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Mechanisms of Sarcomere Protein Mutation-Induced Cardiomyopathies

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Abstract

Purpose of Review The pace of identifying cardiomyopathy-associated mutations and advances in our understanding of sarcomere function that underlies many cardiomyopathies has been remarkable. Here, we aim to synthesize how these advances have led to the promising new treatments that are being developed to treat cardiomyopathies.

Recent Findings The genomics era has identified and validated many genetic causes of hypertrophic and dilated cardiomyopathies. Recent advances in our mechanistic understanding of sarcomere pathophysiology include high-resolution molecular models of sarcomere components and the identification of the myosin super-relaxed state. The advances in our understanding of sarcomere function have yielded several therapeutic agents that are now in development and clinical use to correct contractile dysfunction–mediated cardiomyopathy.

Summary New genes linked to cardiomyopathy include targets with limited clinical evidence and require additional investigation. Large portions of cardiomyopathy with family history remain genetically undiagnosed and may be due to polygenic disease.

Keywords Cardiomyopathy · DCM · HCM · Sarcomere · Myofilament

Introduction

Cardiomyopathy encompasses a group of discrete diseases that result in impaired function of cardiac muscle and can be caused by genetic factors, environmental insults to the heart, or a combination of the two [1–3]. Genetic cardiomyopathies have been described since the 1950s, with clinical documentation of clear familial inheritance of a range of phenotypes [4, 5]. Basic and clinical research categorized these diseases based on their morphological and functional phenotypes [6]. The most common cardiomyopathies are hypertrophic (HCM) that occurs in 1:200–1:500 people [1], dilated (DCM) that occurs in 1:500 people [7], and others including arrhythmogenic cardiomyopathy [8], syndromic cardiomyopathies, and emerging classification like atrial myopathy [9].

Before the genes that caused cardiomyopathy were identified, cardiomyopathy was a diagnosis of exclusion, defined as pathological myocardial structure and functional changes without an obvious cause (termed idiopathic at the time) [6]. The first genes were identified as causative for HCM and DCM in the 1990s [10–14]. Cardiomyopathy mutations were initially considered to be inherited in a monogenic autosomal dominant manner [5]. However, after the birth of genetic testing of cardiomyopathy patients, it was observed that these mutations showed variable penetrance, with carriers of the same cardiomyopathy-causing mutation showing fulminant disease or asymptomatic presentation [15]. Additionally, cardiomyopathies tend to have a long time of onset (pediatric cardiomyopathies excluded [16]) and are typically identified in the second or third decade of life [1]. Genotype-positive individuals with an asymptomatic phenotype require lifelong vigilance, as the mechanisms that enable the pathological manifestation of mutations are not well understood [17•]. However, the gradual onset of disease also presents a long therapeutic window for the prevention or mitigation of disease [17•].

Mutations in the contractile machinery of the heart (i.e., the sarcomere and myofilaments) cause a majority of familial HCM and account for ~30% of familial DCM [17•, 18]. Genomic approaches have yielded a variety of candidate genes in DCM, although the evidence for many of them is still minimal and requires further investigation (Table 1)

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[17•]. Over the past 30 years, the physiological mechanism for many sarcomere proteins has been established and many pathophysiological consequences linked with cardiomyopathy are well understood (Fig. 1) [18, 19]. This knowledge has recently culminated in the development of small molecule therapeutics that specifically target sarcomere proteins to treat cardiomyopathy [20•, 21••]. In this review, we will cover the recent advances in our understanding of the mechanisms and treatment of cardiomyopathy arising from mutations in sarcomere proteins.

Classification of Genetic Cardiomyopathies

HCM is a condition where the ventricular walls thicken without obvious cause [1, 5]. HCM was described in 1957 by Dr. Robert Teare from autopsies of eight young patients with asymmetrical hypertrophy of the heart [4], and Dr. Eugene Braunwald provided a comprehensive description of the HCM phenotype in 1964 [5]. HCM is diagnosed via the hemodynamic and morphological nature of the heart [22]. The left ventricle of HCM patients usually has an elevated ejection fraction and a reduced end-systolic volume that can be diagnosed via echocardiogram and exercise/stress testing [22]. HCM patients can be treated with beta-blockers or antihypertensive drugs to alleviate hemodynamic stress [1]. Seventy percent of HCM cases have an intraventricular septal hypertrophy that causes aortic outflow tract obstruction [1]. Surgical resection or septal ablation has been used to restore the ability of blood to exit the ventricle. A majority of HCM patients can live their lives well but they may be limited by progressing disease and the development of heart failure.

