Review Article

Recent Advances in Induced Pluripotent Stem Cells-based Hypertrophic Cardiomyopathy Study

Julia Shante Petri[1], Emilian Christopher Stamatopoulos[2]

[1]Oaks Christian School 31749 La Tienda Rd, Westlake Village, CA 91362, USA;
[2]El Camino Real Charter High School, 5440 Valley Circle Blvd, Woodland Hills, CA 91376, USA

ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a heritable cardiovascular disorder marked by abnormal thickening of the heart muscle, leading to left ventricular outflow tract obstruction and compromised blood flow. It is associated with a range of symptoms including arrhythmias, chest pain, and sudden cardiac death, with no current effective treatments available. The pathogenic mechanisms underlying HCM remain elusive and induced pluripotent stem cells (iPSCs) reprogrammed from patient somatic cells have emerged as a powerful tool for uncovering pathogenic mechanisms of HCM. This review examines recent advancements in the iPSC-based HCM modeling and highlights how isogenic iPSCs generated by CRISPR/Cas9 overcome genetic background variability associated with the traditional iPSC-HCM models. Isogenic iPSCs not only enhance our understanding of single-causative genetic mechanisms of HCM but also will offer potential insights into complex cases involving multiple genetic mutations in the future.

Keywords: Induced Pluripotent Stem Cells, Hypertrophic Cardiomyopathy, Disease Modeling, CRISPR/Cas9, Isogenic, Mutations, Cardiomyocytes

INTRODUCTION

HCM is a heritable cardiovascular disease characterized by abnormal thickening of the heart muscle, which can obstruct the left ventricular outflow tract and impede effective blood flow (1). Symptoms of HCM can include arrhythmia (irregular heart rhythm), chest pain, shortness of breath, fatigue, fainting, dizziness, pounding in the chest, and heart murmur (2). HCM is the leading cause of sudden cardiac death in the USA, and there is no effective treatment available currently.

HCM is primarily caused by mutations in genes encoding sarcomeric or sarcomere-related proteins , and over 400 autosomal mutations associated with HCM have been identified (3). Among them, the mutations in β-myosin heavy chain (MYH7) and myosin binding protein C (MYBPC3) account for approximately 60-70% of all HCM cases (3, 4). The estimated prevalence of HCM in the general population worldwide is 0.2% and it affects individuals regardless of ethnicity or gender (5). Despite significant research efforts, studying the molecular mechanisms underlying HCM has been challenging due

Corresponding author: Emilian Christopher Stamatopoulos, E-mail: Emilianstam@icloud.com.

Received September 8, 2024**; Accepted** October 3, 2024
https://doi.org/10.70251/HYJR2348.239199

to the difficulty in obtaining human cardiac samples and the inability to effectively propagate human heart tissue in cell culture systems. Although several murine HCM models have been developed and are valuable for studying the disease (6-8), they may not accurately represent human HCM because the mouse heart primarily consists of α-myosin, whereas the human heart is predominantly composed of β-myosin.

The discovery of iPSCs has profoundly revolutionized heritable disease research including HCM (9). iPSCs can be reprogrammed from adult somatic cells by forcing the expression of specific transcription factors (OCT4, SOX2, Klf4 and C-Myc) . Functionally similar to embryonic stem cells (ESCs), iPSCs can be differentiated into all somatic cells in the body including cardiomyocytes (10, 11). However, unlike ESCs, iPSCs are not associated with ethical concerns and potential immune reactions during transplant. Therefore, iPSCs reprogrammed from patient somatic cells have been widely used for disease modeling, drug screening and potential cell therapy. In the disease modeling studies, phenotypic differences observed between iPSC-derived cells from patients and healthy individuals are often considered relevant to the disease pathophysiology. However, this traditional approach does not consider the impact of genetic background differences between healthy controls and patients, which can lead to misleading outcomes (12). Recently, isogenic iPSCs generated using CRISPR/Cas9 technology have been able to eliminate these genetic background differences, offering more reliable research outcomes in iPSC-based disease modeling (13, 14) (Figure 1). In this article, we review recent advances in iPSCs-based HCM disease modeling and discuss the potential future development in this field.

