"My family" by George Bellows. Courtesy National Gallery of Art, Washington.

Emerging Relationships of Sarcomeric Mutations and the Cardiomyocyte Transcriptome in the setting of Familial Hypertrophic Cardiomyopathy

Chandana Kulkarni[1]

[1]Burnett School of Medicine at Texas Christian University, Fort Worth, Texas 76129
Correspondence: C.Kulkarni@TCU.edu

Familial hypertrophic cardiomyopathy (FHC) is thought to be the most common genetically inheritable cardiac disease with a prevalence of 1 in 500 individuals. A classic sign of FHC is inappropriate asymmetrical thickening of the septum with the potential for heart failure and sudden cardiac death, in the absence of mechanical stress, pressure overload, or pathogenic infiltration. Molecular analysis of the thickened septum in the past has revealed that these cardiomyocytes are enlarged and disorganized with interstitial fibrosis, thus causing restricted blood flow out of the left ventricle. Significant causes of idiopathic FHC disease pathogenicity have been linked to sarcomere dysfunction in 8 key genes. Research over the years has identified two major sarcomere mutations such as myosin-binding protein C (*MYBPC3*) and

β-myosin heavy chain (*MYH7*). Together these gene mutations account for over 80% of causes for FHC phenotype presentation. The focus of this review will be to analyze current knowledge regarding the *MYBPC3* and *MYH7* gene mutations in the sarcomere, as well as take look at how they directly and indirectly affect the transcriptome associated with cardiomyocyte hypertrophy and fibrosis. Finally, we will identify current and future potential targets for disease-modifying diagnostics and therapy.

INTRODUCTION

Familial hypertrophic cardiomyopathy (FHC) is the most common monogenic cardiac disease in which the left ventricle (LV) and septum experience hypertrophy generally in the absence of any other cardiac or systemic disease. Clinically this is defined as unexplained LV hypertrophy with a maximum wall thickness greater than 15 mm in adults or a z-score > 3 in children (1). This asymmetric hypertrophy of the left ventricular wall and septum places individuals with FHC at an increased risk for sudden cardiac death (1). With a prevalence of approximately 1 in 500 amongst the general population (1). Familial hypertrophic cardiomyopathy is inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. While the clinical presentation and course display a large degree of heterogeneity, the disease is an important cause of disability and death among patients of diverse age. The estimated penetrance of hypertrophic cardiomyopathy (HCM) after a 15-year follow-up period was found to be 46% (2, 3).

A sarcomeric gene refers to a gene that encodes proteins involved in the structure and function of the sarcomere, which is the basic contractile unit of muscle cells, including cardiomyocytes in the heart. Since the majority of FHC cases have been traced to sarcomeric protein mutations, the focus of this review will be exploring two of the most implicated sarcomeric genes in FHC: β-myosin heavy chain (*MYH7*) and myosin binding protein C (*MYBPC3*), responsible for approximately 80% or greater of FHC (1). As seen in **Table 1**, it should also be noted that while other mutations in the sarcomeric genes such as *TTN*, *MYH6*, *MYL2*, *MYL3*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC*, and *TNNC1* do occur and contribute to FHC, they together only account for less than 10% of cases and will therefore not be explored in this review. The protein produced from the *MYH7* gene is a major component of the thick filament in sarcomeres and is involved with the ATPase function used to generate force (4). The protein produced from the *MYBPC3* gene provides structural support and regulates muscle contractions by associating with the thick filament (4).

Patients with FHC who harbor sarcomeric gene mutations often exhibit a diverse array of clinical manifestations. Common clinical symptoms may include shortness of breath, chest pain, fatigue, and palpitations. It is noteworthy that the disease presentation can vary widely, with some individuals remaining asymptomatic for extended periods, while others may experience severe symptoms, such as heart failure or arrhythmias. Additionally, there is a notable risk of sudden cardiac death, particularly among younger patients, underscoring the importance of early diagnosis and comprehensive clinical management (5). Histological examination shows that FHC causes myofibrillar disarray as well as considerable degrees of tissue and interstitial fibrosis. Activation of the hypertrophic signaling pathways and profibrotic signals in the nonmyocyte cells produce the disease remodeling in FHC. Cytokines, microRNA's, and other cell cycle proteins have all been implicated in

