ANNUAL REVIEWS

Annual Review of Genomics and Human Genetics

Advances in the Genetic Basis and Pathogenesis of Sarcomere Cardiomyopathies

Raquel Yotti,[1,2] Christine E. Seidman,[3,4,5] and Jonathan G. Seidman[3]

[1]Department of Cardiology, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, 28007 Madrid, Spain; email: raquel.yotti@salud.madrid.org

[2]Centro de Investigación Biomédica en Red en Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, 28029 Madrid, Spain

[3]Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; email: cseidman@genetics.med.harvard.edu, seidman@genetics.med.harvard.edu

[4]Cardiovascular Division and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA

[5]Howard Hughes Medical Institute, Chevy Chase, Maryland 20815, USA

Annu. Rev. Genom. Hum. Genet. 2019. 20:129–53

First published as a Review in Advance on April 12, 2019

The *Annual Review of Genomics and Human Genetics* is online at genom.annualreviews.org

https://doi.org/10.1146/annurev-genom-083118-015306

Keywords

hypertrophic cardiomyopathy, dilated cardiomyopathy, sarcomere physiology, gene-based diagnosis, interacting-heads motif, titin

Abstract

Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are common heart muscle disorders that are caused by pathogenic variants in sarcomere protein genes. HCM is characterized by unexplained cardiac hypertrophy (increased chamber wall thickness) that is accompanied by enhanced cardiac contractility and impaired relaxation. DCM is defined as increased ventricular chamber volume with contractile impairment. In this review, we discuss recent analyses that provide new insights into the molecular mechanisms that cause these conditions. HCM studies have uncovered the critical importance of conformational changes that occur during relaxation and enable energy conservation, which are frequently disturbed by HCM mutations. DCM studies have demonstrated the considerable prevalence of truncating variants in titin and have discerned that these variants reduce contractile function by impairing sarcomerogenesis. These new pathophysiologic mechanisms open exciting opportunities to identify new pharmacological targets and develop future cardioprotective strategies.

1. INTRODUCTION

Sarcomeres are the basic unit of contraction in cardiac muscle. Damage to the structural or functional integrity of sarcomeres causes prominent myocardial disorders called cardiomyopathies. These conditions are classically divided into two major subtypes (**Figure 1**): hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) (25, 75). Mutations in genes that encode sarcomere proteins are the most common genetic causes of HCM and DCM. While

