

Elena Matsa, Karim Sallam, and Joseph C. Wu

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### Abstract

Cardiomyopathies are mechanical disorders of the heart muscle that can lead to heart failure and lethal arrhythmias. Still accounting for over 20,000 deaths annually in the United States alone, these disorders are often misdiagnosed and mistreated. The ability to generate human heart cells in vitro via the use of induced pluripotency technologies is now allowing researchers to study cardiomyopathies caused by known genetic mutations. This chapter describes the clinical features, classifications and current treatments for cardiomyopathy and provides a guide of how cardiac cells generated from patient-specific human induced pluripotent stem cells (hiPSCs) have been used to elucidate important pathological mechanisms and propose novel treatments for this class of lethal cardiac conditions.

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### Keywords

Cardiomyopathy • Cardiomyocyte • Induced pluripotency • Stem cells • Disease modelling

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E. Matsa, Ph.D. • J.C. Wu, M.D., Ph.D. (✉)

Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, USA  
e-mail: [joewu@Stanford.edu](mailto:joewu@Stanford.edu)

K. Sallam, M.D.

Department of Medicine, Division of Cardiology, Stanford University School of Medicine, Lorry I. Lokey Stem Cell Research Building, 265 Campus Drive, Rm G1120, Stanford, CA 94305-5111, USA

## 2.1 Introduction to Clinical Features of Cardiomyopathy

Cardiomyopathy is a pathologic disorder of the heart muscle that affects millions of individuals and accounts for approximately 23,000 deaths annually in the United States (NHLBI 2012). It is characterised by defects in the mechanical function of the heart, which often lead to heart failure syndrome or sudden cardiac death due to increased predisposition to lethal arrhythmias. Several classes of cardiomyopathy have been defined, mainly distinguished by their pathologic findings and clinical course, which can be attributed to inherited or acquired causes. It is well documented that acquired cardiomyopathy leads to myocyte injury via ischemia, increased loading conditions or systemic disorders affecting the heart, but the pathophysiology of inherited cardiomyopathy remains poorly understood. Current knowledge of disease classifications, etiologies, diagnosis, risk stratification and treatment of cardiomyopathy are further detailed in this introductory section (Table 2.1).

**Table 2.1** List of abbreviations

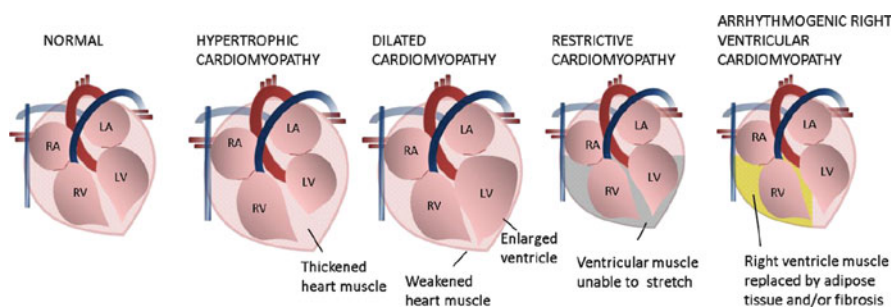
Abbreviation	Explanation
ACTN2	Alpha 2-actinin
ANF	Atrial natriuretic factor
ARVC	Arrhythmogenic right ventricular cardiomyopathy
CALN	Calcineurin
CASP3	Caspase 3
CM	Cardiomyocyte
DAD	Delayed afterdepolarisation
DCM	Dilated cardiomyopathy
EB	Embryoid body
GWAS	Genome-wide association studies
HCM	Hypertrophic cardiomyopathy
hESC	Human embryonic stem cell
hiPSC	Human induced pluripotent stem cell
hPSC	Human pluripotent stem cell
ICD	Implantable cardioverter defibrillators
MEA	Multielectrode array
MYH	Myosin heavy chain
NFAT	Nuclear factor of activated T cells
PKP2	Plakophilin-2
PPAR	Peroxisome proliferator-activated receptor
RCM	Restrictive cardiomyopathy
SERCA2a	Sarcoplasmic reticulum Ca <sup>2+</sup> adenosine triphosphatase
TNNC	Troponin C
TNNT2	Troponin T type 2

