Review

Modeling Hypertrophic Cardiomyopathy: Mechanistic Insights and Pharmacological Intervention

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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.

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-
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 polyplody 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.

**Expression:** 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 (iPSCs) 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).

**Penetration:** 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/m²), with MYH7-mutant samples showing the lowest values when normalized to myofibril density (73 vs 113 kN/m²) [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 (cTnl/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]).

### Pathophysiology:
- Increased LV wall thickness without abnormal loading conditions
- Interstitial fibrosis
- Arrhythmias
- Cardiomyocyte hypertrophy and disarray

### Disease progression
- Compensatory response
  - Hypertrophy
  - Initiation of fetal gene program
  - Metabolic shifts (phosphocreatine → fatty acids → glucose)
  - Fibrosis (interstitial and perivascular)
- End-stage heart failure: energy and functional imbalance

### Genetics:
- Approximately 50% of patients have mutations in one or more of >20 sarcomeric genes
- Complex genetic causation (variable penetrance and expressivity):
  - No mutation
  - MYH7 (β-MHC)
  - MYBPC3
  - TNNT2 (cTnl)
  - TNNT3 (cTnl)
  - TPM1 (troponymyosin)
  - Others

### Diagnosis: increased LV wall thickness
- Echocardiography
- Magnetic resonance imaging (e.g., late gadolinium enhancement)
- Nuclear imaging
- Computerized tomography
- Genetic screening

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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.
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 immunoabsorbed 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
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-1-4c, 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 Ca2+ transients (20% of the cells vs virtually none in healthy controls). Mutant cardiomyocytes showed a 21% increase in resting Ca2+ concentration and 50% lower Ca2+ 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\textsuperscript{2+} sensitivity, and pro-arrhythmic decreased action potential (AP) duration [77]). The reduced systolic Ca\textsuperscript{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 Ca²⁺ 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 frameshift, 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 Ca²⁺, 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-β-MHC 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
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 Ca2+ 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 metipranol,
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 [88]. 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 cTnl [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 hypercontractility 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.
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 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.

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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 (ESCG). 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 Lennard, L.A. (2011) The cell biology of disease: cellular mechanisms of cardiomyopathy. J. Cell Biol. 194, 355-366
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. Cardiavasc. Med. 5, 159-168
6. Martin, J.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, e001520

Outstanding Questions

1. Elliott, P.M. (2011) Hypertrophic cardiomyopathy of the European Society of Cardiology Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy (2006) Prevalence, natural history, and clinical implications. Circ. Res. 100, 158-168
2. Maron, B.J. (2011) Genetic cardiomyopathies causing heart failure. Circ. Res. 113, 660-675
3. Harvey, P.A. and Leinwand, L.A. (2011) The cell biology of disease: cellular mechanisms of cardiomyopathy. J. Cell Biol. 194, 355-366
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. Cardiavasc. Med. 5, 159-168
6. Martin, J.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, e001520

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 Lennard, L.A. (2011) The cell biology of disease: cellular mechanisms of cardiomyopathy. J. Cell Biol. 194, 355-366
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. Cardiavasc. Med. 5, 159-168
6. Martin, J.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, e001520

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 Lennard, L.A. (2011) The cell biology of disease: cellular mechanisms of cardiomyopathy. J. Cell Biol. 194, 355-366
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. Cardiavasc. Med. 5, 159-168
6. Martin, J.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, e001520
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. Pasini, 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. 106, 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. Schramm, L.R. and Campbell, S.G. (2019) Modelling sarco-meric cardiomyopathies with human cardiomyocytes derived from induced pluripotent stem cells. J. Physiol. Published online January 9, 2019. https://doi.org/10.1113/JP276553

17. Schumacker, E.H. et al. (1985) The contractile properties of human heart muscle: studies on myocardial mechanics of surgically excised papillary muscles. J. Clin. Invest. 44, 966-977

18. Vamava, A.M. et al. (2003) Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. Heart 84, 476

19. Schneider, R. and Pfrtzer, P. (1972) Number of nuclei in isolated human myocardial cells. Vinchos Arch. B Cell Pathol. 12, 239-258

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, 489-494

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, 1469-1477

22. Lindemann, G.E. et al. (1971) Some biochemical studies on subcellular systems isolated from fresh recipient human car- diac tissue obtained during transplantation. Am. J. Cardiol. 27, 277-293

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 myocardiopathy from patients with end-stage heart failure. Circ. Res. 61, 70-76