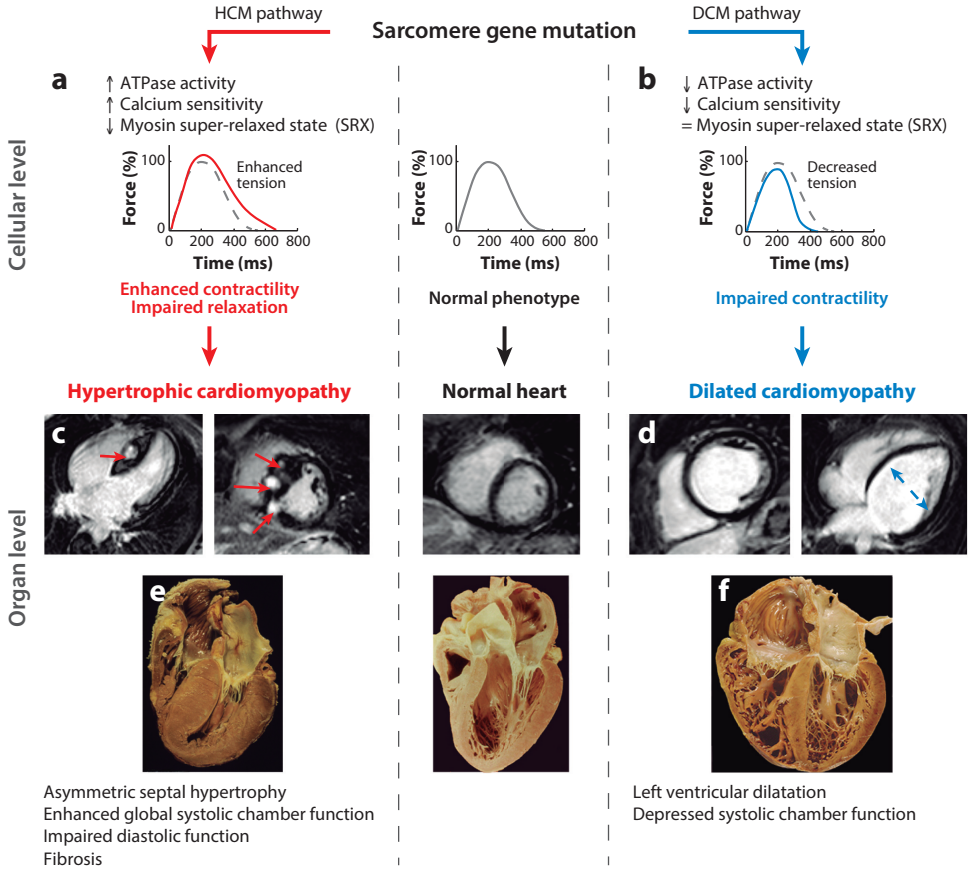

Figure 1

Key phenotypic features of hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). (*a*,*b*) The principal mechanisms by which pathogenic variants lead to HCM and DCM. In HCM (panel *a*), pathogenic variants lead to enhanced contractility and impaired relaxation. In DCM (panel *b*), pathogenic variants have the opposite effects on ATPase activity and calcium sensitivity, leading to impaired contractility. Typical changes in the force–time relationship of cardiac contractility in HCM and DCM are shown. (*c*–*f*) Morphologic features of HCM and DCM, which include characteristic geometrical and functional abnormalities that are recognized by noninvasive cardiac imaging. In panel *c*, the red arrows point to focal extensive fibrosis (identified by late gadolinium enhancement) in a 22-year-old HCM patient with the *MYH7* missense variant p.Arg249Gly. Panel *d* shows cardiac magnetic resonance imaging of a 32-year-old DCM patient with the *MYH7* missense variant p.Arg369Gln, leading to left ventricular dilatation and impaired global systolic function. Panel *e* illustrates anatomic features of HCM, showing asymmetric hypertrophy with prominent involvement of the interventricular septum, and panel *f* illustrates anatomic features of DCM, showing markedly increased ventricular volumes.

both disorders arise from deficits in the same essential core protein complex, HCM and DCM mutations incite profoundly different ventricular morphology, histopathology, and contractile performance (**Figure 1**) that result in distinct clinical presentations and outcomes (77).

HCM is diagnosed in approximately 0.2% of the adult population (24, 36) based on the recognition of ventricular hypertrophy, which is usually asymmetric and occurs in the absence of abnormal loading conditions such as hypertension or valvular abnormalities. Cardiac hypertrophy is clinically diagnosed by echocardiography or cardiac magnetic resonance imaging (MRI) that demonstrates increased left ventricular wall thickness ($\geq$1.5 cm in an adult or a *z*-score >2 in children) (24). The morphologic abnormalities of HCM are accompanied by hypercontractility (increased systolic performance) and poor relaxation (diastolic dysfunction). Restrictive cardiomyopathy (RCM) is an uncommon clinical diagnosis that exhibits severely impaired diastolic function (31) but with normal or only mildly increased left ventricular wall thickness (151). Sarcomere gene mutations are the most common cause for both HCM and RCM (31).

DCM is prevalent in the adult population (42) and a leading cause of heart failure. It is characterized by left (or biventricular) ventricular chamber enlargement (dilatation, or an end-diastolic volume or diameter more than two standard deviations above predicted values adjusted for body surface area, age, and gender) that is accompanied by impaired systolic function. Hypokinetic, nondilated cardiomyopathy is a newly proposed clinical description to more fully capture the earliest clinical spectrum of DCM (96) and includes patients with impaired cardiac contractility regardless of chamber volume. DCM that occurs in the absence of coronary artery disease, endocrine disorders, toxins, or infection was historically denoted as idiopathic; however, molecular discoveries have shown that pathogenic variants in multiple genes that encode a variety of proteins with distinct functions in cardiac biology account for substantial proportions of idiopathic DCM. Among all genetic etiologies of DCM, variants in sarcomere protein genes occur most frequently (78).

