Trends in Molecular Medicine

CellPress REVIEWS

Review

Modeling Hypertrophic Cardiomyopathy: Mechanistic Insights and Pharmacological Intervention

Diogo Mosqueira,[1,*] James G.W. Smith,[2] Jamie R. Bhagwan,[1] and Chris Denning[1]

Hypertrophic cardiomyopathy (HCM) is a prevalent and complex cardiovascular disease where cardiac dysfunction often associates with mutations in sarcomeric genes. Various models based on tissue explants, isolated cardiomyocytes, skinned myofibrils, and purified actin/myosin preparations have uncovered disease hallmarks, enabling the development of putative therapeutics, with some reaching clinical trials. Newly developed human pluripotent stem cell (hPSC)-based models could be complementary by overcoming some of the inconsistencies of earlier systems, whilst challenging and/or clarifying previous findings. In this article we compare recent progress in unveiling multiple HCM mechanisms in different models, highlighting similarities and discrepancies. We explore how insight is facilitating the design of new HCM therapeutics, including those that regulate metabolism, contraction and heart rhythm, providing a future perspective for treatment of HCM.

Highlights

HCM is a prevalent and complex disease governed by multiple molecular mechanisms, and there is currently no efficient cure. New treatment strategies are under development, and several drugs are reaching the final stages of clinical trials, with partial efficacy.

HCM disease models vary in complexity. Furthermore, they are limited by sample availability, demanding logistics, preparation artefacts, oversimplicity, and species-differences relative to the human cardiovascular physiology, often producing conflicting results.

Application of genome-editing technology to hPSCs enables unlimited isogenic sets of heart cells in which the primary mutation is the only change, allowing elucidation of disease phenotypes and genetic causation.

Uncovering new disease mechanisms and targets will pave the way to more efficient HCM therapeutics.

HCM: A Complex Heart Disease

Cardiomyopathies constitute a heterogeneous group of diseases that represent the major cause of heart failure (HF), and are defined by structural or functional perturbations of the myocardium [1]. HCM is the most prevalent cardiac genetic disease, often leading to sudden cardiac death at a young age [2]. Although described by increased left ventricle (LV) wall thickness in the absence of abnormal loading conditions (see Clinician's Corner), HCM shares many hallmarks with other cardiomyopathies [3] and progresses to a compensatory phase. However, a sustained hypertrophic response leads to HF as a result of energy and functional imbalance [4]. Although classically associated with preserved to hyperdynamic ejection fraction (EF), burn-out HCM with systolic dysfunction is also part of the HCM spectrum [1].

Approximately half of HCM patients bear mutations in one or more of >20 genes encoding sarcomeric proteins and associated myofilament elements that are responsible for regulating cardiomyocyte contraction and ultimately cardiac function [5–7]. However, genetic causation is very complex because HCM typically shows variable **penetrance** (see Glossary) and **expressivity**, even in the same family [8] (Figure 1). This implies that factors beyond the single pathogenic mutation (e.g., genetic/epigenetic background, environmental modifiers) influence the phenotype, as verified in nonfamilial HCM patients [9].

Overall, the clinical and genetic complexity of HCM and its manifold molecular mechanisms have hindered the development of effective treatment options. Although noninvasive monitoring of cardiac function in patients has generated diagnostic tools for determining the progression of HCM [10], this approach cannot characterize disease mechanisms. Such under-

[1]Department of Stem Cell Biology, Centre of Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, UK
[2]Faculty of Medicine and Health Sciences, Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich NR4 7UQ, UK

*Correspondence: diogo.mosqueira@nottingham.ac.uk (D. Mosqueira).

standing would allow progress towards better therapeutics. In this regard, several HCM models that enable more refined analyses of cardiac physiology have been generated. These include; (i) intact heart muscle strips, (ii) isolated cardiomyocytes, (iii) myofibrils derived from skinned hearts, and (iv) purified actin/myosin sarcomeric proteins, as well as (v) *in silico* approaches [11–13]. Although these models have contributed greatly to dissecting the hallmarks of HCM (the reader is directed to [14] for an exhaustive analysis), they still pose several challenges such as sample availability, preparation artefacts, and species differences (Figure 2, Key Figure). The recent application of genome editing to **human pluripotent stem cell (hPSC)**-derived cardiomyocytes (hPSC-CMs) has enabled multifaceted investigation of the genetic causation of HCM, complementing previous models (comprehensively reviewed in [15,16]).

In the following, we evaluate the pros and cons of different HCM models, and critically explore recently uncovered discrepancies obtained from their study, to consolidate current knowledge of disease mechanisms with a view towards future therapeutics.

Key Lessons from Heart Muscle Derivatives

Initial studies to dissect the mechanisms underlying HF were performed in cardiac muscle strips from explanted human hearts [17], unveiling hallmarks of HCM. Post-mortem histological analysis of cardiac tissue revealed extensive areas of interstitial fibrosis, myocyte enlargement, and chaotic spatial arrangement in HCM patients [18]. Further investigations showed 2–2.5-fold increased polyploidy in HCM, with inconsistent changes in multinucleation [19–21]. Isolated mitochondria from human hypertrophied hearts exhibited about twofold higher oxygen consumption relative to healthy controls [22].

Preservation of an intact sarcolemma in whole tissue preparations is advantageous for the recapitulation of cardiac tissue architecture and pharmacology *in vitro*. This led to characterization of HCM pathophysiology by the identification of abnormal drug responses, including an attenuated increase in contraction force induced by β-adrenergic agonists [23], and perturbed calcium handling leading to prolonged relaxation during diastole [24]. However, cardiac tissue biopsies are often derived from HF patients who have undergone myectomy to reduce muscle thickening, and therefore require demanding logistics of sample handling (e.g., immediate processing).

Mechanical and enzymatic dissociation of human endocardial tissue generates viable cardiomyocytes that have been used for electrophysiology studies and pharmacological responses [25], revealing arrhythmias (sixfold higher early after-depolarizations) and ~50% higher diastolic Ca^{2+} concentrations. Single-cell transcriptome investigations revealed highly variable mutant versus wild-type (WT) sarcomeric gene expression in heterozygous HCM patients, underlying heterogeneous cell contractility and Ca^{2+} sensitivity [26].

However, isolated human cardiomyocytes dedifferentiate almost immediately after explant and do not proliferate in culture [27]. Thus, human cardiac tissue and its derivatives offer an important, but rarely available, biological source, and this greatly reduces the scope of the physiological parameters that can be investigated.

Myofibrils from Skinned Heart Tissue/Cells: Direct Myofilament Evaluation

Subcellular structures have also been used to model HCM by placing tissue/cell-derived skinned myofibril preparations between a force transducer and length motor, and immersing in solutions with different Ca^{2+} concentrations to stimulate contraction/relaxation [28].

Glossary

Cardiac troponin I/T (cTnI/T): cardiac regulatory sarcomeric proteins that control the calcium-mediated interaction between actin and myosin.
CRISPR/Cas9: genome-editing technology that enables the introduction or correction of SNPs, for example in sarcomeric genes involved in HCM. The technology facilitates the generation of isogenic sets of cardiac cells for disease modeling.
Expressivity: the severity of the phenotype that develops in a patient with a pathogenic mutation.
Heterologous expression: expressing a gene in a host organism that normally does not produce it, by recombinant DNA technology.
Human pluripotent stem cells (hPSCs): these encompass (i) human embryonic stem cells (hESCs) isolated from the preimplantation blastocyst stage embryo, and (ii) human induced pluripotent stem cells (hiPSCs) reprogrammed from somatic cells donated by HCM patients and healthy individuals.
Isogenic sets: cell lines that differ only in the mutation being studied and retain the same genetic background. This allows the effect of the mutation in the disease progression to be isolated (providing a clearer understanding of genetic causation) by directly comparing healthy versus diseased isogenic counterparts.
Myosin-binding protein C (MYBPC): a sarcomeric protein involved in regulating the positions of myosin and actin for interaction, acting as a brake on cardiac contraction.
Myosin heavy chain (MHC): one of the several sarcomeric proteins involved in regulating cardiomyocyte contraction. Can be expressed in two isoforms, α and β, that are encoded by the *MYH6* and *MYH7* genes, respectively. Species differences underlie changes in compartmental expression of α- versus β-MHC expression in the heart (β is the main form expressed in human ventricles)
Myosin light chain 2 (MLC2): also known as regulatory light chain of myosin, MLC2 is a sarcomeric protein involved in modulating cardiac myosin cross-bridge kinetics (its phosphorylation enhances myofilament sensitivity to calcium).
Penetrance: the proportion of individuals carrying a pathogenic mutation who display a phenotype.

This method enables direct access to myofilament function to quantify isometric tension, Ca^{2+} sensitivity, and ATP consumption (Figure 2). Most reports using this methodology show decreased contractile force in human HCM samples. For instance, cardiac explants from patients bearing different mutations in *MYH7 and MYBPC3* genes [encoding **β-myosin heavy chain** (β-MHC), and **myosin-binding protein C**, respectively] have consistently revealed lower tension forces relative to healthy controls (21 vs 36 kN/m^2), with *MYH7*-mutant samples showing the lowest values when normalized to myofibril density (73 vs 113 kN/m^2) [29]. In addition, Kraft *et al.* showed a modest increase in Ca^{2+} sensitivity of skinned myofibers from human cardiac explants bearing the R723G-β-MHC mutation, relative to healthy controls, that was dependent on the hyperphosphorylation state of several sarcomeric proteins [**cardiac troponin I/T (cTnI/T)**, MYBPC, and **myosin light chain 2 (MLC2]** [30].