Table 1 Frequencies of mutations in genes encoding sarcomere proteins in HCM and DCM

Familial HCM (60% of all HCM)		Familial DCM (30% of all DCM)	
Sarcomere P/LP +	**42%**	**Sarcomere**	**19–36%**
MYBPC3	24%	*TTN*	12–25%
MYH7	13%	*MYH7*	3–4%
MYL2	2%	*TNNT2*	1–3%
TPM1	1%	*TPM1*	1–2%
TNNT2	< 1%	*TNNC1*	< 1%
TNNI3	< 1%	*ACTC1*	< 1%
MYL3	< 1%	**Non-sarcomere**	**16–22%**
ACTC1	< 1%	**Other genes/limited evidence**	Unknown
Sarcomere VUS +	**9%**	**Familial DCM +, genotype –**	**40–60%**
Sarcomere P/LP/VUS-	**49%**		

P/LP pathogenic/likely pathogenic, *VUS* variants of uncertain significance

In contrast, DCM manifests with thinning and dilation of the ventricle, increased end-diastolic pressure, reduced stroke volume and cardiac output, and dysfunctional filling of the ventricles that often leads to heart failure [23]. DCM has a prevalence of 1:500 [19]. Genetic DCM accounts for approximately 30% of all DCM cases, as DCM is also caused by structural heart disease, valve disorders, hypertension, and other factors [24]. DCM patients are prone to cardiac remodeling and cardiac fibrosis due to the left ventricular dilation. In order to help DCM patients manage their condition, angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, vasodilators, and aldosterone antagonists can be prescribed [25].

Genetic Testing for Cardiomyopathies

When a patient is diagnosed with cardiomyopathy, genetic panel testing is often employed to identify known pathogenic variants in upwards of 100 genes [17•, 26•, 27]. A genetic diagnosis can help inform treatment of the patient and provide information to the extended family regarding who may be at risk of developing disease or passing on a pathogenic mutation [26•, 27]. The first pathogenic mutation linked to HCM was discovered in 1990, with the affected members of two unrelated families sharing the R403Q missense mutation in the *MYH7* gene [10]. Currently, 33 genes have been linked to HCM with various levels of evidence, with the 8 most definitive being *MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC1*, *MYL3*, and *MYL2* (Table 1) [13, 18, 26•, 28–32]. While many mutations have been clearly linked to an autosomal dominant inheritance pattern, the high variability in penetrance of HCM-causing mutations has led to studies that have shown the impact of polygenic modifiers on disease severity [33•].

In the age of genomics research, many variants are initially categorized one way (benign, likely benign, uncertain significance, likely pathogenic, pathogenic), with additional evidence changing the interpretation [26•]. The *MYBPC3* c.1224-52G > A intronic splice altering variant occurs in 4% of the South Asian population [34]. This variant is associated with HCM, although it is too prevalent to be a fully penetrant pathogenic mutation. Whole-genome sequencing and clinical phenotyping of a South Asian population revealed minimal association between this variant and HCM features. However, a second *MYBPC3* variant, D389V, was found on approximately 10% of the c.1224-52G > A variant alleles and individuals with the D389V variant had significantly more hyperdynamic contractile phenotypes [35]. However, several preclinical models have shown the common c.1224-52G > A to cause sarcomere dysfunction by itself [36]. These findings illustrate the difficulty in understanding the mechanism by which a mutation can impact function.