Sarcomere cardiomyopathies are dominant disorders that exhibit familial clustering with age-related expression of clinical manifestations. Recognition of high familial risk of these disorders has revised the diagnostic criteria for first-degree relatives of affected patients. Among at-risk relatives, a lower ventricular wall thickness ($\geq$1.3 cm) fulfills clinical diagnostic criteria for HCM (24). In addition, clinical evaluations of HCM and DCM family members demonstrate that young carriers of pathogenic sarcomere variants can exhibit subtle contractile abnormalities (48, 62) before the emergence of prototypic changes in cardiac morphology (e.g., increased ventricular wall thickness for HCM and ventricular dilatation for DCM). These data imply that deficits in cellular contractility and/or relaxation (10, 34) are a primary effect of defective sarcomere genes that triggers secondary remodeling of the heart (48, 62).

During the last few years, exciting studies of sarcomere physiology and biophysics have helped to unravel mechanisms that underlie the precise functional abnormalities associated with HCM and DCM pathogenic variants. These insights have established new concepts about sarcomere physiology, defined the contractile apparatus as an unexpected therapeutic target, and advanced the development of novel precision drugs for HCM and DCM. In addition to improving clinical care for cardiomyopathy patients, genetic discoveries have deepened our understanding of fundamental properties of sarcomere biology and muscle mechanics.

In the following sections, we review contemporary knowledge of genetic causes of sarcomere cardiomyopathies and strategies for gene-based diagnosis, explore the longitudinal phenotypes associated with sarcomere cardiomyopathies, consider molecular mechanisms that inform how pathogenic variants evoke clinical phenotypes, and discuss the emergence of new pharmacological targets developed from advances in understanding the pathophysiology of sarcomere cardiomyopathies.

2. THE MOLECULAR GENETICS OF SARCOMERE CARDIOMYOPATHIES

Pathogenic variants in sarcomere genes were first discovered by statistical analyses of the cosegregation of genotype and phenotype in large and unrelated cardiomyopathy families (115). Since the first description of *MYH7* as an HCM disease gene (35, 108), more than 1,000 rare pathogenic variants have been identified in HCM and DCM families and in individual patients. Most variants are private and unique to one or a few families. The high degree of genetic heterogeneity in HCM and DCM is thought to reflect recent de novo mutations, which cause sufficiently adverse consequences to evoke negative selection. By contrast, some HCM founder mutations have been identified (1, 22, 51, 60, 93, 110, 137, 142), and analyses of population haplotypes associated with these variants indicate that many originated thousands of years ago. However, the absence of population spreading of founder variants and clinical studies showing delayed disease onset is interpreted to indicate that founder variants provide no evolutionary advantages and are likely subjected to neutral evolutionary selection.

Contemporary genetic tests for sarcomere variants employ strategies that directly sequence panels of cardiomyopathy genes, exomes, or genomes. These analyses detect three classes of gene variants (103). Pathogenic variants occur in established disease genes (defined by statistically significant segregation of genotype and phenotype in at least two independent families) that disrupt the structure or function of the encoded proteins. Likely pathogenic variants also occur in established disease genes, albeit with less compelling evidence with regard to familial segregation or functional impact. Both pathogenic and likely pathogenic variants are clinically actionable. Benign variants in sarcomere protein genes are common in the general population, whereas pathogenic and likely pathogenic variants have a minor allele frequency of less than 0.1% (144). Rare variants with unknown significance (VUSs) are also identified in definitive cardiomyopathy genes, but with insufficient experimental and/or segregation data for clinical use. Even with large data sets [such as those of the Exome Aggregation Consortium (ExAC) and Genome Aggregation Database (gnomAD)] that inform the population prevalence of variants, VUSs in cardiomyopathy genes remain an unsolved and challenging clinical issue and contribute to the high rates of patients with overt cardiomyopathy being classified as mutation negative (144).

HCM and DCM pathogenic variants in sarcomere genes are expressed as autosomal dominant traits with high lifetime penetrance (>90%) in men and women. Clinical expression of cardiomyopathies is age dependent, and lifelong manifestations can vary considerably even among family members with the same pathogenic variant. HCM variants rarely cause overt manifestations early in childhood (77), but manifestations emerge near adolescence. The age of onset of DCM variants is more variable. Consistent with this, gene-based diagnosis has become an essential clinical tool in HCM to establish a definitive diagnosis and enables cascade testing of at-risk relatives. Genetic testing can avoid unnecessary clinical follow-up of mutation-negative family members and provides significant health-care cost savings (7). Although gene-based diagnosis of DCM occurs in most specialization centers, it is not yet standard of care at many institutions.