Simultaneous measurement of force development and ATPase activity in tissue-extracted myofibrils can be used to quantify the energy cost of contraction. This revealed significant increases for tissues from *MYBPC3*-mutant and *MYH7*-mutant patients, compared with **sarcomere mutation-negative (SMN)** HCM patients, at saturating Ca^{2+} concentrations [31]. This was corroborated in multicellular cardiac myofibrils of human R403Q-β-MHC, showing ~50% lower tension generation relative to SMN-HCM patients, as well as maximum ATPase activity [32]. This results in a higher cost of contraction, indicating inefficient ATP utilization that causes higher cardiac workload, often leading to HF (termed the 'energy depletion model' [4]).

Sarcomeric mutation-negative (SMN): HCM patients who do not exhibit known mutations in sarcomeric genes, constituting approximately 50% of all cases.

Single-nucleotide polymorphisms (SNPs): point mutations that cause a change in the DNA sequence leading to abnormal or truncated proteins with perturbed structure and function, a common cause of HCM.

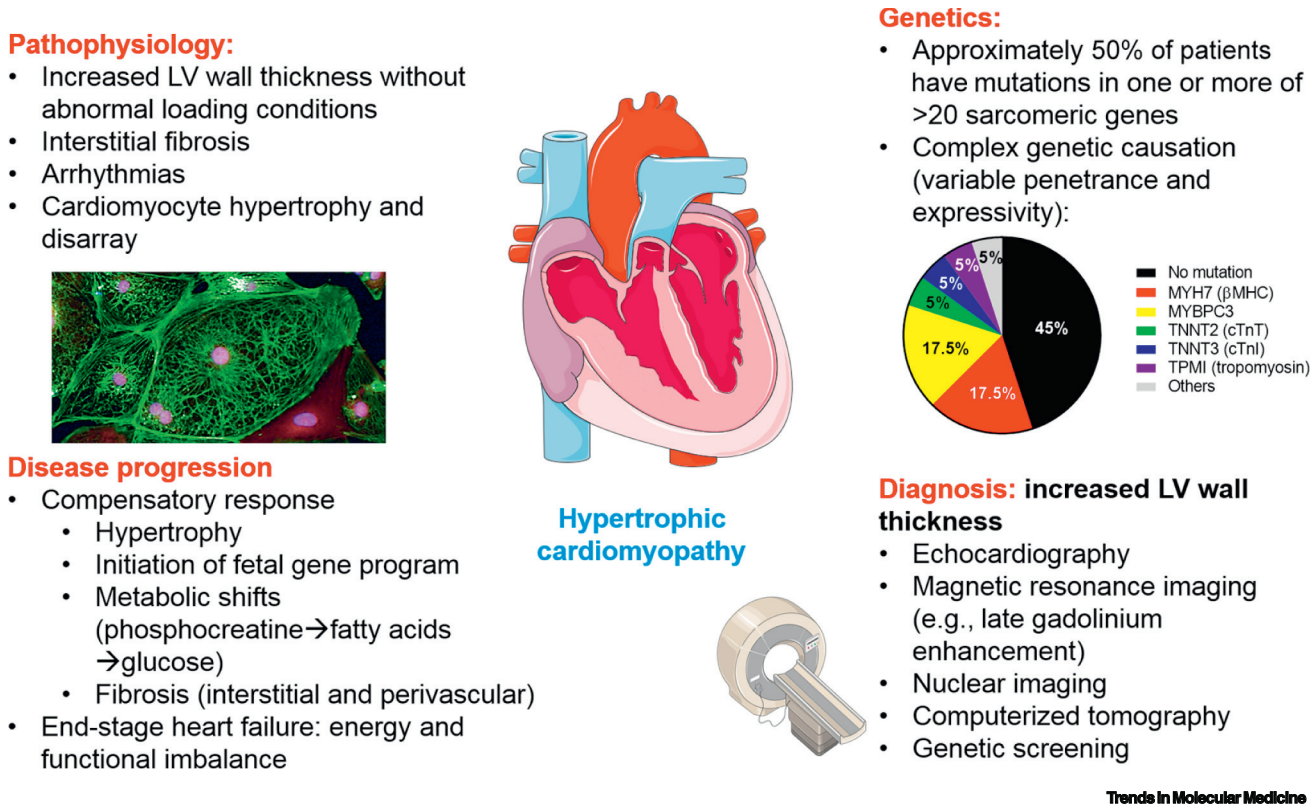
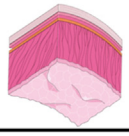
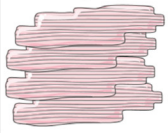
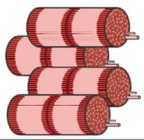
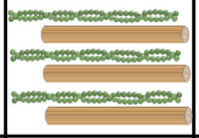
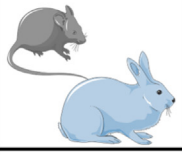
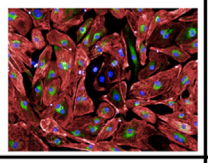


Figure 1. Main Features of Hypertrophic Cardiomyopathy (HCM) Pathophysiology, Genetic Causation, Disease Progression, and Diagnosis. The plethora of disease features and compensatory responses underlying HCM, as well as the overlap with other cardiomyopathies, make HCM a very complex condition to diagnose and treat. Abbreviation: LV, left ventricle.

Key Figure

Disease Models Used to Investigate Hypertrophic Cardiomyopathy (HCM) Phenotypes

	Intact heart muscle strips	Isolated cardiomyocytes	Myofibrils from tissue/cells	Purified actomyosin	Animal models	hPSC-CMs
Disease model						
Advantages	Physiological relevance Pharmacological predictivity	Amenable to measurements of subcellular functions	Direct access to myofilament function Simple logistics (allows sample freezing)	Higher sample availability (heterologous systems) Direct assessment of actin–myosin interactions	Whole organ/ organism studies Genetically tractable Easily available cell and tissue samples	Unlimited source of cardiomyocytes Recaptures patient genotype Gene editing enables isogenic controls
Disadvantages	Demanding logistics Limited sample availability	Limited sample availability Lack of proliferative ability in culture Lack of 3D architecture	Preparation artefacts Limited sample availability	Over-simplistic (nonrepresentative of whole sarcomere) Preparation artefacts (causing contradictory results)	Species differences relative to human Time-consuming genetic manipulation	Immaturity relative to human adult cardiomyocytes Lack of multicellular cardiac composition and neurohormonal control
Main readouts	Histology: fibrosis, myocyte enlargement, and disarray Pharmacological responses (e.g., β-blockers)	Electrophysiology Single-cell transcriptomics	Calcium sensitivity Isometric tension ATP consumption Energy cost of contraction	Kinetics of contraction ATPase activity Surrogate of force generation	Histology: fibrosis, disarray Contraction parameters and calcium sensitivity (integrated in EHTs)	High-throughput molecular and functional phenotyping Genetic causation (isogenic controls)

Trends in Molecular Medicine

Figure 2. Although they offer different advantages, disadvantages, and outputs, recapitulating HCM physiology and complexity at varying degrees, disease models have collectively enabled a deeper molecular understanding of disease progression, paving the way for its treatment. Abbreviations: CM, cardiomyocyte; EHT, engineered heart tissue; hPSC, human pluripotent stem cell.

However, this difference was not observed in single myofibrils derived from the same tissues, indicating that sample preparation affects the endpoint assay [33].

Nevertheless, myofibrils derived from skinned cardiac muscle offer simpler handling logistics because samples can be frozen in a relaxation solution that preserves their functionality for several months. Remarkably, higher energy cost in HCM muscle strips appears to be a feature shared by different mutations. This was recently corroborated in homozygous K280N-troponin T samples, which showed 24–72% higher values than three different control groups, that was ascribed to faster cross-bridge detachment [34].

Purified Actin/Myosin Proteins: Sarcomeric Interactions

Addressing the low sample availability limitations of whole-cell/tissue preparations, *in vitro* motility assays were developed by recording the movement of fluorescently labeled actin filaments over a layer of randomly oriented myosin molecules immunoadsorbed to an antibody-coated surface [35]. Tethering an ultracompliant microneedle to actin filaments enables measurement of average force per crossbridge, facilitating direct assessment of sarcomeric mutations and interactions at the molecular level.

However, studies performed using this technique have produced results conflicting with whole-tissue/cell analysis. For instance, rat tissues with **heterologous expression** of R403Q-α-MHC showed a fourfold decrease in ATPase activity and a fivefold reduction in motility compared with controls [36], whereas the same mutation in myosin isolated from mouse explants led to 2.3-fold higher ATPase activity and 60% higher velocity than WT myosin tissue [37]. These discrepancies extended to human biopsies. Although tissues from patients harboring β-MHC mutations displayed consistently lower sliding velocities (0.11–0.29 μm/s) than healthy controls (0.48 μm/s) [38], another report showed the opposite in R403Q- and L908V-βMHC, which had 30% higher velocities [39].

These differences highlight the main drawback of this method: technical artefacts derived from protein purification procedures. Tissue biopsies contain limited concentrations of myosins that are further degraded by freezing and tissue handling procedures. Alternatively, heterologous production of recombinant proteins often result in changes in structure and expression levels relative to endogenous systems, with functional consequences for the endpoint assay [14].

These limitations are evidenced when analyzing the same protein interactions by different techniques. Laser-trap assays can be used for direct molecular analysis of actin–myosin interactions by measuring the force and displacement resulting from the interaction of a single myosin molecule with an optically trapped actin filament [40]. The same group has shown significant differences between myosin extracted from mice bearing the R403Q-β-MHC mutation vs WT when performing *in vitro* motility assays (2.3-fold higher ATPase activity, 2.2-fold greater force generation, and 1.6-fold faster actin filament sliding), but no changes in force and displacement in the same samples when using the optical trap assay [37].

Taken together, these differences suggest that more reductionist models, where isolated proteins are investigated under unloaded conditions, tend to generate less robust conclusions due to technical constraints and the absence of physiological complexity that is characteristic of highly organized sarcomeres.