Table 1. Genes Associated with Familial Hypertrophic Cardiomyopathy

Gene Name (full)	Gene Symbol	Chromosomal Location	Number of Mutations Associated with FHC
Myosin-binding protein C	*MYBPC3*	11p11.2	Over 500 mutations (Approximate)
β-myosin heavy chain	*MYH7*	14q11.2	Over 300 mutations (Approximate)
Titin	*TTN*	2q31.2	Varied mutations (Less common)
Myosin light chain 2	*MYL2*	12q24.11	Less common mutations
Myosin light chain 3	*MYL3*	3p21.31	Less common mutations
Troponin T	*TNNT2*	1q32.1	Less common mutations
Troponin I	*TNNI3*	19q13.4	Less common mutations
Tropomyosin	*TPM1*	15q22.1	Less common mutations
Cardiac alpha actin	*ACTC*	15q14	Less common mutations
Troponin C	*TNNC1*	3p21.31	Less common mutations

This table provides an overview of genes associated with Familial Hypertrophic Cardiomyopathy (FHC), including their full names, gene symbols, chromosomal locations, and an approximate number of mutations linked to FHC for each gene. The table highlights two major genes, MYBPC3 and MYH7, which are the primary contributors to FHC, along with other genes that also play a role in the disease, albeit less commonly (1, 5).

myocardial necrosis and fibrosis in patients with FHC. Transforming growth factor-β (TGF-β) is one such cell cycle protein that has been implicated as a major pro-fibrotic protein in the pathologic remodeling in FHC (1). MicroRNA's, such as miRNA29, have also been noted to be markedly elevated in FHC and are theorized to serve as markers for cardiomyocyte hypertrophy as well as a potential role in the development of interstitial fibrosis. Pharmacologic inhibition of these targets warrants further exploration as potential therapies for FHC.

This review aims to summarize some of the current knowledge about the pathogenesis of Familial Hypertrophic Cardiomyopathy, analyze new research studying the transcriptome changes underlying the hypertrophic phenotype seen in cardiomyocytes with this disease, and explore a few directions of the new therapeutics.

DISCUSSION

Sarcomeric Mutations

After the discoveries were made in the 1980s regarding the locations of the genes associated with FHC, more studies have been done to pinpoint the specific mutations. The study done in August 2010 by Tanjore et al. showed that there have been 186 mutations identified within the *MYH7* gene to date, mainly within the exons 7, 12, 19, and 20 that have been implicated heavily with the disease progression of FHC (6). While numerous studies are detailing the diverse array of disease severity associated with specific mutations, we will focus on the molecular mechanisms in a broader sense. The majority of genotyped sarcomeric FHC related mutations of *MYH7*, implicated only a single missense nucleotide substitution that results in a mutated protein. As seen in **Figure 1**, normally this protein is involved in interacting with the actin filament and using its ATPase activity required to produce a power stroke necessary in contraction. In an investigation into the *R723Q* mutation of *MYH7* by Kraft et al. in triton-permeabilized cardiomyocytes, it was found that maximum force was significantly lowered, even though the calcium sensitivity remained unchanged