Pathogenic and likely pathogenic sarcomere gene variants predict two distinct mechanisms by which these variants cause disease. Missense variants that encode a stable protein are expected to be incorporated into the sarcomere, disrupt normal mechanical function, and evoke pathologic signaling. Sarcomere proteins that harbor missense residues may also be misfolded and contribute to pathogenesis by overwhelming normal cellular clearance pathways. Gene variants that result in insertions, deletions, or premature stop codons or alter canonical splice sites are expected to encode unstable transcripts or loss-of-function proteins; these pathogenic variants cause cardiomyopathies through a haploinsufficiency mechanism (50, 76).

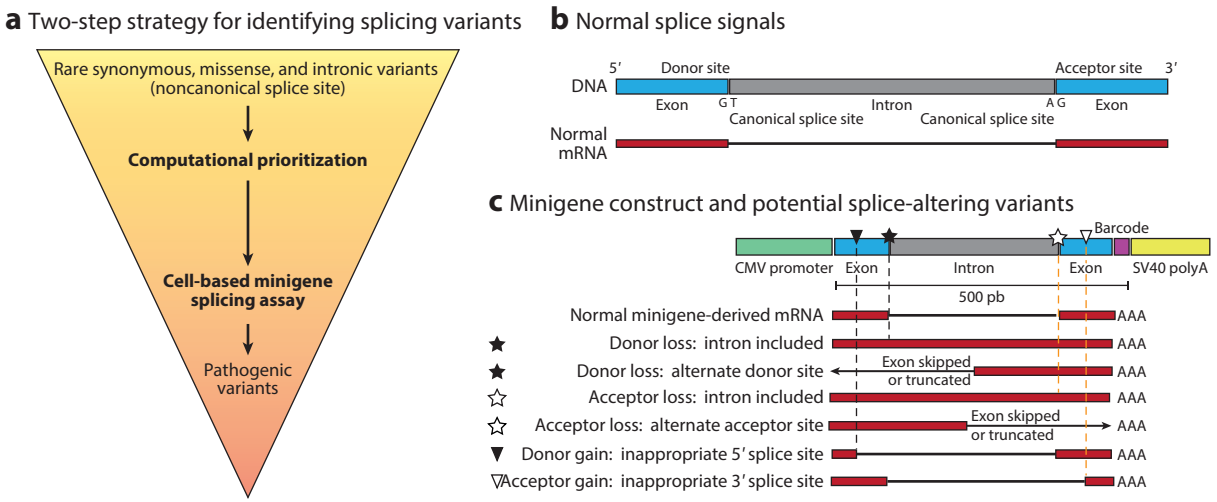

Figure 2

Strategies to improve the interpretation of cardiomyopathy variants of unknown significance (VUSs). (*a*) Outline of a two-step protocol to assess VUSs. This protocol employs a computation prediction tool followed by assays to determine whether the variant alters splicing in dosage-sensitive cardiomyopathy genes. (*b*) Schematic representation of normal splice signals. (*c*) Minigene construct and potential effects of splice-altering variants. Abbreviations: CMV, cytomegalovirus; SV40, simian virus 40. Panels *b* and *c* adapted from Reference 50 with permission.

Contemporary bioinformatic prediction tools can classify variants that are likely to cause loss of function with considerable accuracy, but computational predictions about the consequences of missense and noncoding variants are far less informative. Without experimental assays to interrogate biologic properties in model systems, VUS is the default classification for many missense variants in definitive cardiomyopathy genes. A recent strategy to reclassify VUSs that reside in sequences that flank canonical splice signals combines a computational predictive algorithm with functional assays to assess splicing. From analyses of cardiomyopathy VUSs within exons (including synonymous and missense variants) and introns, this strategy identified a substantial subset that disrupt normal splicing, either incorporating additional sequences or excluding normal residues (50). As these events would severely alter protein structure, these VUSs would be predicted to cause loss of protein function (**Figure 2**). VUSs that disrupt splicing in cardiomyopathy genes that cause disease by haploinsufficiency (e.g., *MYBPC3*) could therefore be reclassified as likely pathogenic. The development of other functional genomic approaches, such as testing of variant effects on splicing and contractile function in culture cardiomyocytes, is expected to further improve variant classification and the utility of gene-based diagnosis.

