



# Human heart disease: lessons from human pluripotent stem cell-derived cardiomyocytes

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**Abstract** Technical advances in generating and phenotyping cardiomyocytes from human pluripotent stem cells (hPSC-CMs) are now driving their wider acceptance as in vitro models to understand human heart disease and discover therapeutic targets that may lead to new compounds for clinical use. Current literature clearly shows that hPSC-CMs recapitulate many molecular, cellular, and functional aspects of human heart pathophysiology and their responses to cardioactive drugs. Here, we provide a comprehensive overview of hPSC-CMs models that have been described to date and highlight their most recent and remarkable contributions to research on cardiovascular diseases and disorders with cardiac traits. We conclude discussing immediate challenges, limitations, and emerging solutions.

## Keywords

Human pluripotent stem cell-derived cardiomyocytes · Disease modeling · Cardiac disease · Cardiovascular disease · Safety pharmacology · Drug screening · Cardiac arrhythmia · Cardiomyopathy

## Abbreviations

|                  |   |
|------------------|---|
| AAV              | Adeno-associated virus  |
| ALC-1            | Atrial myosin essential light chain                                       |
| ALDH2            | Aldehyde dehydrogenase-2  |
| ALPK3            | $\alpha$ -kinase-3  |
| AMPK             | AMP-activated protein kinase  |
| AP               | Action potential  |
| APA              | Action potential amplitude  |
| APD              | Action potential duration   |
| ARVC             | Arrhythmogenic right ventricular cardiomyopathy                           |
| ATTR             | Familial transthyretin amyloidosis  |
| BrS              | Brugada syndrome  |
| BTHS             | Barth syndrome  |
| Ca <sup>2+</sup> | Calcium   |
| CAD              | Coronary artery disease   |
| CaM              | Calmodulin  |
| CaMKII           | Ca <sup>2+</sup> /calmodulin-dependent serine–threonine protein kinase II |
| cAMP             | Cyclic adenosine monophosphate  |
| CASQ2            | Calsequestrin-2   |
| CDI              | Ca <sup>2+</sup> /CaM-dependent inactivation                              |
| CFCS             | Cardiofaciocutaneous syndrome   |
| CPVT             | Catecholaminergic polymorphic ventricular tachycardia                     |
| cTnT             | Cardiac troponin T  |
| DADs             | Delayed after depolarizations   |
| DCM              | Dilated cardiomyopathy  |
| DMD              | Duchenne muscular dystrophy   |
| EBs              | Embryoid bodies   |
| ECC              | Excitation–contraction coupling   |
| ECG              | Electrocardiogram   |
| EHTs             | Engineered heart tissues  |
| ERT              | Enzyme replacement therapy  |
| FDA              | Food and drug administration  |

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|                     |  |
|---------------------|--|
| GAA                 | Acid $\alpha$ -glucosidase                           |
| GCS                 | Glucosylceramide synthase                            |
| GL-3                | Globotriaosylceramide                                |
| HCM                 | Hypertrophic cardiomyopathy                          |
| hECT                | Human engineered cardiac tissue                      |
| hERG                | Ether-a-go-go related gene                           |
| hESCs               | Human embryonic stem cells                           |
| hiPSCs              | Human-induced pluripotent stem cells                 |
| HLHS                | Hypoplastic left heart syndrome                      |
| hPSC-CMs            | Human pluripotent stem cell-derived cardiomyocytes   |
| IHD                 | Ischemic heart damage                                |
| IL-18               | Interleukin-18                                       |
| iPSCs               | Induced pluripotent stem cells                       |
| JLNS                | Jervell and Lange-Nielsen syndrome                   |
| K <sup>+</sup>      | Potassium  |
| LAMP2               | Lysosomal-associated membrane protein type 2         |
| LQTS                | Long-QT syndrome                                     |
| MCB                 | Membranous cytoplasmic body                          |
| MHC                 | Myosin heavy chain                                   |
| MI                  | Myocardial infarction                                |
| MLC                 | Myosin light chain                                   |
| Na <sup>+</sup>     | Sodium   |
| NCX                 | Na <sup>+</sup> /Ca <sup>2+</sup> exchanger          |
| PD                  | Pompe disease  |
| PGD                 | Preimplantation genetic diagnosis                    |
| PKA                 | Protein kinase A                                     |
| PKP2                | Plakophilin-2  |
| PPAR- $\gamma$      | Peroxisome proliferator-activated receptor- $\gamma$ |
| RBM20               | RNA-binding motif protein 20                         |
| rhGAA               | Recombinant human GAA                                |
| RMP                 | Resting membrane potential                           |
| ROS                 | Reactive oxygen species                              |
| RYR2                | Ryanodine receptor-2                                 |
| SERCA2a             | SR Ca <sup>2+</sup> -ATPase                          |
| SR                  | Sarcoplasmic reticulum                               |
| TAZ                 | Tafazzin   |
| TECRL               | <i>Trans</i> -2,3-enoyl-CoA reductase-like           |
| TS                  | Timothy syndrome                                     |
| TTN <sub>trvs</sub> | TTN-truncating variants                              |
| TTX                 | Tetrodotoxin   |
| T2DM                | Type-2 diabetes mellitus                             |
| V <sub>max</sub>    | Maximum upstroke velocity                            |

## Introduction

Human embryonic stem cells, derived from the early human embryos, and human-induced pluripotent stem cells, derived by reprogramming somatic cells (hESCs and hiPSCs, respectively, and collectively called hPSCs) can self-renew

and differentiate into all cell types of the human body, including cardiomyocytes [1–3]. They have potential applications in regenerative medicine but are also becoming a useful tool in cardiovascular research. Most particularly, they offer new opportunities to develop in vitro models of human cardiac development and cardiovascular diseases, as they are able to capture much of the normal and pathological physiology of the human heart, including aspects of congenital defects. In addition, hPSC-derived cardiomyocytes (hPSC-CMs) may be used in cardiac safety pharmacology, drug screening, and drug discovery, to predict the effects of candidate drugs and new compounds and to identify key target pathways in disease. Whilst hESCs can now readily be engineered to carry specific disease mutations, the derivation of hiPSCs from virtually any patient of interest offers some advantages over hESCs for disease modeling, since hiPSCs incorporate individual complex genetic backgrounds of the patients from which they were originated. For this reason, expectations are high on their contribution to precision medicine where the goal is to prevent disease development and find personalized treatments that take genetic variability of patients into account [4].

In this review, we provide comprehensive coverage of hPSC models of human heart disease.

## Generation of hiPSCs and hESCs for cardiac disease modeling

The need for more robust cell models for human disease, including cardiovascular disorders, has led to increasing interest in hPSCs.

hESCs were the first human pluripotent stem cells described. They were derived from the inner cell mass of blastocyst-stage embryos in 1998 by Thomson [1]. These cells could differentiate toward cell lineages of all three germ layers yet to be maintained in a state of self-renewal indefinitely in their undifferentiated state. Multiple hESC lines have been used successfully for studying genetic disorders most often through specific gene knockdown or deletion using homologous recombination [5] or lentiviral transduction [6]. Furthermore, in the case of some potentially fatal of untreatable conditions, hESCs have also been derived from preimplantation embryos genetically diagnosed as defective by single blastomere sampling during Preimplantation Genetic Diagnosis (PGD). Disorders that have been studied using PGD-hESC include a number of severe congenital disorders such as fragile X syndrome [7], Turner syndrome [8], and trisomy 21 [9]. However, hESC lines to investigate multifactorial and complex diseases may not be available through PGD, because they may not be considered sufficiently severe [10], as is the case for many cardiac diseases. Thus, even though hESCs are useful

when there is pre-existing knowledge on the specific mutations causing the disease and the mutations can be introduced into an otherwise healthy line, hiPSCs are preferred where the entire genetic background is relevant.

The use of patient somatic cells to derive hiPSCs is also preferable in some countries, since it circumvents ethical issues that surround the destruction of human embryos for research purposes. The advent of hiPSCs has also superseded efforts to derive cloned embryos by somatic cell nuclear transfer and isolate individual hESC lines from them [2, 11, 12]. Many methods have now been described that allow somatic cell reprogramming [13]. The first and still among the most efficient methods described overexpress the reprogramming factors c-MYC, SOX2, KLF4, and OCT3/4 after retroviral or lentiviral transduction of dermal fibroblasts. This results in the integration of reprogramming genes into the genome and subsequent reactivation of the endogenous counterparts [11, 14]. Alternative non-integrating reprogramming methods are now more widely used and include the use of Sendai viruses [15], plasmids [16], and modified RNA [17]. Small molecules have also been used but have relatively lower efficiencies [18]. Somatic cell sources currently used for reprogramming not only include the original dermal fibroblasts isolated from skin biopsies, but also blood cells [19], keratinocytes from plucked hair [20], and exfoliated renal tubular epithelial cells obtained from urine [21]. Many patient-specific lines have been described that are suitable for cardiovascular disease modeling and are proving of particular value for studying disorders of unknown or complex genetic origin, as will be discussed in this review.

## Differentiation into cardiomyocytes

In vitro differentiation of hPSCs into cardiomyocytes mimics the sequential stages of embryonic cardiac development [22]. In the vertebrate embryo, the heart is one of the first organs to develop; after gastrulation, anterior migrating mesodermal cells intercalate between the ectoderm and the endoderm germ layers in the primitive streak to start generating the heart [23, 24]. Cardiac progenitor cells derive from two small tracts of epiblast cells of the developing primitive streak and take residence in the lateral plate mesoderm [25]. Signals from the surrounding tissues, such as growth factors of the WNT, BMP, and TGF- $\beta$  families, are critical to promote the specification of myocardial fate. Accordingly, many of the successful protocols developed to induce cardiomyogenesis in hPSCs are based on activating and inhibiting these signaling pathways. As an example, stimulation of extraembryonic ectoderm via BMP signaling (by BMP4)

and posterior primitive streak via WNT signaling (by CHIR99201) during the first 24 h of differentiation promotes the exit from self-renewal and the induction of cardiac mesoderm [26]. Moreover, inhibitors of WNT signaling, such as IWR-1, IWP-3, and XAV939, have been shown to induce cardiogenesis when added after mesoderm formation [27–29], while SB-431542, an inhibitor of the TGF- $\beta$  pathway, promotes cardiogenesis when its addition occurs after mesoderm specification [30]. Current methods for cardiac differentiation of hPSCs rely on three different approaches that are summarized in Table 1, embryoid body formation, co-cultures, and monolayer culture [22].

Functional cardiomyocytes can be generated from hPSCs as three-dimensional spheroid-like aggregates termed embryoid bodies (EBs), referring to their similarity with the early post-implantation embryos. Protocols to form EBs were originally developed using fetal bovine serum supplemented culture medium, but a variety of serum-free, defined media formulations are now available. Methods to form EBs from hPSCs range from an enzymatic partial dissociation of hPSC colonies, and to precise control of cell number and size by forced aggregation in microwells, to microwells in which hPSC colonies are first expanded to a defined size, to micropatterned substrates [22].

Alternatively, the early studies also used inductive co-culture of mechanically passaged hESCs with visceral endoderm-like END2 cells derived from mouse P19 embryonal carcinoma cells [31]. Notably, visceral endoderm plays a key role in the induction of cardiogenic precursor cells in development.

For ease of use though, monolayer differentiation protocols have been preferred. Benefits compared to the EB and co-culture systems include higher efficiencies and easy monitoring of outcome. Refinements over the last decade now support the generation of differentiated cell populations containing 85% cardiomyocytes; multiple methods have been described in which cardiomyocytes can be enriched to 95% using, for example, selection in sodium ( $\text{Na}^+$ ) lactate containing medium [32–34] or on the basis of cell surface markers like SIRPA and VCAM1 [28, 35].

Cardiomyocytes derived under all these culture conditions beat spontaneously, express sarcomeric proteins and ion channels, and exhibit cardiac-type action potentials (APs) and calcium ( $\text{Ca}^{2+}$ ) transients. Furthermore, they show similar functional properties to the cardiomyocytes in the developing heart, such as comparable dose-dependent response to cardiac drugs in terms of beating frequency and contractility,  $\beta$ -adrenoreceptor responses, action potential (AP) morphologies, and excitation–contraction coupling mechanisms [36]. Although opportunities still remain for

**Table 1** Methods for differentiating hPSCs into cardiomyocytes (modified from [34])

| Differentiation      | Culture conditions  | Limits  | Efficiency <sup>a</sup> (%) | References |
|----------------------|---|---|-----------------------------|------------|
| EBs                  | Serum-based media   | Low efficiency<br>Serum media   | 5–15                        | [3]        |
|                      | RPMI + B27 supplement<br>ActivinA + BMP4                                  | Medium efficiency<br>Batch-to-batch variability of growth factors<br>Chemically undefined “B27” | 60                          | [232]      |
|                      | Bioreactor suspension culture<br>RPMI + B27 supplement<br>Small molecules | Chemical undefined “B27”  | 90                          | [233]      |
| Inductive co-culture | Serum-based media   | Low efficiency  | 35                          | [22]       |
|                      | Feeder layer<br>Mouse END-2 cells   | Serum media<br>Requirement for mouse feeder cells   |                             |            |
| Monolayer culture    | RPMI + B27 supplement<br>ActivinA + BMP4                                  | Low efficiency<br>Batch-to-batch variability of growth factors<br>Chemically undefined “B27”    | 35                          | [234]      |
|                      | RPMI + B27 supplement<br>Matrigel Sandwich<br>ActivinA + BMP4             | Batch-to-batch variability of Matrigel and growth factors<br>Chemically undefined “B27”         | 90                          | [235]      |
|                      | RPMI + B27 supplement<br>Small molecules                                  | Chemically undefined “B27”  | 90                          | [236]      |
|                      | RPMI + human albumin  |   | 85                          | [32]       |
|                      | L-ascorbic acid 2-phosphate<br>Small molecules                            |   |                             |            |
|                      | Na <sup>+</sup> lactate   |   | 95                          |            |
|                      | ActivinA + BMP4   | Medium efficiency<br>Batch-to-batch variability of growth factors                               | 50                          | [237]      |

<sup>a</sup> Efficiency was calculated from flow cytometry data as the number of cells positive for cardiac troponin T (cTnT), MLC-2 $\alpha$ , and MLC-2 $\nu$ , by immunostaining for MHC- $\beta$  or by determining the percentage of EBs containing contracting areas

improvement of reproducibility in cardiac differentiation between individual hPSC lines, reduction in the cost of reagents and in batch-to-batch variability, and of the yield and purity of required cardiomyocyte types, several protocols now support robust cardiac differentiation and some of these are available commercially as kits.

### Characterization of cardiomyocyte phenotype

The use of hPSC-CMs as a platform to model cardiovascular disorders requires their rigorous molecular and functional characterization. To maximize their potential applications in cardiovascular medicine, a qualitative comparison with adult (or fetal) primary human cardiomyocytes is advisable. Parameters used to characterize the cardiomyocyte phenotype are listed in Table 2 and include size and morphology, sarcomere structure, electrophysiological properties, Ca<sup>2+</sup> handling and contractile force, responses to  $\beta$ -adrenergic stimulation, mitochondrial function and metabolic profile, and conduction velocity.

### Size and morphology

In the adult heart, cardiomyocytes are elongated and rod shaped, and ~65% of them are mononucleated and this percentage does not change significantly throughout life [37, 38]. Furthermore, adult cardiomyocytes align longitudinally in the heart and are connected by intercalated discs that facilitate the electrical conduction and muscle contraction [39]. To date, despite the high differentiation efficiencies now achievable, hPSC-CMs remain small in size and round in shape [40] suggesting an immature or fetal phenotype. Several strategies have been used to mature hPSC-CMs. These include prolonged time in culture (>50 days), where hPSC-CMs become more elongated and less rounded [40] and advanced engineering approaches such as 3D platforms, either as “biowires”, or engineered heart tissues (EHTs), which allows the generation of hiPSC-CMs with improved ultrastructural and electrophysiological properties [41, 42]. Examples of improved ultrastructural properties included cardiomyocyte anisotropy with Z bands

**Table 2** Key features used to characterize the human cardiomyocyte phenotype

| Features  | Measured parameters   | Human adult cardiomyocyte   |
|---|---|---|
| Size and morphology   | Shape (rod, round)  | Elongated   |
|   | Size ( $\mu\text{m}$ )  | Rod shaped  |
|   | Cell capacitance (pF)   | ~65% mononucleated  |
| Sarcomeres  | Alignment   | Organized and aligned   |
|   | Organization (Z lines, H zone, I bands, A bands)  | MYH7 predominant isoform in the ventricle   |
|   | Molecular composition (MYH7:MYH6, MYL2:MYL7, TNNI1:TNNI3)   | MYL7 predominant isoform in the atrium  |
| Electrophysiological properties                                 | AP (APA, RMP, $V_{\text{max}}$ , APD)   | Typical atrial, ventricular, pacemaker, and Purkinje AP shapes [238, 239]   |
|   | Ion current densities and gating properties ( $I_{\text{Na}}$ , $I_{\text{CaL}}$ , $I_{\text{CaT}}$ , $I_{\text{to}}$ , $I_{\text{Kur}}$ , $I_{\text{Ks}}$ , $I_{\text{K1}}$ , $I_{\text{K,Ach}}$ , $I_{\text{K,ATP}}$ , $I_{\text{f}}$ ) | Distinct ion current densities and function in atrial, ventricular, pacemaker, and Purkinje cardiomyocytes [238, 239]   |
| Ca <sup>2+</sup> handling and contractile force                 | Ca <sup>2+</sup> transients   | Efficient Ca <sup>2+</sup> transient induction by Ca <sup>2+</sup> influx through L-type Ca <sup>2+</sup> channels (Ca <sup>2+</sup> -induced Ca <sup>2+</sup> -release) [52] |
|   | Force of contraction  | Force of contraction: 10–50 mN/mm <sup>2</sup> (ventricular myocytes) [240]   |
|   | Ca <sup>2+</sup> sparks and Ca <sup>2+</sup> waves  | Positive force-frequency relationship (Bowditch phenomenon) [241]   |
|   |   | Low rate of spontaneous Ca <sup>2+</sup> release  |
| Response to $\beta$ -adrenergic stimulation (cascade of events) | Chronotropic effect   | Positive chronotropic, inotropic and lusitropic effects   |
|   | Inotropic effect  |   |
|   | Lusitropic effect   |   |
| Mitochondrial function and metabolic profile                    | Oxygen consumption  | Mitochondria occupies one-third of the total volume of CMs<br>ATP production occurs mainly through oxidative metabolism (predominantly fatty acids)                           |
|   | Glycolysis and ATP measurements   |   |
|   | Mitochondrial membrane potential  |   |
|   | Mitochondrial [Ca <sup>2+</sup> ]   |   |
|   | Mitochondrial [Na <sup>+</sup> ]  |   |
|   | Redox state   |   |
|   | Intramitochondrial pH   |   |
|   | ROS generation  |   |
| Conduction velocity   | Conduction velocity maps  | Generation of the electrical signal through Na <sup>+</sup> channels and propagation through gap junctions<br>Localization of gap junction proteins at cell borders           |
|   | Expression level of ion channels and gap junction proteins  |   |
|   | Localization, density, and composition of gap junction proteins   |   |

frequently visible and aligned, pronounced presence of H zones and I bands, and scattered presence of T-tubule-like structures [41, 42]. These methods as well as other maturation strategies are summarized in the “Conclusions” section of this review.

### Sarcomere structure

Human adult cardiomyocytes are characterized by organized and aligned sarcomeres [38], the smallest contractile units of striated muscles. Sarcomeres are composed of contractile proteins, including actin and myosin, which generate the force of contraction, and thin filament proteins, which

calibrate the force generated by contractile proteins. In the adult ventricle, the  $\beta$  isoform of the protein Myosin Heavy Chain (MHC- $\beta$ ), encoded by the gene *MYH7*, is predominant compared to the atrial  $\alpha$  isoform MHC- $\alpha$ , encoded by *MYH6* [43]; in addition, the isoform Myosin Light Chain 2v (MLC-2v), encoded by the gene *MYL2*, is predominant compared to the MLC-2 $\alpha$ , encoded by *MYL7*, which is instead the primary human atrial isoform. Similarly, a genetic switch between the troponin I fetal (*TNNI1*) and adult isoforms (*TNNI3*) in the human heart characterizes the transition from fetal to post-natal development [44].

Sarcomeres in hPSC-CMs are less organized than in adult cardiomyocytes, and MHC- $\alpha$  and MLC-2 $\alpha$  are



generally highly expressed, while MHC- $\beta$  and MLC-2v display relatively low level of expression [45]. In addition, the TNNI1:TNNI3 protein isoform ratio reflects a fetal stage, even after long-term culture [46]. This is partly due to hPSC-CMs being more similar to fetal cardiomyocytes but also to the heterogeneous nature of the hPSC-CMs population, which consists on a mixture of ventricular-, atrial-, and nodal-like cells. Recent engineering approaches have attempted to improve sarcomere organization and myofibril alignment in hPSC-CMs, to allow study of their structural and contractile properties, such as actin–myosin cross-bridge cycling, myofibril tension, and kinetics of activation and relaxation. Examples include the work of Salick and colleagues in which hESC-CMs were seeded onto controlled two-dimensional micropatterned rectangles made with high-resolution photolithography and micro-contact printing [47], and the work of Pioner and colleagues in which hiPSC-CMs were seeded on nano-grooved surfaces and cultured long term (80–100 days) [48]. Importantly, the latter study demonstrated that myofibril tension and kinetics were similar between long-term cultures of hiPSC-CMs and second trimester human fetal ventricular cardiomyocytes. Importantly, the fetal sarcomeric properties of hPSC-CMs may represent an obstacle to faithfully recapitulating cardiomyopathy-associated phenotypes that are linked to sarcomere protein mutations. For example, the force of contraction was decreased in hiPSC-CMs with MYBPC3 mutations compared with wild-type cells, while hypertrophic cardiomyopathy (HCM) due to sarcomeric mutations is usually associated with hypercontractility [49, 50].