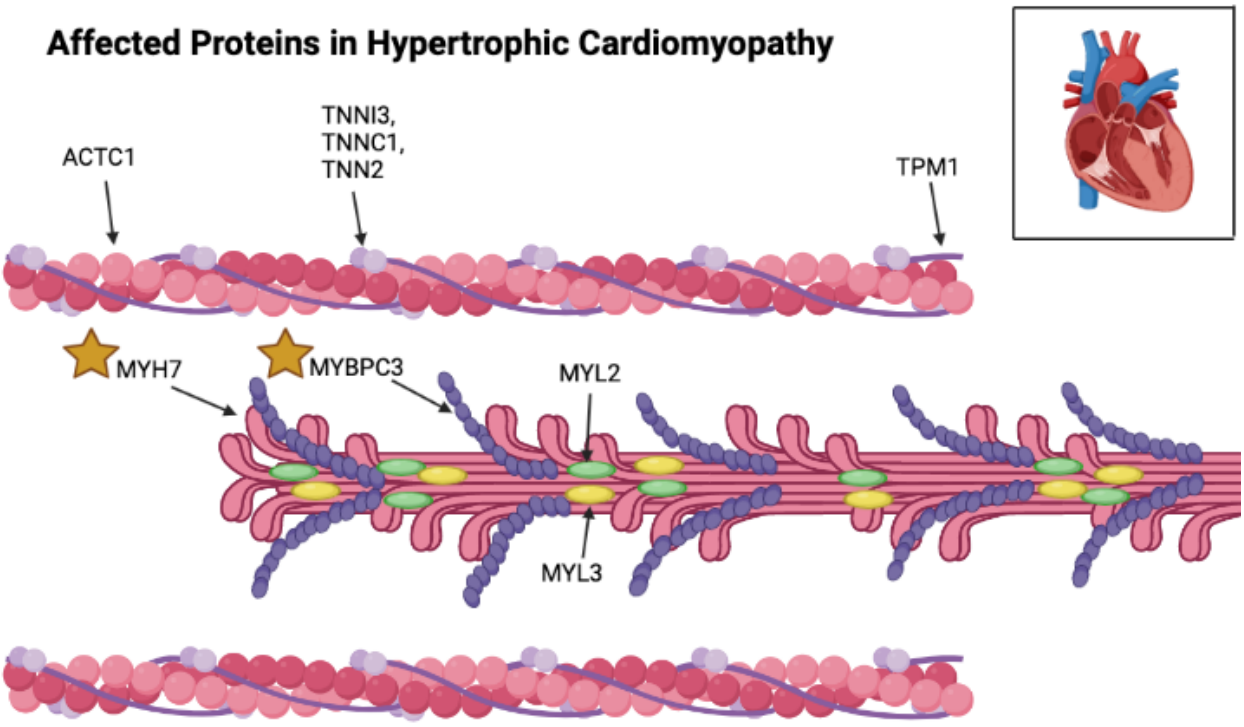

Figure 1. ***Causal Genes for FHC.*** *This schematic shows the structure of the sarcomere and the specific proteins of the thin and thick filament. Each of the labeled proteins are currently established as causal genes for FHC. The starred proteins,* MYH7 *and* MYBPC3*, both represent the elements of the thick filament that are most often implicated in the development of FHC.*

from the normal baseline. Further analysis revealed that protein phosphorylation was decreased in the other proteins in the sarcomere, such as troponin I and T, myosin-binding protein C, and myosin light chain 2 in the *R723Q* cardiomyocytes (7). This is interesting to note since it may provide evidence of secondary cellular effects that a mutation in the *MYH7* gene may cause. Histological sections of the tissue were taken and analyzed which showed that the myofibrillar density was greatly reduced along with irregular Z-discs and variable axes of the sarcomeres within the cardiomyocytes (7). This shows that the low cardiomyocyte force generation capacity in FHC patients can be explained by the reduced myofibril density and myofibrillar disarray. Furthermore, the hypo-contractile sarcomeres may present the primary cause for hypertrophy in patients with *MYH7* mutations. These findings were further corroborated by Witjas-Paalberend's research which studied whether cellular dysfunction is due to intrinsic sarcomere defect or cardiomyocyte remodeling by measuring maximal force-generating capacity (Fmax) in various mutations within the filaments (8). This study showed that *MYH7* mutations reduced force-generating capacity at all Ca2+ concentrations and is explained by hypertrophy and reduced myofibril density.

While missense mutations explain mutations in *MYH7*, *MYBPC3* is primarily affected by frameshift mutations (9). Frameshift mutations of the *MYBPC3* gene result in a truncated cardiac myosin-binding C protein (*cMyBP-C*) in the myocardium from patients with FHC. Normally *cMyBP-C*

interacts with both the myosin and actin via phosphorylation of its head to promote ATPase activity and cross-bridge formation. A study was done by Toepfer et al. investigated how mutations in *MYBPC3* alter cardiac muscle contraction and relaxation by using both mouse models and human fibers. They showed that *MYBPC3* mutations cause FHC by haploinsufficiency, and further demonstrated that cardiomyocyte phenotypes are dependent on *cMyBP-C* quantities by manipulating the levels of the protein present in the cardiomyocyte (9). By testing contractility of the cardiomyocyte sarcomere at varying levels of *cMyBP-C*, confirmed the dose-dependent relationship in disease presentation. *cMyBP-C* truncation and lower overall levels of functional *cMyBP-C* in the cell correlated with the hypercontractility of the sarcomere (9). This is interesting because this is a different pathogenesis than an *MYH7* mutation. Lack of *cMyBP-C* also altered the myosin confirmations during relaxation and encouraged more ATP hydrolysis leading to more thin filament interactions while discouraging the relaxed state of the myosin head. This information posits the theory that myosin dysregulation is the main pathology behind *MYBPC3* mutations. This hypercontractility, failure to properly relax, and increased energy consumption lead to hyperdynamic contraction, diastolic dysfunction, and energy inefficiency observed in FHC cardiomyocytes.