2.1. Hypertrophic Cardiomyopathy

Pathogenic or likely pathogenic variants in sarcomere protein genes account for 30–60% of HCM cases, a range that reflects diagnostic criteria (7, 72) and approaches to variant classification (103). Variant detection rates are highest (>75%) among patients with clinical evidence of familial HCM. Pathogenic and likely pathogenic HCM variants most often reside in one of eight sarcomere genes, but they predominate in *MYH7* and *MYBPC3* [encoding β-myosin heavy chain and cardiac myosin-binding protein C (cMyBP-C), respectively]. Pathogenic variants in *MYL2* and *MYL3* (myosin essential and regulator light chains, respectively), *TPM1* (α-tropomyosin), *TNNT2*

(troponin T), *TNNI3* (troponin I), and *ACTC1* (actin) are collectively identified in approximately 10% of cases (103). Less commonly, variants are identified in genes that encode other sarcomere proteins or in sarcomere-associated proteins, as well as in molecules with other functions, including *CSRP3*, *FHL1*, *PLN*, *ACTN2*, *CRYAB*, *FLNC*, *MYOZ2*, *MYH6*, *TNNC1*, *TRIM55*, and *TRIM63* (143). Whether these variants cause HCM by mechanisms similar to or different from sarcomere protein gene mutations is unknown.

Pathogenic variants in several genes, most often *PRKAG2* (γ_2-subunit of AMP kinase), *GLA* (α-galactosidase A), and *LAMP2* (lysosome-associated membrane protein 2), are identified in approximately 2% of patients misdiagnosed as having HCM (103). Pathogenic variants in these genes activate different pathogenic mechanisms to elicit hypertrophy as well as additional clinical phenotypes that do not occur in HCM.

Most pathogenic and likely pathogenic sarcomere gene variants that cause HCM encode missense residues, with the notable exception of *MYBPC3*, where loss-of-function alleles predominate. *MYBPC3* is also notable for harboring the vast majority of HCM founder variants. Despite these distinctions, recent structural and functional analyses (discussed in Section 4.1.1) demonstrate a shared mechanism by which missense variants in *MYH7*, *MYL2*, and *MYL3* and loss-of-function variants in *MYBPC3* cause HCM.

2.2. Dilated Cardiomyopathy

In comparison with HCM, the genetic architecture of DCM is less well defined. DCM pathogenic and likely pathogenic variants are reported in more than 50 genes (**Figure 3**) that collectively account for approximately 30% of cases (143). As with HCM, the highest rates for pathogenic variants (50%) occur among patients with familial DCM (82). Genes that harbor pathogenic DCM variants encode components of the sarcomere, nuclear membrane, cytoskeleton, outer cellular membrane, and extracellular matrix, as well as ion channels, mitochondrial proteins, and splice-regulating proteins (**Figure 3**). Many of these DCM variants also cause additional phenotypes, most often in skeletal muscles, while pathogenic variants in sarcomere genes cause isolated DCM.

Titin-truncating variants (TTNtvs) are the most common genetic cause of DCM and explain up to 25% of familial and 15% of sporadic cases (41, 105). TTNtvs also occur in the approximately 10% of DCM cases that emerge in the context of another condition, including peripartum cardiomyopathy (147), alcoholic cardiomyopathy (146), and cancer-therapy-induced cardiomyopathy (33, 66). Pathogenic variants in *RBM20* (13), which encodes a regulator of cardiac-specific pre-mRNA splicing of *TTN*, also cause DCM, further substantiating the critical role that titin plays in normal heart structure and function.

Excluding TTNtvs, pathogenic variants in other sarcomere genes (*MYH7*, *TNNT2*, *MYBPC3*, *MYPN*, *TPM1*, and *ACTC1*) collectively occur in less than 5% of DCM cases. Approximately 10% of DCM cases harbor variants in nonsarcomere genes, most commonly in *LMNA* (lamin A/C), *SCN5A* (sodium voltage-gated channel α-subunit 5), and *BAG3* (BCL2-associated athanogene 3) (82, 144).

DCM that occurs early in childhood is a rare disorder that can rapidly progress to fulminant heart failure (9, 92, 130). In most children, the cause is unknown, and the role of genetics remains uncertain. TTNtvs are surprisingly absent in childhood-onset DCM (27), but these are identified when DCM occurs in the context of an additional predisposition, such as treatment for childhood cancers (33, 66). Pathogenic variants in other contractile protein genes sometimes cause childhood-onset DCM but more commonly cause adult-onset disease (87, 101). The mechanisms accounting for why a pathogenic variant is expressed with a wide range of penetrance are unknown.