### Electrophysiological properties

Electrophysiological properties of adult cardiomyocytes can be described by their AP profile, which is widely considered specific for each cardiomyocyte subtype (atrial, ventricular, pacemaker, and Purkinje). However, independent of subtype, AP always starts with a rapid influx of  $\text{Na}^+$  as a rapid depolarizing current ( $I_{\text{Na}}$ ), termed “AP upstroke” (phase 0). Afterwards, phase 1 of the AP is characterized by a transient repolarizing current ( $I_{\text{to1}}$ ) of efflux of potassium ( $\text{K}^+$ ), followed by the inward  $\text{Ca}^{2+}$  current ( $I_{\text{CaL}}$ ) through the L-type depolarization-activated  $\text{Ca}^{2+}$  channels, which is called the plateau phase of the AP (phase 2). Next, two  $\text{K}^+$  currents ( $I_{\text{ks}}$  and  $I_{\text{kr}}$ ) drive the repolarizing phase 3 of the AP. Hence, in adult atrial and ventricular cardiomyocytes, the presence of a rectifying  $\text{K}^+$  current ( $I_{\text{k1}}$ ) stabilizes the resting membrane potential (RMP) at  $-85$  mV; this is termed phase 4 of the AP.

hPSC-CMs are more depolarized compared to adult cardiomyocytes: RMP is less negative ( $-50/-60$  mV),  $\text{Na}^+$  channels are fewer, and phase 0 of the AP is slow. In

addition, hPSC-CMs exhibit spontaneous contractile activity, due to the absence or very low expression of  $I_{\text{k1}}$ , and the presence of a funny current ( $I_f$ ), which is a pacemaker  $\text{Na}^+/\text{K}^+$  hyperpolarizing current [51, 52].

Despite the differences with adult cardiomyocytes (reviewed in [53] and [54]), hPSC-CMs offer the opportunity to study some developmental- and disease-relevant cardiac properties. As an example, arrhythmogenic diseases of the heart have successfully been recapitulated using patient hiPSC-CMs, displaying significant AP changes, such as AP prolongation in the long-QT syndrome [55]. In addition, in 2013, the US Food and Drug Administration (FDA) chose hiPSC-CMs as cell type of choice for testing cardiac effects of novel compounds [51].

### $\text{Ca}^{2+}$ handling and contractile force (excitation–contraction coupling)

The process termed “excitation–contraction coupling” (ECC) consists of the repeated contraction and relaxation of the chambers of the heart, in which  $\text{Ca}^{2+}$  is, perhaps, the most important ion involved.  $\text{Ca}^{2+}$  that enters the cell during the plateau phase of the AP enhances  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) through ryanodine receptor-2 (RyR2) channels. This causes an increase in intracellular  $\text{Ca}^{2+}$ , which binds to the myofilament protein troponin C, activating the mechanism of the contraction. For relaxation,  $\text{Ca}^{2+}$  instead dissociates from troponin C and leaves the cytosol through four different systems: SR  $\text{Ca}^{2+}$ -ATPase (SERCA2a); sarcolemmal  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX); sarcolemmal  $\text{Ca}^{2+}$ -ATPase; and mitochondrial  $\text{Ca}^{2+}$  uniport [52]. T-tubules are invaginations in the cell membrane located where L-type  $\text{Ca}^{2+}$  channels and RyR2 channels are close to each other and represent one of the most important components of the  $\text{Ca}^{2+}$  handling system, contributing to ECC [56]. To date, although hPSC-CMs express NCX at comparable levels of adult cardiomyocytes [57], the SR is still poorly developed and T-tubules have rarely been described. Consequently,  $\text{Ca}^{2+}$  handling kinetics as well as ECC are overall slow in hPSC-CMs [58].

### Responses to $\beta$ -adrenergic stimulation

Sympathetic stimulation of the heart through  $\beta$ -adrenergic receptor agonists, such as epinephrine, activates a membrane stimulatory GTP-binding protein, which stimulates adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP), which, in turn, leads to the subsequent activation of Protein Kinase A (PKA), therefore, potentiating the cardiac  $\text{Ca}^{2+}$  transients. In response to  $\beta$ -adrenergic stimulation, adult cardiomyocytes display positive chronotropic (increase in beating frequency), positive

inotropic (increase in contractility), and positive lusitropic (acceleration of relaxation) effects [52]. Although hPSC-CMs as well as fetal cardiomyocytes do exhibit chronotropic responses to  $\beta$ -adrenergic stimulation [59, 60], they do not show an increase in contraction or acceleration in the relaxation period [61], unless when incorporated in human EHTs as shown by Mannhardt [42]. These considerations need to be taken into account when hPSC-CMs are used for testing the efficiency of  $\beta$ -adrenergic drugs on the cardiovascular system.

### Mitochondrial function and metabolic profile

Due to its incessant contraction, the heart has an extremely high energy demand compared to other tissues of the human body [52]. Mitochondrial biogenesis increases over time during heart development, so that in adult cardiomyocytes, one-third of the cell volume is, indeed, occupied by mitochondria [62]. Due to this change, during development, glucose and lactate represent the predominant substrates for the majority of ATP production in fetal cardiomyocytes, while adult cardiomyocytes mainly use fatty acids [63, 64]. Although hPSC-CMs still display an immature phenotype, they also use fatty acids for the majority of ATP production and mitochondrial density increases over time, recapitulating to a certain extent the development of the human heart [51, 65]. For this reason, hPSC-CMs have successfully been used to recapitulate and study the key aspects of mitochondrial and metabolic diseases in humans, as Drawnel and colleagues have recently showed by modeling diabetic cardiomyopathy and phenotypically screening drugs for a complication of type 2 diabetes [66].

### Conduction velocity

While the parameters above can be evaluated in single cells, the conduction velocity can only be measured in monolayer cultures. Major factors contribute to determine the conduction velocity of cardiomyocytes: propagation of the electrical signal through  $\text{Na}^+$  channels [67]; localization of  $\text{Na}^+$  channels and gap junction proteins [68]; localization, density, and composition of gap junction proteins [69]; and cell size [70]. Although the composition of gap junction proteins is similar in hPSC-CMs and adult cardiomyocytes,  $\text{Na}^+$  channels and gap junctions need to be distributed at the edges of two adjacent cells (adult cardiomyocytes) [71], rather than all around the cell circumference (fetal and hPSC-CMs). This, together with a reduced availability of  $\text{Na}^+$  channels due to a hyperpolarized RMP and cell size, contributes to the slow conduction velocity observed in hPSC-CMs [51]. Of note though,

several groups have addressed this issue by repolarizing the RMP through overexpression or electronic enhancement of  $I_{\text{K1}}$  as a robust method to obtain more physiological electrical behaviour, including increased  $\text{Na}^+$  channel availability and improved  $\text{Ca}^{2+}$  transients profile [72–75]. Importantly,  $I_{\text{K1}}$ -enhanced hiPSC-CMs displayed a stable RMP in the absence of spontaneous beating activity, allowing more accurate quantitative analysis of AP in comparing healthy and diseased myocytes [72–75]. In addition, increased cell size, membrane capacitance, and DNA synthesis were also observed [73].

### Existing hiPSC models of cardiovascular and non-cardiovascular diseases with cardiac traits

To date, hiPSC-CMs have successfully been used not only to recapitulate, but also to better understand and elucidate the disease-relevant cellular and molecular pathological mechanisms of several cardiovascular diseases. They remain one of the few opportunities to study the heart against a background of human gene expression. Below, as well as in Fig. 1 and Table 3, we list most of the hiPSC cardiac models to date and provide specific examples.

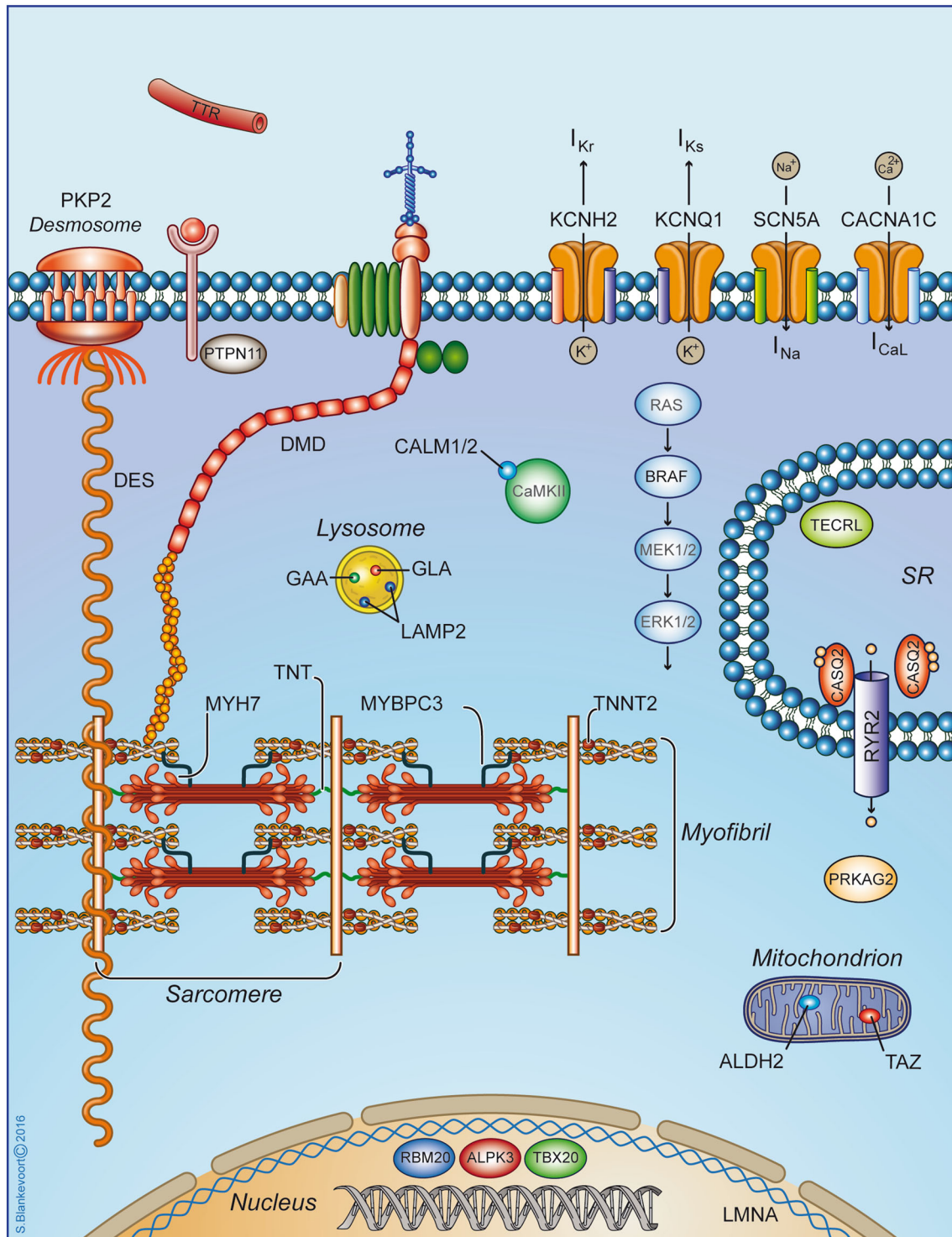
#### Arrhythmias and channelopathies

##### *Familial long-QT syndrome*

Long-QT syndrome (LQTS) is a potentially life-threatening arrhythmia characterized by a prolongation in the ventricular repolarization component (QT interval) of the electrocardiogram (ECG) [76]. Patients affected by LQTS experience polymorphic ventricular tachycardia with a characteristic shape of the ECG also termed “Torsades de Pointes”, syncope, and sudden cardiac death. LQTS includes hereditary variants: the autosomal-dominant form or Romano–Ward syndrome and the recessive form or Jervell and Lange-Nielsen syndrome (JLNS) [77–80]. LQTS is associated with more than 500 mutations in 16 different genes encoding cardiac ion channel proteins and their auxiliary subunits or modulating proteins, and displays a wide range of phenotypes even within members of the same family [81, 82].

##### *LQT1*

LQT1 patients harbor mutations in the *KCNQ1* gene, which encodes the  $\text{K}^+$  channel  $\text{K}_v7.1$  mediating the repolarizing current  $I_{\text{ks}}$  of the AP [83]. To date, several LQT1 hiPSC lines have been generated and characterized from patients carrying distinct mutations in the *KCNQ1* gene, such as R190Q [84, 85], G269S and G345E [85, 86], P631fs/33



**Fig. 1** Schematic representation of cardiomyocyte structure and relevant cellular and molecular components that are mutated in cardiac diseases. This schematic shows the cardiac proteins encoded by mutated genes for which hiPSCs have been generated and reviewed here. Disease genes of interest, which are also listed in

Table 3, are located in different compartments of the cardiomyocyte, such as the extracellular matrix, sarcoplasmic reticulum (SR), cytoskeleton, sarcomere, desmosome, lysosome, mitochondrion, and the nucleus



**Table 3** Existing hiPSC models of cardiovascular diseases and disorders with cardiac traits

| Disease                         | Gene    | Mutation    | References          |
|---------------------------------|---------|-------------|---------------------|
| Arrhythmias and channelopathies |         |             |                     |
| LQT1                            | KCNQ1   | R190Q       | [84]                |
| LQT1                            | KCNQ1   | P631 fs/33  | [87]                |
| LQT1                            | KCNQ1   | Ex7Del      | [88]                |
| LQT1/JLNS                       | KCNQ1   | R594Q       | [111]               |
| LQT1/LQT2                       | KCNQ1   | E160fs+138X | [85]                |
|                                 |         | G269S       |                     |
|                                 |         | G345E       |                     |
|                                 |         | R190Q       |                     |
| LQT2                            | KCNH2   | A614V       | [90, 91]            |
|                                 | KCNH2   | G1681A      |                     |
| LQT2                            | KCNH2   | R176W       | [93]                |
| LQT2                            | KCNH2   | A561V       | [95]                |
| LQT2                            | KCNH2   | N996I       | [94]                |
| LQT2                            | KCNH2   | A614V       | [92]                |
| LQT2                            | KCNH2   | A561P       | [97]                |
| LQT2/LQT3                       | KCNH2   | A422T       | [96]                |
|                                 |         | SCN5A       |                     |
| LQT2                            | TBX20   | R311C       | [98]                |
| LQT3                            | SCN5A   | V1763M      | [100]               |
| LQT3                            | SCN5A   | V240M       | [102]               |
|                                 |         | R535Q       |                     |
| LQT3                            | SCN5A   | F1473C      | [101]               |
|                                 | KCNH2   | K897T       |                     |
| LQT3                            | SCN5A   | R1644H      | [103]               |
| LQT8/TS                         | CACNA1C | G1216A      | [107]               |
| LQT14                           | CALM1   | F142L       | [75]                |
| LQT15                           | CALM2   | D130G       | [110]               |
| BrS/LQT3                        | SCN5A   | 1795insD    | [74, 118, 120, 242] |
| BrS/LQT3                        | SCN5A   | E1784K      | [121]               |
| BrS                             | SCN5A   | R620H/R811H | [122]               |
|                                 |         | 4189delT    |                     |
| CPVT                            | RYR2    | M4109R      | [243]               |
| CPVT                            | RYR2    | F2483I      | [126, 244]          |
| CPVT                            | RYR2    | P2328S      | [245]               |
| CPVT                            | RYR2    | S406L       | [127]               |
| CPVT                            | RYR2    | P2328S      | [128]               |
|                                 |         | EX3del      |                     |
|                                 |         | T2538R      |                     |
|                                 |         | L4115F      |                     |
|                                 |         | Q4201R      |                     |
|                                 |         | V4653F      |                     |
| CPVT                            | RYR2    | L3741P      | [130]               |
| CPVT                            | RYR2    | I4587V      | [131]               |
| CPVT                            | RYR2    | E2311D      | [129]               |
| CPVT                            | CASQ2   | G112+5X     | [132]               |
| CPVT/LQTS                       | TECRL   | SRD5A2L2    | [133]               |
|                                 |         | c.331+1G>A  |                     |

**Table 3** continued

| Disease                  | Gene   | Mutation           | References |
|--------------------------|--------|--------------------|------------|
| Cardiomyopathies         |        |                    |            |
| BTHS                     | TAZ    | 517delG            | [137]      |
| BTHS                     | TAZ    | Gly197Val          | [136]      |
|                          |        | EX2Del             |            |
|                          |        | Arg57Leu           |            |
| Leopard                  | PTPN11 | T468M              | [140]      |
| ARVC                     | PKP2   | Gly828Gly          | [146]      |
|                          |        | R672fsX683         |            |
| ARVC                     | PKP2   | L614P              | [144]      |
| ARVC                     | PKP2   | A324fs335X         | [145]      |
| ARVC                     | SCN5A  | R1898H             | [147]      |
| DCM                      | TNNT2  | R173W              | [152, 153] |
| DCM                      | LMNA   | R225X              | [151]      |
| DCM                      | TTN    | W976R              | [156]      |
|                          |        | A22352fs           |            |
|                          |        | P2258fs            |            |
| DCM                      | DES    | A285V              | [154]      |
| DCM                      | RBM20  | R636S              | [157]      |
| HCM                      | MYBPC3 | C2373dipG          | [50]       |
| HCM                      | MYH7   | Arg663His          | [159]      |
| HCM                      | BRAF   | T599R              | [163]      |
| HCM                      | BRAF   | T599R              | [164]      |
|                          |        | Q257R              |            |
| DCM/HCM                  | ALPK3  | W1264X             | [166]      |
| HCM                      | PRKAG2 | N488I              | [168]      |
|                          |        | R531Q              |            |
| LQT1                     | KCNQ1  | G269S              | [86]       |
| HCM                      | MYH7   | R663H              |            |
| DCM                      | TNNT2  | R173W              |            |
| HCM                      | MYH7   | R442G              | [160]      |
| HCM                      | MYBPC3 | Arg91Cys           | [161]      |
|                          |        | N/A                |            |
|                          |        | Gly999/Gln1004del  |            |
| HLHS                     | N/A    | N/A                | [169]      |
| IHD/CAD                  | ALDH2  | ALDH2*2            | [184]      |
| Cardiometabolic diseases |        |                    |            |
| PD                       | GAA    | Ex18Del            | [190]      |
|                          |        | 1441delT/TRP746TER |            |
| PD                       | GAA    | Arg266Cys/M439K    | [194]      |
| PD                       | GAA    | D645E/D645E        | [189]      |
|                          |        | D645E/2040-1G      |            |
| PD                       | GAA    | Ex18del            | [193]      |
| Danon                    | LAMP2  | 129-130 insAT      | [198]      |
|                          |        | IVS-1 c.64+1 G>A   |            |
| Fabry                    | GLA    | W162X              | [201]      |
| Fabry                    | GLA    | W162X/R220X        | [202]      |
| Fabry                    | GLA    | IVS4+919 G>A       | [203, 204] |
| Diabetic cardiomyopathy  | N/A    | N/A                | [66]       |

**Table 3** continued

| Disease   | Gene | Mutation   | References |
|---|------|------------|------------|
| Non-cardiovascular diseases with cardiac traits |      |            |            |
| DMD   | DMD  | Ex50Del    | [212]      |
| DMD   | DMD  | Ex45-52del | [213]      |
| ATTR  | TTR  | L55P       | [215]      |

A search for original articles published up to February 2017 was performed using PubMed Advanced Search Builder using the following criteria: (i) (human-induced pluripotent stem cells) AND (cardiac disease model) NOT review; (ii) (human-induced pluripotent stem cells) AND (cardiomyocytes) NOT review; (iii) (human-induced pluripotent stem cells) AND (cardiomyocytes) AND (mechanistic insight) NOT review. References on cardiac regeneration were manually excluded. References from some of the most comprehensive reviews of the field were screened and manually added when not present in the above-mentioned search. Limitation of this review relates to selection bias

[87], and a novel heterozygous exon 7 deletion (ex7Del) [88].

In 2010, Moretti and colleagues used retroviral vectors to generate patient-specific hiPSCs from members of a family affected by the autosomal-dominant missense mutation R190Q in the *KCNQ1* gene and differentiated the patient-derived cells into functional cardiomyocytes that recapitulated in vitro electrophysiological features of the LQT1 disease phenotype and the therapeutic approach of  $\beta$ -blockade [84]. In the same study, hiPSC-CMs helped demonstration of a dominant negative trafficking defect of the mutated channel. Similarly, Egashira et al. identified the same molecular mechanism as being responsible of an LQT1 phenotype in P631fs/33-KCNQ1 mutated hiPSC-CMs [87]. In another study, Liang and colleagues generated a library of hiPSC-CMs from healthy individuals and patients with different hereditary cardiac disorders, including LQT1, for recapitulating and predicting drug-induced arrhythmia. Interestingly, these cells displayed a broad spectrum of cardiotoxicity effects suggesting that disease-specific hiPSC-CMs may accurately predict adverse drug-induced cardiotoxicity [86]. Furthermore, in 2014, Wang et al. generated hiPSCs by overexpressing ion channel genes with dominant negative mutations causing LQT1 (G269S, G345E, and R190Q). To achieve stable transgene expression, these genes were integrated into the AAVS1 safe harbor locus using the Zinc Finger Nuclease technology. Next, transgene cells and isogenic unedited controls were differentiated into cardiomyocytes and recapitulated the LQT1 disease phenotype showing a prolongation in the AP duration (APD) [85].

### LQT2

LQT2 patients carry mutations in the *KCNH2* gene, also termed human ether-a-go-go related gene (*hERG*), which encodes the  $K^+$  channel mediating the repolarizing current

$I_{kr}$  of the AP [89]. A panel of LQT2-diseased hiPSCs carrying the following *hERG* mutations has been generated and characterized: G1681A [90, 91], A614V [85, 92], R176W [93], N996I [94], A561V [95], A422T [96], and A561P [97].