25. Coppari, R. et al. (2018) Altered Ca2+ and Na+ homeostasis in human hypertrophic cardiomyopathy: implications for arrhythmogenesis. Front. Physiol. 9, 1391

26. Montani, J. et al. (2018) Burst-like transcription of mutant and wildtype MYH7 alleles as possible origin of cell-to-cell contrac- tility imbalance in hypertrophic cardiomyopathy. Front. Physiol. 9, 269

27. Bird, S.D. et al. (2003) The human adult cardiomyocyte pheno-type. Cardiovasc. Res. 58, 425-434

28. van der Velden, J. et al. (1999) Isometric tension development and its calcium sensitivity in skinned myocyte-sized prepara-tions from different regions of the human heart. Cardiovasc. Res. 42, 706-719

29. Wijns-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: func-tional effects of myosin mutation R723G in cardiomyocytes. J. Mol. Cell. Cardiol. 57, 13-22

31. Wijns-Paalberends, E.R. et al. (2014) Gene-specific increase in the energetic cost of contraction in hypertrophic cardiomyopa-thy caused by thick filament mutations. Cardiovasc. Res. 103, 245-257

32. Wijns-Paalberends, E.R. et al. (2014) Faster cross-bridge detach-ment and increased tension cost in human hypertrophic cardiomyopathy with the R403Q MYH7 mutation. J. Physiol. 592, 2527-2572

33. Pridoti, N. et al. (2007) Tension generation and relaxation in single myofilaments from human atrial and ventricular myocardium. Pflügers Arch. - Eur. J. Physiol. 454, 63-73

34. Pridoti, N. et al. (2019) The homozygous K280N troponin T mutation alters cross-bridge kinetics and energetics in human HCM. J. Gen. Physiol. 151, 19

35. Warnhau, 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 h ypertrophic 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. Palmiet, K.A. et al. (2000) R403Q and L308V 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, 600-620

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 contracture research: advantages and disadvantages. Pharmacol. Ther. 141, 235–249

42. Longer, M. et al. (2013) Myosin-binding protein C DNA variants in domestic cats (A01P, A47T, R82W) and their association with hypertrophic cardiomyopathy. J. Vet. Intern. Med. 27, 270–285

43. Sakamoto, A. et al. (1997) Both hypertrophic and dilated cardiomypathies are caused by mutation of the same gene, β-sarcoglycan, in hamster: an animal model of disrupted dystrophin-associatedglycoproteincomplex. 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. Res. 96, 214–224

45. Friedrich, F.W. et al. (2012) Evidence for FH1 as a novel disease gene for isolated hypertrophic cardiomyopathy. Hum. Mol. Genet. 21, 3237-3254

46. Crochu, 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. Stähli, A. et al. (2013) Contractile abnormalities and altered drug response in engineered heart tissue from Mypc3-targeted knock-in mice. J. Mol. Cell. Cardiol. 63, 189–198

49. Carrier, L. et al. (2004) Asymmetric septal hypertrophy in heterozygous dMYH6-null mice. Cardiovasc. Res. 63, 293-304

50. Stoehr, A. et al. (2014) Automated analysis of contractile force and Ca2+-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, 544-565

52. Lyons, G.E. et al. (1996) Developmental regulation of myosin gene expression in mouse cardiac muscle. J. Cell Biol. 111, 2427–2436

53. Lovell, S. et al. (2008) Functional effects of the hypertrophic cardiomyopathy R403Q mutation are different in α- or β-myosin heavy chain backbones. J. Biol. Chem. 283, 20579-20589

54. Lovell, 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, 14760-14767

55. Lovell, 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, 11298
Trends in Molecular Medicine, Month 2019, Vol. xx, No. xx

56. Denning, C. et al. (2016) Cardiomyocytes from human pluripotent stem cells from laboratory curiosity to industrial biotechnological platform. Biochim. Biophys. Acta Mol. Cell Res. 1855, 1728–1748

57. Talhabhi, 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. (2016) 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. Stem Cell 12, Cell Stem Cell 11, 101–113

61. Han, L. et al. (2014) Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. Cardioren. Rep. 104, 259–265

62. Tanaka, A. et al. (2016) Endothelin-1 induces myocardial disarray and contractile vector variability in hypertrophic cardiomyopathy-induced pluripotent stem cell-derived cardiomyocytes. J. Am. Coll. Cardiol. 67, H1234–H1235

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, 665–676

64. Zhou, W. et al. (2019) Induced pluripotent stem cell-derived cardiomyocytes from a patient with MYL2-R265Q-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/s12265-019-06873-6