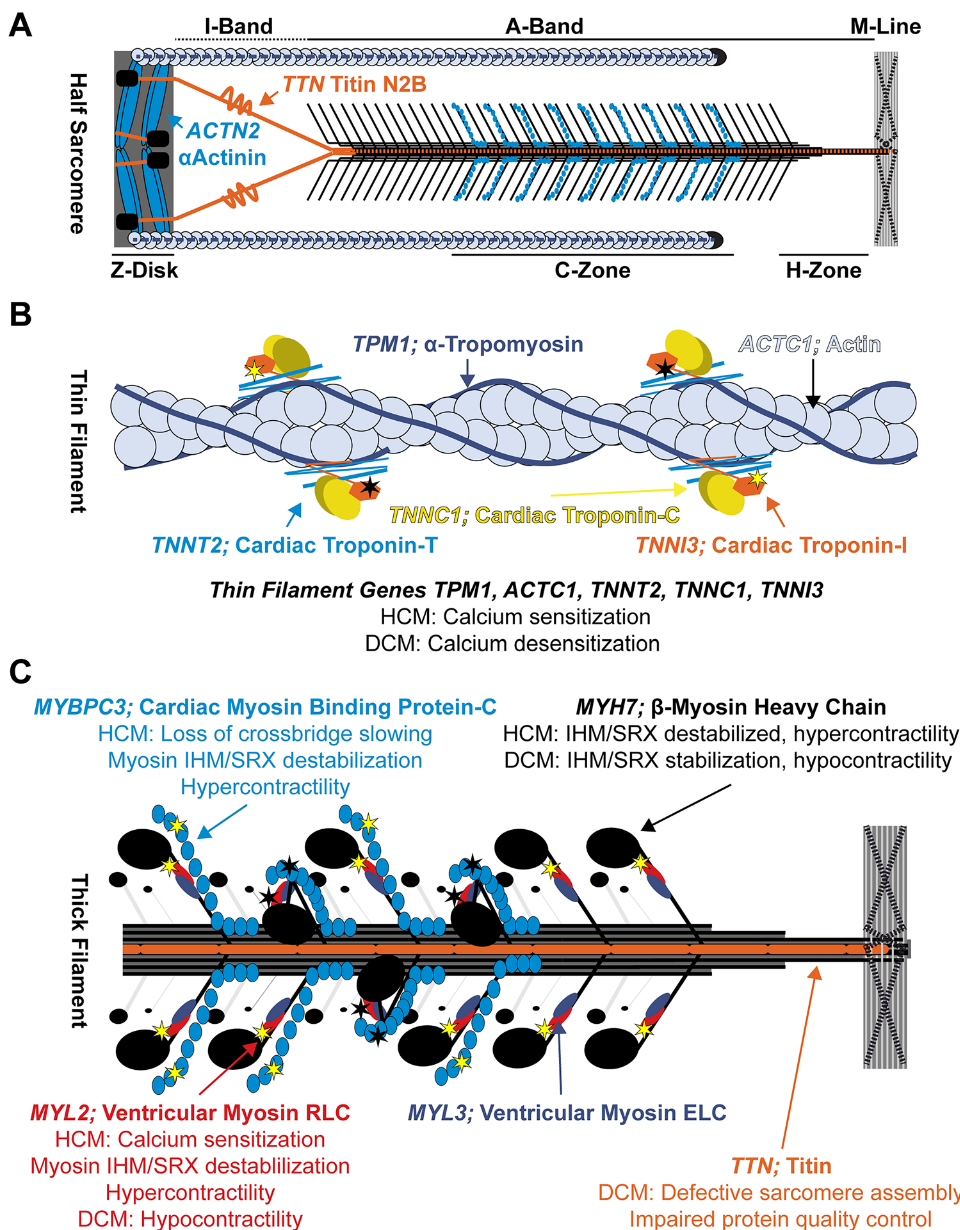

Fig. 1 Schematic of the sarcomere proteins linked with cardiomyopathy. **A** Half sarcomere from Z-disk to M-line with isotropic (I) and anisotropic (A) bands labeled. The C-zone is defined as the ~350-nm-wide portion of the myosin thick filament where cMyBP-C is localized in ventricular sarcomeres. The H-zone is the region with no actin overlap and no myosin heads. **B** Schematic with gene and protein names of the cardiac thin filament with general mechanism of cardiomyopathies listed. **C** Schematic of the cardiac thick filament. Phosphorylatable regulatory regions are marked with 6-point stars, with yellow stars indicating phosphorylation. Mechanisms of cardiomyopathy-causing mutations listed below the gene names

Genetic DCM is not exclusively caused by mutations in sarcomere genes. Mutations in proteins involved in the cytoskeleton, nuclear envelope, sarcolemma, ion channels, calcium handling, and others are also involved [25, 37]. While the genetic causes of DCM are more varied than of HCM (Table 1), 19–36% of cases are linked to mutations in sarcomere proteins, including mutations in titin that accounts for ~20% of familial DCM [38]. The diversity in genetic causes of DCM is remarkable, as is the considerable amount of genetic disease with no known variant associated. In DCM, genetic screening can include over 100 genes but only provides a genetic diagnosis for about 50% of patients [39]. A negative genetic screening can be due to an unknown gene or variant, or a combination of genes that are not pathogenic on their own but combine to ill effect [39]. Variant curation has focused primarily on gene coding regions, while regulatory regions are also capable of contributing to some of the missing genetic linkage for cardiomyopathy [40].