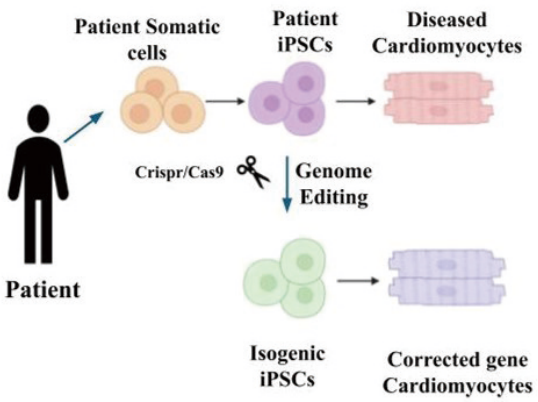

Figure 1. Diagram of CRISPR/Cas9 in iPSCs-Based HCM Disease Modeling. Patient-derived somatic cells are first reprogrammed into iPSCs, followed by CRISPR/Cas9 genome editing to correct the HCM genetic mutations. The resulting corrected, isogenic iPSCs, alongside the original HCM patient iPSCs, are differentiated to both diseased and healthy cardiomyocytes to facilitate comparative studies of HCM pathology.

MUTATIONS INVOLVED IN HCM

Single mutations associated with HCM

HCM was one of the first reported genetic cardiovascular disorders. In 1990, Christine Seidman and colleagues reported a mutation (Arg 403 to Glu) in gene MYH7 encoding the β-myosin heavy chain, a critical component of the sarcomere (15) (Table 1). This mutation, later found to be involved in 30-40% of all HCM cases. It leads to impaired cardiac muscle contraction, and is associated with severe HCM phenotypes, including early onset of symptoms and significant left ventricular hypertrophy. Clinical outcomes show that patients with the MYH7 mutation typically show an earlier onset, severe symptoms, and a poorer prognosis. This landmark discovery provided the initial genetic link to HCM and

Table 1. Prevalence of Gene Mutations Associated with HCM (4)

Gene	Symbol	Frequency of all HCM cases
β-Myosin heavy chain	MYH7	40-44%
Myosin binding protein-C	MYBPC3	35-40%
Cardiac troponin T	TNNT2	5-15%
α-tropomyosin	TPM1	3%
Cardiac troponin I	TNNI3	5%
Myosin light chain, essential	MYL3	1%
Myosin light chain regulatory	MYL2	1-2%
Cardiac α-actin	ACTC	1%
Ankyrin repeat	ACTN2	<1%
Troponin C	TNNC1	<1%
ZASP-LIM binding domain 3	LBD3	1-5%
Alpha-Actinin-2	ACTN2	<1%
Muscle LIM Protein	CSRP3	<1%

opened the door for further research into the genetic basis of HCM. To date, over 400 autosomal mutations have been identified in at least 13 genes encoding sarcomeric or sarcomere-related proteins in HCM patients. Other than MYH7, MyBP-C3 is another most prevalent mutation in HCM, accounting for approximately 30-40% of all identified mutations in HCM. Mutations in MYBPC3 are found to be correlated with a milder HCM phenotype and a later onset.

Additionally, several less common single-gene mutations associated with HCM have been identified, each with varying phenotypes and symptoms. For instance, Pasquale et al. reported on TNNT2 mutations in a large cohort study, which account for approximately 5% of HCM cases (16). Among 20 families with TNNT2 mutations, 12 distinct mutations were identified, including Arg278Cys, Arg92Leu, Arg92Trp, ΔGlu163, IVS15lGA, Ala104Val, Arg278His, Arg92Gln, Arg94Leu, Glu163Lys, Glu83Lys and Ile79Asn. This study showed that disease penetrance is low in children, and a large minority of adults with TNNT2 mutations have normal echoes, but abnormal electrocardiogram was typically seen throughout, even in those with normal echoes. Furthermore, this study concluded that left ventricular hypertrophy was uncommon in children and in an important minority of adults. Also, the frequency of sudden death was similar to other mutations of the sarcomere protein. Moreover, cardiac actin (*ACTC1*) is another gene carrying rare mutations associated with around 1% of all HCM cases. In 2000, Olson et al described in a paper (4), their study on multiple family members with HCM identified two heterozygous missense mutations, Pro164Ala and Ala331Pro in actin, which are located adjacent to regions of actin-actin and actin-myosin interaction (4). Many patients were found to be associated with an apical form of HCM. This study associated single amino acid substitution in actin to cause congestive heart failure or maladaptive cardiac hypertrophy.