65. Yang, X. et al. (2014) Engineering adolescence: maturation of human 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, G.P. 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, 14269

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 monzygotic 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. Proc. Natl. Acad. Sci. U. S. A. 112, 10197–10197

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, eaat5804

73. Kondrashov, A. et al. (2018) Simplified footprint-free Cas9/CRISPR editing of cardiogenic-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, 2291–2308

75. Ben Jehuda, R. et al. (2018) CRISPR correction of the PRKA2 delta mutation in the patient’s induced pluripotent stem cell-derived cardiomyocytes eliminates electrophysiologic 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, 2669–2681

77. Wang, L. et al. (2018) Hypertrophic cardiomyopathy-linked mutation in troponin T causes myofibrillar disarray and proarrhythmic action potential changes in human PSC cardiomyocytes. J. Mol. Cell Cardiol. 114, 300–307

78. Smith, J.G.W. et al. (2018) Isogenic pairs of hiPSC-CMs with hypertrophic cardiomyopathy/LVNC-associated ACTC1 E99K mutation reveal differential functional deficits. Stem Cell Reports 11, 1325–1343

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, 27562–27573

81. Mosquera, 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. Debido, E.P. et al. (2007) Hypertrophic and dilated cardiomyopathy mutations differentially affect the molecular force generation of mouse e-cardiac myosin in the laser trap assay. Am. J. Physiol. Heart Circ. Physiol. 293, H284–H289

83. Sommese, R.F. et al. (2013) Molecular consequences of the R443C 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, 729–740

85. Vikhorev, G.P. and Vikhoreva, N.N. (2018) Cardiomyopathies and related changes in contractility of human heart muscle. Int. J. Mol. Sci. 19, E2294

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) Echoangiographic strain in hypertrophic cardiomyopathy and hypertensive left ventricular hypertrophy. Echoangiography 36, 257–265

90. Tzepier, C.N. et al. (2018) Hypertrophic cardiomyopathy mutations in MYBPC3 deregulate myosin. Sci. Transl. Med. 11, eaax1196

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. Chierchia, G. et al. (1966) Beta-adrenergic blockade in hypertrophic obstructive cardiomyopathy. Br. Med. J. 1, 895–898

94. Sicpato, 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. Cannici, P.G. et al. (2012) Pharmacological treatment options for hypertrophic cardiomyopathy: high time for evidence. Eur. Heart J. 33, 1724–1733

97. Makaros, G. et al. (2019) Hypertrophic cardiomyopathy: an updated review on diagnosis, prognosis, and treatment. Heart Fail. Rev. 24, 433–459

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, 791–792

99. Olivotto, I. et al. (2018) Efficacy of nolambrene in patients with symptomatic hypertrophic cardiomyopathy: the RESTYLE-HCM randomized, double-blind, placebo-controlled study. Circ. Heart Fail. 11, e004124

100. Söhrer, K-D. et al. (2018) 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. 100, 90–102
101. Zhang, L. et al. (2015) Calcium desensitizer catechol 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 cardiac troponin C in complex with the green tea polyphenol, (-)-epigallocatechin gallate. J. Biol. Chem. 284, 20212–20223
103. Messer, A.E. et al. (2016) Mutations in troponin T associated with hypertrophic cardiomyopathy increase Ca2+ sensitivity and suppress the modulation of Ca2+-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, 273–282
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. (2015) 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 mercapib and blebbistatin modulate cardiac contractility by perturbing the regulatory state of the myosin filament. J. Physiol. 596, 31–46
111. Kaplinsky, E. and Mallakjy, G. (2018) Cardiac myosin activators for heart failure therapy: focus on omecamtiv mescarib. Drugs Contert. 7, 21556
112. Kaplinsky, E. and Mallakjy, G. (2018) Cardiac myosin activators for heart failure therapy: focus on omecamtiv mescarib. Drugs Contert. 7, 21556
113. Yang, W. et al. (2015) Microarray profiling of long non-coding RNA (lncRNA) associated with hypertrophic cardiomyopathy. BMC Cardiovasc. Disord. 15, 62
114. Ren, C.W. et al. (2016) RNA-seq profiling of mRNA associated with hypertrophic cardiomyopathy. Mol. Med. Report. 14, 5573–5586
115. Cohn, R. et al. (2019) A contraction stress model of hypertrophic cardiomyopathy due to sarcomere mutations. Stem Cell Reports 12, 71–83
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. Melacni, P. et al. (2018) 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. Liu, 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, E1849–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