In keeping with the hypothesis that a proportion of DCM is polygenic, patients with DCM-causing mutations in *MYH7* have been shown to have significantly more non-synonymous single-nucleotide polymorphisms in approximately 100 cardiomyopathy-related genes than *MYH7* mutation carriers with HCM [41•]. This study provides evidence that cumulative, normally benign changes in proteins linked to contractile regulation may influence the severity of disease elicited from a primary pathogenic mutation [41•]. Polygenic modifiers of

disease may also account for some of the variable penetrance of HCM and DCM mutations [42•]. There are genes that have enriched expression in the atria including *MYL4*, *MYH6*, and *MYBPHL* that may alter atrial function and exacerbate a mildly pathogenic mutation expressed in ventricular cardiomyocytes [43]. Myosin light chain 4 (*MYL4*) frameshift mutations have been demonstrated to impair the actomyosin cross-bridge formation and cause a specific atrial cardiomyopathy and atrial remodeling [44].

Sarcomere Proteins in Hypertrophic Cardiomyopathy

β-Myosin Heavy Chain (*MYH7*) *MYH7* encodes β-myosin heavy chain (β-MHC), the primary myosin motor in the ventricular sarcomere [45]. β-MHC contains an N-terminal motor domain that binds to actin and hydrolyzes ATP to power the cross-bridge cycle and force generation [46]. Mutations can affect the hydrolytic cycle of ATP or the myosin neck region that allows the myosin heads to swing into the interfilament space and interact with actin [47]. *MYH7* mutations are more frequent in the motor domain, particularly in the "myosin mesa" region that is critical for actin binding [46, 48]. The molecular biophysical consequences of *MYH7* mutations are varied, including increased sarcomere force development, higher ATPase activity, increased actomyosin binding, decrease affinity of the myosin interacting head state, and faster cross-bridge cycling (Table 2) [46, 47, 49•]. Many of these processes are linked, and a single *MYH7* mutation can cause a variety of these biophysical changes, but ultimately cause a hypercontractile left ventricle that pathologically hypertrophies [49•, 50•].

There have been several recent advances regarding the so-called super-relaxed state of myosin. The myosin head domain exists in several conformations when not bound to actin, including a disordered relaxed state that resides in the interfilament space, ready to bind to actin [50•, 51, 52]. The super-relaxed state involves the folding back on myosin heads onto the thick filament backbone. Several avenues of research have converged on this mechanism, including cryo-electron microscopy of the myosin head on the thick filament backbone [53], X-ray diffraction data [54••] showing the movement of myosin heads towards the thick filament in association with the low ATPase state, and molecular mechanisms of modulating the super-relaxed state [49•, 51]. Importantly, it has been shown that some *MYH7* HCM-causing mutations destabilize the formation of the super-relaxed state and increase the number of disordered relaxed heads ready to generate cross-bridges, therefore increasing ensemble contractility [50•, 51].

Myosin Light Chains (*MYL2, MYL3*) Sarcomere myosin is a heteromeric complex consisting of a dimer of myosin heavy chains with a coiled-coil tail integrating into the thick filament and two myosin motor domains in the interfilament space, linked with a flexible "neck" region [55]. Each myosin head is associated with two light chains. In the ventricle, these are the ventricular regulatory (RLC, *MYL2*) and essential (ELC, *MYL3*) myosin light chains. The light chains modulate the strength and speed of the myosin heavy chain motor's ability to generate force [56]. The ELCs bind to the alpha helix of the myosin heavy chain neck and participate in cross-bridge formation [57]. The ventricular RLCs contain calcium-binding EF hand motifs and are phosphorylated [55, 58].