In conclusion, understanding single gene mutations is essential for comprehending the pathology of HCM. Mutations in genes such as MYH7, MYBPC3, TNNT2, and ACTC1, which represent the majority of HCM mutations, exhibit diverse phenotypes and frequencies. These variations significantly influence disease severity, age of onset, and clinical outcomes, highlighting the importance of genetic research in the diagnosis and management of HCM.

Multiple mutations associated with HCM

Genetic studies on HCM have indicated that up to 5% of families carry two or more distinct disease-causing gene mutations (3, 17). Compared to individuals with single mutations, HCM patients with double mutations, whether homozygous or compound heterozygous, typically exhibit more severe left ventricular hypertrophy and a higher incidence of sudden cardiac death events, including resuscitated cardiac arrest, among family members (17, 18). For instance, Ho et al genetically evaluated a child with a family history of HCM and sudden deaths (19). DNA sequencing identified a mutation in exon 11 of the cTnT gene, resulting in a substitution of phenylalanine for serine at residue 179 (Ser179Phe). Both parents and three out of four surviving clinically unaffected children were heterozygous for this mutation. Genetic analysis of the deceased child who had profound left and right ventricular hypertrophy revealed he was homozygous for the Ser179Phe mutation (19). This suggests that the Ser179Phe mutation in the cTnT gene is likely responsible for the HCM observed in the family, with homozygosity potentially correlating with a more severe clinical phenotype.

Like those with double homozygous mutations, those with double heterozygous mutations show severe phenotypes. Suzuki et al studied a family with HCM with heterozygous missense variant Pro731Thr in the *MYH7* gene and the heterozygous frameshift variant Lys364fs* in the *MYH6* gene (20). The proband was a 7-year old boy diagnosed with HCM. At age 53, the probands maternal grandmother died of heart failure. In addition, the mother's younger brother died suddenly at age 19 due to HCM. All family members with HCM had the heterozygous mutations accompanied with a Z score >10 in the interventricular septum dimension. The proband and eldest brother had symptoms such as chest pain and systolic ejection murmur at the apex. Furthermore, the proband mother had exercise-induced dyspnea and was diagnosed with dilated phase HCM. In summary, this HCM-affected family with rare double variants in *MYH6* and *MYH7* genes, showed an increase of severity in symptoms and phenotype.

Patients with triple mutations tend to have even more severe clinical manifestations, such as earlier onset of symptoms, more pronounced cardiac hypertrophy, increased risk of heart failure, and a greater overall disease burden. Girolami and colleagues reported 4 hypertrophic cardiomyopathy pedigrees with each pedigree containing one person with triple HCM mutations (17). The mutations were the following: MYH7-Arg869His, MYBPC3-Glu258Lys and TNNI3-Ala86fs in a 32-year-old woman; MYH7-Arg723Cys, MYH7-Glu1455X and MYBPC3-Glu165Asp in a 46-

year old man; MYH7-Arg869His, MYBPC3-Lys1065fs and MYBPC3-Pro371Arg in a 45-year old woman; and MYH7-Arg1079Gln, MYBPC3- Gln969X and MYBPC3-Arg668His in a 50-year old woman (17). In 3 of the pedigrees, those who carried 3 mutations had onset ages of 18, 29, and 24 years which were relatively early. In comparison to the individuals carrying one or two mutations in the family, those with triple mutations show more severe phenotypes and aggressive disease such as heart failure, cardiac arrest (17).

In conclusion, studies examining multiple mutations in HCM have demonstrated that individuals with double mutations, whether homozygous or compound heterozygous, often exhibit more severe phenotype such as more aggressive left ventricular hypertrophy and a higher incidence of sudden cardiac death events compared to those with single mutations. Similarly, patients harboring triple mutations involving genes such as MYH7, MYBPC3, and TNNI3 show even more aggressive disease phenotypes, including earlier symptom onset, pronounced cardiac hypertrophy, and heightened risk of heart failure and cardiac arrest. This concludes that double mutations and multiple gene mutations occurring in HCM families result in a more severe clinical phenotype.