Animal Models: Transgenics and Species Differences

Animal models of heart disease have been crucial in advancing knowledge of pathophysiology towards new therapeutics because the basic principles of cardiac excitation and contraction in the species used are relatively conserved [41]. Although some naturally occurring cardiomyopathies have been detected in animals (e.g., Portuguese waterdogs), transgenic animal models enable detailed physiological and molecular analysis of disease [11,42]. Rodents (e.g., Syrian hamsters [43]) in particular have been extensively employed to model HCM because they overcome cell source limitations and facilitate whole-organism investigation of disease progression over time.

Primary cardiomyocytes isolated from transgenic rats and mice have been integrated into fibrin-based engineered heart tissues (EHTs) that enable assessment of contractility by measuring the displacement of silicon posts to which they are attached [44]. Analysis of the contractile force of rat cardiomyocytes transduced with adeno-associated virus expressing FLH1 variants containing **single-nucleotide polymorphisms (SNPs)** identified in HCM patients revealed hyper- or hypocontractile phenotypes depending on the mutation (K455fs-*Fhl1*, 27% higher force; C276S-*Fhl1*, 23% lower force vs WT controls). Evaluation of the beating kinetics of these tissues showed prolonged contraction and relaxation times in both variants (~18% and ~30% longer, respectively) [45]. The same approach

was applied to *Ankrd1* in rat, showing ~50% higher contractile force as well as contraction and relaxation velocities in T123M-*Ankrd1* EHTs versus controls, but no discernible phenotypes in P52A and I280V variants [46]. This reinforces the notion that HCM phenotypes are mutation-specific because different mutations in the same locus elicit variable effects on contraction.

Furthermore, data from transgenic mouse models have clearly linked mutations in sarcomeric genes with impaired Ca^{2+} handling. Knollmann and colleagues studied isolated cardiomyocytes, perfused hearts, and whole mice bearing the human I79N-cTnT mutation, showing shortened ventricular action potentials at 70% repolarization (14 ms in I79N-cTnT, vs 23 ms in control). Ca^{2+} transients of electrically stimulated ventricular I79N-cTnT myocytes were measured using a fluorescent Ca^{2+} indicator dye (Fura-2-AM) and showed reduced intensity (half the fluorescent amplitude in I79N-cTnT vs control) and twofold slower decay kinetics, consistent with increased Ca^{2+} sensitivity of I79N-cTnT mutant fibers [47]. Moreover, EHTs made from *Mybpc3*-mutant mice displayed higher sensitivity to Ca^{2+}, as evidenced by lower Ca^{2+} EC_{50} values for force generation relative to the WT (0.34 mM for homozygous, 0.48 mM for heterozygous, vs 0.66 mM for WT) [48,49]. This methodology has also shown differences in Ca^{2+} EC_{50} values for contraction between species (0.15, 0.39, and 1.05 mM Ca^{2+} for rat, mouse, and human EHTs, respectively [50]).

The variation in Ca^{2+} EC_{50} values for contraction between species highlights the main drawback of animal models – the existence of striking dissimilarities in cardiovascular physiology relative to humans. These are particularly prominent in the mouse: mice have ~10-fold faster beat rates (500 bpm vs 60 bpm) and 5–10-fold shorter electrocardiogram duration (50–100 ms vs 450 ms) relative to humans [41]. Changes in gene expression are also abundant, such as those pertaining to α/β-MHC expression: whereas in humans the α isoform is mainly located to the atria and the β to the ventricles [51], in the mouse the α-MHC is highly expressed in both compartments [52]. Despite sharing >90% sequence homology, this discrepancy in α versus β-MHC expression is reflected in the animal models generated.

Knock-in R403Q-α-MHC mice exhibited a significant enhancement in ATPase activity and transient kinetics (e.g., 20% increase in ADP release rate) relative to WT littermates, whereas R403Q-β-MHC animals displayed opposite or nonsignificant changes [53,54]. These inconsistencies are further exacerbated when comparing the same mutation in different animals. A recent report characterizing a transgenic rabbit model of the same mutation R403Q-β-MHC showed ~20% lower force generation in comparison with WT littermates in single myofibril analysis by atomic force microscopy [55], whereas transgenic mouse models show the opposite in actin–myosin assays [37]. These incongruities between models make it challenging to translate these findings to the human disease phenotype.

hPSC-CMs: An Unlimited Cell Source

Addressing the limitations of previous samples, hPSC-CMs have been harnessed for disease modeling because they constitute an unlimited cell source [56] that enables multiparametric and detailed studies in patient-relevant genomes (Figure 2). Cardiac differentiation protocols recapitulating heart development can be applied to hPSCs, thereby generating highly pure cardiomyocyte populations [57] (Box 1). To interrogate different features of HCM pathology in hPSC-CMs, several medium- and high-throughput phenotypic assays that measure cardiomyocyte structure, metabolism, electrophysiology, calcium handling, and contractility have been developed [58,59].

Box 1. Methods for Differentiating hPSCs into Cardiomyocytes

To generate LV hPSC-CMs needed for modeling HCM, several protocols were developed based on recapitulation of cardiac development. hPSCs undergo sequential differentiation steps (mesoderm, cardiac mesoderm, cardiac progenitors) that are spatiotemporally regulated by different growth factor combinations to form cardiomyocytes [118]. These protocols can generally be divided into four main classes: (i) inductive coculture, (ii) embryoid body (EB) formation, (iii) monolayer culture, and (iv) suspension culture [57].

Early differentiation protocols relied on coculture of hPSCs with a murine visceral endoderm-like cell line (END-2) which provided the growth factors [activin A, bone morphogenetic protein (BMP)] necessary to control cardiogenesis [119,120]. Although coculture requires few cells, it is a time-consuming and labor-intensive method that results in impure cardiomyocyte populations surrounded by a murine cell line, rendering this protocol unsuitable for HCM modeling.

Methods for generating EBs are based on suspension cultures of hPSCs as single cells or small clumps in a medium that drives cardiogenesis. EBs can be formed in many ways, allowing variable control of EB size and number (e.g., in ultra-low attachment static conditions, hanging-drop method, and forced aggregation in V-shaped wells). Despite being successful, these methods are not amenable to scale-up and require very strict control of parameters such as the initial number of cells, resulting in heterogeneous cell types. EB cardiomyocytes often do not survive the dissociation into single cells that is required for phenotypic analyses [57].

Unlike EB methods where complex diffusional barriers confound the concentration of growth factors hPSCs are exposed to, uniform monolayer 2D cultures provide a more controlled and reproducible differentiation environment. Additional improvements such as temporal exclusion of insulin from the medium and/or the incorporation of a Matrigel™ overlay step resulted in higher cardiomyocyte purities (~90%). To decrease the variability and costs associated with growth factor-based protocols, several small molecules have been explored for driving cardiac differentiation, such as GSK3β inhibitor ChiR and the WNT inhibitors IWR-1-4, KY0211, and XAV939, resulting in cost-effective production of cardiomyocytes [121–123].

Although cardiac monolayer protocols typically result in high numbers of hPSC-CMs, cardiomyocytes suffer inherent heterogeneity (mixed subtype populations), batch variation, limited scalability, and interlaboratory variability [124] because small-scale 2D cultures are sensitive to fluctuations in physicochemical parameters. To greatly increase the scalability of differentiation protocols, several 3D suspension bioreactor systems have produced CMs from hPSCs by using anchorage-dependent (i.e., microcarrier-based) and -independent systems [125–127]. These recent protocols produce high purities of abundant LV cardiomyocytes that are essential for modeling HCM.

In the past 6 years, hPSC-CMs have been extensively used for modeling HCM [15,16]. Lan *et al.* pioneered the field by reporting *in vitro* phenotypes of R663H-β-MHC cardiomyocytes, namely increased multinucleation (48.3%) relative to healthy controls (22.1%), with stark abnormalities in calcium handling (about threefold higher percentage of cells displaying arrhythmias) [60]. Accordingly, R442G-β-MHC hPSC-CMs exhibited irregular Ca^{2+} transients (20% of the cells vs virtually none in healthy controls). Mutant cardiomyocytes showed a 21% increase in resting Ca^{2+} concentration and 50% lower Ca^{2+} release from the sarcoplasmic reticulum (SR) upon caffeine treatment relative to WT controls [61]. Tanaka *et al.* coupled high-speed video microscopy with a motion vector prediction algorithm to investigate myofibrillar disarray in hPSC-CMs derived from three HCM patients, showing an increased percentage of cells exhibiting disorganized sarcomeres (~9%) relative to controls (~5%) [62]. Moreover, myofibrils derived from hPSC-CMs bearing the E848G-β-MHC mutation showed ~56% lower maximal isometric tension forces relative to controls, with faster kinetics of actin–myosin cross-bridge detachment [63]. Finally, R58Q-MYL2 hPSC-CMs recently reported significantly lower $I_{Ca,L}$ current density relative to a control line, which led to irregular beating, suggesting impaired diastolic relaxation [64].

However, although hPSC-CMs enable detailed studies at the molecular, myofibrillar, cellular, and tissue levels (when integrated into EHTs), they are immature in comparison to adult CMs, with differences ranging from morphological and gene expression up to structural and functional properties [65], namely an absence of T tubules [66]. This was evidenced in [67] where induced pluripotent stem cell (iPSC)-derived CMs (iPSC-CMs) bearing truncating mutations in *TTN* displayed striking hypocontractility, TTN haploinsufficiency, and decreased sarcomere length, which were not observed in adult myofibril samples.

Moreover, hPSC-CM disease models fail to recapitulate the physiological complexity of a multicellular intact heart system that encompasses fluid dynamics inherent to the circulatory system as well as neurohormonal control (i.e., metabolic changes) and extracellular matrix alterations (e.g., fibrosis). Recent efforts have included additional cell types such as cardiac fibroblasts or endothelial cells, although these were derived from different sources with multiple genetic backgrounds [68]. Thus, HCM modeling studies using hPSC-CMs should also be interpreted carefully because hallmarks of disease may be under- or overestimated [16]. Nevertheless, these cells offer a direct approach to address the highly complex genetic causation of HCM.