Mutant Cardiomyocyte Transcriptome

While the mechanisms are likely extremely complicated, it is helpful to see what the cardiomyocyte itself may be doing in terms of gene expression and production of transcripts by looking at the transcriptome of the cell. In a study by Farrell et al., they aimed to use a murine model to identify the early genetic mediators in the development of cardiomegaly seen in *cMyBP-C* mutations by studying the mutant cardiomyocyte transcriptome. By performing microarray analysis on left ventricles of wild type and *cMyBP-C* mutant mice at varying post-natal days, they were able to identify genes that were dysregulated in the mutant mice even prior to the hypertrophy phenotype (10). Some of these genes included genes in mechano-sensing pathways and potassium channels linked to arrhythmias (10). One of the genes, *Xirp2*, and its protein are normally regulated during normal growth but show significant upregulation in pre-hypertrophic mutant hearts (10). The researchers also found that transcription factor *Zbtb16* also shows upregulation in pre-hypertrophic mutant hearts (10). The dysregulation of both genes and their protein products even before the hypertrophic phenotype in *MYBPC3* mutant mice hearts may indicate that these are important stress sensing genes early in the development of FHC. It may also provide the door to genetic diagnostics that shed light on the stage of disease presentation. This study also underlines the importance of the extracellular matrix in the hypertrophic phenotype.

The pathologic mechanisms behind the development of FHC are certainly complex. Currently little is known about the upstream regulators that may be affected by sarcomeric mutations and thus cause the disease phenotype. One such protein involved in the metabolic stress response in FHC is p53. A study done by Cohn et al. applied RNA sequencing to the cardiomyocyte samples with *MYH7* and *MYBPC3* mutations. The results from this study implicated p53 signaling as a common molecular consequence of the thick filament mutations (11). RNA sequencing data for p53 dependent gene expression revealed an increased concentration of BBC3, BAX, and FAS transcripts within mutant cardiomyocytes (11). These transcripts all play a role in cytotoxicity and function in the regulation of cell death. Given the previously