Differentiation of iPSCs into Cardiomyocytes

Significant efforts have been devoted to developing effective methods for differentiating human iPSCs into cardiomyocytes, which is crucial for understanding the molecular mechanisms underlying HCM. Zhang et al. successfully utilized the embryoid body method to differentiate human iPSCs into cardiomyocytes (21). RT-PCR analysis confirmed the expression of cardiac-specific genes and the downregulation of pluripotency markers OCT4 and NANOG during differentiation (21). Their result demonstrated that the iPSC-CMs exhibited well-formed sarcomeric structures with nodal-, atrial-, and ventricular-like phenotypes and responded to β-adrenergic stimulation with increased spontaneous beating rates and decreased action potential durations (21). Furthermore, a refined differentiation method by combining the matrix sandwich with sequential application of growth factors (Activin A, bone morphogenetic protein 4, and basic fibroblast growth factor) was reported for the differentiation of human iPSCs to cardiomyocytes (22). This approach significantly enhanced the efficiency of generating CMs, achieving up to 98% purity (22).

In contrast to growth factors, small molecules are relatively stable and inexpensive, and they can pass through cell membranes to reach intracellular targets. Therefore, several groups have developed novel small molecules-based methods for the iPSC-CMs differentiation. For instance, Aguilar et al reported a small molecules-based approach which consists in activation of the Wnt signaling at day 0-1 with small molecule CHIR99021, followed by inhibition of bone morphogenetic protein signaling at day 1-4 with DMH1 (5). This method significantly promotes cardiac formation from human iPSCs, leading to about 75% cardiomyocytes of the total cells within 7 days (5). In addition, Lian et al. and Burridge et al. independently developed small molecule-based cardiac differentiation protocols. They demonstrated that the upregulation of the Wnt signaling with small molecule CHIR99021 during the early stage of differentiation, followed by the inhibition of the Wnt signaling with small molecules WntC59 and XAV939, efficiently produces over 95% cardiomyocytes (6, 7).

Nevertheless, the cardiomyocytes induced by the aforementioned approaches are normally immature, similar to those at the fetal stage. These immature cardiomyocytes exhibit distinct characteristics in morphology, electrophysiology, calcium handling, metabolism, and transcriptional signatures (8). For instance, immature iPSC-CMs are smaller, rounder, and mononucleated with fewer mitochondria, whereas mature cardiomyocytes are rod-shaped, longer, sometimes binucleated, and possess a high density of mitochondria (8, 9). Additionally, in iPSC-CMs, glycolysis is the primary ATP production pathway suitable for the hypoxic environment of the developing heart, whereas in adult cardiomyocytes, fatty acid oxidation predominates, providing a more efficient ATP yield to meet the high-energy demands of the adult heart (10).

Immature iPSC-CMs cause many problems when used in disease modeling and therapeutic applications. For instance, animals with the transplanted immature iPSC-CMs could develop arrhythmias and even myocardial infarctions (23). Therefore, significant efforts have been made to generate mature iPSC-CMs that function similar to adult cardiomyocytes. Hsueh et al. discovered that Secreted Frizzled Related Protein 2 (Sfrp2), a more selective Wnt inhibitor, induced mature cardiomyocyte. They form gap junctions and contain longer sarcomeres with a lower beating frequency and less circular cardiomyocyte shape while being physiologically normal with a lengthened action potential. This matured phenotype did not dedifferentiate and stayed stable in culture for up to 40 days (24). For more information of iPSC-CM maturation, readers may refer to other review articles (25, 26).