Isogenic Sets to Comprehend Genetic Causation

A limitation of earlier studies employing hPSC-CMs was that the impact of (epi)genetic background on phenotype can exceed that caused by the pathogenic mutation, as evidenced by variations in action potential durations between 100–700 ms among different lines described as 'healthy controls' [69]. This was also verified in patients, as recently reported for monozygotic twins carrying the same mutation (G768-β-MHC) but showing differences in myocardial fibrosis [70].

Gene-editing technologies such as **CRISPR/Cas9** enable the generation of **isogenic sets** wherein precise changes can be made to the genome in an otherwise constant genetic background. Changes can range from large insertions or deletions (e.g., the ~65 kb *Dip2a* gene [71]) to single bases leading to point mutations [72,73]. This means that, by coupling hPSCs with genome-editing technology, sets of disease-related and healthy isogenic hPSC-CMs can be compared to isolate the effect of the mutation on disease progression [74].

This 'isogenic' strategy has only very recently been used to model HCM. Isogenic sets of R302Q-PRKAG2 versus CRISPR-corrected hPSC-CMs were used to evaluate beating parameters by microelectrode array analysis [75]. This model identified arrhythmogenic events in diseased hPSC-CMs which were abolished upon CRISPR-mediated correction. Beat rate variability was much higher in diseased lines (coefficient of variation ~30%), although there were differences between the two healthy controls (unrelated ~5% vs CRISPR-corrected ~12%), reinforcing the need for isogenic sets.

Genome editing enables a wider range of genotypes to be generated. This was explored in [76], wherein hPSCs from an asymptomatic patient carrying a heterozygous A57N-MYL3 mutation were further engineered to generate WT (corrected), homozygous mutant, and heterozygous frameshift mutant. Despite analyzing several parameters (morphology, transcriptome, sarcomeric structure, contractility, action potentials, and calcium handling), none of these lines exhibited a phenotype, unlike another known heterozygous pathogenic mutation (A57G-MYL3).

Wang *et al.* characterized isogenic sets of I79N-cTnT hPSC-CMs, and these recapitulated the phenotypes described in the transgenic mouse model bearing the same mutation {hypercontractility, impaired relaxation, increased Ca^{2+} sensitivity, and pro-arrhythmic decreased action potential (AP) duration [77]}. The reduced systolic Ca^{2+} release upon caffeine addition is likely to be due to calcium buffering by the calcium-sensitive mutant cTnT because treatment with calcium sensitizer, EMD57033, precipitates AP instability and arrhythmias in both models.

Smith *et al.* generated isogenic sets of E99K-ACTC1 variants, either by correcting or inserting the mutation in patient-derived hPSCs [78]. Notably, inserting the E99K-ACTC1 mutation in healthy

lines did not induce the pathological phenotypes observed in patients with the same genotype (3.6-fold higher contraction force in EHTs, increased Ca^{2+} sensitivity, and double the prevalence of arrhythmogenic events). Genomic correction of the diseased line restored the abnormalities back to baseline levels, which renders this mutation as 'necessary but not sufficient' to cause HCM. These results are in line with studies on a previous transgenic mouse model [79,80] which also reported enlarged atria and increased interstitial fibrosis. However, it is likely that the noncardiomyocyte cell types and full embryonic development that are absent from the hPSC-CM model play an important role in developing HCM phenotypes. Nevertheless, isogenic sets allowed a deeper understanding of the genetic causation of the disease by suggesting that background (epi)genetics acts as an important modulator of the pathogenic phenotype.

Engineering the same mutation in several hPSC lines and analyzing disease phenotypes facilitates direct evaluation of its penetrance. This approach was explored by CRISPR/Cas9-mediated engineering of the R453C-β-MHC change in three independent hPSC lines, generating 11 different genetic variants in total (entailing heterozygous, homozygous, homozygous plus α-MHC frame-shift, and knockout genotypes) [81]. Extensive molecular and functional evaluation of hPSC-CMs showed recapitulation of the main hallmarks of HCM in all three isogenic sets, with differences in magnitude that were correlated with the mutant:WT allele expression ratio in the heterozygous lines. In the most diseased line (~60% mutant:WT allele expression), hypertrophy (51% increase in cell volume), sarcomeric disarray, increased mitochondrial respiration, arrhythmias, and hypocontractility (16.3–81.3% lower force) were reported. The mutation load was associated with the level of phenotypic perturbation, and the energy-depletion phenotype likely hampers the energetically demanding SERCA2a pump, resulting in higher levels of intracellular Ca^{2+}, leading in turn to increased risk of arrhythmia. This human model corroborated energy depletion as an underlying response to HCM mutations [4,22].

Unveiling HCM Complexity: Hypercontractility versus Hypocontractility

Contraction changes reported in R453C-βMHC hPSC-CMs are opposite to the response previously reported in transgenic mouse models, which showed 80% higher force in homozygous mutant cardiac myosins [82] and ~50% increase in maximal force in heterologous mouse cells [83] (Table 1). However, the hPSC-CM data are consistent with isometric tension analysis from explanted human HCM tissue [29] and E848-βMHC hPSC-CMs [84]. This discrepancy is probably due to species differences between mouse and human hearts, namely pertaining to the ventricular predominance of α versus β-MHC expression [51,52].

Accordingly, a recent review compiled all the data from human skinned muscle strips, isolated cardiomyocytes, and myofibrils obtained from frozen HCM hearts bearing mutations in the *MYBPC3*, *MYH7*, *TPM1*, *TNNT2*, or *TNNI3* sarcomeric genes. Across the 15 studies reviewed, spanning tens of gene–mutation combinations, maximal force developed by cardiomyocytes averaged ~40% lower than control hearts in almost all cases [85].

Arguably, hypo- versus hypercontractile effects are mutation-specific, accounting for the highly complex genetic causation of HCM, which often shows overlapping features with other cardiomyopathy forms such as dilated cardiomyopathy, thwarting understanding of disease progression [86].

A hypocontractile phenotype in HCM can be clinically explained by the concentric nature of the hypertrophy: thickening of the ventricular walls without increase in heart size leads to a smaller LV end-diastolic volume. This causes an apparent hypercontraction [resulting in similar or higher LV ejection fraction, (LVEF)] but not when normalized to cardiomyocyte or myofibril density

Table 1. Discrepancies in HCM Characterization between Different Models

Model	Species	System	Mutation	Assay	Result (vs control)[a]	Refs
Purified actomyosin	Rat	Heterologous expression	R403Q-αMHC	*In vitro* motility	↓ ATPase activity (fourfold) ↓ Motility (fivefold)	[36]
Purified actomyosin	Mouse	Transgenic tissue explant	R403Q-αMHC	*In vitro* motility	↑ ATPase activity (2.3-fold) ↑ Motility (60%)	[37]
Purified actomyosin	Human	Tissue explant	R403Q-βMHC	*In vitro* motility	↓ Sliding velocity (73%)	[38]
Purified actomyosin	Human	Tissue explant	R403Q-βMHC	*In vitro* motility	↑ Sliding velocity (30%)	[39]
Purified actomyosin	Mouse	Transgenic tissue explant	R403Q-βMHC	*In vitro* motility	↑ ATPase activity (2.3-fold) ↑ Force generation (2.2-fold) ↑ Sliding velocity (1.6-fold)	[37]
Purified actomyosin	Mouse	Transgenic model	R403Q-αMHC (homozygous)	Laser trap	↑ Force generation (50%) ↑ Sliding velocity (24%)	[82]
Purified actomyosin	Mouse	Transgenic tissue explant	R403Q-βMHC	Laser trap	= Force generation	[37]
Purified actomyosin	Rabbit	Transgenic model	R403Q-βMHC	*In vitro* motility	= ATPase activity ↓ Sliding velocity (50%)	[55]
Single myofibril analysis	Rabbit	Transgenic model	R403Q-βMHC	Atomic force microscopy	↓ Force generation (17%)	[55]
Multicellular myofibril analysis	Human	Tissue explant	R403Q-βMHC	Tension (force/area)	↓ Tension generation (53%)	[32]
Purified actomyosin	Mouse	Transgenic model	R453C-αMHC (homozygous)	Laser trap	↑ Force generation (80%) = Sliding velocity	[82]
Purified actomyosin	Mouse	Cell line heterologous expression	R453C-βMHC	*In vitro* motility	↓ ATPase activity (30%) ↑ Force generation (50%)	[83]
hPSC-CM-EHTs	Human	hPSC-CMs	R453C-βMHC	EHT (contractile force)	↓ Force generation (16–81%)	[81]

[a]Key: ↓, decreased; ↑, increased; =, no change.

[87,88]. Interestingly, echo-based strain imaging has consistently reported hypocontractile function in HCM patients [89].

These findings question the reliability of over-simplistic assays of surrogate measurements of contractility solely based on the interaction of two myofilament proteins. They also question the physiological relevance of at least some murine models which are known to have striking species differences relative to humans [52]. In others, such as mouse and human muscle fibers bearing the F764L-MYBPC3 HCM mutation, a hypercontractile phenotype was consistently reported in both [90]. Notwithstanding, whole-organism *in vivo* models do not simply reflect situations *in vitro*. Importantly, it is conceivable that variations in contractile force in HCM change with disease course, in other words it may be hyperdynamic early (compensatory phase) but hypocontractile later (end-stage).

Pharmacological Intervention Strategies for Treating HCM

Disease models that recapitulate HCM phenotypes offer a powerful platform for screening pharmacological rescue strategies. However, the varied phenotypes and molecular mechanisms associated with different HCM-associated mutations prevent a consensus on the most efficient treatment strategies. Thus, pharmacological interventions with diverse modes of actions can be effective in HCM rescue (Figure 3). Treatments include β-blockers, L-type Ca^{2+} channel blockers, antiarrhythmic drugs, calcium desensitizers, and metabolic and contractility modulators [91,92].