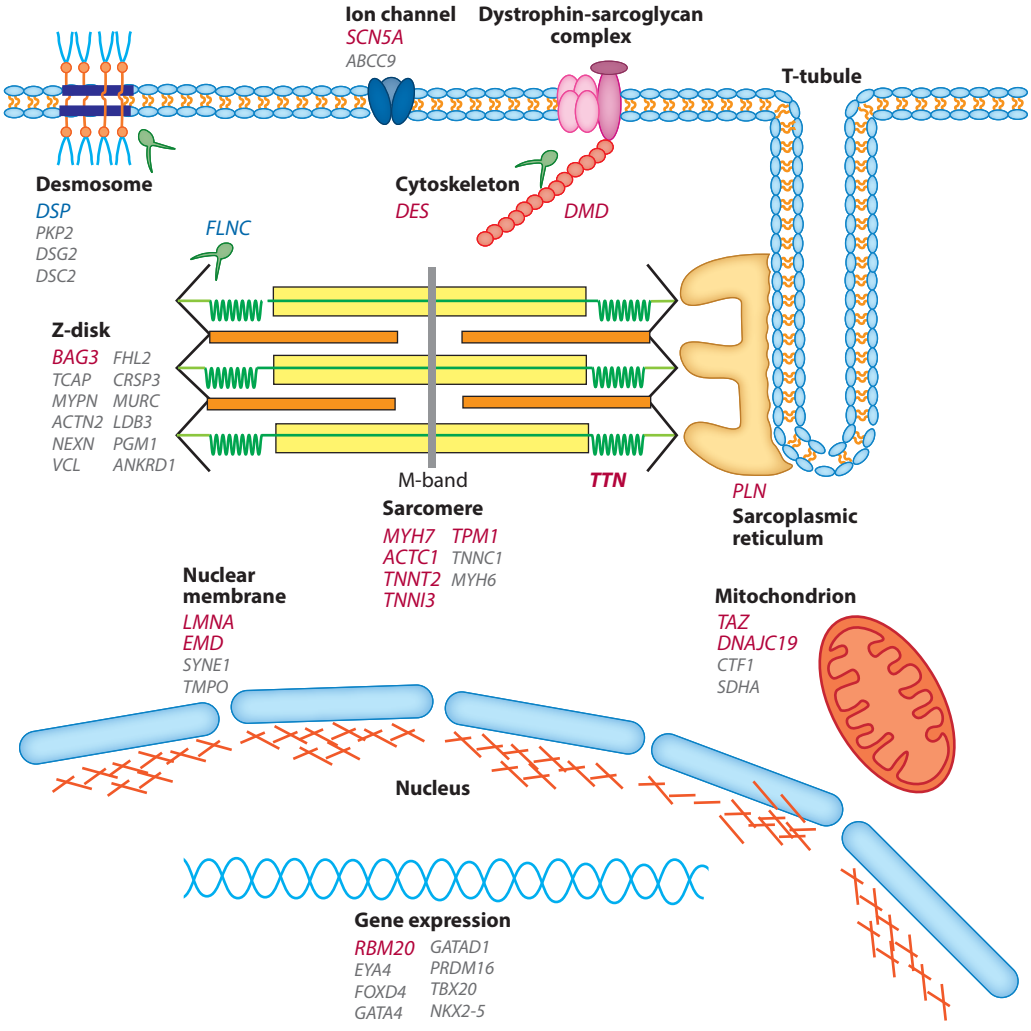

Figure 3

Genes associated with dilated cardiomyopathy (DCM), showing schematic representations and subcellular locations of the proteins encoded by these genes. Definitive DCM genes are shown in red, while posited DCM genes are shown in gray. DCM genes that have overlapping phenotypes with arrhythmogenic ventricular cardiomyopathy (52) are shown in blue.