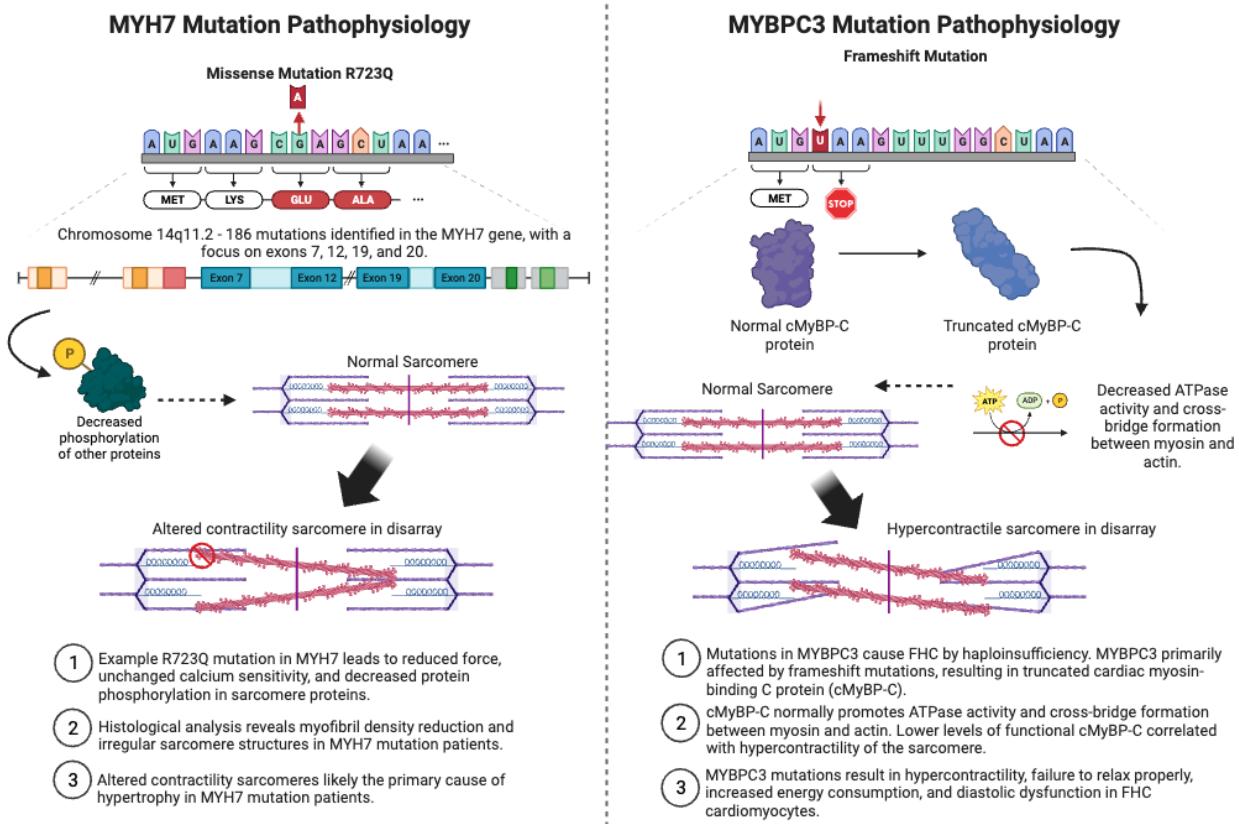

Figure 2. ***Key findings related to MYH7 and MYBPC3 mutations in Familial Hypertrophic Cardiomyopathy (FHC).*** *MYH7 mutations in FHC reduce maximum force generation despite normal calcium sensitivity, leading to hypo-contractile sarcomeres and hypertrophy. MYBPC3 mutations cause cMyBP-C haploinsufficiency, resulting in hypercontractility, impaired relaxation, and diastolic dysfunction in cardiomyocytes. These insights are vital for developing FHC therapies.*

established energy inefficiency problem of FHC cardiomyocytes, this is in line with p53 become activated as a problem solver of metabolic stress. It was also found that due to the increased energy usage and higher ADP:ATP ratio, mutant cardiomyocytes also contain higher mitochondrial-derived ROS (11). This also provides background on why p53 may be provoked in FHC cardiomyocytes.

Another important part of any cell transcriptome is microRNAs. Myocardial miRNA's may modulate the processes of cardiomyocyte hypertrophy, excitation-contraction coupling, and apoptosis. A study done by Roncarati et al. showed that 12 miRNAs were significantly increased in HCM plasma, however, only 3 of those miRNAs were found to be correlated with hypertrophy (12). Of those, it was significant that miRNA-29a was the only one correlated with fibrosis (12). Another study around the same time done by Kuster et al. studied the microRNA expression profile of FHC patients carrying *MYBPC3* mutations. The interesting thing here is that the 13 miRNA's that were found to be correlated with FHC hypertrophy originated from an intron in the *TRPM3* gene (13). RT-PCT analysis showed that the *TRPM3* gene was upregulated in FHC compared to the normal myocardium (13). These studies indicate that *MYBPC3*

mutations produce a specific miRNA expression profile which could be useful in understanding signaling pathways and designing therapeutics that target these specific miRNAs.

Current Recommendations and Novel Therapies

Since the characterization of FHC almost 60 years ago, the diagnosis and management of patients have moved forward with cardiac imaging and previous serious arrhythmias, and interventional cardiology measures. Concurrent with Landstrom et al. it's understood that it is not possible yet to determine prognosis based on the mutation (14). Given the sheer number of mutations that can lead to FHC with so many different modifying factors, it is difficult to establish a genotype to the phenotype endpoint with precision. Given the same mutation in two patients, it is almost certain that the phenotype will differ. The current pharmacotherapeutic recommendations for the management of FHC are aimed at alleviating symptoms, preventing complications, and enhancing cardiac function. Individualized treatment plans are essential, and specialized healthcare teams, including cardiologists and genetic counselors, play a pivotal role in providing comprehensive care.

Commonly used pharmacological interventions include beta-blockers like metoprolol and atenolol to reduce heart rate, relieve chest pain, shortness of breath, and palpitations, as well as to prevent arrhythmias. Calcium channel blockers such as verapamil or diltiazem may be employed, either alone or in conjunction with beta-blockers, to enhance heart muscle relaxation and reduce stiffness. In certain cases, anti-arrhythmic medications like disopyramide are used to manage abnormal heart rhythms. Patients at risk of atrial fibrillation or blood clots may be prescribed anticoagulants like warfarin or newer oral anticoagulants. Diuretics, such as furosemide, may help alleviate fluid retention and congestion in heart failure. ACE inhibitors or ARBs may be considered to manage blood pressure and reduce cardiac workload. Symptomatic relief for angina can be achieved using nitrates. Genetic testing and counseling are often recommended to identify specific gene mutations associated with FHC and assess familial risk. In severe cases with a high risk of sudden cardiac death due to arrhythmias, implantable cardioverter defibrillators (ICDs) may be implanted for continuous monitoring and intervention. For refractory symptoms and severe obstruction, septal reduction therapies like septal myectomy or alcohol septal ablation may be considered (5). Collaboration with healthcare providers specializing in FHC management and a multidisciplinary approach are essential for optimal care.