### 2.1.1 Disease Classification

Multiple classification systems have been proposed for cardiomyopathy, but the most widely adopted system is based on structural and pathologic features of the disorder that tend to also dictate much of the therapeutic approach for each class (Richardson et al. 1996). As illustrated in Fig. 2.1, *hypertrophic cardiomyopathy* (HCM) is notable for increased relative thickness of (mostly) the left ventricle, which is often asymmetric and associated with hemodynamic significance. *Dilated cardiomyopathy* (DCM) is characterised by progressive dilation of one or both ventricular cavities, whereas *restrictive cardiomyopathy* (RCM) is characterised by impaired diastolic filling of one or both ventricles. *Arrhythmogenic right ventricular cardiomyopathy* (ARVC) is recognised most commonly by fibrofatty infiltration of the right ventricle leading to arrhythmic disorders and sudden death.

The same classification system also identifies certain subgroups as **specific cardiomyopathies**, i.e. cardiomyopathies in which a clear underlying cause is defined but is often unrelated to cardiac muscle. Some of these disorders include ischemic, hypertensive and toxic cardiomyopathy, whose pathologies and treatments may overlap with DCM, HCM or RCM while still maintaining features specific to the disorder.

Some cardiomyopathies such as *noncompaction cardiomyopathy* are *unclassified* under the various categories described above. To fill this gap, some researchers have proposed classifying cardiomyopathies as being *primary*, *secondary* or *mixed*, based on underlying molecular and cellular pathophysiology causing the disorder rather than on end-stage features. Under this system, primary cardiomyopathies are those involving the heart and can be genetic, acquired or both, whereas secondary cardiomyopathies involve the heart as part of a broader systemic disorder. This new classification system may enable identification of common pathways involved in various cardiomyopathies, potentially improving our understanding of the disorders and leading to better therapies (Maron et al. 2006).



**Fig. 2.1** Classification of cardiomyopathies. Schematic diagram depicting structural changes that occur in cardiac muscle during cardiomyopathy. In *dilated cardiomyopathy*, progressive weakness in ventricular muscle is observed, accompanied by enlargement of one or both ventricular cavities, whereas in *hypertrophic cardiomyopathy*, enlargement is mostly in the left ventricular muscle, constricting the ventricle(s). *Restrictive cardiomyopathy* is characterised by impaired diastolic filling of one or both ventricles. Finally, *arrhythmogenic right ventricular cardiomyopathy* is typically characterised by fibrofatty deposition in the right ventricle

### 2.1.2 Familial Cardiomyopathy

Familial cardiomyopathies are those of genetic origin, frequently identified as “primary” in the most recent classification system (Sect. 2.1.1). Over 2,000 mutations in 30 genes have thus far been implicated in cardiomyopathy, including the genes troponin C, T and I (*TNNC1*, *TNNT2*, *TNNI3*), myosin-6 and myosin-7 (*MYH6*, *MYH7*),  $\alpha$ -actinin (*ACTN2*), lamin (*LMNA/C*) and dystrophin (*DMD*) (Maron and Maron 2013). Although inheritance is typically autosomal dominant, X-linked and autosomal recessive patterns have also been observed (Hershberger et al. 2010; Maron et al. 2012). HCM is most commonly linked to sarcomeric gene mutations which together account for 75 % of identifiable inherited HCMs. Familial cases of RCM are mainly attributed to *cTNNI3* gene mutations (Sen-Chowdhry et al. 2010), whereas DCM is far more genetically heterogeneous, with rarer mutations also found in cytoskeletal, nucleoskeletal, mitochondrial and calcium-handling protein-encoding genes (McNally et al. 2013). Over 50 % of ARVC cases are linked to mutations in desmosome protein-encoding genes (e.g. plakophilin-2, *PKP2*; plakoglobin, *PKG*; desmoplakin; *DSP*) (Moric-Janiszewska and Markiewicz-Loskot 2007).