3. GENETIC INSIGHTS INTO HYPERTROPHIC AND DILATED CARDIOMYOPATHY PHENOTYPES

Patients with HCM typically exhibit increased contractility (left ventricular ejection fraction >65%) and impaired cardiac relaxation (**Figure 1*a***), as well as increased energy consumption. DCM patients exhibit reduced contractility (left ventricular ejection fraction <45%) with mild deficits in myocardial relaxation and increased energy consumption (**Figure 1*b***). Each of these distinct functional profiles occurs in the setting of abnormal myocardial dimensions. Hypertrophy reduces chamber volumes in HCM, while ventricular dilatation increases chamber volume in DCM. Myocardial fibrosis progressively increases in both cardiomyopathies but is more often clinically detected in HCM due to focal accumulations that are identified by cardiac MRI

with late gadolinium enhancement (**Figure 1*c,d***). The clinical emergence of these mechanical, morphologic, and histologic abnormalities is highly integrated, which confounds the interpretation of whether functional deficits in HCM and DCM are the primary effects of sarcomere mutations or occur secondary to ventricular remodeling processes. Insights into these relationships have emerged from the detailed phenotypic evaluations of preclinical individuals who carry a pathogenic variant but do not have hypertrophy or dilation. Preclinical individuals are often young and identified through cascade genetic testing after a pathogenic variant is found in a family member with overt cardiomyopathy.

Advanced imaging studies of preclinical HCM individuals with pathogenic variants in *MYH7* and *MYBPC3* demonstrate a significantly increased left ventricular ejection fraction and reduced relaxation velocities despite normal cardiac dimensions (48, 91). Cardiac MRI combined with late gadolinium enhancement is typically normal, but longitudinal relaxation time analyses (T1 mapping) can reveal increased extracellular volumes (43, 47). Consistent with these findings, analyses of high signal intensity coefficients indicate abnormalities in microstructures of the heart (46). Energetics—deficits detected by phosphorus-31 magnetic resonance spectroscopy (20)—are also abnormal. Collectively, these observations demonstrate that functional deficits and increased energy consumption precede overt hypertrophic remodeling in HCM. Hence, hypercontractility in HCM hearts is not solely a hemodynamic response to a diminished ventricular cavity due to increased wall thickness, nor are the relaxation deficits due only to increases in myocardial fibrosis. Rather, these preclinical phenotypes imply that functional abnormalities and increased energy demand are primary or at least proximal effects of pathogenic sarcomere gene variants.

The emergence of morphologic remodeling in HCM increases functional and morphologic abnormalities. Ventricular hypertrophy that characteristically occurs in the proximal interventricular septum and accumulation of interstitial and focal fibrosis (**Figure 1*e***) distort cardiac geometry, which further affects systolic performance, relaxation, and energy consumption. Fibrosis can result from microvascular abnormalities in hypertrophic regions, ischemia, and outflow obstruction (140) and activates stress signals and further morphologic remodeling. Analyses also find that increased contractility in isolated cardiomyocytes (109) and in HCM mice begets cardiac remodeling (94, 125). Progression of these events can propel the development of end-stage HCM with hypocontractility and heart failure (14). Progressive fibrosis is also prominent in RCM and contributes to increased chamber stiffness; impaired relaxation; and, in late stages of disease, systolic dysfunction (151).

Detailed evaluations of preclinical individuals with DCM pathogenic variants demonstrate normal cardiac dimensions with subtle functional defects. Carriers of pathogenic variants in *MYH7*, *TPM1*, or *TNNT2* have a normal ventricular ejection fraction and diastolic function, but tissue Doppler and strain echocardiography demonstrate reduced systolic myocardial velocity, strain, and strain rates (62). Although parallel studies have not yet characterized preclinical phenotypes in DCM families harboring pathogenic TTNtvs, cardiac MRI studies of unrelated healthy volunteers with TTNtvs revealed unexpected abnormalities. Healthy volunteers with versus those without TTNtvs had larger left ventricular end-diastolic (8%) and systolic (15%) volumes, resulting in a 2.8% lower ejection fraction—a subtle depression that would not fulfill criteria for overall reduced contractile performance. Moreover, the changes in ventricular volumes reflected mild morphologic dilation with significant outward displacement of the endocardial border in systole and diastole (79% and 47% of ventricular surfaces, respectively) (112).

The recognition that pathogenic variants cause cardiac dysfunction before the onset of overt morphologic features of HCM and DCM has propelled studies to define molecules and pathways that incite concentric hypertrophy, which increases cardiomyocyte and wall thickness, and eccentric remodeling, which increases cardiomyocyte length and expands chamber volume, as in DCM

(**Figure 1*c–f***). Although mouse models that carry pathogenic variants in cardiomyopathy genes have been studied to define signaling pathways, their relevance to human disease is complicated by considerable differences in cardiac physiology. Major differences include heart rates (mouse heart rates are 10 times faster than those of humans) and reciprocal expression of cardiac myosin isoforms (predominantly α-myosin heavy chain in adult mice and β-myosin heavy chain in humans). There are also differences in the regulation of calcium cycling and the dependency on phospholamban (29, 67, 68, 114, 132), which is an indispensable essential inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase in humans but not in mice (111).