Table 2 Molecular mechanisms associated with sarcomere protein mutations in HCM and DCM

Effect	Genes	References
Hypertrophic cardiomyopathy		
Hypercontractility	*MYH7*, *MYL2*, *MYPBC3*, *TPM1*	[46, 60•, 71••, 95]
IHM/SRX destabilization	*MYH7*, *MYBPC3*	[49•, 52, 60•, 70]
Protein degradation stress	*MYBPC3*	[96•]
Atrial dysfunction	*TNNT2*, *TPM1*	[31, 75]
Arrhythmia	*TNNT2*	[75]
Ca sensitization	*TNNI3*, *TNNT2*, *TNNC1*, *TPM1*, *ACTC1*	[74, 76, 79, 97, 98]
Increased ATPase activity	*TNNC1*	[49•, 50•, 71••, 97]
Reduced MHC/cMyBP-C affinity	*MYH7*	[99•]
Dilated cardiomyopathy		
Hypocontractility	*MYH7*, *MYL2*, *TNNT2*, *TNNI3*, *TPM1*	[29, 92, 93•]
IHM/SRX stabilization	*MYH7*	[52]
Reduced ATPase activity	*TNNT2*, *TNNI3*, *TMP1*	[93•]
Ca desensitization	*MYH7*, *TNNI3*, *TNNT2*, *TNNC1*, *TPM1*, *ACTC1*	[78, 91, 93•, 94, 98]
Protein quality control	*TTN*	[100•]
Defective sarcomerogenesis	*TTN*	[87]

Mutations in the ventricular myosin light chains have been associated with hypertrophic and restrictive cardiomyopathies [30, 59]. The mechanisms for this include altered flexibility of the myosin neck [14] and altered localization of the myosin heads in the interfilament space [57]. The HCM-related *MYL2* A57G mutation increases RLC phosphorylation levels and increases the percent of myosin heads in the disordered relaxed state, whereas the restrictive cardiomyopathy-associated *MYL2* E143K mutation reduced RLC phosphorylation and promoted the super-relaxed state of myosin [60•]. The ability of the myosin light chains to regulate the myosin super-relaxed and their ability to be phosphorylated and dephosphorylated makes them attractive targets for drug development.

Cardiac Myosin Binding Protein-C (*MYBPC3*) The first *MYBPC3* mutations linked to HCM were identified in 1995 [11, 12]. In the following years, it was established that approximately 70% of *MYBPC3* mutations associated with HCM were nonsense mutations that truncated portions of the C′-terminal domains of cMyBP-C [61]. MyBP-C is comprised of a linear series of immunoglobulin and fibronectin-like domains, with most of the penultimate C′-terminal domain required for sarcomere incorporation [36, 62]. *MYBPC3* truncating mutations cause haploinsufficiency, expressing an insufficient amount of cMyBP-C to maintain normal cross-bridge regulation that results in hypercontractile sarcomeres and HCM [63–66]. Additional evidence suggests that the truncated allele causes cellular stress by compromising the protein quality control system [67, 68•]. While nonsense mutations in *MYBPC3* all result in a similar impairment, the pathogenic effect of *MYBPC3* missense mutations depends on the residues affected. These include altering myosin binding affinity [69], regulation of the super-relaxed state [70], ATP hydrolysis [71••], and actin interaction [72], which lead to hypercontractile sarcomeres.

Cardiac MyBP-C can be phosphorylated by a variety of kinase pathways and its function shifts from inhibiting cross-bridge cycling to activating cross-bridge cycling when phosphorylated [73]. Dephosphorylated cMyBP-C promotes the formation of the super-relaxed state whereas phosphorylated cMyBP-C primes myosin heads for cross-bridge formation by promoting the myosin disordered relaxed state [70, 71••]. The myosin S2 region is bound by cMyBP-C to mediate this function, and mutations in *MYH7* that alter residues in this region reduce the ability of cMyBP-C to bind to and regulate β-MHC [64]. These interactions between β-MHC and cMyBP-C provide a coherent picture of how both *MYH7* and *MYBPC3* mutations can converge on a similar HCM phenotype (Table 2).

Thin Filament HCM Mutations Sarcomere thin filament function consists of a chain of events starting with calcium binding to troponin-C (cTnC, *TNNC1*) causing a conformational change through troponin T (cTnT, *TNNT2*) that allows alpha-tropomyosin (α-TM, *TPM1*) to move, exposing myosin binding sites on actin (*ACTC1*), allowing cross-bridge formation. This chain of events is regulated by troponin-I (cTnI, *TNNI3*) and depends on many key residues and protein regions to correctly translate calcium binding to cross-bridge formation. Mutations in thin filament proteins that cause HCM are typically missense mutations that cause an increase in thin filament calcium sensitivity, allowing force development at relatively lower calcium concentrations and resulting in hypercontractility (Table 2) [74–76].