By performing multi-electrode array, patch-clamp electrophysiology, and drug testing, Matsa et al. demonstrated that hiPSC-CMs from two patients carrying the G1681A *KCNH2* mutation showed prolonged APs but displayed different drug-induced sensitivity [90, 91]. Two independent laboratories applied similar strategies for modeling LQT2 by generating hiPSCs from patients carrying the missense A614V [92] and R176W [93] mutations on the hERG channel. However, despite the novelty of using patient hiPSC-CMs for modeling LQT2, these studies were performed under genetically non-defined conditions and, therefore, genetic background variations were not taken into account. To address this limitation, we modeled LQT2 syndrome by generating hiPSCs from a patient carrying the N996I hERG missense mutation and corrected the mutation by homologous recombination. Next, we introduced the same mutation in hESCs, generating two genetically distinct isogenic pairs of LQTS and control lines [94]. This approach allowed the electrophysiological changes to be attributed to the specific mutation. In another study, hiPSCs were derived using a virus-free method from patients with the A561V missense mutation in the *KCNH2* gene and they differentiated them into beating cardiomyocytes. Notably, this study provided an approach to rescue the diseased LQT2 phenotype correcting hERG trafficking defects with the pharmacological agent ALLN, demonstrating with patient-specific hiPSC-CMs that re-trafficking of the mutated channels might represent an alternative approach for some *KCNH2* mutations [95].

Recently, the use of hiPSC-CMs for modeling LQT2 helped revealing a key role for the transcription factor

TBX20 in the regulation of *KCNH2* expression [98]. In this study, Caballero and colleagues investigated the electrophysiological effects of the R311C-TBX20 mutation, which is found in individuals affected by LQTS, in hiPSC-CMs. The authors showed that the R311C mutation specifically disables the posttranscriptional activity of TBX20 over *KCNH2*, which decreases the  $I_{Kr}$  and prolongs the AP, therefore, identifying TBX20 as an LQT2-modifying gene [98].

### LQT3

LQT3 patients usually carry gain-of-function mutations in the *SCN5A* gene, which encodes the  $\text{Na}^+$  channel  $\text{Na}_v1.5$  mediating the fast depolarizing current  $I_{\text{Na}}$  during AP [99]. To date, several *SCN5A* mutations have been modeled with patient-specific hiPSC-CMs: V1763M [100], F1473C [101], V240M and R535Q [102], and R1644H [103].

In 2013, Ma and colleagues derived hiPSC-CMs from an LQT3 patient harboring a V1763M-*SCN5A* mutation and recapitulated the biophysical abnormalities (prolonged APD, increased tetrodotoxin (TTX)-sensitive late or persistent  $\text{Na}^+$  current, positive shift of steady-state inactivation, and faster recovery from inactivation) of the disease. In this study, the hiPSC line was generated from dermal fibroblasts of the patient and control-hiPSC-CMs were derived from the healthy sister of the patient [100]. However, LQTS may occur in families whose members are affected by multiple mutations and complex genetics. Such disease phenotypes are difficult to recapitulate in vitro; moreover, the development of patient-specific clinical regimens remains challenging. To address these limitations, hiPSC-CMs have been generated from family members with complex genetics, such as reported by Terrenoire et al. [101]. In this study, hiPSCs were derived from an LQTS patient harboring the F1473C *SCN5A* mutation and the K897T *KCNH2* polymorphism. Notably, analysis of the biophysics and molecular pharmacology of ion channels expressed in cardiomyocytes differentiated from these cells displayed a primary LQT3  $\text{Na}^+$  channel defect responsible for the patient's arrhythmias, which was not influenced by the *KCNH2* polymorphism. In a similar manner, Fatima et al. reported the generation of hiPSCs from two LQT3 patients carrying two distinct mutations in *SCN5A* (V240M and R535Q), which resulted in defective biophysical properties of  $\text{Na}_v1.5$  [102]. Furthermore, in a large family affected by congenital LQT3 syndrome, 15 out of the 23 available individuals were identified as heterozygous carriers of the missense mutation R1644H in *SCN5A*. Of note, Malan and colleagues obtained skin biopsies from one member of this family affected by LQT3, as well as from one healthy control individual of the same family [103]. Of particular interest, after addition of mexiletine, a  $\text{Na}^+$  channel inhibitor

commonly used in LQT3 therapy, a shortening in the APD was noticed in LQT3 hiPSC-CMs, which successfully rescued the disease phenotype of the patient.

### LQT8/Timothy syndrome (TS)

LQT8, also known as Timothy syndrome (TS), is a complex multi-system disorder characterized by QT prolongation, webbed fingers and toes, flattened nasal bridge, low-set ears, small upper jaw, thin upper lip, and typical autism traits [104, 105]. TS patients carry mutations in the *CACNA1C* gene, which encodes the  $\text{Ca}^{2+}$  channel  $\text{Ca}_v1.2$ , the main L-type  $\text{Ca}^{2+}$  channel in the mammalian heart responsible for the plateau phase of the AP and essential for ECC [106]. Yazawa and colleagues successfully modeled the cardiac phenotype of TS including irregular contraction and electrical activity, and abnormal  $\text{Ca}^{2+}$  handling by generating hiPSC from a patient harboring a G1216A missense mutation in *CACNA1C* [107]. Of particular interest, the small molecule roscovitine proved successful in restoring normal electrical and  $\text{Ca}^{2+}$  properties.

### LQT14

Patients carrying mutations in one of the three genes encoding calmodulin (CaM, a multifunctional intermediate  $\text{Ca}^{2+}$ -binding messenger protein essential for the functionality of the heart, immune system, and brain) manifest cardiac arrhythmias associated with severe LQTS, as well as catecholaminergic polymorphic ventricular tachycardia and idiopathic ventricular fibrillation [108–110]. Mutations in the *CALM1* gene, encoding CaM, are associated with type 14 LQTS (LQT14). In this regard, Rocchetti and colleagues recently investigated the unclear arrhythmogenic effect of the heterozygous F142L mutation in *CALM1* by studying patient-specific hiPSC-CMs electrophysiology with addition of stimulated  $I_{k1}$  by Dynamic-Clamp [75]. Mutated hiPSC-CMs displayed loss of  $I_{\text{CaL}}$  inactivation and abnormal APD, whilst  $I_{\text{ks}}$  and  $I_{\text{NaL}}$  remained unaltered.  $I_{\text{CaL}}$  blockage rescued the disease phenotype. Importantly, these findings demonstrated that F142L-CaM arrhythmogenesis is caused by loss of  $I_{\text{CaL}}$  inactivation [75].

### LQT15

*CALM2* mutations are associated with type 15 LQTS (LQT15). In a recent study, Limpitikul and colleagues generated hiPSC-CMs from a patient carrying the D130G-CaM mutation within the *CALM2* gene. Notably, the patient-derived iPSC-CMs showed prolongation of the APD and disruption of  $\text{Ca}^{2+}$ /CaM-dependent inactivation (CDI) of L-type  $\text{Ca}^{2+}$  channels. Importantly, allele-specific

suppression of the mutated *CALM2* gene using CRISPR interference resulted in functional rescue in the hiPSC-CMs, with normalization of APD and CDI after treatment [110].

### JLNS

The Jervell and Lange-Nielsen syndrome is inherited as an autosomal recessive trait and is characterized by a severe QT interval prolongation at the ECG and by deafness [78]. JLNS patients harbor homozygous or compound heterozygous mutations in *KCNQ1* or *KCNE1* genes. In one study, both patient-derived and engineered hiPSCs carrying the E160fs + 138X or the R594Q *KCNQ1* mutations recapitulated the severe JLNS electrophysiological phenotype including APD prolongation and drug-induced arrhythmia susceptibility [111].

### Brugada syndrome

Brugada syndrome (BrS) is an inheritable channelopathy characterized by a coved-type ST-segment elevation in the right precordial leads of ECG and increased risk of sudden cardiac death from ventricular fibrillation [112, 113]. Loss-of-function mutations in the *SCN5A* gene encoding the  $\text{Na}^+$  channel responsible for the cardiac  $I_{\text{Na}}$  are associated with BrS; they account for ~20% of cases [114, 115]. Genetic alterations in additional genes encoding  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  channels or associated proteins have been linked to BrS [116]; however, ~70% of BrS patients remain genetically unsolved, suggesting that additional factors, such as copy number variations, mutations in yet-unknown genes, epigenetic factors, and post-translational modifications may contribute to this disease [117].

The 1795insD *SCN5A* mutation underlying both BrS and LQT3 was identified in a large Dutch family with ECG features of bradycardia and ventricular and atrial conduction slowing [118, 119]. In a study performed by Davis and colleagues, hiPSC were generated from a patient carrying the 1795insD mutation and differentiated toward cardiomyocytes that displayed the overlapped  $I_{\text{Na}}$  and AP properties of both BrS and LQT3 channelopathies (decrease in  $I_{\text{Na}}$  density, large persistent  $I_{\text{Na}}$ , reduced upstroke velocity, and prolonged APD) [120]. Similarly, Okata et al. generated hiPSCs from a patient carrying the E1784K *SCN5A* mutation, which has previously been associated with the mixed phenotype of LQT3/BrS. Interestingly, electrophysiological analysis showed that LQT3/BrS-hiPSC-CMs recapitulated the phenotype of LQT3 but not BrS. Due to the fact that *SCN3B* is the predominant  $\text{Na}^+$  channel  $\beta$ -subunit in fetal hearts as well in hiPSC-CMs, while *SCN1B* is the predominant  $\beta$ -subunit in the adults, the knockdown of *SCN3B* in the LQT3/BrS-hiPSC-CMs successfully unmasked the phenotype of BrS. Moreover,

corrected-LQT3/BrS-hiPSC-CMs exhibited the normal electrophysiological phenotype [121].

In another study of interest, Liang and colleagues generated hiPSC-CMs from two patients affected by BrS; the first patient carrying the double missense mutation (R620H and R811H) in *SCN5A* and the second patient carrying one base-pair deletion mutation in *SCN5A* (4189delT) [122]. Importantly, BrS hiPSC-CMs successfully recapitulated features of the BrS disease, such as the reduction of inward  $\text{Na}^+$  current density and reduction of maximal upstroke velocity, increased triggered activity and abnormal  $\text{Ca}^{2+}$  handling [122].

However, a dysfunction in the cardiac  $\text{Na}^+$  channel may not always represent a prerequisite for BrS phenotype in vitro, as demonstrated by Veerman and colleagues [74]. In this study, a comparison of electrophysiological properties between hiPSC-CMs generated from three patients affected by BrS and two unrelated controls revealed no significant differences in  $I_{\text{Na}}$  and in upstroke velocity, therefore, indicating that the BrS phenotype here could not be recapitulated in the hiPSC model. These results led to the hypothesis that other mechanisms than ion channel defects might underlie the phenotype in these patients, such as fibrosis, decreased cardiomyocyte coupling, and environmental factors; alternatively, or in addition, immaturity of hiPSC-CMs might have hampered the detection of the disease phenotype.

### Catecholaminergic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited cardiac disorder characterized by ventricular tachyarrhythmia, syncope and sudden cardiac death usually induced by emotional and physical stress [105, 123]. CPVT is caused by mutations in the *RYR2* gene, which leads to the CPVT1 variant, or by mutations in the calsequestrin-2 gene (*CASQ2*), which leads to the CPVT2 variant [124]. As previously mentioned, *RYR2* encodes the principal  $\text{Ca}^{2+}$  releasing channel expressed in the membrane of the SR, while *CASQ2* encodes a high-capacity and low-affinity  $\text{Ca}^{2+}$ -binding glycoprotein of the SR, both key players in ECC [125].

To date, several models of patient-specific hiPSC-CMs carrying *RYR2* mutations have been generated. Importantly, all these studies successfully demonstrated that hiPSC-CMs can recapitulate some of the  $\text{Ca}^{2+}$  handling abnormalities typical of CPVT1 and, therefore, opened new opportunities for the investigation of the disease mechanisms in vitro as well as for drug testing. As an example, Fatima and colleagues demonstrated that patient-specific hiPSC-CMs harboring the F2483I mutation in the *RYR2* channel displayed arrhythmias and delayed after depolarizations (DADs) post-catecholaminergic



stimulation, and higher amplitudes and longer durations of spontaneous  $\text{Ca}^{2+}$  release events at basal state when compared to healthy controls. Of note, these  $\text{Ca}^{2+}$  release events continued even after repolarization and were abolished by increasing the cytosolic concentration of cAMP with forskolin, an adrenergic stimulator that acts via production of cAMP [126]. In another study of interest, Jung and colleagues successfully restored normal  $\text{Ca}^{2+}$  spark properties and rescued the arrhythmogenic S406L RYR2 phenotype by addition of dantrolene, a drug against malignant hyperthermia. Moreover, their findings suggested that the pathogenesis of the S406L mutation is due to a defect of inter-domain interactions within the RYR2 channel [127]. The antiarrhythmic effect of dantrolene was also assessed by Penttinen and colleagues in six patients carrying various RYR2 mutations and in their corresponding hiPSC-CM models [128]. This study showed similar patient-to-patient variation in dantrolene effects both in the patients and in the corresponding iPSC-CMs, suggesting that it may be possible to predict personalized drug-dose responses in vitro without predisposing the patient to the potentially severe side-effects of a drug [128]. In another study, Di Pasquale et al. developed a model of CPVT1 by generating hiPSCs from a patient harboring the E2311D RYR2 mutation. Treatment of hiPSC-CMs with KN-93, a specific antiarrhythmic drug that inhibits  $\text{Ca}^{2+}$ /calmodulin-dependent serine–threonine protein kinase II (CaMKII), decreased DADs, and successfully rescued the arrhythmic phenotype induced by catecholaminergic stress [129]. Interestingly, a recent study performed by Preininger and colleagues revealed the inadequacy of  $\beta$ -blocker treatment by nadolol in one patient affected by a novel mutation in RYR2 that causes CPVT1 [130]. hiPSC-CMs generated from the patient showed persistent ventricular arrhythmias during  $\beta$ -blockade with nadolol, whereas no arrhythmias were observed during treatment with the  $\text{Na}^+$  channel blocker flecainide. In detail, nadolol treatment during  $\beta$ -adrenergic stimulation achieved negligible reduction of  $\text{Ca}^{2+}$  wave frequency and failed to rescue the  $\text{Ca}^{2+}$  spark defects in diseased hiPSC-CMs. On the other hand, flecainide reduced both frequency and amplitude of  $\text{Ca}^{2+}$  waves and restored the  $\text{Ca}^{2+}$  sparks to the baseline levels [130], closely recapitulating drug treatment in the patient. In a similar manner, Sasaki and colleagues combined electrical pacing with CPVT- and control-hiPSC-CMs to validate S107, a drug that stabilizes the closed state of the RYR2, as potential therapeutic agent for CPVT1 [131].

After proving the efficacy of Adeno-associated virus (AAV)-mediated CASQ2 gene replacement therapy for CPVT2 in mouse models, Lodola and colleagues investigated the efficacy of this strategy in hiPSC-CMs generated from a patient carrying the homozygous G112+5XCASQ2 mutation [132]. HiPSC-CMs infection

with AAV carrying the wild-type CASQ2 gene revealed to be sufficient to restore the physiological expression of CASQ2 protein, and to observe decrease in the percentage of DADs following adrenergic stimulation as well as normalization of  $\text{Ca}^{2+}$  transient amplitude and  $\text{Ca}^{2+}$  sparks. These findings show the potential of gene therapy as curative approach in patients affected by some CPTV mutations [132].

**CPVT/LQTS**—A recent study by Devalla and colleagues was carried out on hiPSC-CMs from patients from three different families with clinical arrhythmias and high risk of sudden cardiac death [133]. Precisely, two of these patients were diagnosed with LQTS, whereas the third patient belongs to a family diagnosed with the early onset and highly malignant form of CPVT. Of note, all of them carried mutations in the gene encoding the *trans*-2,3-enoyl-CoA reductase-like protein (*TECRL* gene), whereas no mutations in the most common LQTS and CPVT genes. Analysis of intracellular  $\text{Ca}^{2+}$  dynamics, AP measurements, stimulation by noradrenaline, and treatment with the antiarrhythmic drug flecainide in the patient-specific hiPSC-CMs recapitulated the clinical phenotypes of LQTS and CPVT, showing, for the first time, that mutations in the *TECRL* gene are associated with inherited arrhythmias with clinical features of both LQTS and CPVT [133].

## Cardiomyopathies

### Barth syndrome

Barth syndrome (BTHS) is an X-linked cardiac and skeletal mitochondrial myopathy caused by mutations of the gene *Tafazzin* (*TAZ*) [134] responsible for remodeling cardiolipin, the major phospholipid of the mitochondrial inner membrane [135]. To date, two independent studies generated BTHS hiPSCs [136, 137]. Interestingly, Wang and colleagues recapitulated the pathophysiology of BTHS cardiomyopathy by combining patient-derived hiPSCs with genome editing, modified RNAs, and “heart on a chip” technologies [137]. They demonstrated that a mutation in *TAZ* gene (517delG) is sufficient to disassemble the structure of the cardiomyocyte sarcomeres. Furthermore, they demonstrated that BTHS cardiomyopathy can be reversed by either reintroducing the wild-type *TAZ* gene, or by suppressing the level of reactive oxygen species (ROS) produced by BTHS mitochondria. In another study of interest, Dudek and colleagues studied mitochondrial oxidative phosphorylation in BTHS-hiPSC-CMs, which displayed a severe decrease in basal oxygen consumption rate and in the maximal respiratory capacity when compared to wild-type cells, leading to a dramatic increase of ROS production [136].

### *Leopard syndrome*

LEOPARD is the acronym of “Lentigines, Electrocardiographic abnormalities, Ocular hypertelorism, Pulmonary valve stenosis, Abnormal genitalia, Retardation of growth, Deafness”, an autosomal-dominant disease that belongs to a class of disorders associated with RAS–mitogen-activated protein kinase signaling [138, 139]. Approximately 90% of LEOPARD syndromes are caused by missense mutations in the *PTPN11* gene, which encodes the ubiquitously expressed tyrosine phosphatase protein SHP2, although hypertrophic cardiomyopathy remains the most common abnormality in patients affected by LEOPARD syndrome [139]. Against this background, Carvajal-Vergara and colleagues generated hiPSCs from two patients with the heterozygous T468M mutation in the *PTPN11* gene and highlighted important molecular mechanisms in the signaling pathways responsible for the cardiac hypertrophic phenotype in LEOPARD syndrome, such as the increased phosphorylation of specific proteins such as MEK1 in LEOPARD-hiPSC-CMs compared to wild-type, demonstrating that RAS-MAPK signaling is perturbed in LEOPARD syndrome [140].

### *Arrhythmogenic right ventricular cardiomyopathy*

Another inherited cardiac disorder that has been modeled with hPSC-CMs is the arrhythmogenic right ventricular cardiomyopathy (ARVC), characterized by the replacement of cardiomyocytes with fatty or fibrofatty tissue [141]. Approximately half of the patients affected by ARVC carry a mutation in one of the genes encoding for key components of the desmosome, the intercellular junction of cardiac muscle [142, 143]. Of note, different laboratories studied ARVC hiPSCs from patients with mutations in the *PKP2* gene, which encodes the plakophilin-2 desmosomal protein [144–146]. In these studies, fibroblasts were reprogrammed into hiPSC via retrovirus infection and cardiomyocytes were generated using 3D protocols of differentiation. Gene expression profiling, immunofluorescence staining of desmosomal proteins, transmission electron microscopy, and exposure of the cells to adipogenic stimuli allowed these scientists to successfully recapitulate the ARVC phenotype in vitro and provided mechanistic insights into the early disease pathogenesis, such as the association of ARVC phenotype with the upregulation of the pro-adipogenic transcription factor peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) [145].

Notably, it has recently been suggested an interaction between the desmosome and the Na<sup>+</sup> channel protein Na<sub>v</sub>1.5 encoded by the *SCN5A* gene, raising the hypothesis that mutations in this Na<sup>+</sup> channel complex may lead to

ARVC cardiomyopathy [147]. On this note, Riele and colleagues generated hiPSC-CMs from an ARVC patient harboring the rare mutation (R1898H) in *SCN5A* and no desmosomal mutations. In this study, the authors demonstrated reduced Na<sup>+</sup> current and Na<sub>v</sub>1.5/N-Cadherin clusters at junctional sites in the patient-derived hiPSC-CMs, suggesting that Na<sub>v</sub>1.5 may be part of a functional complex with adhesion molecules such as N-Cadherin, which reveals a non-canonical mechanism by which *SCN5A* mutations lead to ARVC cardiomyopathy [147].

### *Familial dilated cardiomyopathy*

Dilated cardiomyopathy (DCM) is an inherited cardiac disorder that mostly affects the myocardium. It is characterized by left or biventricular dilatation, which is sufficient to cause global systolic impairment [148]. DCM is a genetically heterogeneous disease that can be caused by mutations in many different genes [149]. One of the key genes identified in familial DCM is *LMNA*, which encodes intermediate filament proteins of the nuclear lamina, the “lamin A/C proteins” [150]. Two different *LMNA* mutations, the autosomal-dominant non-sense R225X and a frame shift mutation, were investigated in a work from Siu and colleagues [151]. This study revealed that haploinsufficiency due to R225X mutation was associated with accelerated nuclear senescence and apoptosis of patient-specific hiPSC-CMs under electrical stimulation, which was attenuated by pharmacological blocking of ERK1/2 signaling pathway. Another gene associated with DCM is *TNNT2*. So far, three independent groups succeeded in showing hypertrophic signatures in hiPSC-CMs carrying the R173W *TNNT2* mutation [86, 152, 153]. Furthermore, as demonstrated by Tse and colleagues, patient-specific hiPSC-CMs can be used to confirm histological and functionally suspected genetic bases for DCM [154]. In this study, using whole-exome sequencing, Tse et al. identified the novel heterozygous mutation A285V in the muscle-specific intermediate filament protein Desmin (encoded by the *DES* gene) responsible for the cytoskeletal organization between cardiomyocytes and striated muscle cells [155]. Nevertheless, the most common genetic cause for DCM consists of mutations that truncate the massive sarcomeric protein Titin (encoded by the *TNN* gene), the so-called “TTN-truncating variants” (TTNts), such as the W976R, A22352fs, and P2258fs mutations, studied by Hinson and colleagues [156]. Here, RNA sequencing and functional analyses were combined with cardiac engineered micro-tissues from healthy, mutated, and isogenic hPSC lines to demonstrate that truncations in the A-band domain of *TTN* cause DCM, whereas truncations in the I band are better tolerated, because alternative splicing excludes I-band exons from most mature *TTN* transcripts [156]. Finally, by

investigating stage-specific cardiogenesis in hiPSC carrying mutations in the RNA-binding motif protein 20 gene (*RBM20*), Wyles et al. showed that in this specific case, DCM is a developmental disorder [157].