β-Blockers were first used to treat symptomatic HCM in the 1960s [93] because, by modulating heart rate, ventricular contractility, and stiffness, they can improve ventricular relaxation in HCM patients [94,95]. A range of β-blockers have been used, including propranolol and metipranolol,

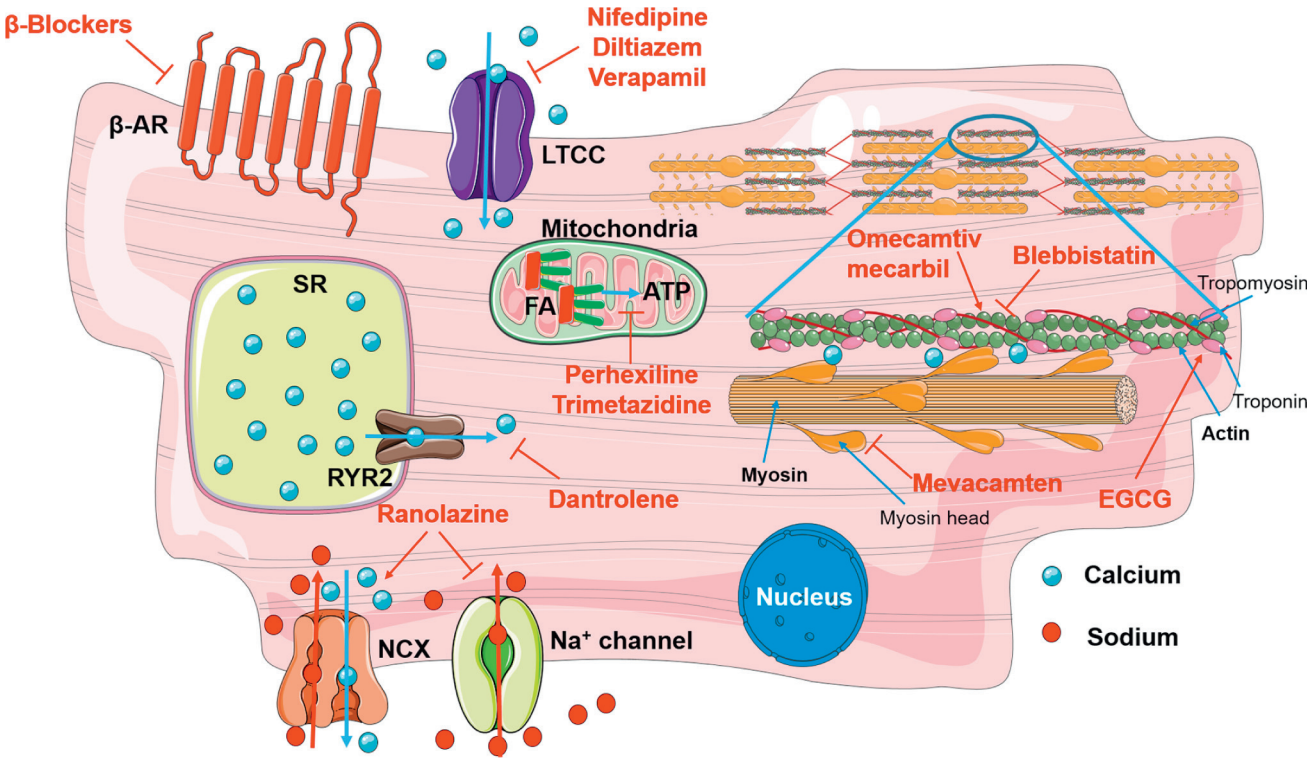


Figure 3. Ongoing Pharmacological Strategies to Treat Hypertrophic Cardiomyopathy (HCM). Disease models have uncovered several molecular players in cardiomyocytes that can be targeted by drugs (in red). β-Blockers prevent adrenergic signaling, leading to reduced heart rate and improved ventricular relaxation. Drugs targeting LTCC, RYR2, and NCX reduce the intracellular calcium build-up that underlies arrhythmogenic events associated with HCM. Metabolic modulators such as perhexiline and trimetazidine inhibit fatty acid oxidation, thereby promoting the use of glucose as an energy substrate and fostering improved energy efficiency by reversing the energy depletion phenotype. Blebbistatin and epigallocatechin-3-gallate (EGCG) alter myofilament calcium sensitivity. Mevacamten reduces contractility by decreasing the ATPase activity of sarcomeric myosin heavy chain. Omecamtiv mecarbil increases actin–myosin binding, which results in enhanced cardiomyocyte contraction. Abbreviations: β-AR, β-adrenoreceptor; FA, fatty acid; LTCC, L-type calcium channel; NCX, sodium/calcium exchange pump; SR, sarcoplasmic reticulum; RYR2, ryanodine receptor.

but it remains unclear why some are more effective than others [96]. Patient specific hPSC-CMs have demonstrated their efficacy: metoprolol significantly decreased beating irregularity and arrhythmias in an R442G-β-MHC model [61].

Elevated intracellular Ca^{2+} and dysfunctional Ca^{2+} cycling are commonly reported to be central to the pathogenesis of HCM, and inhibition of Ca^{2+} entry by L-type Ca^{2+} channel blockers has been investigated for the treatment of HCM [97]. In a hPSC-CM model of R663H-β-MHC mutation, diltiazem abolished calcium-handling abnormalities and arrhythmias, whereas verapamil also prevented myocyte hypertrophy, fully alleviating the HCM phenotype [60]. Another Ca^{2+} blocker, nifedipine, reduced arrhythmia in R453C-β-MHC hPSC-CMs [81].

Intracellular Ca^{2+} levels can be targeted indirectly through antiarrhythmic drugs that inhibit Na^{+} influx, such as mexiletine and ranolazine [98]. These reduce intracellular Ca^{2+} by targeting the Na^{+}/Ca^{2+} exchanger and promoting Ca^{2+} efflux to restore intracellular Na^{+} levels. Ranolazine halved the number of arrhythmias in R453C-β-MHC hPSC-CMs [81] and reduced hypertrophic brain natriuretic peptide (BNP) signaling in E99K-ACTC1 hPSC-CMs [78]. Hypertrophic signaling was reduced further in combination with dantrolene, a drug that blocks SR Ca^{2+} release through inhibiting

the ryanodine receptor (RYR2) [78]. However, Phase II clinical trials (clinicaltrialsregister.eu 2011-004507-20) have not shown efficacy of ranolazine to improve functional capacity in HCM [99]. This is possibly due to other organ-level features such as myocardial fibrosis that may overrule the cellular effects or ranolazine, or to the high variability in late sodium current expression in HCM cardiomyocytes [100].

An alternative therapeutic strategy for reducing intracellular Ca^{2+} is to target myofilament Ca^{2+} sensitivity with desensitizing drugs. The myosin inhibitor blebbistatin alters myofilament sensitivity via an inhibitory effect on actomyosin cross-bridge formation [101], and 3 μM treatment prevented pro-arrhythmic AP triangulation in I79N-TnT hiPSC-CMs [77]. Epigallocatechin-3-gallate (EGCG) is a calcium desensitizer that acts through the formation of a ternary complex with cTnC and cTnI [102]. EGCG treatment completely reversed abnormal Ca^{2+} sensitivity analyzed by an *in vitro* motility assay of seven HCM-related mutations in cTnT [103].

Energy depletion also has therapeutic potential in HCM. Metabolic modulators that shift the substrate preference from fatty acids towards glucose are being increasingly investigated [103] because they increase ATP production with the same oxygen consumption (thus improving myocardial efficiency). Perhexiline promotes the use of carbohydrates as the substrate for myocardial energy by inhibiting carnitine-palmitoyltransferase [104], increasing exercise capacity in Phase II trials for HCM patients (ClinicalTrials.gov NCT00500552) [105]. However, other metabolic modulators such as trimetazidine, a direct inhibitor of fatty acid β-oxidation, have shown limited effectiveness in recent HCM clinical trials (ClinicalTrials.gov NCT01696370) [106], although its use for preventive therapy is now being investigated [107].

Because disease models have shown changes in contraction force, modulators of contractility are under investigation. The allosteric modulator of cardiac myosin, mevacamten, reduces contractility by decreasing the ATPase activity of cardiac MHC in a mouse model of HCM [101], also showing effectiveness in reversing the hypercontactility seen in truncated MYBPC hPSC-CM mutants [108]. Phase III clinical trials are now ongoing to test mevacamten efficacy in adults with symptomatic obstructive HCM (ClinicalTrials.gov NCT03470545), with completion expected in June 2020 [97]. Conversely, omecamtiv mecarbil (OM) is being tested to treat hypocontractility in patients by augmenting the speed of ATP hydrolysis, thus increasing myosin head binding to actin, which results in an enhanced force-producing state [109]. Interestingly, the effects of OM are dependent on intracellular Ca^{2+} levels (increasing contractility at low Ca^{2+} concentrations, and decreasing it at higher concentrations [110]). Despite displaying limited effectiveness in R453C-β-MHC hPSC-CMs [81] (possibly due to the inherent immaturity of these cells), OM has shown encouraging clinical results, progressing to Phase III trials (ClinicalTrials.gov NCT02929329) that are due for completion in January 2021 [111].

Altogether, data from earlier models revealed that HCM is governed by multiple pathways, leading to progression of new drugs into clinical trials, although some are showing disappointing efficacy. HCM disease models based on isogenic hPSC-CMs offer a high-throughput system for investigating the relative efficacy of a range of combinatorial pharmacological interventions in diverse HCM genetic backgrounds. Data from such screening projects may inform more effective and mutation-specific use of therapeutic drugs. These studies highlight the importance of greater mechanistic understanding to facilitate therapeutics for HCM.