Because relatively few clinical clues guide the diagnosis of inherited cardiomyopathies, genetic testing can help identify patients at risk of accelerated disease progression, congestive heart failure and arrhythmia (Ackerman et al. 2011). However, over 70 % of patients with familial DCM and 40 % of patients with HCM have no identifiable genetic testing variants (Hershberger et al. 2010; Lakdawala et al. 2012; Van Driest et al. 2005). Such cardiomyopathy cases are considered *idiopathic*, a decision that potentially bears huge prognostic and diagnostic implications for affected individuals and family members. With recent arrival of next-generation sequencing methods and completion of the Human Genome Project, genome-wide association studies (GWAS) have enabled the identification of a growing number of genetic variants as myopathy-causing. This has changed the cases of DCM thought to be familial from 2 % in 1982 to 25–30 % in 2012 (Lenfant 2013), allowing timely screening of family members and early initiation of therapy (Hershberger and Siegfried 2011).

### 2.1.3 Clinical Diagnosis and Risk Stratification

The diagnosis of cardiomyopathy is based on a constellation of clinical findings and tests, including pathological examinations, electrocardiography and echocardiography. Advanced imaging modalities such as magnetic resonance imaging (MRI) have enhanced our ability to identify pathological changes in the absence of symptoms or when the clinical presentation is unclear (Steeds 2013). Once diagnosis is made, patients can have variable clinical courses in terms of response to therapy and risk for arrhythmic complications. A major challenge in treating all classes of cardiomyopathy is the ability to stratify the arrhythmic risk associated with each given case. General clinical and imaging parameters guide the use of implantable cardioverter defibrillators (ICDs) in DCM and HCM (Goldberger

et al. 2011; Epstein et al. 2013), but while some of these guidelines work well on a population level, data show that only 17–64 % of patients receive appropriate shocks (Germano et al. 2006). Notably, at least 30 % of patients suffer risks associated with ICD implantation without benefit. This creates a significant cost burden for the health care system, and highlights the clear need for improved risk stratification tools to better identify arrhythmic risks in cardiomyopathy patients.

### 2.1.4 Currently Available Therapies

The mainstay of medical therapy for DCM includes  $\beta$ -blockade, angiotensin-converting enzyme inhibition or angiotensin receptor blockade and aldosterone antagonists. Inhibition of catecholamine and renin-angiotensin-aldosterone axis effects on the heart, combined with mechanical unloading, has also provided substantial improvement in patient survival on a population level (Jessup et al. 2009). Nevertheless, current treatment mainly relies on retarding deleterious cardiac remodelling and providing symptomatic relief through modulating the hemodynamic consequences of myopathy such as reducing preload or reducing intracardiac obstruction, rather than combating disease causation (Gersh et al. 2011; Kushwaha et al. 1997). Moreover, there is a vast range of responsiveness to pharmacotherapy, with some patients experiencing near complete recovery and others deriving little or no benefit.

For other classes of cardiomyopathy such as HCM and RCM, there has been little progress in pharmacotherapy that offers significant mortality improvement. Therefore, substantial efforts are needed to identify more effective drug treatments for these types of cardiomyopathy. For example, it would be highly beneficial to rapidly identify patients who might not respond to traditional therapy, so they could be timely referred to cardiac transplant or ICD therapy (Parry et al. 2013). This would also reduce the \$40 billion socioeconomic burden currently spent in management of cardiomyopathies (Heidenreich et al. 2011).

