypoplasia of the human hearts. Hum Mol Genet 2008;17: 1397–1405.

99 Hinton RB, Martin LJ, Rame-Gowda S et al. Hypoplastic left heart syndrome links to chromosomes 10q and 6q and is genetically related to bicuspid aortic valve. J Am Coll Cardiol 2009;53:1065–1071.

100 Iascone M, Ciccone R, Galletti L et al. Identification of de novo mutations and rare variants in hypoplastic left heart syndrome. Clin Genet 2012;81:542–554.

101 Jiang Y, Habibollah S, Tilgner K et al. An induced pluripotent stem cell model of hypoplastic left heart syndrome (HLHS) reveals multiple expression and functional differences in HLHS-derived cardiomyocytes. Stem Cells Transl Med 2014;3:416–423.

102 Maron BJ, Gardin JM, Flack JM et al. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. Circulation 1995;92:785–789.

103 Geisterfer-Lowrance AA, Kass S, Tanigawa G et al. A molecular basis for familial hypertrophic cardiomyopathy: A beta cardiac myosin heavy chain gene missense mutation. Cell 1990;62:999–1006.

104 Seidman JG, Seidman C. The genetic basis for cardiomyopathy: From mutation identification to mechanistic paradigms. Cell 2001;104:557–567.

105 Lan F, Lee AS, Liang P et al. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. Cell Stem Cell 2013;12: 101–113.