#### *Familial hypertrophic cardiomyopathy*

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder that can be caused by more than 1400 mutations in at least 11 genes encoding the thick and thin contractile myofilaments or the Z-discs of the sarcomere, leading to an abnormal thickness of the myocardial left ventricle [158]. Although the majority of individuals affected by HCM are asymptomatic or manifest mild symptoms, they are equally exposed to a high risk of progressive heart failure, arrhythmia, and sudden cardiac death [105]. However, the pathways by which sarcomeric mutations induce cardiomyocyte hypertrophy and electrophysiological abnormalities are still not completely clear [159]. Therefore, the generation of patient-specific hiPSC-CMs to model HCM may help to elucidate and, maybe, in the future, to predict the onset and the development of HCM, as demonstrated by Lan and colleagues [159]. In this study, hiPSC-CMs were generated from patients harboring the missense R663H *MYH7* mutation. These cells showed enlarged cell size and contractile arrhythmia at the single-cell level. Furthermore,  $\text{Ca}^{2+}$  analysis revealed deregulation of  $\text{Ca}^{2+}$  cycling and  $\text{Ca}^{2+}$  intracellular concentration, and key mechanisms of HCM pathogenesis. Similarly, two other groups recapitulated the disease phenotype of HCM by generating hiPSC-CMs from patients carrying mutations in the *MYH7* gene [86, 160].

In two other studies, hPSC-CMs carrying a mutation in *MYBPC3*, the gene encoding the cardiac myosin-binding protein C, were generated [50, 161]. After generating hPSC-CMs from three patients with HCM, Tanaka and colleagues demonstrated that the HCM phenotype as well as the contractile variability observed in the three classes of HCM hPSC-CMs were caused by interactions between the patient's genetic backgrounds and the cardiomyocyte hypertrophy-promoting factor endothelin-1 [161]. In another study of interest, Birket and colleagues showed that, under optimized conditions for cardiomyocyte function, which included the presence of thyroid hormone, insulin growth factor-1, and dexamethasone, single HCM hPSC-CMs showed lower contractile force when compared to controls [50].

HCM can also affect individuals with cardiofaciocutaneous syndrome (CFCS), a genetic disease characterized by abnormal RAS/MAPK signaling in multiple populations of cardiac cell progenitors [162]. In a recent study, Cashman et al. generated a 3D model of human engineered cardiac tissue, termed "hECT", using hiPSC-CMs from

patients carrying BRAF mutations and presenting with CFCS and HCM [163]. After 1 week in culture, BRAF-hECTs exhibited several structural, molecular, and functional features of hypertrophic phenotype when compared to hECTs derived from healthy individuals (larger cross-sectional area, increased expression level of the hypertrophic marker *ANP*, increased expression of the hypertrophic marker *BNP*, and the  $\text{Ca}^{2+}$  regulatory marker *SERCA2a*, as well as greater developed force, shorter twitch duration, and higher maximum rates of contraction and relaxation). Furthermore, a model consisting on BRAF-mutated hiPSC-CMs not only recapitulated the disease phenotype of HCM, but also helped elucidating the role of RAS/MAPK signaling in HCM pathogenesis [164]. Here, Josowitz and colleagues demonstrate that activation of this pathway through TGF $\beta$  signaling leads to cardiomyocyte hypertrophy driven by both autonomous and non-autonomous cardiomyocyte defects. Importantly, these findings suggest a potential therapeutic use of TGF $\beta$  inhibitors in HCM and CFCS patients, for which no curative options exist to date [164].

Another study conducted on three unrelated families demonstrated that pediatric HCM can be caused by biallelic truncating mutations in the gene encoding the  $\alpha$ -kinase-3 (*ALPK3*) [165]. Notably, several features of DMC, such as alterations in the systolic function, were also found in the same individuals, suggesting a role for the *ALPK3* pathway in the pathogenesis of a mixed DCM/HCM phenotype. Subsequently, Phelan et al. derived cardiomyocytes from a consanguineous family harboring a novel biallelic truncating mutation, and from hESCs lacking *ALPK3*. Ultrastructural analysis, multi-electrode array, and  $\text{Ca}^{2+}$  imaging on these cells revealed disorganized sarcomere structures and intercalated discs, extended field potential duration, and increased irregular  $\text{Ca}^{2+}$  transients (arrhythmia) indicative of abnormal  $\text{Ca}^{2+}$  handling. Collectively, this study suggests that mutations in *ALPK3* can cause familial cardiomyopathy, identifying abnormal  $\text{Ca}^{2+}$  handling as a potential feature of cardiomyocytes lacking *ALPK3* [166].

In addition, several missense mutations causing HCM have been observed in the gene encoding *PRKAG2*, one of the three regulatory subunits of the AMP-activated protein kinase (AMPK) that is highly expressed in the heart and involved in glucose handling and mitochondrial biogenesis [167]. Using hiPSC-CMs, three-dimensional cardiac microtissues, RNA sequencing, and metabolomics, Hinson and colleagues recently revealed key links between AMPK and cardiomyocyte survival and metabolism with TGF $\beta$  signaling. By demonstrating that AMPK inhibits TGF $\beta$  production and fibrosis in vivo, the authors suggest that molecules that activate AMPK may be beneficial for the treatment of fibrosis and HCM [168].

### Hypoplastic left heart syndrome

Hypoplastic left heart syndrome (HLHS) is characterized by underdevelopment of the left side of the heart which can lead to variable complications like hypoplasia or atresia of the left ventricle, ascending aorta, and aortic and mitral valves [169]. It has been suggested that HLHS may be due to a diminished blood flow through the left side of the heart [170, 171], or to the disruption of specific genetic networks required for left ventricular chamber development [172, 173]. In a study from Jiang and colleagues, dermal fibroblasts were obtained from the skin biopsy of one HLHS patient and were reprogrammed to hiPSCs [169]. Interestingly, mutated hiPSC-CMs displayed gene expression and functional differences when compared to healthy control cardiomyocytes: reduced expression of CX43 and cTnT; higher expression of *CD31* and embryonic atrial myosin essential light chain (*ALC-1*); higher expression of *MYH6* and decreased expression of *MYH7*; lower numbers and beating rates of contractile areas; accelerated rate of  $\text{Ca}^{2+}$  transient decay; RYR2 dysfunction; and upregulation of IP3-receptor expression. Collectively, these findings demonstrated that HLHS-disease hPSC-CMs show developmental and/or functional defects that could compromise their ability to contribute to normal cardiogenesis in vivo.

### Ischemic heart damage and coronary artery disease

A decrease of oxygen concentration in the heart tissue dramatically alters the metabolism of cardiomyocytes by producing high oxidative stress. To date, it is known that oxidative stress and ROS play a key role in Ischemic Heart Damage (IHD) and Coronary Artery Disease (CAD) pathogenesis [174]. Indeed, during Myocardial Infarction (MI), ROS cause oxidative damage such as lipid peroxidation and enhanced production of toxic aldehydes [175–177]. Moreover, the high concentration of ROS during ischemia–reperfusion triggers apoptosis and necrosis in the heart tissue [178].

Because of the more complex nature of IHD and CAD compared with cell-autonomous genetic cardiac diseases, IHD and CAD are more difficult to recapitulate in vitro with hiPSC-CMs [179]. Nevertheless, some examples are starting to emerge, suggesting that some aspects might be recapitulated and elucidated in a culture dish. Interestingly, IHD and increased risk of CAD have been linked to the single-nucleotide polymorphism E487K in the cardioprotective enzyme aldehyde dehydrogenase-2 (ALDH2\*2) [180–183]. Ebert et al. generated hiPSC-CMs carrying the heterozygous *ALDH2\*2* allele and showed that, under ischemic conditions, these cells displayed high levels of ROS and toxic aldehydes, which led to cell cycle arrest and activation of apoptotic signaling pathways

[184]. These findings highlighted the key role of ALDH2 in modulating cell survival decisions. Overall, these insights into molecular mechanisms of ALDH2\*2-related ischemic damage might be useful for the development of patient-specific diagnostic methods and therapies against IHD and CAD.

### Cardiometabolic diseases

#### *Pompe disease*

Pompe disease (PD) is an autosomal recessive disorder caused by mutations in the gene encoding the lysosomal glycogen-degrading enzyme, acid  $\alpha$ -glucosidase (GAA) [185, 186]. Patients affected by PD manifest reduced GAA activity, increased cytoplasmic glycogen level, mitochondrial aberrance, and progressive autophagy [187]. PD can be classified either as infantile-onset form, characterized by progressive weakness of skeletal muscle and cardiac hypertrophic cardiomyopathy, or late-onset form, and characterized by later and slower progressive weakness of skeletal muscle [188]. Importantly, the first hiPSC model of PD was generated by Huang and colleagues [189]. Since the heart is one of the most affected organs especially in the infantile-onset form of PD, Huang et al. examined whether cardiomyocytes derived from infantile PD-hiPSCs exhibited the pathophysiological features of the disease by comparing their GAA activity, glycogen content, mitochondrial function, and ultrastructural changes with healthy hiPSCs-CMs. PD-hiPSC-CMs displayed depressed GAA activity, higher glycogen content, lower oxygen consumption rate, lower extracellular acidification rate, and some but not all the ultrastructural abnormalities, such as freely dispersed glycogen [189]. Since the mechanism by which loss of GAA activity causes cardiomyopathy in the infantile-onset form of PD is not well understood, Raval and colleagues reprogrammed fibroblasts from patients affected by infantile PD and generated additional hiPSCs-CMs to gain further insight into the molecular mechanisms. Unexpectedly, they found that the lysosome-associated membrane proteins LAMP1 and LAMP2 from PD-hiPSC-CMs displayed higher electrophoretic mobility compared with healthy hiPSC-CMs. Collectively, this study suggested that PD-hiPSC-CMs produce LAMPs lacking appropriate glycosylation and that misglycosylation in these proteins may contribute to the pathophysiology of Pompe cardiomyopathy [190]. Although it has been reported that cardiovascular complications mostly affect the infantile-onset form of PD, several groups demonstrated that late-onset PD patients can also be affected, although in a less severe and frequent manner [191, 192]. To investigate this, Sato and colleagues generated late-onset PD-hiPSCs and successfully differentiated



cardiomyocytes from both PD and control hiPSCs. Importantly, massive accumulation of glycogen in the lysosome of cardiomyocytes derived from PD-hiPSCs, not from control, was observed, but there were no significant differences in the structure of the cardiomyocyte fiber, such as disarray and hypertrophy. In another study of interest, Higuchi et al. compared hiPSCs generated from patients with infantile- and late-onset forms of PD [193]. Notably, ultrastructural features of these hiPSCs revealed massive accumulation of glycogen granules in the lysosomes of patients affected by infantile PD, and a few lysosomes in patients affected by the late-onset form of the disease. Collectively, these data show that cellular pathology of late-onset PS is reflected in patient-specific hiPSC-CMs [194]. Furthermore, when treated with recombinant human GAA (rhGAA), glycogen granules of infantile hiPSCs significantly decreased in a dose-dependent manner, confirming that enzyme replacement therapy improves the survival period as well as the muscle symptoms in some PD patients [195].

#### *Danon disease*

Danon disease is a familial cardiomyopathy characterized by impaired autophagy due to mutations in the gene encoding the lysosomal-associated membrane protein type 2 (*LAMP2*) [196, 197]. Patients affected by Danon disease display severe cardiac and skeletal muscle abnormalities resulting in heart failure and consequent sudden cardiac death [198].

Hashem and colleagues generated five independent hiPSC lines from two patients affected by Danon disease and compared them with two wild-type hiPSC lines derived from healthy unrelated individuals [198]. Importantly, all healthy and disease hiPSC-CMs expressed the cardiac-specific contractile protein  $\alpha$ -actinin, but only Danon hiPSC-CMs lacked LAMP2 protein. Next, size, gene expression and functionality of hiPSC-CMs were examined to investigate whether they recapitulated the heart failure phenotype observed in Danon patients. Cytological analysis revealed that Danon hiPSC-CMs were significantly larger compared to healthy hiPSC-CMs, therefore, recapitulating the hypertrophy observed in the patients. Furthermore, some but not all Danon hiPSC-CMs exhibited longer  $\text{Ca}^{2+}$  decay compared to healthy controls, consistent with the decrease of systolic and diastolic function typical of heart failure [199, 200].

#### *Fabry disease*

Fabry disease is a rare X-linked metabolic disorder characterized by deficiency of the enzyme  $\alpha$ -galactosidase and encoded by the *GLA* gene, causing progressive lysosomal

accumulation of globotriaosylceramide (GL-3) in the kidney, heart, and other tissues throughout the body [201].

In 2013, Kawagoe and colleagues generated hiPSCs from human fibroblasts of patients affected by Fabry disease. Electron microscopic analysis indicated that Fabry-hiPSCs exhibited massive accumulation of membranous cytoplasmic body (MCB) in the lysosomes, which is typical of Fabry disease, and they could not be easily differentiated into cardiomyocytes due to the continuous damages of the intracellular architecture [201]. By contrast, in a study by Itier and colleagues, hiPSCs generated from Fabry patients were successfully differentiated toward the cardiac fate [202]. Importantly, GL-3 resulted accumulated over time in the lysosomes of these cardiomyocytes and typical features of Fabry disease were observed (displacement of cardiac myofibrils to the periphery of the cells, focal areas of myofibrillar lysis, and myofilament degradation with troponin I degradation products). Furthermore, this in vitro model also demonstrated that substrate reduction therapy via inhibition of the enzyme glucosylceramide synthase (GCS) prevented accumulation of GL-3 in hiPSC-CMs.

Since enzyme replacement therapy (ERT) is currently the only efficient therapy in Fabry disease, there is a need to identify pathogenetic biomarkers and therapeutic targets in ERT-treated patients. On this note, Chien and colleagues recently constructed an iPSC-based disease model from patients carrying a *GLA* mutation (IVS4+919 G>A) responsible for Fabry disease [203] and demonstrated for the first time that Interleukin-18 (IL-18), a pro-hypertrophic inflammatory cytokine involved in several cardiac diseases, is involved in the pathogenesis of the disease [204]. Interestingly, these findings suggest that targeting IL-18 might be a potential adjunctive therapy combined with ERT in Fabry patients with the IVS4+919 G>A mutation [204].

#### *Diabetes-induced cardiomyopathy*

Patients affected by type-2 diabetes mellitus (T2DM) can be more easily affected by coronary artery disease, a condition that can progress to dilated cardiomyopathy and heart failure [205, 206]. Importantly, T2DM alters the cardiomyocyte-metabolic profile [207], which results in the decrease of ATP production followed by reduction of myocardial efficiency and accumulation of toxic lipid metabolites [208]. Furthermore, mitochondrial dysfunction and ROS production activate ROS-sensitive proteases that cleave myofilament proteins [209], whereas proteolytic damage and inadequate protein production cause sarcomere disorganization [66].

In 2014, Drawnel and colleagues investigated diabetes-dependent changes in cardiomyocyte functionality by



developing an in vitro DCM model using T2DM-hiPSCs [66]. In such study, the diabetes-induced cardiomyopathy phenotype was recapitulated in hiPSCs-CMs after exposure of the cells to a diabetic environment, consisting on persistent insulin signaling in the absence of glucose, to force the adaptation to fatty acids. Treated cells showed disorganized sarcomeres, altered  $\text{Ca}^{2+}$  transients, cellular hypertrophy, lipid intracellular accumulation, oxidative stress, and decreased expression of genes controlling protein production. Moreover, treated cardiomyocytes were exposed to a library of 480 compounds to identify small molecules that could prevent the development of the diabetic phenotype. Interestingly, small molecules involved in  $\text{Ca}^{2+}$  homeostasis and  $\text{Na}^+$  and  $\text{K}^+$  channel blockers, as well as multikinase inhibitors and protein synthesis inhibitors were identified as candidate protective drugs from diabetes-induced cardiomyopathy [66].

### Non-cardiovascular diseases with cardiac traits

#### *Duchenne muscular dystrophy*

Duchenne muscular dystrophy (DMD) is an X-linked genetic disease caused by frameshift mutations in the *dystrophin* gene, which results in the translation of a truncated and non-functional dystrophin protein [210]. Dystrophin is part of the dystrophin–glycoprotein complex, which connects the actin cytoskeleton to the extracellular matrix, providing cellular stability [211]. In patients affected by DMD, myocytes are particularly sensitive to mechanical stress and rupture, which contributes to muscle degeneration, fibrotic tissue deposition, and premature death. Patients affected by DMD display diastolic dysfunction, arrhythmias, and cardiomyopathy [212].

In 2015, Lin and colleagues generated hiPSC-CMs from healthy individuals and patients affected by DMD. Notably, DMD-hiPSC-CMs recapitulated key features of the disease phenotype (dystrophin deficiency, cytosolic  $\text{Ca}^{2+}$  overload, mitochondrial damage, and cell apoptosis). Moreover, this study showed that the membrane sealant Poloxamer 188 can suppress the cytosolic  $\text{Ca}^{2+}$  overload, repress Caspase-3 activation, and decrease cardiomyocyte apoptosis in DMD-hiPSC-CMs [213].

To detect cell structure- and contractile function-properties typical of the DMD disease phenotype, Macadangang and colleagues cultured healthy and diseased DCM-hiPSC-CMs on a novel engineered platform termed “anisotropically nanofabricated substrata” [212]. This nanopatterned model consisted of 800 nm parallel arrays of grooves and ridges for mimicking the structure of the myocardial extracellular matrix. Although structural differences between healthy and DMD-hiPSC-CMs were masked on the conventional flat substrates, DMD-hiPSC-

CMs cultured on the nanotopographic substrate displayed lower structural and functional responses to the underlying nanotopography when compared to healthy cardiomyocytes, probably due to a lower level of actin cytoskeleton turnover, suggesting that DMD-hiPSC-CMs are less adaptable to changes in their extracellular environment [212].

#### *Familial transthyretin amyloidosis*

Familial transthyretin amyloidosis (ATTR) is a lethal, autosomal-dominant disorder caused by single base-pair mutations in the *TTR* gene encoding for the 55 kDa transport protein transthyretin secreted by the liver [214]. However, the liver is not a clinically relevant site of amyloid deposition in vivo, whilst the brain and the heart are the major organs that are affected, suggesting a need for a multi-lineage model capable of recapitulating the complexity of ATTR disease phenotype in vitro. To model the three major tissues involved in this disease, Leung et al. generated ATTR patient-specific hiPSCs and differentiated them into hepatocytes, neurons, and cardiomyocytes [215]. hiPSC-derived neurons and cardiomyocytes displayed oxidative stress and increased cell death when exposed to TTR produced by patient-matched hiPSC-derived hepatocytes. Moreover, small molecule stabilizers of TTR, such as diflunisal and flufenamic acid, confirmed their efficacy in this model. Collectively, this study recapitulated key aspects of the ATTR disease phenotype in vitro, demonstrating that hiPSCs can also model disorders in which multiple tissues are affected [215].

### Conclusions

hiPSC-CMs already have diverse applications, ranging from studying human heart development to cardiac disease modeling and drug testing. They are perceived as having significant value. However, before the technology becomes widely accepted in the cardiovascular disease field as clinically relevant and predictive in human drug testing applications, some crucial hurdles need to be addressed. First, directed differentiation of hiPSCs in vitro to specific cardiomyocyte subtypes is still somewhat of a challenge, even though a number of studies have reported specific derivation of atrial-, ventricular-, and pacemaker-like cells. This is due to the limited understanding of later cardiac development in vivo, sometimes continued use of poorly defined (serum-containing) or uncontrolled (such as growth factors not optimally titrated) differentiation culture conditions in vitro. Nevertheless, increased knowledge of heart formation together with deeper understanding of signaling

pathways involved in cardiomyocyte development is now leading to the establishment of more defined methods for differentiation that are applicable over multiple hPSC lines and enrich for specific cardiomyocyte subtypes [29, 58, 65, 216–219]. Second, in most standard culture conditions, hPSC-CMs do not display all of the morphological and functional characteristics of adult cardiomyocytes. This needs to be taken into account when studying late-onset cardiovascular diseases but also mechanisms that are based on the highly specialized contraction machinery or gene splicing variants only expressed postnatally. Of note in this context, recent strategies based on biochemical, molecular, or bioengineering approaches [220, 221] have been developed to enhance hPSC-CM maturation. In the biochemical approaches, hormones or adrenergic agonists have been added to improve cardiomyocyte functionality [222]. In the molecular approaches, cardiac ion channels (such as  $I_{K1}$ ) and microRNAs have been overexpressed to improve electrophysiology and  $Ca^{2+}$  handling [57, 72, 73, 223–225]. In the bioengineering approaches, controlled substrate stiffness, topography, and electrical/mechanical conditioning, as well as integrated systems to deliver nutrients, such as microsystems and bioreactors, all improved sarcomeric organization and contractility [220]. In this regard, additional signatures based on gene expression switches during heart development have been used to track the maturation status of hiPSC-CMs [226]. Among these, inactivation of the fetal *TNNI1* isoform and its replacement by the adult *TNNI3* isoform have proven valuable in quantifying cardiomyocyte maturation in differentiated cultures [46, 226]. Third, the 2D microenvironment in which hPSC-CMs are cultured does not entirely recapitulate the complex dynamics and properties of the human heart [34]. hPSC-CMs can be cultured in 3D either on scaffolds that serve as a platform for cell attachment [227], or in scaffold-free systems in which cells self-organize into structures termed “cardiac microtissues” [228–230]. In this context, several microphysiological systems that use hiPSC-CMs have been developed for drug screening and cardiotoxicity testing [34]. Finally, it is becoming clear that including non-cardiomyocyte cell types to generate multicellular in vitro tissues is essential to advance current disease models, which primarily focus on monotypic cultures of cardiomyocytes, neglecting other cellular components of the myocardium. Endothelial cells, cardiac fibroblasts and smooth muscle cells all provide essential contributions to myocardial structure and function and also play crucial roles in drug-induced cardiovascular toxicity [229, 231]. Providing a system that more closely approximates human heart biology and physiology will allow the generation of more efficient and predictive platforms for modeling complex diseases, for the

development of new drug candidates, and also for rescuing (or rehabilitating) molecules that have been withdrawn because of negative outcomes in toxicity assays.