Clinician's Corner

HCM is a prevalent and complex cardiomyopathy that often leads to heart failure when cardiac function is unable to meet the metabolic requirements of the body [1]. HCM is defined by thickening of the left ventricle (LV) that is associated with preserved to increased ejection fraction [1]. Diagnosis of HCM relies on detection of increased LV wall thickness by several imaging technologies as well as by genetic screening [1,97].

HCM exhibits intricate genetic causation: its effects are mutation-specific, and clinical outcomes range from asymptomatic to sudden cardiac death [115–117].

There is no current effective treatment for HCM, and heart transplantation is the only long-term solution [97].

Previous HCM models have highlighted hallmarks of disease: hypertrophy, perturbations in calcium handling (arrhythmias), contractility, and energy imbalance [3]. Gene-edited human pluripotent stem cell-derived cardiomyocytes enable more refined analysis of the genetic causation of HCM and multiparametric investigation of disease mechanisms.

Developing better models of HCM will enable deeper understanding of HCM molecular mechanisms leading to more efficient treatment options.

Concluding Remarks

HCM is an 'umbrella' term used to describe a tough-to-treat and very complex disease associated with an intricate genetic causation. It exhibits a heterogeneous set of clinical manifestations with a wide spectrum of molecular mechanisms.

Earlier disease models have greatly contributed to understanding HCM progression, and have proved to be useful as a collective to advance treatment. However, they have often failed to faithfully phenocopy HCM *in vitro* owing to its intricate and multifarious nature. Unlimited production of hPSC-CMs has facilitated deeper mechanistic studies, complementing approaches from previous models. Genome-edited isogenic cell models have enabled detailed investigation of the genetic causation of HCM, and their further study is expected to unearth new gene modifiers, mechanisms, and therapeutic opportunities (see Outstanding Questions).

Importantly, transcriptomic data are available and are expected to highlight new molecular targets for therapy. Two reports have performed RNA sequencing analysis from nongenetically profiled patient-derived tissue explants, identifying hundreds of differentially expressed genes relative to unrelated healthy controls [112,113]. Data on transcriptomic analysis of isogenic sets of HCM hPSC-CM lines have recently been published [81,114] providing genetically precise comparisons for identifying new molecular mechanisms of disease.

The unsatisfactory efficacy of drug management of HCM [96] is likely due to the use of suboptimal preclinical testing carried out in physiologically irrelevant animals [56] and/or by lack of consideration of the complex genotype–phenotype relationship characteristic of HCM. The development of refined models of HCM will further elucidate this complex disease by recapitulating human adult conditions of pathology. Although HCM is primarily a condition of the cardiomyocytes, incorporating multiple cardiac cell lines in *in vitro* models will improve *in vivo* pathophysiological relevance. Optimizing technical constraints associated with human tissue explant analysis and solving the relative immaturity of hPSC-CMs will also foster the generation of more sophisticated HCM models that could accurately predict drug responses to treat this multifaceted disease.

Outstanding Questions

Refinement of disease models: the heart comprises cardiomyocytes, cardiac fibroblasts, smooth muscle cells, and endothelial cells, with additional neurohormonal control. Therefore, what cell types should be used to recapitulate HCM *in vitro*, and in what proportions, while managing the balance between practical use and over-complexity? Are there protocols to efficiently derive all these cell types from hPSCs bearing HCM mutations? Will these cells integrate in functional tissues?

How can the whole-tissue/organ interstitial and perivascular fibrosis characteristic of HCM be reproduced *in vitro*?

What is the best strategy to improve maturation of hPSC-derived cardiac cell types while maintaining compatibility with high-throughput phenotypic assays?

Less explored mechanisms of disease: what is the contribution of autophagy to HCM progression? What is the molecular mechanism linking telomere shortening to HCM progression?

Is heart tissue polyploidy associated with the compensatory response to HCM?

Is there an underlying/unifying phenotype of HCM among patients with different sarcomeric mutations?

What are the most relevant gene modifiers/candidates in HCM that aggravate/attenuate phenotypes caused by primary structural mutations?

Can various gene-editing tools be efficiently used to engineer additional variants of sarcomeric mutant hPSCs to investigate the role of less studied mutations in HCM progression so as to clarify the involvement of secondary gene modifiers?

Treatment of HCM: can HCM be treated with single drugs directed at individual phenotypes? Are combinational therapies and lifestyle interventions more promising approaches?

Will drugs targeting novel/secondary disease mechanisms be more efficient in treating HCM?

Acknowledgments

The authors would like to thank the British Heart Foundation (BHF) [grant numbers: SP/15/9/31605, PG/14/59/31000, RG/14/1/30588, RM/13/30157, P47352/CRM], Britain Israel Research and Academic Exchange Partnership (BIRAX) [04BX14CDLG], Medical Research Council (MRC) [MR/M017354/1], Engineering and Physical Sciences Research Council (EPSRC) [DM's Doctoral Prize Research Fellowship], National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) [CRACK-IT:35911-259146, NC/K000225/1, NC/S001808/1], Heart Research UK, German Research Foundation [DFG-Es-88/12-1, HA3423/5-1], European Research Council [ERC-AG-IndivuHeart], European Commission [FP7- Biodesign], German Centre for Cardiovascular Research (DZHK), German Ministry of Education and Research, and the Freie und Hansestadt Hamburg for funding.

References

1. Elliott, P.M. *et al.* (2014) 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy. The Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur. Heart J.* 35, 2733–2779
2. Harris, K.M. *et al.* (2006) Prevalence, clinical profile, and significance of left ventricular remodeling in the end-stage phase of hypertrophic cardiomyopathy. *Circulation* 114, 216–225
3. Harvey, P.A. and Leinwand, L.A. (2011) The cell biology of disease: cellular mechanisms of cardiomyopathy. *J. Cell Biol.* 194, 355–365
4. Ashrafian, H. *et al.* (2003) Hypertrophic cardiomyopathy: a paradigm for myocardial energy depletion. *Trends Genet.* 19, 263–268
5. Keren, A. *et al.* (2008) Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression. *Nat. Clin. Pract. Cardiovasc. Med.* 5, 158–168
6. Marston, S.B. (2011) How do mutations in contractile proteins cause the primary familial cardiomyopathies? *J. Cardiovasc. Transl. Res.* 4, 245–255
7. Maron, B.J. *et al.* (2012) Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J. Am. Coll. Cardiol.* 60, 705–715
8. Cahill, T.J. *et al.* (2013) Genetic cardiomyopathies causing heart failure. *Circ. Res.* 113, 660–675
9. Ingles, J. *et al.* (2017) Nonfamilial hypertrophic cardiomyopathy - prevalence, natural history, and clinical implications. *Circ. Cardiovasc. Genet.* 10, e001620