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## 2.2 Animal Models of Cardiomyopathy

As mentioned earlier, the precise disease mechanisms of familial cardiomyopathy are not well comprehended. This poses major challenges in understanding cellular and systemic pathophysiologies involved in cardiomyopathy and identifying potential targets for treatment. Laboratory investigations of cardiomyopathy could shed light into these mechanisms, but have been limited by the inability to obtain and sustainably culture adult human heart cells. Genetically modified mice are the most widely utilised research animals for studying cardiomyopathy, and have thus far allowed mechanistic studies on DCM, HCM and ARVC (Recchia and Lionetti 2007; Maass and Leinwand 2000; Pilichou et al. 2011). These models have proven to be critical in the discovery of novel pathophysiological mechanisms, but have offered little advancement in diagnosis and therapy. This is attributed to differences

in gene expression and physiology between humans and animal species. For example, the murine heart beats nearly eight times faster than the human (500 bpm vs. 60 bpm) (Doevendans et al. 1998). Furthermore, mouse and human hearts rely on separate sets of ion channels for their contraction and relaxation and exhibit differences in use of regulatory proteins (e.g. phospholamban) (Davis et al. 2011), distribution of structural genes (e.g. alpha- and beta-myosin heavy chains,  $\alpha$ - $\beta$ -MHC) (Morano 1999) and expression of surface markers (e.g. SIRPA) (Dubois et al. 2011). Such differences mean that extrapolation of research findings from mouse to human can be tenuous, something that has long posed need for development of novel in vitro humanised systems for studying cardiomyopathies.

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## **2.3 De Novo Generation of Human Cardiomyocytes Using Pluripotent Stem Cells**

Recent discovery of hiPSC technologies has now brought us closer to human laboratory models of cardiomyopathy. Tissue samples (e.g. skin, blood) can be collected from cardiomyopathy patients with relative ease and subsequently converted to human cardiomyocytes retaining appropriate genetic variant(s) to closely mimic disease pathology. This ability may offer insights into cardiomyopathy that could identify novel diagnostic, pharmacogenomic and therapeutic targets. Methods for generating and studying hiPSC-derived cardiomyocytes (hiPSC-CMs) in the laboratory are further discussed in this section.

### **2.3.1 Methods for In Vitro Cardiac Differentiation**

Similar to pluripotent human embryonic stem cells (hESCs), hiPSCs have the remarkable potential of differentiation into many cell types of the adult body (Reubinoff et al. 2000; Takahashi et al. 2007), including spontaneously contracting cardiomyocytes (Burridge et al. 2007). During in vitro differentiation, the first crucial step towards cardiogenesis is mesoderm formation, a step heavily dependent on NODAL and BMP4 signalling pathways (Mordwinkin et al. 2013). Cardiac specification then proceeds with formation of committed cardiac mesoderm and subsequently cardiac progenitors of the first and second heart fields (giving rise to endothelium and myocardium, and cardiac chambers, respectively) (Buckingham et al. 2005). Finally, progenitors mature into cardiomyocytes that can be identified by spontaneous contraction and expression of sarcomeric proteins, such as TNNT2 and ACTN2 (Rajala et al. 2011). Three key methods are available for mesoderm induction and cardiac specification of pluripotent stem cells to cardiomyocytes (Burridge et al. 2012). These include (1) coculture with END2 mouse visceral endoderm-like cells, a relatively inefficient method that nevertheless provides ~85 % ventricular cardiomyocytes (Mummery et al. 2003); (2) embryoid body (EB) formation via forced aggregation in 96-well plates, an efficient but technically demanding method; and (3) monolayer differentiation of pluripotent cultures,

which is to date the most efficient, reproducible and scalable method available (BurrIDGE et al. 2012). Growth factors Activin-A, BMP4 and FGF2 have been shown to enhance cardiac specification of hiPSCs, in a time and dose-dependent manner (Kattman et al. 2011; Xu et al. 2011). It has also been shown that growth factors can be replaced by small molecules such as CHIR99021, a selective inhibitor of glycogen synthase kinase 3 beta (GSK3B) that activates the canonical WNT signalling pathway, thus inducing mesoderm formation (Hu et al. 2013).