A new interesting approach has identified a small molecule MYK-461 (15). This small molecule was studied by Green et al. showed that it reduces the contractility by decreasing the ATPase activity of the cardiac myosin heavy chain. This study also shows that chronic heavy chain administration of MYK-461 suppresses the development of ventricular hypertrophy, cardiomyocyte disarray and prevents myocardial fibrosis by blocking fibrotic gene expression (15). Given that the hyperdynamic contraction and induction of profibrotic genes are a central tenet for the development of FHC, this new molecule presents an extremely promising therapeutic approach. Further research was done by Toepfer et al also showed that this molecule had the ability to attenuate myosin activity in cardiomyocytes with *MYBPC3* mutations. Mavacamten, a synthetic version of MYK-461, is the first in its selective allosteric inhibitor of cardiac myosin ATPase which serves to reduce actin-myosin cross-bridge formation and reduce cardiomyocyte

energy usage (16). This new drug was approved for use in the US in April 2022.

Mavacamten achieves its therapeutic effects by inhibiting the ATPase rate of beta myosin, shifting its equilibrium away from its activated state towards a super relaxed state. This reduction in beta myosin activity results in the inhibition of contractility and a decrease in excitotoxic calcium handling. Preclinical studies in rodent models demonstrated several beneficial effects, including the reduction of myocardial contractility, prevention of left ventricular hypertrophy, reduction of myocardial fibrosis, and suppression of pro-fibrotic signaling pathways. These effects translated into improved functional capacity and the prevention of hypertrophic remodeling in animal models. Positive results from the Phase II PIONEER-HCM (Hypertrophic Cardiomyopathy) trial paved the way for the Phase III EXPLORER-HCM trial, which assessed mavacamten's efficacy and safety in patients with obstructive HCM. The trial met its primary endpoint, with a significant improvement in New York Heart Association (NYHA) functional class and peak oxygen consumption. Subsequent studies, such as VALOR-HCM, explored mavacamten's benefits in patients eligible for septal reduction therapy, demonstrating a significant decrease in LV outflow tract gradients and NYHA class. Additionally, ongoing open-label extension studies suggest the potential for long-term benefits, including the reduction of LV wall thickness and myocardial fibrosis. Other cardiac myosin inhibitors, like aficamten, are under development and have shown promise in preliminary trials, offering additional therapeutic options for FHC (5).

As of now, the genetics aspect of this disease has remained largely diagnostic, rather than be wielded as a therapeutic tool, but it is now emerging as a promising strategy to target the genetic origins of this disease. Several approaches, including gene replacement using adeno-associated viral vectors, gene editing, allele-specific silencing, trans-splicing, and exon skipping, are being explored. Recent advancements, such as base editing to correct specific HCM-causing variants, have demonstrated potential in rescuing the disease phenotype in preclinical models. Gene therapy methods, like gene replacement, have shown potential in studies using special cells that lacked *MYBPC31718*. More recently, a technique called base editing was able to correct a common disease-causing variant in HCM, known as *MYH7* p.R403Q, and reverse the HCM symptoms in both lab-grown heart cells and a mouse model (19). However, early-phase human trials face ethical challenges, patient selection issues, outcome identification, and the management of off-target effects. Transcriptomic studies such as the ones above provide us incredible opportunities to control or prevent the disease progression in with the help of small molecule therapeutics like siRNAs to silence pathologic phenotypes.

CONCLUSIONS

FHC is just one subtype of an incredibly complex pathology collectively known as hypertrophic cardiomyopathy. The clinical phenotypes, histological presentation, and genetic causes of FHC are extremely diverse. They are the consequences of a large of mediating factors, ranging from causal genetic mutation to lifestyle and other genetic predeterminants. Progress in understanding the genetic basis for the disease has led to the identification of important causative mutations. Greater knowledge of the pathogenic pathways incriminated in sarcomeric mutations, cell cycle and regulatory proteins, and miRNAs will elucidate the way to treat the causes of the disease rather than symptoms. Ideally, this will also present solutions to shift from

treating myocyte hypertrophy, fibrosis, and obstruction to using genetic and phenotypic analysis to provide individual solutions for each patient. Insights into these processes from culture studies, murine models, and human clinical trials will advance the field of cardiology.

DISCLOSURES

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