10. Jenni, R. *et al.* (2001) Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. *Heart* 86, 666–671
11. Duncker, D.J. *et al.* (2015) Animal and *in silico* models for the study of sarcomeric cardiomyopathies. *Cardiovasc. Res.* 105, 439–448
12. Romero, L. *et al.* (2015) *In silico* screening of the impact of hERG channel kinetic abnormalities on channel block and susceptibility to acquired long QT syndrome. *J. Mol. Cell. Cardiol.* 87, 271–282
13. Passini, E. *et al.* (2017) Human *in silico* drug trials demonstrate higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. *Front. Physiol.* 8, 668
14. Eschenhagen, T. *et al.* (2015) Modelling sarcomeric cardiomyopathies in the dish: from human heart samples to iPSC cardiomyocytes. *Cardiovasc. Res.* 105, 424–438
15. Eschenhagen, T. and Carrier, L. (2018) Cardiomyopathy phenotypes in human-induced pluripotent stem cell-derived cardiomyocytes – a systematic review. *Pflügers Arch.* 471, 755–768
16. Sewanan, L.R. and Campbell, S.G. (2019) Modelling sarcomeric cardiomyopathies with human cardiomyocytes derived from induced pluripotent stem cells. *J. Physiol.* Published online January 9, 2019. https://doi.org/10.1113/JP276753
17. Sonnenblick, E.H. *et al.* (1965) The contractile properties of human heart muscle: studies on myocardial mechanics of surgically excised papillary muscles. *J. Clin. Invest.* 44, 966–977
18. Varnava, A.M. *et al.* (2000) Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. *Heart* 84, 476
19. Schneider, R. and Pfitzer, P. (1972) Number of nuclei in isolated human myocardial cells. *Virchows Arch. B Cell Pathol.* 12, 238–258
20. Vliegen, H.W. *et al.* (1991) Myocardial changes in pressure overload-induced left ventricular hypertrophy. A study on tissue composition, polyploidization and multinucleation. *Eur. Heart J.* 12, 488–494
21. Olivetti, G. *et al.* (1996) Aging, cardiac hypertrophy and ischemic cardiomyopathy do not affect the proportion of mononucleated and multinucleated myocytes in the human heart. *J. Mol. Cell. Cardiol.* 28, 1463–1477
22. Lindenmayer, G.E. *et al.* (1971) Some biochemical studies on subcellular systems isolated from fresh recipient human cardiac tissue obtained during transplantation. *Am. J. Cardiol.* 27, 277–283
23. Bristow, M.R. *et al.* (1982) Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N. Engl. J. Med.* 307, 205–211
24. Gwathmey, J.K. *et al.* (1987) Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. *Circ. Res.* 61, 70–76
25. Coppini, R. *et al.* (2018) Altered Ca^{2+} and Na^{+} homeostasis in human hypertrophic cardiomyopathy: implications for arrhythmogenesis. *Front. Physiol.* 9, 1391
26. Montag, J. *et al.* (2018) Burst-like transcription of mutant and wildtype MYH7-alleles as possible origin of cell-to-cell contractile imbalance in hypertrophic cardiomyopathy. *Front. Physiol.* 9, 359
27. Bird, S.D. *et al.* (2003) The human adult cardiomyocyte phenotype. *Cardiovasc. Res.* 58, 423–434
28. van der Velden, J. *et al.* (1999) Isometric tension development and its calcium sensitivity in skinned myocyte-sized preparations from different regions of the human heart. *Cardiovasc. Res.* 42, 706–719
29. Witjas-Paalberends, E.R. *et al.* (2013) Mutations in MYH7 reduce the force generating capacity of sarcomeres in human familial hypertrophic cardiomyopathy. *Cardiovasc. Res.* 99, 432–441
30. Kraft, T. *et al.* (2013) Familial hypertrophic cardiomyopathy: functional effects of myosin mutation R723G in cardiomyocytes. *J. Mol. Cell. Cardiol.* 57, 13–22
31. Witjas-Paalberends, E.R. *et al.* (2014) Gene-specific increase in the energetic cost of contraction in hypertrophic cardiomyopathy caused by thick filament mutations. *Cardiovasc. Res.* 103, 248–257
32. Witjas-Paalberends, E.R. *et al.* (2014) Faster cross-bridge detachment and increased tension cost in human hypertrophic cardiomyopathy with the R403Q MYH7 mutation. *J. Physiol.* 592, 3257–3272
33. Piroddi, N. *et al.* (2007) Tension generation and relaxation in single myofibrils from human atrial and ventricular myocardium. *Pflugers Arch. - Eur. J. Physiol.* 454, 63–73
34. Piroddi, N. *et al.* (2019) The homozygous K280N troponin T mutation alters cross-bridge kinetics and energetics in human HCM. *J. Gen. Physiol.* 151, 18
35. Warshaw, D.M. (1996) The in vitro motility assay: a window into the myosin molecular motor. *News Physiol. Sci.* 11, 1–7
36. Sweeney, H.L. *et al.* (1994) Heterologous expression of a cardiomyopathic myosin that is defective in its actin interaction. *J. Biol. Chem.* 269, 1603–1605
37. Tyska, M.J. *et al.* (2000) Single-molecule mechanics of R403Q cardiac myosin isolated from the mouse model of familial hypertrophic cardiomyopathy. *Circ. Res.* 86, 737–744
38. Cuda, G. *et al.* (1997) The *in vitro* motility activity of beta-cardiac myosin depends on the nature of the beta-myosin heavy chain gene mutation in hypertrophic cardiomyopathy. *J. Muscle Res. Cell Motil.* 18, 275–283
39. Palmiter, K.A. *et al.* (2000) R403Q and L908V mutant beta-cardiac myosin from patients with familial hypertrophic cardiomyopathy exhibit enhanced mechanical performance at the single molecule level. *J. Muscle Res. Cell Motil.* 21, 609–620
40. Finer, J.T. *et al.* (1994) Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* 368, 113–119
41. Milani-Nejad, N. and Janssen, P.M.L. (2014) Small and large animal models in cardiac contraction research: advantages and disadvantages. *Pharmacol. Ther.* 141, 235–249
42. Longeri, M. *et al.* (2013) Myosin-binding protein C DNA variants in domestic cats (A31P, A74T, R820W) and their association with hypertrophic cardiomyopathy. *J. Vet. Intern. Med.* 27, 275–285
43. Sakamoto, A. *et al.* (1997) Both hypertrophic and dilated cardiomyopathies are caused by mutation of the same gene, δ-sarcoglycan, in hamster: an animal model of disrupted dystrophin-associated glycoprotein complex. *Proc. Natl. Acad. Sci.* 94, 13873–13878
44. Eder, A. *et al.* (2016) Human engineered heart tissue as a model system for drug testing. *Adv. Drug Deliv. Rev.* 96, 214–224
45. Friedrich, F.W. *et al.* (2012) Evidence for FHL1 as a novel disease gene for isolated hypertrophic cardiomyopathy. *Hum. Mol. Genet.* 21, 3237–3254
46. Crocini, C. *et al.* (2013) Impact of ANKRD1 mutations associated with hypertrophic cardiomyopathy on contraction parameters of engineered heart tissue. *Basic Res. Cardiol.* 108, 349
47. Knollmann, B.C. *et al.* (2003) Familial hypertrophic cardiomyopathy-linked mutant troponin T causes stress-induced ventricular tachycardia and calcium-dependent action potential remodeling. *Circ. Res.* 92, 428–436
48. Stöhr, A. *et al.* (2013) Contractile abnormalities and altered drug response in engineered heart tissue from Mybpc3-targeted knock-in mice. *J. Mol. Cell. Cardiol.* 63, 189–198
49. Carrier, L. *et al.* (2004) Asymmetric septal hypertrophy in heterozygous cMyBP-C null mice. *Cardiovasc. Res.* 63, 293–304
50. Stoehr, A. *et al.* (2014) Automated analysis of contractile force and Ca^{2+} transients in engineered heart tissue. *Am. J. Physiol. Heart Circ. Physiol.* 306, H1353–H1363
51. Morano, I. (1999) Tuning the human heart molecular motors by myosin light chains. *J. Mol. Med.* 77, 544–555
52. Lyons, G.E. *et al.* (1990) Developmental regulation of myosin gene expression in mouse cardiac muscle. *J. Cell Biol.* 111, 2427–2436
53. Lowey, S. *et al.* (2008) Functional effects of the hypertrophic cardiomyopathy R403Q mutation are different in an α- or β-myosin heavy chain backbone. *J. Biol. Chem.* 283, 20579–20589
54. Lowey, S. *et al.* (2013) Transgenic mouse α- and β-cardiac myosins containing the R403Q mutation show isoform-dependent transient kinetic differences. *J. Biol. Chem.* 288, 14780–14787
55. Lowey, S. *et al.* (2018) Hypertrophic cardiomyopathy R403Q mutation in rabbit β-myosin reduces contractile function at the molecular and myofibrillar levels. *Proc. Natl. Acad. Sci. U. S. A.* 115, 11238

Can CRISPR/Cas9 genome-editing technology overcome its current hurdles to clinical application (fidelity and biodistribution to the heart) to enable gene therapy in HCM patients?