Cardiac specification of pluripotent stem cells was until recently a largely inefficient process, generating low purity populations of cardiomyocytes that need to be further refined by either labour-intensive genetic selection processes or use of surface markers (e.g. SIRPA) and mitochondrial dyes (e.g. MitoTracker) (Matsa and Denning 2012). More recent protocols relying on monolayer differentiation into cardiomyocytes have alleviated the need for purification as they typically yield >90 % cardiomyocytes. This has been a significant step towards generating high quality cardiomyocyte populations that can be used in disease modelling and drug screening. Generating purified populations of each cardiomyocyte subtype, however, presents a major remaining hurdle as in vitro preparations of cardiac myocytes typically contain proportions of pacemaker and working chamber (atrial and ventricular) cells.

### 2.3.2 In Vitro Characterisation of Cardiomyocytes

The use of in vitro-derived hiPSC-CMs for disease modelling requires their characteristics to be physiologically analogous to human cardiomyocytes in vivo. Gene and protein expression assays are typically performed to assure contracting cells form striated sarcomeres and express terminal cardiac markers and structural proteins such as ACTN2, TNNT2, MYH6 and MYH7. In vitro characterisation also relies on detailed electrophysiological analysis, mainly comprising patch-clamp and multielectrode arrays (MEAs; see Box 2.1) (Navarrete et al. 2013; Matsa et al. 2011). These methods can be exploited to determine the percentage of pacemaker-, atrial- and ventricular-like cells that are formed, as well as to measure drug effects on contractility and ion channel currents. Calcium transients (see Box 2.1), an important property for contraction of cardiomyocytes, can also be visualised in the laboratory using real-time microscopy in the presence of calcium-sensitive dyes (Jung et al. 2012).

#### Box 2.1: Electrophysiology Terms

**Multielectrode arrays (MEAs)** are laboratory electronic devices containing a set of microelectrode wires in a fixed spatial arrangement, that are capable of detecting voltage changes in extracellular environment/culture medium caused by excitation of cells adhering to the surface of the devices.

(continued)

**Box 2.1** (continued)

**Patch-clamp** is a technique used in laboratory electrophysiology in which a single excitable cell is perforated with a glass needle to measure changes in membrane voltage, in order to study the electrical behaviour of single ion channels found across cell membranes.

**Excitation-contraction coupling** is the relationship between the electrical depolarisation of the cardiac cell membrane and the activation of the contractile myofilaments.

**Calcium transients** refer to the amount of calcium ions ( $\text{Ca}^{2+}$ ) present for a short time period in the sarcoplasm of an excitable cell, while the cell undergoes cycles of excitation and relaxation.

**Chronotropic effect (Greek chronos = time)** is one that changes the rate of muscle contraction.

**Inotropic effect (Greek ina = fibre)** is one that changes the force of muscle contraction.

Previous molecular and electrophysiological studies have revealed that hiPSC-CMs have normal cardiomyocyte functional properties, including contraction regulated by physiologic intracellular signalling such as excitation-contraction coupling (Zhang et al. 2009; Anderson et al. 2007). However, cells are also reported to have some immature developmental properties; for instance, their spontaneous contraction in culture is an indication of immature automaticity typically observed in fetal hearts. They also exhibit fetal-type ion channel expression, electrophysiological signals, gene expression patterns and physical phenotypes (Davis et al. 2011; Cao et al. 2008). Prolonged passaging of 3 months up to 1 year and temporal 3D culture have been shown to moderately increase maturation (Fu et al. 2008; Otsuji et al. 2010) by improving myofibril density and alignment and increasing the fraction of multinucleated cardiomyocytes observed (Lundy et al. 2013). However, more cost and time-effective methods for maturation have not been described to date. Regardless of their immaturity, the potential ability of hiPSC-CMs to “capture the entire patient’s genome in a dish” offers unique opportunities to identify genetic loci or cellular pathways related to predisposition towards cardiac disorders.

## 2.4 Recapitulating Cardiomyopathies in a Dish

Just 8 years after the first report of somatic cell reprogramming to pluripotency, hiPSCs have been used to generate over 80 models of human disease. Conditions affecting the heart, smooth muscle, skeletal muscle, immune system, skin, central nervous system, blood and eye, as well as imprinting, metabolic and multi-organ disorders have been recapitulated in the lab and used to gain insightful information regarding disease mechanism and potential novel therapies (Rajamohan et al. 2013; Matsa et al. 2014). This section focuses on reports of cardiomyopathy disease modelling using cardiomyocytes derived from hiPSCs as an in vitro platform.