TRADITIONAL DISEASE MODELING HCM WITH IPSC-CMS

MYH7 gene mutations in HCM disease modeling

The advent of iPSCs reprogrammed from patient somatic cells followed by differentiation into cardiomyocytes has proven to be a valuable tool to study HCM. Mutations in MYH7 gene accounts for ~ 35% HCM, and they have been extensively studied by using iPSC-CMs. Lan et al. generated patient-specific iPSC-CMs from a ten-member family cohort with a hereditary HCM caused by $MYH7^{Arg663His}$ gene mutation (27). The iPSC-CMs exhibited key HCM phenotypes, including cellular enlargement and contractile arrhythmias at the single-cell level. Additionally, Ca^{2+} imaging showed that dysregulation of Ca^{2+} cycling and elevated intracellular Ca^{2+} are closely associated with HCM (27). When incubated for 5 days with isoproterenol, a β-adrenergic agonist known to trigger for myocyte hypertrophy, the patient iPSC-CMs increased in cell size by 1.7-fold between day 30 and 35 in comparison to control counterparts which typically did not exhibit cellular hypertrophy until day 40. In consistence, treatment with propranolol, a β-adrenergic blocker with isoproterenol significantly ameliorated the exacerbation of hypertrophy, Ca^{2+} handling deficiencies and arrhythmia (27). Diastolic dysfunction frequently occurs to HCM patients, leading to major morbidity and mortality, and recently Wu et al examined diastolic dysfunction in HCM by using iPSC-CMs derived from HCM patient carrying $MYH7^{Arg663His}$ mutation (28). Their results have demonstrated the diseased iPSC-CMs exhibited impaired diastolic function such as prolonged relaxation time, decreased relaxation rate, and shortened diastolic sarcomere length (28). Furthermore, Ca^{2+} imaging results have shown elevated diastolic Ca^{2+} and abnormal Ca^{2+} handling, worsened by β-adrenergic challenge. Han et al. studied the $MYH7^{Arg442Gly}$ gene mutant in HCM by using patient iPSC-CMs (29). In comparison to the healthy iPSC-CMs, there are a significant increase in genes related to 'Cell Proliferation' in HCM iPSC-CMs evidenced by the whole transcriptome sequencing and pathway enrichment analysis (29). Moreover, HCM iPSC-CMs also exhibited disorganized sarcomeres and electrophysiological abnormalities (29).

MYBPC3 mutations in HCM disease modeling

MYBPC3 is the other most commonly mutated gene in HCM, accounting for 34-40% of cases. Mutations in *MYBPC3* frequently result in a premature termination codon which leads to a deleterious truncated peptide and haploinsufficiency characterized by a decreased production of full-length protein in cardiac myocytes compared to normal levels (30). This reduction in functional protein is thought to be a key factor contributing to HCM in cases involving MYBPC3 truncation mutations (31). Ojala et al generated iPSCs from Finnish patients carrying the MYBPC3-Gln1061X nonsense mutation (32). After differentiation of the patient-specific iPSCs into CMs, the authors could not detect mutant allele on mRNA expression level or truncated MYBPC protein in the patient-specific iPSC-CMs, suggesting that the mutant mRNA and the truncated MYBPC3 protein might be degraded. They further demonstrated that the patient-specific iPSC-CMs with MYBPC3-Gln1061X displayed larger cellular size than those derived from healthy control iPSCs (32). Increased Ca2+ sensitivity is thought to be a common characteristic across various HCM mutations (33, 34). Interestingly, the Ca2+ handling abnormalities observed in the patient-specific iPSC-CMs with MYBPC3-Gln1061X were comparable to those found in the control CMs (35).

Other gene mutations in HCM disease modeling

Other than MYH7 and MYBPC3, some other gene mutations are involved in HCM, which have been studied as well. For instance, α-tropomyosin (TPM1-Asp175Asn) gene mutation is the other important founder mutation for HCM in Finland. Ojala et al compared the iPSC-CMs carrying TPM1-Asp175Asn mutation and the cells harboring the MYBPC3-Gln1061X nonsense mutation (32). They found that the iPSC-CMs with TPM1-Asp175Asn mutation displayed pathological phenotypes of HCM similar to the cells with the MYBPC3-Gln1061X mutation (32). But there are differences between CMs carrying either MYBPC3-Gln1061X or TPM1-Asp175Asn gene mutation in their cellular size, $Ca^{(2+)}$ handling, and electrophysiological properties, as well as their gene expression profiles (32). These findings suggest that even though the clinical phenotypes of the patients carrying either MYBPC3-Gln1061X or TPM1-Asp175Asn gene mutation are similar, the genetic background as well as the functional properties on the cellular level might be different, indicating that the pathophysiological mechanisms behind the two mutations would be divergent as well. Moreover, Zhou et al reprogrammed diseased iPSCs from a HCM patient carrying Arg58Gln mutation in the myosin regulatory light chain (MYL2), followed by cardiac differentiation (36). They showed that iPSC-CMs harboring MYL2- Arg58Gln were nearly 30% larger than controls at day 60. The MYL2-Arg58Gln