In conclusion, the past few years have witnessed remarkable advances in developmental biology, cell reprogramming, tissue engineering techniques, and in the establishment of innovative molecular assays. Patient-specific hiPSC-CMs and tissue models hold the potential to further advance basic research, on one hand, and personalized and regenerative medicine, on the other hand.

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## References

1. Thomson JA (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145–1147. doi:[10.1126/science.282.5391.1145](https://doi.org/10.1126/science.282.5391.1145)
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861–872. doi:[10.1016/j.cell.2007.11.019](https://doi.org/10.1016/j.cell.2007.11.019)
3. Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L (2001) Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 108:407–414. doi:[10.1172/JCI200112131](https://doi.org/10.1172/JCI200112131)
4. Collins FS, Varmus H (2015) A new initiative on precision medicine. *N Engl J Med* 372:793–795. doi:[10.1056/NEJMp1500523](https://doi.org/10.1056/NEJMp1500523)
5. Urbach A (2004) Modeling for Lesch–Nyhan disease by gene targeting in human embryonic stem cells. *Stem Cells* 22:635–641. doi:[10.1634/stemcells.22-4-635](https://doi.org/10.1634/stemcells.22-4-635)
6. Tulpule A, Daley GQ (2009) Efficient gene knockdowns in human embryonic stem cells using lentiviral-based RNAi. Patient-specific induced pluripotent stem cell models. Humana Press, Totowa, pp 35–42
7. Eiges R, Urbach A, Malcov M, Frumkin T, Schwartz T, Amit A, Yaron Y, Eden A, Yanuka O, Benvenisty N, Ben-Yosef D (2007) Developmental study of fragile X syndrome using human embryonic stem cells derived from preimplantation genetically diagnosed embryos. *Stem Cell* 1:568–577. doi:[10.1016/j.stem.2007.09.001](https://doi.org/10.1016/j.stem.2007.09.001)
8. Urbach A, Benvenisty N (2009) Studying early lethality of 45, XO (Turner’s syndrome) embryos using human embryonic stem

- cells. *PLoS One* 4:e4175–e4179. doi:[10.1371/journal.pone.0004175](https://doi.org/10.1371/journal.pone.0004175)
9. Bittles AH, Bower C, Hussain R, Glasson EJ (2007) The four ages of Down syndrome. *Eur J Public Health* 17:221–225. doi:[10.1093/eurpub/ckl1103](https://doi.org/10.1093/eurpub/ckl1103)
  10. Lengerke C, Daley GQ (2009) Disease models from pluripotent stem cells. *Ann N Y Acad Sci* 1176:191–196. doi:[10.1111/j.1749-6632.2009.04962.x](https://doi.org/10.1111/j.1749-6632.2009.04962.x)
  11. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920. doi:[10.1126/science.1151526](https://doi.org/10.1126/science.1151526)
  12. Bellin M, Marchetto MC, Gage FH, Mummery CL (2012) Induced pluripotent stem cells: the new patient? *Nat Rev Mol Cell Biol* 13:713–726. doi:[10.1038/nrm3448](https://doi.org/10.1038/nrm3448)
  13. Raab S, Klingenstein M, Liebau S, Linta L (2014) A comparative view on human somatic cell sources for iPSC generation. *Stem Cells Int* 2014:768391–768412. doi:[10.1155/2014/768391](https://doi.org/10.1155/2014/768391)
  14. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676. doi:[10.1016/j.cell.2006.07.024](https://doi.org/10.1016/j.cell.2006.07.024)
  15. Ban H, Nishishita N, Fusaki N, Tabata T, Saeki K, Shikamura M, Takada N, Inoue M, Hasegawa M, Kawamata S, Nishikawa SI (2011) Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proc Natl Acad Sci USA* 108:14234–14239. doi:[10.1073/pnas.1103509108](https://doi.org/10.1073/pnas.1103509108)
  16. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S (2008) Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 322:949–953. doi:[10.1126/science.1164270](https://doi.org/10.1126/science.1164270)
  17. Warren L, Manos PD, Ahfeldt T, Loh Y-H, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Stem Cell* 7:618–630. doi:[10.1016/j.stem.2010.08.012](https://doi.org/10.1016/j.stem.2010.08.012)
  18. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol* 26:795–797. doi:[10.1038/nbt1418](https://doi.org/10.1038/nbt1418)
  19. Loh YH, Agarwal S, Park IH, Urbach A, Huo H, Heffner GC, Kim K, Miller JD, Ng K, Daley GQ (2009) Generation of induced pluripotent stem cells from human blood. *Blood* 113:5476–5479. doi:[10.1182/blood-2009-02-204800](https://doi.org/10.1182/blood-2009-02-204800)
  20. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilić J, Pekarik V, Tiscornia G, Edel M, Boué S, Belmonte JCI (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 26:1276–1284. doi:[10.1038/nbt.1503](https://doi.org/10.1038/nbt.1503)
  21. Zhou T, Benda C, Duzinger S, Huang Y, Li X, Li Y, Guo X, Cao G, Chen S, Hao L, Chan YC, Ng KM, Cy Ho J, Wieser M, Wu J, Redl H, Tse HF, Grillari J, Grillari-Voglauer R, Pei D, Esteban MA (2011) Generation of induced pluripotent stem cells from urine. *J Am Soc Nephrol* 22:1221–1228. doi:[10.1681/ASN.2011010106](https://doi.org/10.1681/ASN.2011010106)
  22. Mummery CL, Zhang J, Ng ES, Elliott DA, Elefanty AG, Kamp TJ (2012) Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. *Circ Res* 111:344–358. doi:[10.1161/CIRCRESAHA.110.227512](https://doi.org/10.1161/CIRCRESAHA.110.227512)
  23. Kaufman MH, Navaratnam V (1981) Early differentiation of the heart in mouse embryos. *J Anat* 133:235–246. doi:[10.1111/\(ISSN\)1469-7580](https://doi.org/10.1111/(ISSN)1469-7580)
  24. Kirby ML, Waldo WIBKL (2002) Molecular embryogenesis of the heart. *Pediatr Dev Pathol* 5:516–543. doi:[10.1007/s10024-002-0004-2](https://doi.org/10.1007/s10024-002-0004-2)
  25. Abu-Issa R, Kirby ML (2007) Heart field: from mesoderm to heart tube. *Annu Rev Cell Dev Biol* 23:45–68. doi:[10.1146/annurev.cellbio.23.090506.123331](https://doi.org/10.1146/annurev.cellbio.23.090506.123331)
  26. Rao J, Pfeiffer MJ, Frank S, Adachi K, Piccini I, Quaranta R, Araúzo-Bravo M, Schwarz J, Schade D, Leidel S, Schöler HR, Seebohm G, Greber B (2015) Stepwise clearance of repressive roadblocks drives cardiac induction in human ESCs. *Cell Stem Cell* 18:1–14. doi:[10.1016/j.stem.2015.11.019](https://doi.org/10.1016/j.stem.2015.11.019)
  27. Willems E, Spiering S, Davidovics H, Lanier M, Xia Z, Dawson M, Cashman J, Mercola M (2011) Small-molecule inhibitors of the Wnt pathway potently promote cardiomyocytes from human embryonic stem cell-derived mesoderm. *Circ Res* 109:360–364. doi:[10.1161/CIRCRESAHA.111.249540](https://doi.org/10.1161/CIRCRESAHA.111.249540)
  28. Elliott DA, Braam SR, Koutsis K, Ng ES, Jenny R, Lagerqvist EL, Biben C, Hatzistavrou T, Hirst CE, Yu QC, Skelton RJP, Ward-van Oostwaard D, Lim SM, Khammy O, Li X, Hawes SM, Davis RP, Goulburn AL, Passier R, Prall OWJ, Haynes JM, Pouton CW, Kaye DM, Mummery CL, Elefanty AG, Stanley EG (2011) NKX2-5eGFP/w hESCs for isolation of human cardiac progenitors and cardiomyocytes. *Nat Methods* 8:1037–1040. doi:[10.1038/nmeth.1740](https://doi.org/10.1038/nmeth.1740)
  29. Karakikes I, Senyei GD, Hansen J, Kong CW, Azeloglu EU, Stillitano F, Lieu DK, Wang J, Ren L, Hulot JS, Iyengar R, Li RA, Hajjar RJ (2014) Small molecule-mediated directed differentiation of human embryonic stem cells toward ventricular cardiomyocytes. *Stem Cells Transl Med* 3:18–31. doi:[10.5966/sctm.2013-0110](https://doi.org/10.5966/sctm.2013-0110)
  30. Acimovic I, Vilotic A, Pesl M, Lacampagne A, Dvorak P, Rotrekl V, Meli AC (2014) Human pluripotent stem cell-derived cardiomyocytes as research and therapeutic tools. *Biomed Res Int* 2014:1–14. doi:[10.1155/2014/512831](https://doi.org/10.1155/2014/512831)
  31. Mummery C, Ward D, van den Brink CE, Bird SD, Doevendans PA, Ophof T, la Riviere de AB, Tertoolen L, van der Heyden M, Pera M (2002) Cardiomyocyte differentiation of mouse and human embryonic stem cells. *J Anat* 200:233–242. doi:[10.1046/j.1469-7580.2002.00031.x](https://doi.org/10.1046/j.1469-7580.2002.00031.x)
  32. BurrIDGE PW, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, Lan F, Diecke S, Huber B, Mordwinkin NM, Plews JR, Abilez OJ, Cui B, Gold JD, Wu JC (2014) Chemically defined generation of human cardiomyocytes. *Nat Methods* 11:855–860. doi:[10.1038/nmeth.2999](https://doi.org/10.1038/nmeth.2999)
  33. Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, Hashimoto H, Suzuki T, Yamashita H, Satoh Y, Egashira T, Seki T, Muraoka N, Yamakawa H, Ohgino Y, Tanaka T, Yoichi M, Yuasa S, Murata M, Suematsu M, Fukuda K (2013) Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. *Cell Stem Cell* 12(1):127–137. doi:[10.1016/j.stem.2012.09.013](https://doi.org/10.1016/j.stem.2012.09.013)
  34. Mathur A, Ma Z, Loskill P, Jeeawoody S, Healy KE (2015) In vitro cardiac tissue models: current status and future prospects. *Adv Drug Deliv Rev* 96:1–11. doi:[10.1016/j.addr.2015.09.011](https://doi.org/10.1016/j.addr.2015.09.011)
  35. Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanty AG, Gramolini A, Keller G (2011) SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. *Nat Biotechnol* 29:1011–1018. doi:[10.1038/nbt.2005](https://doi.org/10.1038/nbt.2005)
  36. Khan JM, Lyon AR, Harding SE (2013) The case for induced pluripotent stem cell-derived cardiomyocytes in pharmacological screening. *Br J Pharmacol* 169:304–317. doi:[10.1111/j.1476-5381.2012.02118.x](https://doi.org/10.1111/j.1476-5381.2012.02118.x)
  37. Mollova M, Bersell K, Walsh S, Savla J, Das LT, Park S-Y, Silberstein LE, dos Remedios CG, Graham D, Colan S, Kühn B (2013) Cardiomyocyte proliferation contributes to heart growth in young humans. *Proc Natl Acad Sci USA* 110:1446–1451. doi:[10.1073/pnas.1214608110](https://doi.org/10.1073/pnas.1214608110)

38. Bird S (2003) The human adult cardiomyocyte phenotype. *Cardiovasc Res* 58:423–434. doi:[10.1016/S0008-6363\(03\)00253-0](https://doi.org/10.1016/S0008-6363(03)00253-0)
39. Peters NS, Green CR, Poole-Wilson PA, Severs NJ (1993) Reduced content of connexin43 gap junctions in ventricular myocardium from hypertrophied and ischemic human hearts. *Circulation* 88:864–875. doi:[10.1161/01.cir.88.3.864](https://doi.org/10.1161/01.cir.88.3.864)
40. Snir M, Kehat I, Gepstein A, Coleman R, Itskovitz-Eldor J, Livne E, Gepstein L (2003) Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. *Am J Physiol Heart Circ Physiol* 285:H2355–H2363. doi:[10.1152/ajpheart.00020.2003](https://doi.org/10.1152/ajpheart.00020.2003)
41. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, Zhang B, Jiang J, Massé S, Gagliardi M, Hsieh A, Thavandiran N, Laflamme MA, Nanthakumar K, Gross GJ, Backx PH, Keller G, Radisic M (2013) Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat Methods* 10:781–787. doi:[10.1038/nmeth.2524](https://doi.org/10.1038/nmeth.2524)
42. Mannhardt I, Breckwoldt K, Letuffe-Brenière D, Schaaf S, Schulz H, Neuber C, Benzin A, Werner T, Eder A, Schulze T, Klampe B, Christ T, Hirt MN, Huebner N, Moretti A, Eschenhagen T, Hansen A (2016) Human engineered heart tissue: analysis of contractile force. *Stem Cell Rep* 7:29–42. doi:[10.1016/j.stemcr.2016.04.011](https://doi.org/10.1016/j.stemcr.2016.04.011)
43. Reiser PJ, Portman MA, Ning XH, Schomisch Moravec C (2001) Human cardiac myosin heavy chain isoforms in fetal and failing adult atria and ventricles. *Am J Physiol Heart Circ Physiol* 280:H1814–H1820. doi:[10.1152/ajpheart.00810.2013](https://doi.org/10.1152/ajpheart.00810.2013)
44. Bhavsar PK, Dhoot GK, Cumming D (1991) Developmental expression of troponin I isoforms in fetal human heart. *FEBS Lett*. doi:[10.1016/0014-5793\(91\)80820-S](https://doi.org/10.1016/0014-5793(91)80820-S)
45. Xu XQ, Soo SY, Sun W, Zweigerdt R (2009) Global expression profile of highly enriched cardiomyocytes derived from human embryonic stem cells. *Stem Cells* 27:2163–2174. doi:[10.1002/stem.166](https://doi.org/10.1002/stem.166)
46. Bedada FB, Chan SS-K, Metzger SK, Zhang L, Zhang J, Garry DJ, Kamp TJ, Kyba M, Metzger JM (2014) Acquisition of a quantitative, stoichiometrically conserved ratiometric marker of maturation status in stem cell-derived cardiac myocytes. *Stem Cell Rep* 3:594–605. doi:[10.1016/j.stemcr.2014.07.012](https://doi.org/10.1016/j.stemcr.2014.07.012)
47. Salick MR, Napiwocki BN, Sha J, Knight GT, Chindhy SA, Kamp TJ, Ashton RS, Crone WC (2014) Micropattern width dependent sarcomere development in human ESC-derived cardiomyocytes. *Biomaterials* 35:4454–4464. doi:[10.1016/j.biomaterials.2014.02.001](https://doi.org/10.1016/j.biomaterials.2014.02.001)
48. Pioner JM, Racca AW, Klaiman JM, Yang K-C, Guan X, Pabon L, Muskheli V, Zaunbrecher R, Macadangang J, Jeong MY, Mack DL, Childers MK, Kim D-H, Tesi C, Poggesi C, Murry CE, Regnier M (2016) Isolation and mechanical measurements of myofibrils from human induced pluripotent stem cell-derived cardiomyocytes. *Stem Cell Rep* 6:885–896. doi:[10.1016/j.stemcr.2016.04.006](https://doi.org/10.1016/j.stemcr.2016.04.006)
49. Spudich JA (2014) Hypertrophic and dilated cardiomyopathy: four decades of basic research on muscle lead to potential therapeutic approaches to these devastating genetic diseases. *Biophys J* 106:1236–1249. doi:[10.1016/j.bpj.2014.02.011](https://doi.org/10.1016/j.bpj.2014.02.011)
50. Birket MJ, Ribeiro MC, Kosmidis G, Ward D, Leitoguinho AR, van de Pol V, Dambrot C, Devalla HD, Davis RP, Mastroberardino PG, Atsma DE, Passier R, Mummery CL (2015) Contractile defect caused by mutation in MYBPC3 revealed under conditions optimized for human PSC-cardiomyocyte function. *Cell Rep* 13:733–745. doi:[10.1016/j.celrep.2015.09.025](https://doi.org/10.1016/j.celrep.2015.09.025)
51. Veerman CC, Kosmidis G, Mummery CL, Casini S, Verkerk AO, Bellin M (2015) Immaturity of human stem-cell-derived cardiomyocytes in culture: fatal flaw or soluble problem? *Stem Cells Dev* 24:1035–1052. doi:[10.1089/scd.2014.0533](https://doi.org/10.1089/scd.2014.0533)
52. Bers DM (2002) Cardiac excitation–contraction coupling. *Nature* 415:198–205. doi:[10.1038/415198a](https://doi.org/10.1038/415198a)
53. Hoekstra M, Mummery CL, Wilde AAM, Bezzina CR, Verkerk AO (2012) Induced pluripotent stem cell derived cardiomyocytes as models for cardiac arrhythmias. *Front Physiol*. doi:[10.3389/fphys.2012.00346](https://doi.org/10.3389/fphys.2012.00346)
54. Barbuti A, Benzoni P, Camprostrini G, Dell’Era P (2016) Human derived cardiomyocytes: a decade of knowledge after the discovery of induced pluripotent stem cells. *Dev Dyn* 245:1145–1158. doi:[10.1002/dvdy.24455](https://doi.org/10.1002/dvdy.24455)
55. Sinnecker D, Goedel A, Dorn T, Dirschinger RJ, Moretti A, Laugwitz KL (2012) Modeling long-QT syndromes with iPSC cells. *J Cardiovasc Transl Res* 6:31–36. doi:[10.1007/s12265-012-9416-1](https://doi.org/10.1007/s12265-012-9416-1)
56. Ferrantini C, Crocini C, Coppini R, Vanzi F, Tesi C, Cerbai E, Poggesi C, Pavone FS, Sacconi L (2013) The transverse-axial tubular system of cardiomyocytes. *Cell Mol Life Sci* 70:4695–4710. doi:[10.1007/s00018-013-1410-5](https://doi.org/10.1007/s00018-013-1410-5)
57. Fu J-D, Rushing SN, Lieu DK, Chan CW, Kong C-W, Geng L, Wilson KD, Chiamvimonvat N, Boheler KR, Wu JC, Keller G, Hajjar RJ, Li RA (2011) Distinct roles of microRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. *PLoS One* 6:e27417–e27515. doi:[10.1371/journal.pone.0027417](https://doi.org/10.1371/journal.pone.0027417)
58. Karakikes I, Ameen M, Termglinchan V, Wu JC (2015) Human induced pluripotent stem cell-derived cardiomyocytes: insights into molecular, cellular, and functional phenotypes. *Circ Res* 117:80–88. doi:[10.1161/CIRCRESAHA.117.305365](https://doi.org/10.1161/CIRCRESAHA.117.305365)
59. Pillekamp F, Hausteiner M, Khalil M, Emmelheinz M, Nazzari R, Adelman R, Nguemo F, Rubenzyk O, Pfannkuche K, Matzkies M, Reppel M, Bloch W, Brockmeier K, Hescheler J (2012) Contractile properties of early human embryonic stem cell-derived cardiomyocytes: beta-adrenergic stimulation induces positive chronotropy and lusitropy but not inotropy. *Stem Cells Dev* 21:2111–2121. doi:[10.1089/scd.2011.0312](https://doi.org/10.1089/scd.2011.0312)
60. Chang TD, Cumming GR (1972) Chronotropic responses of human heart tissue cultures. *Circ Res* 30:628–633. doi:[10.1161/01.res.30.6.628](https://doi.org/10.1161/01.res.30.6.628)
61. Brito-Martins M, Harding SE, Ali NN (2008)  $\beta$  1- and  $\beta$  2-adrenoceptor responses in cardiomyocytes derived from human embryonic stem cells: comparison with failing and non-failing adult human heart. *Br J Pharmacol* 153:751–759. doi:[10.1038/sj.bjph.0707619](https://doi.org/10.1038/sj.bjph.0707619)
62. Barth E (1992) Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. *J Mol Cell Cardiol* 24:669–681. doi:[10.1016/0022-2828\(92\)93381-S](https://doi.org/10.1016/0022-2828(92)93381-S)
63. Lopaschuk GD, Collins-Nakai RL, Itoi T (1992) Developmental changes in energy substrate use by the heart. *Cardiovasc Res* 26:1172–1180. doi:[10.1093/cvr/26.12.1172](https://doi.org/10.1093/cvr/26.12.1172)
64. Lopaschuk GD, Jaswal JS (2010) Energy metabolic phenotype of the cardiomyocyte during development, differentiation, and postnatal maturation. *J Cardiovasc Pharmacol* 56:130–140. doi:[10.1097/FJC.0b013e3181e74a14](https://doi.org/10.1097/FJC.0b013e3181e74a14)
65. Birket MJ, Casini S, Kosmidis G, Elliott DA, Gerencser AA, Baartscheer A, Schumacher C, Mastroberardino PG, Elefanti AG, Stanley EG, Mummery CL (2013) PGC-1  $\alpha$  and reactive oxygen species regulate human embryonic stem cell-derived cardiomyocyte function. *Stem Cell Rep* 1:560–574. doi:[10.1016/j.stemcr.2013.11.008](https://doi.org/10.1016/j.stemcr.2013.11.008)
66. Drawnel FM, Boccardo S, Prummer M, Delobel F, Graff A, Weber M, Gérard R, Badi L, Kam-Thong T, Bu L, Jiang X, Hoflack J-C, Kiiialainen A, Jeworutzki E, Aoyama N, Carlson C, Burcin M, Gromo G, Boehringer M, Stahlberg H, Hall BJ, Magnone MC, Kolaja K, Chien KR, Bailly J, Iacone R (2014) Disease modeling and phenotypic drug screening for diabetic