### 2.4.1 Dilated Cardiomyopathy

In 2012, Sun et al. were able to obtain skin tissue samples from DCM family members carrying a p.Arg173Trp point mutation in the gene-encoding sarcomeric cardiac TNNT2 (Sun et al. 2012). Following the reprogramming of skin cells to hiPSCs, they generated cardiomyocytes which, compared to control genetically matched healthy samples, exhibited irregular organisation of sarcomeric cardiac ACTN2, scattered distribution pattern of Z bodies, reduction in contractile force and altered regulation of calcium ions ( $\text{Ca}^{2+}$ ), as observed by calcium imaging. DCM hiPSC-CMs also expressed reduced levels of calcium related key molecules, such as CASQ, TMEM38, NFAT and NECAB. When challenged with the  $\beta$ -adrenergic agonist, norepinephrine, DCM hiPSC-CMs showed increased susceptibility to cellular stress. Using patch-clamp electrophysiology, susceptibility was reported as a positive inotropic and negative chronotropic effect (see Box 2.1). Fluorescence imaging determined that adrenergic stimulation also increased the number of cells with abnormal sarcomeric ACTN2 distribution. These findings demonstrated that hiPSC-CMs from DCM patients could closely recapitulate the morphological and functional phenotypes of DCM.

Furthermore, the same study demonstrated that prolonged treatment with the  $\beta$ -adrenergic blocker, metoprolol, or overexpression of sarcoplasmic reticulum  $\text{Ca}^{2+}$  adenosine triphosphatase (SERCA2a) improved the function of DCM hiPSC-CMs, causing negative chronotropic effects and improving global  $\text{Ca}^{2+}$  transients. It was suggested that metoprolol might lead to reduction in contraction force as well as contraction frequency, thus alleviating cardiomyocytes of mechanical stress. Microarray analysis demonstrated that gene therapy by SERCA2a overexpression was capable of improving abnormal cardiomyocyte function by acting on the  $\text{Ca}^{2+}$ , protein kinase K, G-protein coupled receptor, integrin, cytoskeletal and ubiquitination signalling pathways. Although it is still not clear whether altered  $\text{Ca}^{2+}$  handling is the primary factor that contributes to the disease or merely a secondary consequence of disease progression, the study demonstrated that hiPSC-CMs can provide an important platform to investigate treatments that might clinically benefit DCM disease cardiomyocyte function in culture and further refine our understanding of specific disease mechanisms of DCM.

### 2.4.2 Hypertrophic Cardiomyopathy

Similar to DCM discussed above, Lan et al. were able to recapitulate the pathophysiology of HCM using induced pluripotency technologies. They obtained skin samples from a ten-member family cohort carrying a hereditary HCM-related missense mutation (p.Arg663His) in MYH7. Diseased cardiomyocytes generated from hiPSCs were able to mimic numerous aspects of the HCM clinical characteristics, including 60 % cell enlargement and 26 % increased multinucleation, 105 % increased myofibril content, increased expression of atrial natriuretic factor (ANF), elevation of beta-myosin (MYH7)/alpha-myosin

(MYH6) ratio, calcineurin (CALN) activation and nuclear localisation of nuclear factor of activated T cells (NFAT), as evidenced by immunofluorescence staining. Patch-clamp electrophysiology revealed contractile arrhythmias in the form of delayed afterdepolarisations (DADs) at the single-cell level, whereas calcium imaging demonstrated perturbations in  $\text{Ca}^{2+}$  cycling and elevation in intracellular  $\text{Ca}^{2+}$  concentrations. To confirm the p.Arg663His mutation caused the disease pathology, the study showed that normal hESC-CMs genetically engineered to carry the same mutation were able to recapitulate the calcium-handling abnormalities of HCM hiPSC-CMs.