cells exhibited significantly higher levels of myofibrillar disarray and irregular beating compared to the controls. Calcium signaling was impaired, with delayed decay time. Overall, the MYL2- Arg58Gln iPSC-CMs successfully recapitulated the key HCM phenotypes (36).

In summary, iPSC technology is emerging as a key tool for HCM disease modeling. This approach has provided insights into the effects of genetic mutations, including MYH7 and MYBPC3 genes. These studies have demonstrated that patient-specific iPSC-CMs exhibit hallmark HCM traits like hypertrophy, contractile abnormalities and calcium dysregulation.

Isogeneic iPSCs for HCM modeling

While iPSC-based modeling of HCM provides valuable insights into disease mechanisms, it often overlooks the impacts of confounding factors such as genetic background, which can lead to misleading outcomes. For example, in iPSC-based dyskeratosis congenita disease modeling, two independent groups reported conflicting results: one demonstrated telomere regrowth, while the other observed telomere decay, accounting for in dyskeratosis congenita (37, 38). This discrepancy is primarily due to the confounding effects of genetic variability among iPSC lines generated from different patients and controls. To ensure accurate comparative analysis in iPSC-based disease studies, it is crucial to eliminate genetic and other variabilities between iPSC lines reprogrammed from patients and healthy individuals.

The recent advent of clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) provides a viable solution to the genetic variabilities between the diseased and healthy iPSC lines (39). CRISPR/Cas9 is a gene editing tool adapted from a natural system used by bacteria to defend against viruses, offering ability to make specific changes to the DNA of living organisms in an inexpensive and fast way (39). In this system, a single guide RNA (sgRNA) directs the Cas9, an endonuclease enzyme, to a specific DNA sequence, where it creates a double-strand break (DSB). This break is then repaired by the cell machinery through two mechanisms, the Non-Homologous End Joining (NHEJ) and the Homology-Directed Repair (HDR) (40). NHEJ is an error-prone repair process often resulting in small insertions or deletions (indels) to disrupt gene function. While HDR enables precise genetic modifications by utilizing a DNA template for accurate repair (40). CRISPR/Cas9 can be more beneficial and advantageous than the average gene editing tool because it can target multiple genes at the same time.