- cardiomyopathy using human induced pluripotent stem cells. *Cell Rep* 9:810–820. doi:[10.1016/j.celrep.2014.09.055](https://doi.org/10.1016/j.celrep.2014.09.055)
67. Kléber AG, Rudy Y (2004) Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiol Rev* 84:431–488. doi:[10.1152/physrev.00025.2003](https://doi.org/10.1152/physrev.00025.2003)
  68. Jansen JA, van Veen TAB, de Bakker JMT, van Rijen HVM (2010) *J Mol Cell Cardiol* 48:76–82. doi:[10.1016/j.yjmcc.2009.08.018](https://doi.org/10.1016/j.yjmcc.2009.08.018)
  69. Chen S-C, Davis LM, Westphale EM, Beyer EC, Saffitz JE (2006) Expression of multiple gap junction proteins in human fetal and infant hearts. *Pediatr Res* 36:1–6. doi:[10.1203/00006450-199411000-00002](https://doi.org/10.1203/00006450-199411000-00002)
  70. Wiegerinck RF (2006) Larger cell size in rabbits with heart failure increases myocardial conduction velocity and QRS duration. *Circulation* 113:806–813. doi:[10.1161/CIRCULATIONAHA.105.565804](https://doi.org/10.1161/CIRCULATIONAHA.105.565804)
  71. Vreeker A, van Stuijvenberg L, Hund TJ, Mohler PJ, Nikkels PGJ, van Veen TAB (2014) Assembly of the cardiac intercalated disk during pre- and postnatal development of the human heart. *PLoS One* 9:e94722–e94729. doi:[10.1371/journal.pone.0094722](https://doi.org/10.1371/journal.pone.0094722)
  72. Meijer van Putten RME, Mengarelli I, Guan K, Zegers JG, van Ginneken ACG, Verkerk AO, Wilders R (2015) Ion channelopathies in human induced pluripotent stem cell derived cardiomyocytes: a dynamic clamp study with virtual IK1. *Front Physiol* 6:4875. doi:[10.3389/fphys.2015.00007](https://doi.org/10.3389/fphys.2015.00007)
  73. Vaidyanathan R, Markandeya YS, Kamp TJ, Makielski JC, January CT, Eckhardt LL (2016) IK1-enhanced human-induced pluripotent stem cell-derived cardiomyocytes: an improved cardiomyocyte model to investigate inherited arrhythmia syndromes. *Am J Physiol Heart Circ Physiol* 310:H1611–H1621. doi:[10.1152/ajpheart.00481.2015](https://doi.org/10.1152/ajpheart.00481.2015)
  74. Veerman CC, Mengarelli I, Guan K, Stauske M, Barc J, Tan HL, Wilde AAM, Verkerk AO, Bezzina CR (2016) hiPSC-derived cardiomyocytes from Brugada syndrome patients without identified mutations do not exhibit clear cellular electrophysiological abnormalities. *Sci Rep* 6:1–10. doi:[10.1038/srep30967](https://doi.org/10.1038/srep30967)
  75. Rocchetti M, Sala L, Dreizehnter L, Crotti L, Sinnecker D, Mura M, Pane LS, Altomare C, Torre E, Mostacciolo G, Severi S, Porta A, De Ferrari GM, George AL Jr, Schwartz PJ, Gnechchi M, Moretti A, Zaza A (2017) Elucidating arrhythmogenic mechanisms of long-QT syndrome CALM1-F142L mutation in patient-specific induced pluripotent stem cell-derived cardiomyocytes. *Cardiovasc Res*. doi:[10.1093/cvr/cvx006](https://doi.org/10.1093/cvr/cvx006)
  76. Crotti L, Celano G, Dagradi F, Schwartz PJ (2008) Congenital long QT syndrome. *Orphanet J Rare Dis* 3:18–26. doi:[10.1186/1750-1172-3-18](https://doi.org/10.1186/1750-1172-3-18)
  77. Schwartz PJ, Periti M, Malliani A (1975) The long Q-T syndrome. *Am Heart J* 89:378–390. doi:[10.1016/0002-8703\(75\)90089-7](https://doi.org/10.1016/0002-8703(75)90089-7)
  78. Anton Jervell FL-N (1957) Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. *Am Heart J* 54:59–68. doi:[10.1016/0002-8703\(57\)90079-0](https://doi.org/10.1016/0002-8703(57)90079-0)
  79. Romano C, Gemme G, Pongiglione R (1963) Rare cardiac arrhythmias of the pediatric age. II. Syncopal attacks due to paroxysmal ventricular fibrillation. *La Clin Pediatr* 45:656–683
  80. Oc W (1964) A new familial cardiac syndrome in children. *J Ir Med Assoc* 54:103–106
  81. Schwartz P (2013) Practical issues in the management of the long QT syndrome: focus on diagnosis and therapy. *Swiss Med Wkly*. doi:[10.4414/smw.2013.13843](https://doi.org/10.4414/smw.2013.13843)
  82. Giudicessi JR, Ackerman MJ (2013) Arrhythmia risk in long QT syndrome: beyond the disease-causative mutation. *Circ Cardiovasc Genet* 6:313–316. doi:[10.1161/CIRCGENETICS.113.000260](https://doi.org/10.1161/CIRCGENETICS.113.000260)
  83. Morita H, Wu J, Zipes DP (2008) The QT syndromes: long and short. *Lancet* 372:750–763. doi:[10.1016/S0140-6736\(08\)61307-0](https://doi.org/10.1016/S0140-6736(08)61307-0)
  84. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, Dorn T, Goedel A, Höhnke C, Hofmann F, Seyfarth M, Sinnecker D, Schömig A, Laugwitz KL (2010) Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med* 363:1397–1409. doi:[10.1056/NEJMoa0908679](https://doi.org/10.1056/NEJMoa0908679)
  85. Wang Y, Liang P, Lan F, Wu H, Lisowski L, Gu M, Hu S, Kay MA, Urnov FD, Shinnawi R, Gold JD, Gepstein L, Wu JC (2014) Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. *J Am Coll Cardiol* 64:451–459. doi:[10.1016/j.jacc.2014.04.057](https://doi.org/10.1016/j.jacc.2014.04.057)
  86. Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, Wang Y, Diecke S, Sallam K, Knowles JW, Wang PJ, Nguyen PK, Bers DM, Robbins RC, Wu JC (2013) Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 127:1677–1691. doi:[10.1161/CIRCULATIONAHA.113.001883](https://doi.org/10.1161/CIRCULATIONAHA.113.001883)
  87. Egashira T, Yuasa S, Suzuki T, Aizawa Y, Yamakawa H, Matsuhashi T, Ohno Y, Tohyama S, Okata S, Seki T, Kuroda Y, Yae K, Hashimoto H, Tanaka T, Hattori F, Sato T, Miyoshi S, Takatsuki S, Murata M, Kurokawa J, Furukawa T, Makita N, Aiba T, Shimizu W, Horie M, Kamiya K, Kodama I, Ogawa S, Fukuda K (2012) Disease characterization using LQTS-specific induced pluripotent stem cells. *Cardiovasc Res* 95:419–429. doi:[10.1093/cvr/cvs206](https://doi.org/10.1093/cvr/cvs206)
  88. Ma D, Wei H, Lu J, Huang D, Liu Z, Loh LJ, Islam O, Liew R, Shim W, Cook SA (2015) Characterization of a novel KCNQ1 mutation for type 1 long QT syndrome and assessment of the therapeutic potential of a novel IKs activator using patient-specific induced pluripotent stem cell-derived cardiomyocytes. *Stem Cell Res Ther* 6:39. doi:[10.1186/s13287-015-0027-z](https://doi.org/10.1186/s13287-015-0027-z)
  89. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 80:795–803. doi:[10.1016/0092-8674\(95\)90358-5](https://doi.org/10.1016/0092-8674(95)90358-5)
  90. Matsa E, Dixon JE, Medway C, Georgiou O, Patel MJ, Morgan K, Kemp PJ, Staniforth A, Mellor I, Denning C (2014) Allele-specific RNA interference rescues the long-QT syndrome phenotype in human-induced pluripotency stem cell cardiomyocytes. *Eur Heart J* 35:1078–1087. doi:[10.1093/eurheartj/ehu067](https://doi.org/10.1093/eurheartj/ehu067)
  91. Matsa E, Rajamohan D, Dick E, Young L, Mellor I, Staniforth A, Denning C (2011) Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. *Eur Heart J* 32:952–962. doi:[10.1093/eurheartj/ehr073](https://doi.org/10.1093/eurheartj/ehr073)
  92. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos M, Gepstein L (2012) Modelling the long QT syndrome with induced pluripotent stem cells. *Nature* 471:225–229. doi:[10.1038/nature09747](https://doi.org/10.1038/nature09747)
  93. Lahti AL, Kujala VJ, Chapman H, Koivisto AP, Pekkanen-Mattila M, Kerkela E, Hyttinen J, Kontula K, Swan H, Conklin BR, Yamanaka S, Silvennoinen O, Aalto-Setälä K (2012) Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. *Dis Models Mech* 5:220–230. doi:[10.1242/dmm.008409](https://doi.org/10.1242/dmm.008409)
  94. Bellin M, Casini S, Davis RP, D'Aniello C, Haas J, Ward-van Oostwaard D, Tertoolen LGJ, Jung CB, Elliott DA, Welling A, Laugwitz KL, Moretti A, Mummery CL (2013) Isogenic human pluripotent stem cell pairs reveal the role of a KCNH2 mutation in long-QT syndrome. *EMBO J* 32:3161–3175. doi:[10.1038/emboj.2013.240](https://doi.org/10.1038/emboj.2013.240)



95. Mehta A, Sequiera GL, Ramachandra CJA, Sudibyo Y, Chung Y, Sheng J, Wong KY, Tan TH, Wong P, Liew R, Shim W (2014) Re-trafficking of hERG reverses long QT syndrome 2 phenotype in human iPSC-derived cardiomyocytes. *Cardiovasc Res* 102:497–506. doi:[10.1093/cvr/cvu060](https://doi.org/10.1093/cvr/cvu060)
96. Spencer CI, Baba S, Nakamura K, Hua EA, Sears MAF, Fu C-C, Zhang J, Balijepalli S, Tomoda K, Hayashi Y, Lizarraga P, Wojciak J, Scheinman MM, Aalto-Setälä K, Makielski JC, January CT, Healy KE, Kamp TJ, Yamanaka S, Conklin BR (2014) Calcium transients closely reflect prolonged action potentials in iPSC models of inherited cardiac arrhythmia. *Stem Cell Rep* 3:269–281. doi:[10.1016/j.stemcr.2014.06.003](https://doi.org/10.1016/j.stemcr.2014.06.003)
97. Jouni M, Si-Tayeb K, Es-Salah-Lamoureaux Z, Martin Latypova X, Champon B, Runcoat A, Charpentier F, Loussouarn G, Zibara K, Lemarchanda P, Gaborita N (2015) 0134: using cardiomyocytes differentiated from urine-derived hiPSCs to recapitulate electrophysiological characteristics of LQT2 syndrome. *Arch Cardiovasc Dis Suppl* 7:165–166. doi:[10.1016/S1878-6480\(15\)30097-5](https://doi.org/10.1016/S1878-6480(15)30097-5)
98. Caballero R, Utrilla RG, Amorós I, Matamoros M, Pérez-Hernández M, Tinaquero D, Alfayate S, Nieto-Marín P, Guerrero-Serna G, Liu Q-H, Ramos-Mondragón R, Ponce-Balbuena D, Herron T, Campbell KF, Filgueiras-Rama D, Peinado R, López-Sendón JL, Jalife J, Delpón E, Tamargo J (2017) Tbx20 controls the expression of the KCNH2 gene and of hERG channels. *Proc Natl Acad Sci USA* 114:E416–E425. doi:[10.1073/pnas.1612383114](https://doi.org/10.1073/pnas.1612383114)
99. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT (1995) SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 80:805–811. doi:[10.1016/0092-8674\(95\)90359-3](https://doi.org/10.1016/0092-8674(95)90359-3)
100. Ma D, Wei H, Zhao Y, Lu J, Li G, Sahib NBE, Tan TH, Wong KY, Shim W, Wong P, Cook SA, Liew R (2013) Modeling type 3 long QT syndrome with cardiomyocytes derived from patient-specific induced pluripotent stem cells. *Int J Cardiol* 168:5277–5286. doi:[10.1016/j.ijcard.2013.08.015](https://doi.org/10.1016/j.ijcard.2013.08.015)
101. Terrenoire C, Wang K, Chan Tung KW, Chung WK, Pass RH, Lu JT, Jean J-C, Omari A, Sampson KJ, Kotton DN, Keller G, Kass RS (2012) Induced pluripotent stem cells used to reveal drug actions in a long QT syndrome family with complex genetics. *J Gen Physiol* 141:61–72. doi:[10.1085/jgp.201210899](https://doi.org/10.1085/jgp.201210899)
102. Fatima A, Kaifeng S, Dittmann S, Xu G, Gupta MK, Linke M, Zechner U, Nguemo F, Milting H, Farr M, Hescheler J, Šarić T (2013) The disease-specific phenotype in cardiomyocytes derived from induced pluripotent stem cells of two long QT syndrome type 3 patients. *PLoS One* 8:e83005–e83011. doi:[10.1371/journal.pone.0083005](https://doi.org/10.1371/journal.pone.0083005)
103. Malan D, Zhang M, Stallmeyer B, Müller J, Fleischmann BK, Schulze-Bahr E, Sasse P, Greber B (2016) Human iPSC cell model of type 3 long QT syndrome recapitulates drug-based phenotype correction. *Basic Res Cardiol* 111:1–11. doi:[10.1007/s00395-016-0530-0](https://doi.org/10.1007/s00395-016-0530-0)
104. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, Napolitano C, Schwartz PJ, Joseph RM, Condouris K, Tager-Flusberg H, Priori SG, Sanguinetti MC, Keating MT (2004) CaV1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 119:19–31. doi:[10.1016/j.cell.2004.09.011](https://doi.org/10.1016/j.cell.2004.09.011)
105. Dell’Era P (2015) Cardiac disease modeling using induced pluripotent stem cell-derived human cardiomyocytes. *WJSC* 7:329–415. doi:[10.4252/wjsc.v7.i2.329](https://doi.org/10.4252/wjsc.v7.i2.329)
106. Shaw RM, Colecraft HM (2013) L-type calcium channel targeting and local signalling in cardiac myocytes. *Cardiovasc Res* 98:177–186. doi:[10.1093/cvr/cvt021](https://doi.org/10.1093/cvr/cvt021)
107. Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, Dolmetsch RE (2012) Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature* 471:230–234. doi:[10.1038/nature09855](https://doi.org/10.1038/nature09855)
108. Crotti L, Johnson CN, Graf E, De Ferrari GM, Cuneo BF, Ovod M, Papagiannis J, Feldkamp MD, Rathi SG, Kunic JD, Pedrazzini M, Wieland T, Lichtner P, Beckmann B-M, Clark T, Shaffer C, Benson DW, Käb S, Meitinger T, Strom TM, Chazin WJ, Schwartz PJ, George AL (2013) Calmodulin mutations associated with recurrent cardiac arrest in infants. *Circulation* 127:1009–1017. doi:[10.1161/CIRCULATIONAHA.112.001216](https://doi.org/10.1161/CIRCULATIONAHA.112.001216)
109. Nakano Y, Shimizu W (2016) Genetics of long-QT syndrome. *J Hum Genet* 61:51–55. doi:[10.1038/jhg.2015.74](https://doi.org/10.1038/jhg.2015.74)
110. Limpitkul WB, Dick IE, Tester DJ, Boczek NJ, Limphong P, Yang W, Choi MH, Babich J, DiSilvestre D, Kanter RJ, Tomaselli GF, Ackerman MJ, Yue DT (2017) A precision medicine approach to the rescue of function on malignant calmodulinopathic long-QT syndrome. *Circ Res* 120(1):39–48. doi:[10.1161/CIRCRESAHA.116.309283](https://doi.org/10.1161/CIRCRESAHA.116.309283)
111. Zhang M, D’Aniello C, Verkerk AO, Wrobel E, Frank S, Wardvan Oostwaard D, Piccini I, Freund C, Rao J, Seeböhm G, Atsma DE, Schulze-Bahr E, Mummery CL, Greber B, Bellin M (2014) Recessive cardiac phenotypes in induced pluripotent stem cell models of Jervell and Lange-Nielsen syndrome: disease mechanisms and pharmacological rescue. *Proc Natl Acad Sci USA* 111:E5383–E5392. doi:[10.1073/pnas.141953111](https://doi.org/10.1073/pnas.141953111)
112. Brugada P, Brugada J (1992) Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. *J Am Coll Cardiol* 20:1391–1396. doi:[10.1016/0735-1097\(92\)90253-J](https://doi.org/10.1016/0735-1097(92)90253-J)
113. Mizusawa Y, Wilde AAM (2012) Brugada syndrome. *Circ Arrhythm Electrophysiol* 5:606–616. doi:[10.1161/CIRCEP.111.964577](https://doi.org/10.1161/CIRCEP.111.964577)
114. Schwartz PJ, Crotti L, Insolia R (2012) Long-QT syndrome: from genetics to management. *Circ Arrhythm Electrophysiol* 5:868–877. doi:[10.1161/CIRCEP.111.962019](https://doi.org/10.1161/CIRCEP.111.962019)
115. Le Scouarnec S, Karakachoff M, Gourraud JB, Lindenbaum P, Bonnaud S, Portero V, Duboscq-Bidot L, Daumy X, Simonet F, Teusan R, Baron E, Violleau J, Persyn E, Bellanger L, Barc J, Chatel S, Martins R, Mabo P, Sacher F, Haissaguerre M, Kyndt F, Schmitt S, Bezieau S, Le Marec H, Dina C, Schott JJ, Probst V, Redon R (2015) Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome. *Hum Mol Genet* 24:2757–2763. doi:[10.1093/hmg/ddv036](https://doi.org/10.1093/hmg/ddv036)
116. Fernández-Falgueras A, Sarquella-Brugada G, Brugada J, Brugada R, Campuzano O (2017) Cardiac channelopathies and sudden death: recent clinical and genetic advances. *Biology* 6:7. doi:[10.3390/biology6010007](https://doi.org/10.3390/biology6010007)
117. Brugada R, Campuzano O, Sarquella-Brugada G, Brugada J, Brugada P (2014) Brugada syndrome. *Methodist DeBakey Cardiovasc J* 10:25–28. doi:[10.14797/mdcj-10-1-25](https://doi.org/10.14797/mdcj-10-1-25)
118. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van Der Hout AH, Mannens MM, Wilde AA (1999) A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ Res* 85:1206–1213. doi:[10.1161/01.res.85.12.1206](https://doi.org/10.1161/01.res.85.12.1206)
119. van Den Berg MP, Wilde AA, Viersma TJW, Brouwer J, Haaksma J, van der Hout AH, Stolte-Dijkstra I, Bezzina TCR, Van Langen IM, Beaufort-Krol GC, Cornel JH, Crijns HJ (2001) Possible Bradycardic mode of death and successful pacemaker treatment in a large family with features of long QT syndrome type 3 and Brugada syndrome. *J Cardiovasc Electrophysiol* 12:630–636. doi:[10.1046/j.1540-8167.2001.00630.x](https://doi.org/10.1046/j.1540-8167.2001.00630.x)
120. Davis RP, Casini S, van den Berg CW, Hoekstra M, Remme CA, Dambrot C, Salvatori D, Oostwaard DWV, Wilde AAM, Bezzina CR, Verkerk AO, Freund C, Mummery CL (2012)