The biophysical interaction of the linker between cTnT and α-TM is a major regulatory region and mutations that alter the rigidity or position of the linker dysregulate activation of the thin filaments [77]. Each troponin/tropomyosin regulatory complex spans ~ 38 nm, with neighboring complexes exhibiting cooperativity of activation that is translated through the overlap region of neighboring α-TM molecules [78]. These thin filament mutations also result in hypercontractility due to enhanced cooperativity [79].

Another consequence of HCM-causing thin filament mutations is an increased propensity for arrhythmias due to alteration of the calcium buffering properties of the thin filament. Mutations in the troponin complex can prolong sarcomere calcium release in diastole and promote arrhythmia [75, 80•]. β-Adrenergic signaling causes phosphorylation of cTnI, desensitizing the thin filament to calcium, and increasing the rate of relaxation; thin filament mutations that affect this regulatory effect cause cardiomyopathy [76, 81].

Sarcomere Proteins in Dilated Cardiomyopathy

Titin (*TTN*) Titin is a sarcomere protein that interacts at its N-terminus with telethonin (*TCAP*) and α-actinin in the Z-disk, and then spans nearly 1 micron across a half sarcomere where its C-terminal interacts with myomesin in M-line [82, 83]. Titin is the largest protein, comprised of approximately 17,000–26,000 amino acids depending on isoform and what exons are included in various transcripts. Titin contains a molecular spring region in the I-band that can include an N2B region, common in the ventricle, or the N2B and N2A regions, which are common in the atria. The longer N2B and N2A isoform (N2BA) produces a longer titin molecule that exhibits longer resting sarcomere lengths and reduced sarcomere compliance [84].

DCM has been clearly linked with titin-truncating variants. However, as titin variants were rapidly identified in the early 2010s, an alarming number of titin-truncating variants were identified, enough that it would be impossible for titin truncation alone to cause disease [38]. This mystery was solved by the discovery that titin undergoes high levels of exon exclusion, primarily in exons that encode the I-band of titin, and truncating mutations in these frequently excluded exons are not included in a sufficient amount of transcripts to cause disease [85]. Bona-fide titin-truncating variants are often found in the A-band domains and are generally considered to be pathogenic due to a haploinsufficiency, although fragments of truncated titin have recently been identified, which may cause dysfunction [86, 87].

Titin's A-band region contains several super-domains composed of 7–11 immunoglobulin-like and fibronectin-like domains that are repeated across the A-band [83]. These repeats provide a molecular ruler that sets some of the thick filament's periodic protein localization patterns [88]. Because of the staggering size and repetitive nature of A-band titin and our limited understanding of how these domains regulate binding of sarcomere components, missense variants in this region are both plentiful and difficult to interpret [89•]. Understanding the pathogenicity of titin missense mutations will be a challenging new frontier for sarcomere protein research.

β-Myosin Heavy Chain (*MYH7*) *MYH7* mutations that cause HCM result in hypercontractility, whereas *MYH7* mutations that occur in some of the same functional locations cause loss of function and result in DCM. DCM mutations in MYH7 are spread throughout the molecule but are slightly enriched in residues associated with the nucleotide binding pocket on the myosin head [52, 90]. Mechanistically, DCM mutations in *MYH7* cause defects in the cross-bridge ATPase cycle [91] resulting in depressed function and hypocontractile outcome [92].

Thin Filaments HCM-causing thin filament mutations result in calcium sensitization whereas DCM mutations exhibit the opposite effect, with decreased sensitivity of thin filament activation from calcium [93•]. This includes mutations in *TNNC1*, *TNNT2*, *TNNI3*, *TPM1*, and *ACTC1* [78, 93•, 94]. Interestingly, mutations in these genes cause decreased in calcium sensitivity that is uncoupled from the normal phosphorylation-dependent calcium desensitization mediated from cTnI (i.e., the mutations cause structural changes that result in cTnI to adopt its calcium desensitizing interactions) [76, 94]. Cooperativity of thin filament activation is also oppositely impacted, with DCM mutations in *TPM1* that compact the α-TM overlap regions resulting in decreased cooperativity [78].