With the available CRISPR/Cas9 tool, scientists can effectively generate isogenic iPSC lines with identical genomes except for specific gene mutation(s), allowing for precise studies of diseases including HCM without noises from genetic background difference between the diseased iPSCs and healthy controls. $MYH7^{Arg453Cys}$ is one of the most common HCM mutations, and MYH7 is the sarcomeric gene encoding the beta myosin heavy chain to regulate the myosin actin interaction during cardiomyocyte contraction (41). Using the CRISPR/Cas9 editing system, Mosqueira et al created 11 polymorphic variants of this mutation in 3 different iPSC lines. Isogenic cell line sets were identified and then differentiated into iPSC-CMs which displayed HCM characteristics such as multinucleation, sarcomeric disarray, hypertrophy, and hypertrophic marker expressions (42). Functionally, iPSC-CMs also resembled the HCM model as they displayed energy depletion, arrhythmias, calcium handling and contraction force abnormalities, along with higher metabolic respiration/ ATPase activity. Their study indicated that the *MYH7:MYH6* ratio in the HCM diseased iPSC-CMs was ~5-15 fold higher than that in the normal iPSC-CMs, which is consistent with the increase found in hypertrophied human hearts (42). This suggests a compensatory feedback mechanism that the diseased iPSC-CMs are in favor of the more 'energy-efficient' βMHC isoform over the 'energy-demanding' fast αMHC isoform. Furthermore, Smith et al. reprogrammed diseased specific iPSC lines from three HCM donors of the same family consisting of a father and two sons carrying E99K in cardiac actin ACTC1 protein (43). They then corrected the mutation in the diseased iPSC lines to make the isogeneic health control lines for study comparison by using the CRISPR/Cas9 genomic editing (43). Their result demonstrated that all the HCM iPSC-CMS showed arrhythmogenesis, abnormal calcium handling and contractility, along with hypertrophic signaling. Particularly, the iPSC-CMs from father displayed more pronounced HCM phenotypes as compared to those from his sons, which could be attributed to the older age and genetic background of the father. By using the dual treatment of ranolazine and dantrolene Ca^{2+} handling modifier pharmaceuticals, the hypertrophy was reduced in HCM iPSC-CMs dramatically, highlighting the effectiveness of isogenic iPSC-CMs for understanding HCM disease. Recently, Wu et al. generated isogenic iPSC lines with HCM mutations, demonstrating that diastolic Ca^{2+} overload, slowed $[Ca^{2+}]i$ recycling, and increased myofilament Ca^{2+} sensitivity collectively impair HCM iPSC-CM relaxation (28). Partial blockade of Ca^{2+} or late Na^{+} current improved diastolic function and long-term

survival. Elevated expression of L-type Ca^{2+} channels and transient receptor potential cation channels in HCM iPSC-CMs likely contributed to diastolic [Ca^{2+}] overload (28).

Prodzynski et al discovered a rare T247M mutation in the ACTN2 gene, which encodes α-actinin 2, in a HCM patient (44). The patient exhibited left ventricular hypertrophy, outflow tract obstruction, and atrial fibrillation. iPSC-CMs derived from the patient recapitulate key HCM features, such as hypertrophy, myofibrillar disarray, hypercontractility, impaired relaxation, increased myofilament calcium sensitivity, prolonged action potentials, and elevated L-type calcium currents (44). Treatment with the L-type calcium channel blocker diltiazem significantly reduced force amplitude, improved relaxation, and shortened action potential duration in the HCM iPSC-CMs compared to isogenic controls. Clinical translation of these findings demonstrated that diltiazem improved the prolonged QTc interval in HCM-affected son and sister (44).

Isogenic iPSCs offer a solution to the variability in iPSC-based HCM modeling caused by genetic background differences. By utilizing CRISPR/Cas9 to create iPSC lines with identical genomes except for specific mutations, researchers can generate accurate disease models and study the impact of these mutations without confounding genetic factors.

CONCLUSION AND FUTURE DIRECTIONS

HCM is one of the most prevalent genetic heart conditions that lead to sudden cardiac death among young individuals. Current treatments focus on symptom relief and preventing severe complications, largely due to the elusive underlying mechanisms of HCM. While animal models have provided some insights, they do not accurately reflect human HCM. The advent of iPSCs offers an excellent tool to study HCM in vitro, and many studies have demonstrated iPSC-CMs recapitulate the disease's key features, including hypertrophy, myofibrillar disarray, and altered calcium handling. Nevertheless, traditional iPSC-CM based HCM studies have often overlooked the impact of individual genetic background differences and other variables, potentially leading to inaccurate conclusions. This issue can be addressed through the use of isogenic iPSCs generated by CRISPR/Cas9, which eliminate genetic background differences. Recent studies using isogenic iPSCs have successfully replicated HCM phenotypes. Comparative research between isogenic HCM iPSC-CMs and control iPSC-CMs offers a promising path to better understanding the true pathogenic mechanisms of HCM and could provide a powerful platform for identifying drugs that can reduce or reverse cardiac hypertrophy or fibrosis in HCM.

Additionally, current iPSC studies almost exclusively focus on single causative mutations of HCM, despite evidence that individuals with double or triple causative mutations often present with much more severe clinical phenotypes of the disease. Future studies should explore these complex genotypes, which could provide deeper insights into the mechanisms driving HCM progression and lead to the development of more effective therapeutic strategies tailored to patients with multiple genetic mutations.

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