- Cardiomyocytes derived from pluripotent stem cells recapitulate electrophysiological characteristics of an overlap syndrome of cardiac sodium channel disease. *Circulation* 125:3079–3091. doi:[10.1161/CIRCULATIONAHA.111.066092](https://doi.org/10.1161/CIRCULATIONAHA.111.066092)
121. Okata S, Yuasa S, Suzuki T, Ito S, Makita N, Yoshida T, Li M, Kurokawa J, Seki T, Egashira T, Aizawa Y, Kodaira M, Motoda C, Yozu G, Shimojima M, Hayashiji N, Hashimoto H, Kuroda Y, Tanaka A, Murata M, Aiba T, Shimizu W, Horie M, Kamiya K, Furukawa T, Fukuda K (2016) Embryonic type Na(+) channel  $\beta$ -subunit, SCN3B masks the disease phenotype of Brugada syndrome. *Sci Rep* 6:34198. doi:[10.1038/srep34198](https://doi.org/10.1038/srep34198)
  122. Liang P, Sallam K, Wu H, Li Y, Itzhaki I, Garg P, Zhang Y, Vermglinchan V, Lan F, Gu M, Gong T, Zhuge Y, He C, Ebert AD, Sanchez-Freire V, Churko J, Hu S, Sharma A, Lam CK, Scheinman MM, Bers DM, Wu JC (2016) Patient-specific and genome-edited induced pluripotent stem cell-derived cardiomyocytes elucidate single-cell phenotype of Brugada syndrome. *J Am Coll Cardiol* 68:2086–2096. doi:[10.1016/j.jacc.2016.07.779](https://doi.org/10.1016/j.jacc.2016.07.779)
  123. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P (1995) Catecholaminergic polymorphic ventricular tachycardia in children: a 7-year follow-up of 21 patients. *Circulation* 91:1512–1519. doi:[10.1161/01.CIR.91.5.1512](https://doi.org/10.1161/01.CIR.91.5.1512)
  124. Priori SG, Chen SRW (2011) Inherited dysfunction of sarcoplasmic reticulum Ca<sup>2+</sup> handling and arrhythmogenesis. *Circ Res* 108:871–883. doi:[10.1161/CIRCRESAHA.110.226845](https://doi.org/10.1161/CIRCRESAHA.110.226845)
  125. Beard NA, Laver DR, Dulhunty AF (2004) Calsequestrin and the calcium release channel of skeletal and cardiac muscle. *Prog Biophys Mol Biol* 85:33–69. doi:[10.1016/j.pbiomolbio.2003.07.001](https://doi.org/10.1016/j.pbiomolbio.2003.07.001)
  126. Fatima A, Xu G, Shao K, Papadopoulos S, Lehmann M, Arnáiz-Cot JJ, Rosa AO, Nguemo F, Matzkies M, Dittmann S, Stone SL, Linke M, Zechner U, Beyer V, Hennies HC, Rosenkranz S, Klauke B, Parwani AS, Haverkamp W, Pfitzer G, Farr M, Cleemann L, Morad M, Milting H, Hescheler J, Šarić T (2011) In vitro modeling of ryanodine receptor 2 dysfunction using human induced pluripotent stem cells. *Cell Physiol Biochem* 28:579–592. doi:[10.1159/000335753](https://doi.org/10.1159/000335753)
  127. Jung CB, Moretti A, Mederos y Schnitzler M, Iop L, Storch U, Bellin M, Dorn T, Ruppenthal S, Pfeiffer S, Goedel A, Dirschinger RJ, Seyfarth M, Lam JT, Sinnecker D, Gudermann T, Lipp P, Laugwitz KL (2012) Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. *EMBO Mol Med* 4:180–191. doi:[10.1002/emmm.201100194](https://doi.org/10.1002/emmm.201100194)
  128. Penttinen K, Swan H, Vanninen S, Paavola J, Lahtinen AM, Kontula K, Aalto-Setälä K (2015) Antiarrhythmic effects of dantrolene in patients with catecholaminergic polymorphic ventricular tachycardia and replication of the responses using iPSC models. *PLoS One* 10:e0125366. doi:[10.1371/journal.pone.0125366](https://doi.org/10.1371/journal.pone.0125366)
  129. Di Pasquale E, Lodola F, Miragoli M, Denegri M, Avelino-Cruz JE, Buonocore M, Nakahama H, Portararo P, Bloise R, Napolitano C, Condorelli G, Priori SG (2013) CaMKII inhibition rectifies arrhythmic phenotype in a patient-specific model of catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis* 4:e843–e911. doi:[10.1038/cddis.2013.369](https://doi.org/10.1038/cddis.2013.369)
  130. Preininger MK, Jha R, Maxwell JT, Wu Q, Singh M, Wang B, Dalal A, Mceachin ZT, Rossoll W, Hales CM, Fischbach PS, Wagner MB, Xu C (2016) A human pluripotent stem cell model of catecholaminergic polymorphic ventricular tachycardia recapitulates patient-specific drug responses. *Dis Models Mech* 9:dmm.026823–dmm.026838. doi:[10.1242/dmm.026823](https://doi.org/10.1242/dmm.026823)
  131. Sasaki K, Makiyama T, Yoshida Y, Wuriyanghai Y, Kamakura T, Nishiuchi S, Hayano M, Harita T, Yamamoto Y, Kohjitani H, Hirose S, Chen J, Kawamura M, Ohno S, Itoh H, Takeuchi A, Matsuoka S, Miura M, Sumitomo N, Horie M, Yamanaka S, Kimura T (2016) Patient-specific human induced pluripotent stem cell model assessed with electrical pacing validates S107 as a potential therapeutic agent for catecholaminergic polymorphic ventricular tachycardia. *PLoS One* 11:e0164795. doi:[10.1371/journal.pone.0164795](https://doi.org/10.1371/journal.pone.0164795)
  132. Lodola F, Morone D, Denegri M, Bongianino R, Nakahama H, Rutigliano L, Gosetti R, Rizzo G, Vollero A, Buonocore M, Napolitano C, Condorelli G, Priori SG, Di Pasquale E (2016) Adeno-associated virus-mediated CASQ2 delivery rescues phenotypic alterations in a patient-specific model of recessive catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis* 7:e2393. doi:[10.1038/cddis.2016.304](https://doi.org/10.1038/cddis.2016.304)
  133. Devalla HD, Gélinas R, Aburawi EH, Beqqali A, Goyette P, Freund C, Chaix M-A, Tadros R, Jiang H, Le Béché C, Monshouwer-Kloots JJ, Zwetsloot T, Kosmidis G, Latour F, Alikashani A, Hoekstra M, Schlaepfer J, Mummery CL, Stevenson B, Kutalik Z, de Vries AA, Rivard L, Wilde AA, Talajic M, Verkerk AO, Al-Gazali L, Rioux JD, Bhuiyan ZA, Passier R (2016) TECRL, a new life-threatening inherited arrhythmia gene associated with overlapping clinical features of both LQTS and CPVT. *EMBO Mol Med* 8:1390–1408. doi:[10.15252/emmm.201505719](https://doi.org/10.15252/emmm.201505719)
  134. Bione S, D'Adamo P, Maestrini E, Gedeon AK, Bolhuis PA, Toniolo D (1996) A novel X-linked gene, G4.5, is responsible for Barth syndrome. *Nat Genet* 12:385–389. doi:[10.1038/ng0496-385](https://doi.org/10.1038/ng0496-385)
  135. Houtkooper RH, Turkenburg M, Poll-The BT, Karall D, Pérez-Cerdá C, Morrone A, Malvagia S, Wanders RJ, Kulik W, Vaz FM (2009) The enigmatic role of tafazzin in cardiolipin metabolism. *BBA—Biomembr* 1788:2003–2014. doi:[10.1016/j.bbamem.2009.07.009](https://doi.org/10.1016/j.bbamem.2009.07.009)
  136. Dudek J, Cheng I-F, Balleininger M, Vaz FM, Streckfuss-Bömeke K, Hübscher D, Vukotic M, Wanders RJA, Rehling P, Guan K (2013) Cardiolipin deficiency affects respiratory chain function and organization in an induced pluripotent stem cell model of Barth syndrome. *Stem Cell Res* 11:806–819. doi:[10.1016/j.scr.2013.05.005](https://doi.org/10.1016/j.scr.2013.05.005)
  137. Wang G, McCain ML, Yang L, He A, Pasqualini FS, Agarwal A, Yuan H, Jiang D, Zhang D, Zangi L, Geva J, Roberts AE, Ma Q, Ding J, Chen J, Wang D-Z, Li K, Wang J, Wanders RJA, Kulik W, Vaz FM, Laflamme MA, Murry CE, Chien KR, Kelley RI, Church GM, Parker KK, Pu WT (2014) Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. *Nat Med* 20:616–623. doi:[10.1038/nm.3545](https://doi.org/10.1038/nm.3545)
  138. Gorlin RJ, Anderson RC, Moller JH (1971) The leopard (multiple lentiginos) syndrome revisited. *Laryngoscope* 81:1674–1681. doi:[10.1288/00005537-197110000-00015](https://doi.org/10.1288/00005537-197110000-00015)
  139. Sarkozy A, Digilio M, Dallapiccola B (2008) Leopard syndrome. *Orphanet J Rare Dis* 3:13–18. doi:[10.1186/1750-1172-3-13](https://doi.org/10.1186/1750-1172-3-13)
  140. Carvajal-Vergara X, Sevilla A, D'Souza SL, Ang Y-S, Schaniel C, Lee D-F, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B, Waghray A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR (2010) Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature* 465:808–812. doi:[10.1038/nature09005](https://doi.org/10.1038/nature09005)
  141. Basso C, Corrado D, Marcus FI, Nava A, Thiene G (2009) Seminar arrhythmogenic right ventricular cardiomyopathy. *Lancet* 373:1289–1300. doi:[10.1016/S0140-6736\(09\)60256-7](https://doi.org/10.1016/S0140-6736(09)60256-7)
  142. Sen-Chowdhry S, Syrris P, Ward D, Asimaki A, Sevdalis E, McKenna WJ (2007) Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease

- expression. *Circulation* 115:1710–1720. doi:[10.1161/CIRCULATIONAHA.106.660241](https://doi.org/10.1161/CIRCULATIONAHA.106.660241)
143. Garrod D, Chidgey M (2008) Desmosome structure, composition and function. *Biochim Biophys Acta (BBA)—Biomembr* 1778:572–587. doi:[10.1016/j.bbamem.2007.07.014](https://doi.org/10.1016/j.bbamem.2007.07.014)
  144. Ma D, Wei H, Lu J, Ho S, Zhang G, Sun X, Oh Y, Tan SH, Ng ML, Shim W, Wong P, Liew R (2013) Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 34:1122–1133. doi:[10.1093/eurheartj/ehs226](https://doi.org/10.1093/eurheartj/ehs226)
  145. Caspi O, Huber I, Gepstein A, Arbel G, Maizels L, Boulos M, Gepstein L (2013) Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. *Circ Cardiovasc Genet* 6:557–568. doi:[10.1161/CIRCGENETICS.113.000188](https://doi.org/10.1161/CIRCGENETICS.113.000188)
  146. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen H-SV (2014) Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 494:105–110. doi:[10.1038/nature11799](https://doi.org/10.1038/nature11799)
  147. Rielete ASJM, Agullo-Pascual E, James CA, Leo-Macias A, Cerrone M, Zhang M, Lin X, Lin B, Sobreira NL, Amat-Alarcon N, Marsman RF, Murray B, Tichnell C, van der Heijden JF, Dooijes D, van Veen TAB, Tandri H, Fowler SJ, Hauer RNW, Tomaselli G, van den Berg MP, Taylor MRG, Brun F, Sinagra G, Wilde AAM, Mestroni L, Bezzina CR, Calkins H, Peter van Tintelen J, Bu L, Delmar M, Judge DP (2017) Multilevel analyses of SCN5A mutations in arrhythmogenic right ventricular dysplasia/cardiomyopathy suggest non-canonical mechanisms for disease pathogenesis. *Cardiovasc Res* 113:102–111. doi:[10.1093/cvr/cvw234](https://doi.org/10.1093/cvr/cvw234)
  148. Ku L (2003) Familial dilated cardiomyopathy. *Circulation* 108:118e–121e. doi:[10.1161/01.CIR.0000097493.70422.50](https://doi.org/10.1161/01.CIR.0000097493.70422.50)
  149. Kimura A (2015) Molecular genetics and pathogenesis of cardiomyopathy. *J Hum Genet* 61:41–50. doi:[10.1038/jhg.2015.83](https://doi.org/10.1038/jhg.2015.83)
  150. Parks SB, Kushner JD, Nauman D, Burgess D, Ludwigsen S, Peterson A, Li D, Jakobs P, Litt M, Porter CB, Rahko PS, Hershberger RE (2008) Lamin A/C mutation analysis in a cohort of 324 unrelated patients with idiopathic or familial dilated cardiomyopathy. *Am Heart J* 156:161–169. doi:[10.1016/j.ahj.2008.01.026](https://doi.org/10.1016/j.ahj.2008.01.026)
  151. Siu C-W, Lee Y-K, Ho JC-Y, Lai W-H, Chan Y-C, Ng K-M, Wong L-Y, Au K-W, Lau Y-M, Zhang J, Lay KW, Colman A, Tse H-F (2012) Modeling of lamin A/C mutation premature cardiac aging using patient-specific induced pluripotent stem cells. *Aging* 4:803–822. doi:[10.18632/aging.100503](https://doi.org/10.18632/aging.100503)
  152. Sun N, Yazawa M, Liu J, Han L, Sanchez-Freire V, Abilez OJ, Navarrete EG, Hu S, Wang L, Lee A, Pavlovic A, Lin S, Chen R, Hajjar RJ, Snyder MP, Dolmetsch RE, Butte MJ, Ashley EA, Longaker MT, Robbins RC, Wu JC (2012) Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. *Sci Transl Med* 4:130ra47. doi:[10.1126/scitranslmed.3003552](https://doi.org/10.1126/scitranslmed.3003552)
  153. Wu H, Lee J, Vincent LG, Wang Q, Gu M, Lan F, Churko JM, Sallam KI, Matsa E, Sharma A, Gold JD, Engler AJ, Xiang YK, Bers DM, Wu JC (2015) Epigenetic regulation of phosphodiesterases 2A and 3A underlies compromised  $\beta$ ; -adrenergic signaling in an iPSC model of dilated cardiomyopathy. *Stem Cell* 17:89–100. doi:[10.1016/j.stem.2015.04.020](https://doi.org/10.1016/j.stem.2015.04.020)
  154. Tse HF, Ho JCY, Choi SW, Lee YK, Butler AW, Ng KM, Siu CW, Simpson MA, Lai WH, Chan YC, Au KW, Zhang J, Lay KWJ, Esteban MA, Nicholls JM, Colman A, Sham PC (2013) Patient-specific induced-pluripotent stem cells-derived cardiomyocytes recapitulate the pathogenic phenotypes of dilated cardiomyopathy due to a novel DES mutation identified by whole exome sequencing. *Hum Mol Genet* 22:1395–1403. doi:[10.1093/hmg/ddt556](https://doi.org/10.1093/hmg/ddt556)
  155. Goldfarb LG, Dalakas MC (2009) Tragedy in a heartbeat: malfunctioning desmin causes skeletal and cardiac muscle disease. *J Clin Invest* 119:1806–1813. doi:[10.1172/JCI38027](https://doi.org/10.1172/JCI38027)
  156. Hinson JT, Chopra A, Nafissi N, Polacheck WJ (2015) Titin mutations in iPSC cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. *Science* 349:982–986. doi:[10.1126/science.aaa5458](https://doi.org/10.1126/science.aaa5458)
  157. Wyles SP, Li X, Hrstka SC, Reyes S, Oommen S, Beraldi R, Edwards J, Terzic A, Olson TM, Nelson TJ (2016) Modeling structural and functional deficiencies of RBM20 familial dilated cardiomyopathy using human induced pluripotent stem cells. *Hum Mol Genet* 25:254–265. doi:[10.1093/hmg/ddv468](https://doi.org/10.1093/hmg/ddv468)
  158. Kebed KY, Bos JM, Anavekar NS (2015) Hypertrophic cardiomyopathy, athlete's heart, or both a case of hypertrophic cardiomyopathy regression. *Circulation*. doi:[10.1016/j.jacc.2014.05.003](https://doi.org/10.1016/j.jacc.2014.05.003)
  159. Lan F, Lee AS, Liang P, Sanchez-Freire V, Nguyen PK, Wang L, Han L, Yen M, Wang Y, Sun N, Abilez OJ, Hu S, Ebert AD, Navarrete EG, Simmons CS, Wheeler M, Pruitt B, Lewis R, Yamaguchi Y, Ashley EA, Bers DM, Robbins RC, Longaker MT, Wu JC (2013) Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Stem Cell* 12:101–113. doi:[10.1016/j.stem.2012.10.010](https://doi.org/10.1016/j.stem.2012.10.010)
  160. Han L, Li Y, Tchao J, Kaplan AD, Lin B, Li Y, Mich-Basso J, Lis A, Hassan N, London B, Bett GCL, Tobita K, Rasmuson RL, Yang L (2014) Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. *Cardiovasc Res* 104:258–269. doi:[10.1093/cvr/cvu205](https://doi.org/10.1093/cvr/cvu205)
  161. Tanaka A, Yuasa S, Mearini G, Egashira T, Seki T, Kodaira M, Kusumoto D, Kuroda Y, Okata S, Suzuki T, Inohara T, Arimura T, Makino S, Kimura K, Kimura A, Furukawa T, Carrier L, Node K, Fukuda K (2014) Endothelin-1 induces myofibrillar disarray and contractile vector variability in hypertrophic cardiomyopathy-induced pluripotent stem cell-derived cardiomyocytes. *J Am Heart Assoc* 3:e001263–e001264. doi:[10.1161/JAHA.114.001263](https://doi.org/10.1161/JAHA.114.001263)
  162. Konno T, Chang S, Seidman JG, Seidman CE (2010) Genetics of hypertrophic cardiomyopathy. *Curr Opin Cardiol* 25:205–209. doi:[10.1097/HCO.0b013e3283375698](https://doi.org/10.1097/HCO.0b013e3283375698)
  163. Cashman TJ, Josowitz R, Johnson BV, Gelb BD, Costa KD (2016) Human engineered cardiac tissues created using induced pluripotent stem cells reveal functional characteristics of BRAF-mediated hypertrophic cardiomyopathy. *PLoS One* 11:e0146697. doi:[10.1371/journal.pone.0146697](https://doi.org/10.1371/journal.pone.0146697)
  164. Josowitz R, Mulero-Navarro S, Rodriguez NA, Falce C, Cohen N, Ullian EM, Weiss LA, Rauen KA, Sobie EA, Gelb BD (2016) Autonomous and non-autonomous defects underlie hypertrophic cardiomyopathy in BRAF-mutant hiPSC-derived cardiomyocytes. *Stem Cell Rep* 7:355–369. doi:[10.1016/j.stemcr.2016.07.018](https://doi.org/10.1016/j.stemcr.2016.07.018)
  165. Almomani R, Verhagen JMA, Herkert JC, Brosens E, van Spaendonck-Zwarts KY, Asimaki A, van der Zwaag PA, Frohn-Mulder IME, Bertoli-Avella AM, Boven LG, van Slegtenhorst MA, van der Smagt JJ, van Ijcken WFJ, Timmer B, van Stuijvenberg M, Verdijk RM, Saffitz JE, Plessis du FA, Michels M, Hofstra RMW, Sinke RJ, van Tintelen JP, Wessels MW, Jongbloed JDH, van de Laar IMBH (2016) Biallelic truncating mutations in ALPK3 cause severe pediatric cardiomyopathy. *J Am Coll Cardiol* 67:515–525. doi:[10.1016/j.jacc.2015.10.093](https://doi.org/10.1016/j.jacc.2015.10.093)
  166. Phelan DG, Anderson DJ, Howden SE, Wong RCB, Hickey PF, Pope K, Wilson GR, Pébay A, Davis AM, Petrou S, Elefanti AG, Stanley EG, James PA, Macciocia I, Bahlo M, Cheung MM, Amor DJ, Elliott DA, Lockhart PJ (2016) ALPK3-deficient



- cardiomyocytes generated from patient-derived induced pluripotent stem cells and mutant human embryonic stem cells display abnormal calcium handling and establish that ALPK3 deficiency underlies familial cardiomyopathy. *Eur Heart J* 37:2586–2590. doi:10.1093/eurheartj/ehw160
167. Lang T, Yu L, Tu Q, Jiang J, Chen Z, Xin Y, Liu G, Zhao S (2000) Molecular cloning, genomic organization, and mapping of PRKAG2, a heart abundant  $\gamma$ 2 subunit of 5'-AMP-activated protein kinase, to human chromosome 7q36. *Genomics* 70:258–263. doi:10.1006/geno.2000.6376
  168. Hinson JT, Chopra A, Lowe A, Sheng CC, Gupta RM, Kuppusamy R, O'Sullivan J, Rowe G, Wakimoto H, Gorham J, Zhang K, Musunuru K, Gerszten RE, Wu SM, Chen CS, Seidman JG, Seidman CE (2016) Integrative analysis of PRKAG2 cardiomyopathy iPS and microtissue models identifies AMPK as a regulator of metabolism, survival, and fibrosis. *Cell Rep* 17:3292–3304. doi:10.1016/j.celrep.2016.11.066
  169. Jiang Y, Habibollah S, Tilgner K, Collin J, Barta T, Al-Aama JY, Tesarov L, Hussain R, Trafford AW, Kirkwood G, Sernagor E, Eleftheriou CG, Przyborski S, Stojkovi M, Lako M, Keavney B, Armstrong L (2014) An induced pluripotent stem cell model of hypoplastic left heart syndrome (HLHS) reveals multiple expression and functional differences in HLHS-derived cardiac myocytes. *Stem Cells Transl Med* 3:416–423. doi:10.5966/sctm.2013-0105
  170. Harh JY, Paul MH, Gallen WJ, Friedberg DZ, Kaplan S (1973) Experimental production of hypoplastic left heart syndrome in the chick embryo. *Am J Cardiol* 31:51–56. doi:10.1016/0002-9149(73)90810-2
  171. deAlmeida A, McQuinn T, Sedmera D (2007) Increased ventricular preload is compensated by myocyte proliferation in normal and hypoplastic fetal chick left ventricle. *Circ Res* 100:1363–1370. doi:10.1161/01.RES.0000266606.88463.cb
  172. Hinton RB Jr, Martin LJ, Tabangin ME, Mazwi ML, Cripe LH, Benson DW (2007) Hypoplastic left heart syndrome is heritable. *J Am Coll Cardiol* 50:1590–1595. doi:10.1016/j.jacc.2007.07.021
  173. Iascone M, Ciccone R, Galletti L, Marchetti D, Seddio F, Lincesso AR, Pezzoli L, Vetro A, Barachetti D, Boni L, Federici D, Soto AM, Comas JV, Ferrazzi P, Zuffardi O (2011) Identification of de novo mutations and rare variants in hypoplastic left heart syndrome. *Clin Genet* 81:542–554. doi:10.1111/j.1399-0004.2011.01674.x
  174. Hori A, Yoshida M, Shibata T, Ling F (2009) Reactive oxygen species regulate DNA copy number in isolated yeast mitochondria by triggering recombination-mediated replication. *Nucleic Acids Res* 37:749–761. doi:10.1093/nar/gkn993
  175. Chen K, Kirber MT, Xiao H, Yang Y, Keaney JF Jr (2008) Regulation of ROS signal transduction by NADPH oxidase 4 localization. *J Cell Biol* 181:1129–1139. doi:10.1083/jcb.200709049
  176. Dickinson BC, Chang CJ (2011) Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol* 7:504–511. doi:10.1038/nchembio.607
  177. Lucas MM, Van de Sype G, Hérouart D, Hernández MJ, Puppo A, de Felipe MR (1998) Immunolocalization of ferritin in determinate and indeterminate legume root nodules. *Protoplasma* 204:61–70. doi:10.1007/BF01282294
  178. Madamanchi NR, Runge MS (2013) Redox signaling in cardiovascular health and disease. *Free Radic Biol Med* 61:473–501. doi:10.1016/j.freeradbiomed.2013.04.001
  179. Bellin M, Mummery CL (2016) Inherited heart disease—what can we expect from the second decade of human iPS cell research? *FEBS Lett* 590:2482–2493. doi:10.1002/1873-3468.12285
  180. Guo Y-J, Chen L, Bai Y-P, Li L, Sun J, Zhang G-G, Yang T-L, Xia J, Li Y-J, Chen X-P (2010) The ALDH2 Glu504Lys polymorphism is associated with coronary artery disease in Han Chinese: relation with endothelial ADMA levels. *Atherosclerosis* 211:545–550. doi:10.1016/j.atherosclerosis.2010.03.030
  181. Takagi S, Iwai N, Yamauchi R, Kojima S, Yasuno S, Baba T, Terashima M, Tsutsumi Y, Suzuki S, Morii I, Hanai S, Ono K, Baba S, Tomoike H, Kawamura A, Miyazaki S, Nonogi H, Goto Y (2002) Aldehyde dehydrogenase 2 gene is a risk factor for myocardial infarction in Japanese men. *Hypertens Res* 25(5):677–681. doi:10.1291/hyres.25.677
  182. Takeuchi F, Yokota M, Yamamoto K, Nakashima E, Katsuya T, Asano H, Isono M, Nabika T, Sugiyama T, Fujioka A, Awata N, Ohnaka K, Nakatomi M, Kitajima H, Rakugi H, Nakamura J, Ohkubo T, Imai Y, Shimamoto K, Yamori Y, Yamaguchi S, Kobayashi S, Takayanagi R, Ogiwara T, Kato N (2011) Genome-wide association study of coronary artery disease in the Japanese. *Eur J Hum Genet* 20:333–340. doi:10.1038/ejhg.2011.184
  183. Zhang Y, Babcock SA, Hu N, Maris JR, Wang H, Ren J (2012) Mitochondrial aldehyde dehydrogenase (ALDH2) protects against streptozotocin-induced diabetic cardiomyopathy: role of GSK3 $\beta$  and mitochondrial function. *BMC Med* 10:1–17. doi:10.1186/1741-7015-10-40
  184. Ebert AD, Kodo K, Liang P, Wu H, Huber BC, Riegler J, Churko J, Lee J, de Almeida P, Lan F, Diecke S, Burrridge PW, Gold JD, Mochly-Rosen D, Wu JC (2014) Characterization of the molecular mechanisms underlying increased ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism using a human induced pluripotent stem cell model system. *Sci Transl Med* 6:255. doi:10.1126/scitranslmed.3009027
  185. Hers HG (2005)  $\alpha$ -Glucosidase deficiency in generalized glycogen-storage disease (Pompe's disease). *Biochem J* 86:1–6. doi:10.1042/bj0860011
  186. Beratis NG, LaBadie GU, Hirschhorn K (1978) Characterization of the molecular defect in infantile and adult acid alpha-glucosidase deficiency fibroblasts. *J Clin Invest* 62:1264–1274. doi:10.1172/JCI109247
  187. Thurberg BL, Lynch Maloney C, Vaccaro C, Afonso K, Tsai AC-H, Bossen E, Kishnani PS, O'Callaghan M (2006) Characterization of pre- and post-treatment pathology after enzyme replacement therapy for pompe disease. *Lab Invest* 86:1208–1220. doi:10.1038/labinvest.3700484
  188. Kishnani PS, Howell RR (2004) Pompe disease in infants and children. *J Pediatr* 144:S35–S43. doi:10.1016/j.jpeds.2004.01.053
  189. Huang HP, Chen PH, Hwu WL, Chuang CY, Chien YH, Stone L, Chien CL, Li LT, Chiang SC, Chen HF, Ho HN, Chen CH, Kuo HC (2011) Human Pompe disease-induced pluripotent stem cells for pathogenesis modeling, drug testing and disease marker identification. *Hum Mol Genet* 20:4851–4864. doi:10.1093/hmg/ddr424
  190. Raval KK, Tao R, White BE, De Lange WJ, Koonce CH, Yu J, Kishnani PS, Thomson JA, Mosher DF, Ralphe JC, Kamp TJ (2015) Pompe disease results in a Golgi-based glycosylation deficit in human induced pluripotent stem cell-derived cardiomyocytes. *J Biol Chem* 290:3121–3136. doi:10.1074/jbc.M114.628628
  191. Forsha D, Li JS, Smith PB, van der Ploeg AT, Kishnani P, Pasquali SK (2011) Cardiovascular abnormalities in late-onset Pompe disease and response to enzyme replacement therapy. *Genet Med* 13:625–631. doi:10.1097/GIM.0b013e3182142966
  192. Hobson-Webb LD, Proia AD, Thurberg BL, Banugaria S, Prater SN, Kishnani PS (2012) Autopsy findings in late-onset Pompe disease: a case report and systematic review of the literature. *Mol Genet Metab* 106:462–469. doi:10.1016/j.ymgme.2012.05.007