Prolonged treatment of HCM hiPSC-CMs with the  $\beta$ -adrenergic agonist, isoproterenol, was found to provoke  $\text{Ca}^{2+}$  transient irregularities and arrhythmias, mirroring the development of arrhythmias in HCM patients under sympathetic stimulation. Importantly, co-administration of the  $\beta$ -adrenergic blocker, propranolol, with isoproterenol significantly reduced catecholamine-induced exacerbation of hypertrophy,  $\text{Ca}^{2+}$  handling deficiencies and arrhythmia. Moving one step further, Lan et al. were able to show that pharmacological restoration of  $\text{Ca}^{2+}$  homeostasis with the L-type  $\text{Ca}^{2+}$  channel blocker, verapamil, prevented development of cell enlargement and electrophysiological abnormalities completely. The investigators also tested 13 pharmacological agents on HCM iPSC-CMs. Only those capable of blocking  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  entry alleviated DADs in HCM hiPSC-CMs. It is believed that reduction of  $\text{Na}^{+}$  influx limits  $\text{Ca}^{2+}$  concentration by allowing  $\text{Na}^{+}/\text{Ca}^{2+}$  exchange to remove  $\text{Ca}^{2+}$  more readily. Therefore, these results showed that perturbations in  $\text{Ca}^{2+}$  cycling and elevation in intracellular  $\text{Ca}^{2+}$  concentrations can be considered as central mechanisms for disease development at the cellular level, and demonstrated the potential of patient-specific hiPSC-CMs as a powerful tool for identification of novel pharmaceutical agents to treat HCM.

A separate study was able to show that hiPSC-CMs generated from patients suffering with *LEOPARD syndrome*, 80 % of whom present hypertrophic cardiomyopathy as the most life-threatening aspect of the disorder, had a significantly increased median surface, higher degree of sarcomeric organisation, and 50 % increased preferential localisation of nuclear factor of activated T cells (NFATC4) in the nucleus when compared with hESC-CMs or hiPSC-CMs from healthy family members of the patients (Carvajal-Vergara et al. 2010). These features correlate well with the potential hypertrophic state observed in *LEOPARD syndrome* patients. Using a phosphoproteomic microarray chip containing approximately 600 pan and phospho-specific antibodies, this study also demonstrated that phosphorylation perturbations in proteins involved in the RAS-MAPK signal transduction pathway might be involved in development of disease pathophysiology. Once again, these findings demonstrate that hiPSC-CMs can provide the required characteristics to precisely determine the pathology behind these familial hypertrophic disorders, laying a foundation for studying the molecular mechanisms of these diseases and investigating novel treatment interventions.

### 2.4.3 Arrhythmogenic Right Ventricular Cardiomyopathy

Modelling of ARVC using induced pluripotency technologies has been somewhat more challenging than for DCM and HCM above. This is because, in many cases, ARVC is an adult-onset disorder with a median age of presentation of 26 years that would typically not be manifested in the fetal-like cardiomyocytes generated from hiPSCs. Indeed, hiPSC-CMs derived from ARVC patients carrying plakophilin-2 (*PKP2*) mutations c.C2484T and c.2013delC were only able to recapitulate mild disease pathologies in vitro, such as abnormal plakoglobin nuclear translocation and decreased  $\beta$ -catenin activity. Induction of adult-like metabolic energetics in hiPSC-CMs from an embryonic glycolytic state to an adult fatty acid oxidation state, via the use of an adipogenic cell culture environment, produced a 31.6 % increase in lipogenesis and 39.6 % increase in apoptosis of ARVC hiPSC-CMs compared to unaffected controls. In conjunction, expression of genes involved in fatty acid oxidation, such as peroxisome proliferator-activated receptor- $\alpha$  and - $\gamma$  (*PPAR- $\alpha$*  and - $\gamma$ ) was increased, whereas pro-survival genes such as Caspase 3 (*CASP3*) were detected at lower levels. Thus, this model sufficiently recapitulated the exaggerated lipogenesis and apoptosis features observed clinically in ARVC patients. Furthermore, treatment of hiPSC-CMs with *PPAR- $\alpha$*  and - $\gamma$  antagonists, GW9662, T0070907 and GW6471, was able to prevent lipogenesis and apoptosis in ARVC hiPSC-CMs, largely reverting ARVC pathogenesis features. The study concluded that induction of adult-like metabolic energetics in hiPSC-CMs, together with abnormal *PPAR- $\alpha$*  and - $\gamma$  activation, underlies the pathogenesis of ARVC. Significantly, this was the first report to demonstrate modelling of an adult-onset disease using patient-specific hiPSCs and reveal crucial mechanistic insights of disease pathologies that enabled the proposal of novel disease-modifying therapeutic strategies.