56. Denning, C. *et al.* (2016) Cardiomyocytes from human pluripotent stem cells: from laboratory curiosity to industrial biomedical platform. *Biochim. Biophys. Acta Mol. Cell Res.* 1863, 1728–1748
57. Talkhabi, M. *et al.* (2016) Human cardiomyocyte generation from pluripotent stem cells: a state-of-art. *Life Sci.* 145, 98–113
58. Shaheen, N. *et al.* (2017) Pluripotent stem cell-based platforms in cardiac disease modeling and drug testing. *Clin. Pharmacol. Ther.* 102, 203–208
59. Sala, L. *et al.* (2018) MUSCLEMOTION: a versatile open software tool to quantify cardiomyocyte and cardiac muscle contraction *in vitro* and *in vivo*. *Circ. Res.* 122, e5–e16
60. Lan, F. *et al.* (2013) Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell Stem Cell* 12, 101–113
61. Han, L. *et al.* (2014) Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. *Cardiovasc. Res.* 104, 258–269
62. Tanaka, A. *et al.* (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
63. Pioner, J.M. *et al.* (2016) Isolation and mechanical measurements of myofibrils from human induced pluripotent stem cell-derived cardiomyocytes. *Stem Cell Reports* 6, 885–896
64. Zhou, W. *et al.* (2019) Induced pluripotent stem cell-derived cardiomyocytes from a patient with MYL2-R58Q-mediated apical hypertrophic cardiomyopathy show hypertrophy, myofibrillar disarray, and calcium perturbations. *J. Cardiovasc. Transl. Res.* Published online February 22, 2019. https://doi.org/10.1007/s12265-019-09873-6
65. Yang, X. *et al.* (2014) Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ. Res.* 114, 511
66. Li, S. *et al.* (2013) Calcium signalling of human pluripotent stem cell-derived cardiomyocytes. *J. Physiol.* 591, 5279–5290
67. Vikhorev, P.G. *et al.* (2017) Abnormal contractility in human heart myofibrils from patients with dilated cardiomyopathy due to mutations in TTN and contractile protein genes. *Sci. Rep.* 7, 14829
68. Archer, C.R. *et al.* (2018) Characterization and validation of a human 3D cardiac microtissue for the assessment of changes in cardiac pathology. *Sci. Rep.* 8, 10160
69. Sala, L. *et al.* (2017) Integrating cardiomyocytes from human pluripotent stem cells in safety pharmacology: has the time come? *Br. J. Pharmacol.* 174, 3749–3765
70. Wang, J. *et al.* (2019) Different clinical presentation and tissue characterization in a monozygotic twin pair with MYH7 mutation-related hypertrophic cardiomyopathy. *Int. Heart J.* 60, 477–481
71. Zhang, L. *et al.* (2015) Large genomic fragment deletions and insertions in mouse using CRISPR/Cas9. *PLoS One* 10, e0120396
72. Long, C. *et al.* (2018) Correction of diverse muscular dystrophy mutations in human engineered heart muscle by single-site genome editing. *Sci. Adv.* 4, eaap9004
73. Kondrashov, A. *et al.* (2018) Simplified footprint-free Cas9/CRISPR editing of cardiac-associated genes in human pluripotent stem cells. *Stem Cells Dev.* 27, 391–404
74. Ran, F.A. *et al.* (2013) Genome engineering using the CRISPR-Cas9 system. *Nat. Protocols* 8, 2281–2308
75. Ben Jehuda, R. *et al.* (2018) CRISPR correction of the PRKAG2 gene mutation in the patient's induced pluripotent stem cell-derived cardiomyocytes eliminates electrophysiological and structural abnormalities. *Heart Rhythm.* 15, 267–276
76. Ma, N. *et al.* (2018) Determining the pathogenicity of a genomic variant of uncertain significance using CRISPR/Cas9 and human-induced pluripotent stem cells. *Circulation* 138, 2666–2681
77. Wang, L. *et al.* (2018) Hypertrophic cardiomyopathy-linked mutation in troponin T causes myofibrillar disarray and pro-arrhythmic action potential changes in human iPSC cardiomyocytes. *J. Mol. Cell. Cardiol.* 114, 320–327
78. Smith, J.G.W. *et al.* (2018) Isogenic pairs of hiPSC-CMs with hypertrophic cardiomyopathy/LVNC-associated ACTC1 E99K mutation unveil differential functional deficits. *Stem Cell Reports* 11, 1226–1243
79. Song, W. *et al.* (2013) Mechanical and energetic properties of papillary muscle from ACTC E99K transgenic mouse models of hypertrophic cardiomyopathy. *Am. J. Physiol. Heart Circ. Physiol.* 304, H1513–H1524
80. Song, W. *et al.* (2011) Molecular mechanism of the E99K mutation in cardiac actin (ACTC Gene) that causes apical hypertrophy in man and mouse. *J. Biol. Chem.* 286, 27582–27593
81. Mosqueira, D. *et al.* (2018) CRISPR/Cas9 editing in human pluripotent stem cell-cardiomyocytes highlights arrhythmias, hypocontractility, and energy depletion as potential therapeutic targets for hypertrophic cardiomyopathy. *Eur. Heart J.* 39, 3879–3892
82. Debold, E.P. *et al.* (2007) Hypertrophic and dilated cardiomyopathy mutations differentially affect the molecular force generation of mouse α-cardiac myosin in the laser trap assay. *Am. J. Physiol. Heart Circ. Physiol.* 293, H284–H291
83. Sommese, R.F. *et al.* (2013) Molecular consequences of the R453C hypertrophic cardiomyopathy mutation on human β-cardiac myosin motor function. *Proc. Natl. Acad. Sci. U. S. A.* 110, 12607–12612
84. Yang, K-C. *et al.* (2018) Novel adult-onset systolic cardiomyopathy due to MYH7 E848G mutation in patient-derived induced pluripotent stem cells. *JACC Basic Transl. Sci.* 3, 728–740
85. Vikhorev, G.P. and Vikhoreva, N.N. (2018) Cardiomyopathies and related changes in contractility of human heart muscle. *Int. J. Mol. Sci.* 19, E2234
86. Davis, J. *et al.* (2016) A tension-based model distinguishes hypertrophic versus dilated cardiomyopathy. *Cell* 165, 1147–1159
87. Katz, D.H. *et al.* (2013) Prevalence, clinical characteristics, and outcomes associated with eccentric versus concentric left ventricular hypertrophy in heart failure with preserved ejection fraction. *Am. J. Cardiol.* 112, 1158–1164
88. Marian, A.J. (2000) Pathogenesis of diverse clinical and pathological phenotypes in hypertrophic cardiomyopathy. *Lancet* 355, 58–60
89. Sun, J-P. *et al.* (2019) Echocardiographic strain in hypertrophic cardiomyopathy and hypertensive left ventricular hypertrophy. *Echocardiography* 36, 257–265
90. Toepfer, C.N. *et al.* (2019) Hypertrophic cardiomyopathy mutations in MYBPC3 dysregulate myosin. *Sci. Transl. Med.* 11, eaat1199
91. Sequeira, V. *et al.* (2018) Metabolic changes in hypertrophic cardiomyopathies: scientific update from the Working Group of Myocardial Function of the European Society of Cardiology. *Cardiovasc. Res.* 114, 1273–1280
92. Stefania, P. *et al.* (2013) Adult stem cells and biocompatible scaffolds as smart drug delivery tools for cardiac tissue repair. *Curr. Med. Chem.* 20, 3429–3447
93. Cherian, G. *et al.* (1966) Beta-adrenergic blockade in hypertrophic obstructive cardiomyopathy. *Br. Med. J.* 1, 895–898
94. Spirito, P. *et al.* (1997) The management of hypertrophic cardiomyopathy. *N. Engl. J. Med.* 336, 775–785
95. Marian, A.J. (2009) Contemporary treatment of hypertrophic cardiomyopathy. *Tex. Heart Inst. J.* 36, 194–204
96. Camici, P.G. *et al.* (2012) Pharmacological treatment options for hypertrophic cardiomyopathy: high time for evidence. *Eur. Heart J.* 33, 1724–1733
97. Makavos, G. *et al.* (2019) Hypertrophic cardiomyopathy: an updated review on diagnosis, prognosis, and treatment. *Heart Fail. Rev.* 24, 439–459
98. Dan, G.A. *et al.* (2018) Antiarrhythmic drugs – clinical use and clinical decision making: a consensus document from the European Heart Rhythm Association (EHRA) and European Society of Cardiology (ESC) Working Group on Cardiovascular Pharmacology, endorsed by the Heart Rhythm Society (HRS), Asia-Pacific Heart Rhythm Society (APHRS) and International Society of Cardiovascular Pharmacotherapy (ISCP). *Europace* 20, 731–732
99. Olivotto, I. *et al.* (2018) Efficacy of ranolazine in patients with symptomatic hypertrophic cardiomyopathy: the RESTYLE-HCM randomized, double-blind, placebo-controlled study. *Circ. Heart Fail.* 11, e004124
100. Söhren, K-D. *et al.* (2015) Ranolazine antagonizes catecholamine-induced dysfunction in isolated cardiomyocytes, but lacks long-term therapeutic effects *in vivo* in a mouse model of hypertrophic cardiomyopathy. *Cardiovasc. Res.* 109, 90–102

101. Zhang, L. *et al.* (2015) Calcium desensitizer catechin reverses diastolic dysfunction in mice with restrictive cardiomyopathy. *Arch. Biochem. Biophys.* 573, 69–76
102. Robertson, I.M. *et al.* (2009) Solution structure of human vardiac troponin C in complex with the green tea polyphenol, (–)-epigallocatechin 3-gallate. *J. Biol. Chem.* 284, 23012–23023
103. Messer, A.E. *et al.* (2016) Mutations in troponin T associated with hypertrophic cardiomyopathy increase Ca^{2+}-sensitivity and suppress the modulation of Ca^{2+}-sensitivity by troponin I phosphorylation. *Arch. Biochem. Biophys.* 601, 113–120
104. Kennedy, J.A. *et al.* (1996) Inhibition of carnitine palmitoyltransferase-1 in rat heart and liver by perhexiline and amiodarone. *Biochem. Pharmacol.* 52, 273–280
105. Abozguia, K. *et al.* (2010) Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy. *Circulation* 122, 1562–1569
106. Coats, C.J. *et al.* (2019) Effect of trimetazidine dihydrochloride therapy on exercise capacity in patients with nonobstructive hypertrophic cardiomyopathy: a randomized clinical trial. *JAMA Cardiol.* 4, 230–235
107. van Driel, B.O. *et al.* (2019) Extra energy for hearts with a genetic defect: ENERGY trial. *Neth. Heart J.* 27, 200–205
108. Toepfer, C.N. *et al.* (2019) SarcTrack. *Circ. Res.* 124, 1172–1183
109. Malik, F.I. *et al.* (2011) Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science* 331, 1439–1443
110. Kampourakis, T. *et al.* (2018) Omecamtiv mercabil and blebbistatin modulate cardiac contractility by perturbing the regulatory state of the myosin filament. *J. Physiol.* 596, 31–46
111. Kaplinsky, E. and Mallarkey, G. (2018) Cardiac myosin activators for heart failure therapy: focus on omecamtiv mecarbil. *Drugs Context* 7, 215518
112. Yang, W. *et al.* (2015) Microarray profiling of long non-coding RNA (lncRNA) associated with hypertrophic cardiomyopathy. *BMC Cardiovasc. Disord.* 15, 62
113. Ren, C.W. *et al.* (2016) RNA-seq profiling of mRNA associated with hypertrophic cardiomyopathy. *Mol. Med. Report.* 14, 5573–5586
114. Cohn, R. *et al.* (2019) A contraction stress model of hypertrophic cardiomyopathy due to sarcomere mutations. *Stem Cell Reports* 12, 71–83
115. Towbin, J.A. (2014) Inherited cardiomyopathies. *Circ. J.* 78, 2347–2356
116. Sedaghat-Hamedani, F. *et al.* (2017) Clinical outcomes associated with sarcomere mutations in hypertrophic cardiomyopathy: a meta-analysis on 7675 individuals. *Clin. Res. Cardiol.* 107, 30–41
117. Melacini, P. *et al.* (2010) Clinicopathological profiles of progressive heart failure in hypertrophic cardiomyopathy. *Eur. Heart J.* 31, 2111–2123
118. Mummery, C.L. *et al.* (2012) Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. *Circ. Res.* 111, 344–358
119. Mummery, C. *et al.* (2003) Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation* 107, 2733–2740
120. Passier, R. *et al.* (2005) Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures. *Stem Cells* 23, 772–780
121. Lian, X. *et al.* (2012) Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc. Natl. Acad. Sci. U. S. A.* 109, E1848–E1857
122. Minami, I. *et al.* (2012) A small molecule that promotes cardiac differentiation of human pluripotent stem cells under defined, cytokine- and xeno-free conditions. *Cell Rep.* 2, 1448–1460
123. Burridge, P.W. *et al.* (2014) Chemically defined generation of human cardiomyocytes. *Nat. Meth.* 11, 855–860
124. Moretti, A. *et al.* (2013) Pluripotent stem cell models of human heart disease. *Cold Spring Harb. Perspect. Med.* 3, a014027
125. Ting, S. *et al.* (2014) An intermittent rocking platform for integrated expansion and differentiation of human pluripotent stem cells to cardiomyocytes in suspended microcarrier cultures. *Stem Cell Res.* 13, 202–213
126. Fonoudi, H. *et al.* (2015) A universal and robust integrated platform for the scalable production of human cardiomyocytes from pluripotent stem cells. *Stem Cell Transl. Med.* 4, 1482–1494
127. Breckwoldt, K. *et al.* (2017) Differentiation of cardiomyocytes and generation of human engineered heart tissue. *Nat. Protocols* 12, 1177–1197