193. Higuchi T, Kawagoe S, Otsu M, Shimada Y, Kobayashi H, Hirayama R, Eto K, Ida H, Ohashi T, Nakauchi H, Eto Y (2014) The generation of induced pluripotent stem cells (iPSCs) from patients with infantile and late-onset types of Pompe disease and the effects of treatment with acid- $\alpha$ -glucosidase in Pompe's iPSCs. *Mol Genet Metab* 112:44–48. doi:10.1016/j.ymgme.2014.02.012
194. Sato Y, Kobayashi H, Higuchi T, Shimada Y, Era T, Kimura S, Eto Y, Ida H, Ohashi T (2015) Disease modeling and lentiviral gene transfer in patient-specific induced pluripotent stem cells from late-onset Pompe disease patient. *Mol Ther Methods Clin Dev* 2:15023–15028. doi:10.1038/mtm.2015.23
195. Kishnani PS, Corzo D, Nicolino M, Byrne B, Mandel H, Hwu WL, Leslie N, Levine J, Spencer C, McDonald M, Li J, Dumontier J, Halberthal M, Chien YH, Hopkin R, Vijayaraghavan S, Gruskin D, Bartholomew D, van der Ploeg A, Clancy JP, Parini R, Morin G, Beck M, la Gastine De GS, Jokic M, Thurberg B, Richards S, Bali D, Davison M, Worden MA, Chen YT, Wraith JE (2007) Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 68:99–109. doi:10.1212/01.wnl.0000251268.41188.04
196. Nishino I, Fu J, Tanji K, Yamada T, Shimojok S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y, Sue CM, Yamamoto A, Murakami N, Shanske S, Byrne E, Bonilla E, Nonaka I, Hirano SDM (2016) Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature* 47:1–5. doi:10.1002/chin.201618208
197. Boucek D, Jirikowic J, Taylor M (2011) Natural history of Danon disease. *Genet Med* 13:563–568. doi:10.1097/GIM.0b013e31820ad795
198. Hashem SI, Perry CN, Bauer M, Han S, Clegg SD, Ouyang K, Deacon DC, Spinharney M, Panopoulos AD, Izpisua Belmonte JC, Frazer KA, Chen J, Gong Q, Zhou Z, Chi NC, Adler ED (2015) Brief report: oxidative stress mediates cardiomyocyte apoptosis in a human model of Danon disease and heart failure. *Stem Cells* 33:2343–2350. doi:10.1002/stem.2015
199. Alto LE, Dhalla NS (1981) Role of changes in microsomal calcium uptake in the effects of reperfusion of  $Ca^{2+}$ -deprived rat hearts. *Circ Res* 48:17–24. doi:10.1161/01.res.48.1.17
200. Ito Y, Suko J, Chidsey CA (1974) Intracellular calcium and myocardial contractility. V. Calcium uptake of sarcoplasmic reticulum fractions in hypertrophied and failing rabbit hearts. *J Mol Cell Cardiol* 6:237–247. doi:10.1016/0022-2828(74)90053-4
201. Kawagoe S, Higuchi T, Otaka M, Shimada Y, Kobayashi H, Ida H, Ohashi T, Okano HJ, Nakanishi M, Eto Y (2013) Morphological features of iPSCs generated from Fabry disease skin fibroblasts using Sendai virus vector (SeVdp). *Mol Genet Metab* 109:386–389. doi:10.1016/j.ymgme.2013.06.003
202. Itier J-M, Ret G, Viale S, Sweet L, Bangari D, Caron A, Le-Gall F, Bénichou B, Leonard J, Deleuze J-F, Orsini C (2014) Effective clearance of GL-3 in a human iPSC-derived cardiomyocyte model of Fabry disease. *J Inherit Metab Dis* 37:1013–1022. doi:10.1007/s10545-014-9724-5
203. Chou S-J, Yu W-C, Chang Y-L, Chen W-Y, Chang W-C, Chien Y, Yen J-C, Liu Y-Y, Chen S-J, Wang C-Y, Chen Y-H, Niu D-M, Lin S-J, Chen J-W, Chiou S-H, Leu H-B (2017) Energy utilization of induced pluripotent stem cell-derived cardiomyocyte in Fabry disease. *Int J Cardiol*. doi:10.1016/j.ijcard.2017.01.009
204. Chien Y, Chien C-S, Chiang H-C, Huang W-L, Chou S-J, Chang W-C, Chang Y-L, Leu H-B, Chen K-H, Wang K-L, Lai Y-H, Liu Y-Y, Lu K-H, Li H-Y, Sung Y-J, Jong Y-J, Chen Y-J, Chen C-H, Yu W-C (2016) Interleukin-18 deteriorates Fabry cardiomyopathy and contributes to the development of left ventricular hypertrophy in Fabry patients with GLA IVS4+919 G>A mutation. *Oncotarget* 7:87161–87179. doi:10.18632/oncotarget.13552
205. Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, Fabsitz RR, Robbins D, Rhoades ER, Howard BV (2000) Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation* 101:2271–2276. doi:10.1161/01.CIR.101.19.2271
206. Mandavia CH, Aroor AR, DeMarco VG, Sowers JR (2013) Molecular and metabolic mechanisms of cardiac dysfunction in diabetes. *Life Sci* 92:601–608. doi:10.1016/j.lfs.2012.10.028
207. Heather LC, Clarke K (2011) Metabolism, hypoxia and the diabetic heart. *J Mol Cell Cardiol* 50:598–605. doi:10.1016/j.yjmcc.2011.01.007
208. Lorenzo O, Ramírez E, Picatoste B, Egidio J, Tuñón J (2013) Alteration of energy substrates and ROS production in diabetic cardiomyopathy. *Mediat Inflamm* 2013:1–11. doi:10.1155/2013/461967
209. Steinberg SF (2013) Oxidative stress and sarcomeric proteins. *Circ Res* 112:393–405. doi:10.1161/CIRCRESAHA.111.300496
210. Deconinck N, Dan B (2007) Pathophysiology of duchenne muscular dystrophy: current hypotheses. *Pediatr Neurol* 36:1–7. doi:10.1016/j.pediatrneurol.2006.09.016
211. Ervasti JM (2007) Dystrophin, its interactions with other proteins, and implications for muscular dystrophy. *Biochim Biophys Acta* 1772:108–117. doi:10.1016/j.bbadis.2006.05.010
212. Macadangdang J, Guan X, Smith AST, Lucero R, Czerniecki S, Childers MK, Mack DL, Kim D-H (2015) Nanopatterned human iPSC-based model of a dystrophin-null cardiomyopathic phenotype. *Cell Mol Bioeng* 8:320–332. doi:10.1007/s12195-015-0413-8
213. Lin B, Li Y, Han L, Kaplan AD, Ao Y, Kalra S, Bett GCL, Rasmusson RL, Denning C, Yang L (2015) Modeling and study of the mechanism of dilated cardiomyopathy using induced pluripotent stem cells derived from individuals with Duchenne muscular dystrophy. *Development* 142:e0905–e0906. doi:10.1242/dev.125161
214. Connors LH, Lim A, Prokaeva T, Roskens VA, Costello CE (2003) Tabulation of human transthyretin (TTR) variants, 2003. *Amyloid* 10:160–184. doi:10.3109/13506120308998998
215. Leung A, Nah SK, Reid W, Ebata A, Koch CM, Monti S, Geneux JC, Wiseman RL, Wolozin B, Connors LH, Berk JL, Seldin DC, Mostoslavsky G, Kotton DN, Murphy GJ (2013) Induced pluripotent stem cell modeling of multisystemic, hereditary transthyretin amyloidosis. *Stem Cell Rep* 1:451–463. doi:10.1016/j.stemcr.2013.10.003
216. Talkhabi M, Aghdami N, Baharvand H (2016) Human cardiomyocyte generation from pluripotent stem cells: a state-of-art. *Life Sci* 145:1–16. doi:10.1016/j.lfs.2015.12.023
217. Zhang Q, Jiang J, Han P, Yuan Q, Zhang J, Zhang X, Xu Y, Cao H, Meng Q, Chen L, Tian T, Wang X, Li P, Hescheler J, Ji G, Ma Y (2010) Direct differentiation of atrial and ventricular myocytes from human embryonic stem cells by alternating retinoid signals. *Nat Publ Group* 21:579–587. doi:10.1038/cr.2010.163
218. Devalla HD, Schwach V, Ford JW, Milnes JT, El-Haou S, Jackson C, Gkatzis K, Elliott DA, de Sousa Chuva, Lopes SM, Mummery CL, Verkerk AO, Passier R (2015) Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. *EMBO Mol Med* 7:394–410. doi:10.15252/emmm.201404757
219. Protze SI, Liu J, Nussinovitch U, Ohana L, Backx PH, Gepstein L, Keller GM (2017) Sinoatrial node cardiomyocytes derived from human pluripotent cells function as a biological pacemaker. *Nat Biotechnol* 35:56–68. doi:10.1038/nbt.3745

220. Tzatzalos E, Abilez OJ, Shukla P, Wu JC (2015) Engineered heart tissues and induced pluripotent stem cells: macro- and microstructures for disease modeling, drug screening, and translational studies. *Adv Drug Deliv Rev* 96:1–11. doi:[10.1016/j.addr.2015.09.010](https://doi.org/10.1016/j.addr.2015.09.010)
221. van Meer BJ, Tertoolen LGJ, Mummery CL (2016) Concise review: measuring physiological responses of human pluripotent stem cell derived cardiomyocytes to drugs and disease. *Stem Cells* 34:2008–2015. doi:[10.1002/stem.2403](https://doi.org/10.1002/stem.2403)
222. Yang X, Pabon L, Murry CE (2014) Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Res* 114:511–523. doi:[10.1161/CIRCRESAHA.114.300558](https://doi.org/10.1161/CIRCRESAHA.114.300558)
223. Lieu DK, Fu JD, Chiamvimonvat N, Tung KC, McNERNEY GP, Huser T, Keller G, Kong CW, Li RA (2013) Mechanism-based facilitated maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Arrhythm Electrophysiol* 6:191–201. doi:[10.1161/CIRCEP.111.973420](https://doi.org/10.1161/CIRCEP.111.973420)
224. Kuppusamy KT, Jones DC, Sperber H, Madan A, Fischer KA, Rodriguez ML, Pabon L, Zhu W-Z, Tulloch NL, Yang X, Snidecki NJ, Laflamme MA, Ruzzo WL, Murry CE, Ruohola-Baker H (2015) Let-7 family of microRNA is required for maturation and adult-like metabolism in stem cell-derived cardiomyocytes. *Proc Natl Acad Sci USA* 112:E2785–E2794. doi:[10.1073/pnas.1424042112](https://doi.org/10.1073/pnas.1424042112)
225. Bett GCL, Kaplan AD, Lis A, Cimato TR, Tzanakakis ES, Zhou Q, Morales MJ, Rasmusson RL (2013) Electronic “expression” of the inward rectifier in cardiocytes derived from human-induced pluripotent stem cells. *Heart Rhythm* 10:1903–1910. doi:[10.1016/j.hrthm.2013.09.061](https://doi.org/10.1016/j.hrthm.2013.09.061)
226. Bedada FB, Wheelwright M, Metzger JM (2016) Maturation status of sarcomere structure and function in human iPSC-derived cardiac myocytes. *Biochim Biophys Acta* 1863:1829–1838. doi:[10.1016/j.bbamcr.2015.11.005](https://doi.org/10.1016/j.bbamcr.2015.11.005)
227. Garg T, Singh O, Arora S, Murthy RSR (2012) Scaffold: a novel carrier for cell and drug delivery. *Crit Rev Ther Drug Carrier Syst* 29:1–63. doi:[10.1615/critrevtherdrugcarriersyst.v29.i1.10](https://doi.org/10.1615/critrevtherdrugcarriersyst.v29.i1.10)
228. Fennema E, Rivron N, Rouwkema J, van Blitterswijk C, de Boer J (2013) Spheroid culture as a tool for creating 3D complex tissues. *Trends Biotechnol* 31:108–115. doi:[10.1016/j.tibtech.2012.12.003](https://doi.org/10.1016/j.tibtech.2012.12.003)
229. Ravenscroft SM, Pointon A, Williams AW, Cross MJ, Sidaway JE (2016) Cardiac non-myocyte cells show enhanced pharmacological function suggestive of contractile maturity in stem cell derived cardiomyocyte microtissues. *Toxicol Sci* 152:99–112. doi:[10.1093/toxsci/kfw069](https://doi.org/10.1093/toxsci/kfw069)
230. Giacomelli E, Bellin M, Sala L, van Meer BJ, Tertoolen LGJ, Orlova VV, Mummery CL (2017) Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Development* 144:1017. doi:[10.1242/dev.143438](https://doi.org/10.1242/dev.143438)
231. Tirziu D, Giordano FJ, Simons M (2010) Cell communications in the heart. *Circulation* 122:928–937. doi:[10.1161/CIRCULATIONAHA.108.847731](https://doi.org/10.1161/CIRCULATIONAHA.108.847731)
232. Kattman SJ, Witty AD, Gagliardi M, Dubois NC, Niapour M, Hotta A, Ellis J, Keller G (2011) Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. *Cell Stem Cell* 8:228–240. doi:[10.1016/j.stem.2010.12.008](https://doi.org/10.1016/j.stem.2010.12.008)
233. Chen VC, Couture SM, Ye J, Lin Z, Hua G, Huang H-IP, Wu J, Hsu D, Carpenter MK, Couture LA (2012) Scalable GMP compliant suspension culture system for human ES cells. *Stem Cell Res* 8:388–402. doi:[10.1016/j.scr.2012.02.001](https://doi.org/10.1016/j.scr.2012.02.001)
234. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O’Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE (2007) Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 25:1015–1024. doi:[10.1038/nbt1327](https://doi.org/10.1038/nbt1327)
235. Zhang J, Klos M, Wilson GF, Herman AM, Lian X, Raval KK, Barron MR, Hou L, Soerens AG, Yu J, Palecek SP, Lyons GE, Thomson JA, Herron TJ, Jalife J, Kamp TJ (2012) Extracellular matrix promotes highly efficient cardiac differentiation of human pluripotent stem cells: the matrix sandwich method. *Circ Res* 111:1125–1136. doi:[10.1161/CIRCRESAHA.112.273144](https://doi.org/10.1161/CIRCRESAHA.112.273144)
236. Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP (2012) Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/ $\beta$ -catenin signaling under fully defined conditions. *Nat Protoc* 8:162–175. doi:[10.1038/nprot.2012.150](https://doi.org/10.1038/nprot.2012.150)
237. van den Berg CW, Elliott DA, Braam SR, Mummery CL, Davis RP (2016) Differentiation of Human Pluripotent Stem Cells to Cardiomyocytes Under Defined Conditions. *Methods Mol Biol* 1353:163–180. doi:[10.1007/978-1-4939-9178-1\\_178](https://doi.org/10.1007/978-1-4939-9178-1_178)
238. Nerbonne JM (2005) Molecular physiology of cardiac repolarization. *Physiol Rev* 85:1205–1253. doi:[10.1152/physrev.00002.2005](https://doi.org/10.1152/physrev.00002.2005)
239. Antzelevitch C, Dumaine R (2011) Electrical heterogeneity in the heart: physiological. *Pharmacol Clin Implic*. doi:[10.1002/cphy.cp020117](https://doi.org/10.1002/cphy.cp020117)
240. Mulieri LA, Hasenfuss G, Leavitt B, Allen PD, Alpert NR (1992) Altered myocardial force-frequency relation in human heart failure. *Circulation* 85:1743–1750. doi:[10.1161/01.CIR.85.5.1743](https://doi.org/10.1161/01.CIR.85.5.1743)
241. Wiegnerinck RF, Cojoc A, Zeidenweber CM, Ding G, Shen M, Joyner RW, Fernandez JD, Kanter KR, Kirshbom PM, Kogon BE, Wagner MB (2009) Force frequency relationship of the human ventricle increases during early postnatal development. *Pediatr Res* 65:414–419. doi:[10.1203/PDR.0b013e318199093c](https://doi.org/10.1203/PDR.0b013e318199093c)
242. van den Berg MP, Wilde AA, Viersma TJW, Brouwer J, Haaksma J, van der Hout AH, Stolte-Dijkstra I, Bezzina TCR, Van Langen IM, Beaufort-Krol GC, Cornel JH, Crijns HJ (2003) Possible bradycardic mode of death and successful pacemaker treatment in a large family with features of long QT syndrome type 3 and Brugada syndrome. *J Cardiovasc Electrophysiol* 12:1–7. doi:[10.1046/j.1540-8167.2001.00630.x](https://doi.org/10.1046/j.1540-8167.2001.00630.x)
243. Itzhaki I, Maizels L, Huber I, Gepstein A, Arbel G, Caspi O, Miller L, Belhassen B, Nof E, Glikson M, Gepstein L (2012) Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. *J Am Coll Cardiol* 60:990–1000. doi:[10.1016/j.jacc.2012.02.066](https://doi.org/10.1016/j.jacc.2012.02.066)
244. Zhang XH, Haviland S, Wei H, Šarić T, Fatima A, Hescheler J, Cleemann L, Morad M (2013) Ca<sup>2+</sup> signaling in human induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) from normal and catecholaminergic polymorphic ventricular tachycardia (CPVT)-afflicted subjects. *Cell Calcium* 54:57–70. doi:[10.1016/j.ceca.2013.04.004](https://doi.org/10.1016/j.ceca.2013.04.004)
245. Kujala K, Paavola J, Lahti A, Larsson K, Pekkanen-Mattila M, Viitasalo M, Lahtinen AM, Toivonen L, Kontula K, Swan H, Laine M, Silvennoinen O, Aalto-Setälä K (2012) Cell model of catecholaminergic polymorphic ventricular tachycardia reveals early and delayed afterdepolarizations. *PLoS One* 7:e44660–e44710. doi:[10.1371/journal.pone.0044660](https://doi.org/10.1371/journal.pone.0044660)