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## 2.5 Conclusions and Future Directions

This chapter outlines clinical manifestations of cardiomyopathies, as well as difficulties currently facing physicians when classifying and treating these disorders. It also highlights the three major classes of familial cardiomyopathy that thus far have been successfully recapitulated in vitro, with observed phenotypes closely resembling clinical symptoms described in patients. These studies show that hiPSC-based models of cardiomyopathy provide suitable tools for investigation of pathophysiological mechanisms, as well as for identification of novel drug therapies that could ameliorate observed pathologies and benefit future management and risk stratification for cardiomyopathies.

Another important use for hiPSC-based models of cardiomyopathy has been in drug toxicity screening. Notably, HCM iPSC-CMs (Liang et al. 2013) have been used to demonstrate increased susceptibility to arrhythmias when treated with the drug cisapride, now withdrawn from the market due to adverse side effects in cardiac repolarisation. Similarly, DCM hiPSC-CMs were found to exhibit drug-

induced proarrhythmias when treated with high doses of Nicorandil, a drug clinically used for treatment of angina that presents palpitations as adverse side effects. Thus, disease-specific hiPSC-CMs demonstrate higher susceptibilities to cardiotoxicity as compared to healthy hiPSC-CMs. This correlates well with observations that patients with HCM and DCM are particularly sensitive to drug-induced cardiotoxicity, and are susceptible to lethal arrhythmias. Therefore, disease-specific hiPSC-CMs are not only a good model for disease modelling and drug discovery but also can serve as a valuable platform for drug toxicology studies that could provide timely and accurate prediction of adverse drug side effects on the cardiovascular system. This should benefit the pharmaceutical industry by reducing drug withdrawal at preclinical and clinical stages, as well as cardiomyopathy patients by increasing confidence in safety and efficacy of administered drugs (Rajamohan et al. 2013).

Major efforts are under way to identify more sophisticated subtype differentiation, maturation and purification protocols for hiPSC-CMs that can create accurate physiologic cellular conditions to reflect human adult disease phenotypes. Steady progress is being made towards this direction, and it is anticipated that future studies will shed light on details regarding the precise cellular pathways which are impaired in cardiomyopathy, making possible the identification of novel therapeutic small molecules, including large scale screening of chemical libraries. Developing scaled automated platforms for hiPSC-CM generation could enable future assessment of drug toxicity at an industrial level and bring us closer to generation of cardiac myocytes in appropriate quantity and quality for autologous (hiPSC-CM) and allogeneic (hESC-CM) clinical transplantations.

Finally, recent advances in gene-editing technologies utilising nuclease enzymes to enable targeted DNA modification for gene disruptions or genetic repair are also anticipated to significantly alter the field of disease modelling (Wang et al. 2012; Soldner and Jaenisch 2012). Advantages of these technologies include the ability to correct or introduce disease-causing mutations in hiPSCs to allow creation of genetically matched experimental controls. This could substantially simplify analysis of correlation between genomic variants and disease phenotypes. Along with the dawn of next-generation sequencing technologies, it is anticipated that these tools will enable identification of novel mutations implicated in cardiomyopathy and help explain why mutations in distinct locations of the same gene (e.g. *TNNT2*) might preferentially cause different types of cardiomyopathy. Such studies hold the potential to substantially improve future phenotype-to-genotype correlation analysis and clinical treatments for cardiomyopathies, as well as for other cardiovascular disorders.

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