Novel Pharmaceutical Treatments for Cardiomyopathy

The advances in our understanding of the molecular and biophysical mechanisms of disease in sarcomere proteins have led to the rapid advancement of treatments to target these mechanisms. In the early 2010s, several companies set out to develop small molecules that could selectively modulate sarcomere function. Some companies have targeted the hypercontractile mechanism of disease in HCM [101]. A first-in-class compound, originally known as MYK-461, or mavacamten, showed successful reduction in the obstructive phenotype of HCM by reducing myosin contractility without serious adverse effects [21••]. After these promising phase III trial results, mavacamten received FDA approval in April of 2022 for treatment of obstructive HCM, with further trials ongoing. Aficamten is another myosin inhibitor that has shown positive results in phase II clinical trials [102].

Many HCM-causing mutations in *MYH7* have been shown to disrupt the super-relaxed state, and HCM-causing *MYBPC3*-truncating mutations prevent cMyBP-C from promoting the myosin super-relaxed state [50•, 51, 52, 70]. The super-relaxed state can be stabilized or disrupted to sequester or liberate populations of myosin heads to meet the contractile demand [50•, 60•, 103]. This provides a common mechanism of action for mutations in these two proteins. Mavacamten reduces contractility by stabilizing the super-relaxed state, making mavacamten and other small molecules that target this pathway ideal candidates to address a common mechanism of disease [21••, 103, 104].

Myosin activators have also been developed with the goal of increasing contractility. One compound, omecamtiv mecarbil (OM), was designed to increase cardiomyocyte contractility in patients with heart failure with reduced ejection fraction [20•, 105]. OM did not meet its endpoint for improving contractility for heart failure patients in phase III trials [20•] but did significantly improve function for individuals with the lowest contractile function [106•]. While OM failed to gain FDA approval in December of 2022, other myosin activators, such as danicamtiv, are also in development for treating patients with heart failure with reduced ejection fraction [107].

Treating Cardiomyopathy with Genetic Medicine

As titin-truncating variants comprise ~ 20% of genetic DCM, efforts have been made to identify therapeutic approaches to correct these mutations [108•]. Recent advances in exon skipping therapies and CRISPR-dependent reading frame repair, especially in Duchene muscular dystrophy [109], may

be applicable to correct *TTN*-truncating variant expression. Romano et al. observed pathogenic allele dose-dependent decreases in full-length titin protein isoforms for A- and I-band *TTN*-truncating variants, showing *TTN* haploinsufficiency. Cardiomyocyte genome editing by SpCas9 restored the *TTN* protein reading frame, increasing full-length *TTN* protein levels, and diminishing *TTN* truncating peptides [108•]. Exon skipping approaches use antisense oligonucleotides to facilitate skipping of one or more exons that contain a mutation and have been used to repair a frameshift mutation in *TTN* exon 326 given (Ser14450fsX4) [110]. Skipping of *TTN* exon 326 improved myofibril assembly and stability and ameliorated the DCM phenotype.

Variations on single base editing approaches have also recently been used by two groups to correct a mutation associated with HCM [111, 112]. Adenine base editing (ABE) uses a catalytically dead CRISPR-Cas9, a guide RNA, and a deaminase to convert an adenine to a guanine [113, 114]. The dominant-negative *MYH7* R403Q causes HCM via increasing cardiac contractility and is caused by a 1208G > A mutation. These simultaneous publications both showed effective A > G nucleotide editing that restored the *MYH7* wild-type sequence, with minimal bystander and off-target editing [111, 112]. These approaches used adeno-associated virus to deliver the editing constructs, and the clinical utility of this approach and base editing are still being evaluated.

Conclusions

Over 30 years have passed since the identification of the first genetic causes of HCM and DCM. In this time, we have acquired a vast, albeit incomplete, understanding of the genes that drive these diseases. Biophysical mechanisms and contractile regulation have matured into therapeutic targets, although successfully translating this wealth of knowledge to the development of therapeutics will require extensive research.

While gene therapy holds great promise for correcting disease-causing mutations directly, the implementation and scalability of these approaches remain to be established. Even the most perfect gene therapy approach would be limited by our identification of all the pathogenic and modifying genes responsible for disease. Therefore, identifying "missing" genetic causes of cardiomyopathy and establishing the role of polygenic factors in the development of disease are crucial.

Basic science research has also delineated the mechanisms responsible for many of the proteins and mutations linked to cardiomyopathy. As demonstrated with therapeutically targeting the myosin super-relaxed state, targeting sarcomere protein interactions can be effective for modulating contractility to counter pathological processes. Additionally, signaling pathways like phosphorylation of ventricular myosin light chains to alter the myosin super-relaxed state provide additional upstream targets that could be leveraged for therapeutic development.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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• Of importance
•• Of major importance

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