Calcium is a critical regulator of cardiac remodeling, through its dynamic influence on myocardial tension (19) (**Figure 1*a,b***). Experimental data in mouse models demonstrate that the duration and magnitude of the myocardial mechanical tension developed incites myocardial remodeling (21). Myocardial tension is abnormal in cardiomyopathies. Pathogenic HCM variants increase the calcium sensitivity of myofilaments (107), delaying calcium reuptake in the sarcoplasmic reticulum, prolonging relaxation, and increasing tension (32, 40, 106). RCM variants have similar effects (151). By contrast, DCM variants desensitize myofilaments to calcium (83, 100, 149), reducing tension and promoting faster relaxation for the same amount of calcium (32, 40, 106). Enhancement and prolongation of mechanical tension in cardiomyocytes activate a pathway for concentric hypertrophy, whereas decreases in the magnitude and duration of mechanical tension activate a pathway leading to eccentric hypertrophy (21).

Increases in cardiac mass may also reflect calcium-induced activation of calcineurin signaling, with the directionality of cardiac growth mediated by MEK1-ERK1/2 signaling. In cardiomyocytes, this pathway promotes the addition of sarcomeres in parallel, while pathway inhibition adds sarcomeres in series, which elongates cardiomyocyte (21). Despite these mechanistic insights, the direct relevance of calcineurin signaling in HCM is less certain. Analyses of human HCM tissues indicate that hypertrophy is mediated by posttranslational activation of the calmodulin pathway, independent of calcineurin (40). In addition, while calcineurin activation mediates hypertrophy in response to pressure overload and exercise in mice (84), pharmacological suppression of calcineurin exacerbates rather than attenuates cardiac hypertrophy in HCM mice (28).

4. FUNCTIONAL CONSEQUENCES OF PATHOGENIC VARIANTS

4.1. Hypertrophic Cardiomyopathy

Consideration of the biophysical consequences of HCM pathogenic variants has been extensively informed by detailed understanding of the chemomechanical contraction cycle (121). The development of assays that assess the sliding velocities of myosin along actin filaments (in vitro motility assays), ATPase activity, or the force generated by single actomyosin interactions (117) has revealed novel features of myosin molecules as the sarcomere progresses through the chemomechanical cycle. Because HCM variants in *MYH7* are dispersed throughout the 2,000-amino-acid polypeptide, including in proximity to the ATP-binding pocket and actin-binding domain and within the hinge region (85, 133), an important question emerged: How did amino-acid substitutions at these different locations evoke similar functional consequences of hypercontractility, poor relaxation, and increased energy consumption? Several models address this question.

4.1.1. The myosin interacting-heads motif. Based on observations that, under in vitro experimental low-load conditions, only 5–10% of myosin heads are engaged in the chemomechanical cycle (121), Spudich (122) proposed that HCM variants might cause hypercontractility by increasing the number of myosin heads that participate in force production. This hypothesis is supported by studies of pathogenic HCM variants in the thin-filament protein troponin T

(encoded by *TNNT2*) that alter contractile function by increasing calcium sensitivity and decreasing affinity for tropomyosin (32). Notably, pathogenic *TNNT2* variants that cause DCM have the opposite effect (32, 119). However, a confounding issue for this model relates to loss-of-function variants in *MYBPC3*, which are a leading cause of HCM (76, 139). As experimental systems demonstrate that the amino terminus of cMyBP-C activates the thin filament (104), diminished levels would be expected to reduce activation and impair rather than augment contractility, as occurs in HCM.

A second model is predicated on the myosin interacting-heads motif (IHM) and conformational changes in myosins that occur during cardiac relaxation. Each myosin molecule contains a globular head, a flexible head-rod junction or hinge region, and an inflexible rod to the thick filament. The amino-terminal globular head contains a nucleotide-binding pocket with ATPase activity that drives actomyosin interactions and force production by the sarcomere. Myosin heads also associate with myosin essential and regulatory light chains. The rod portion of myosin forms α-helical coiled coils to allow packing of myosin rods into the cylindrical backbone of the sarcomere, from which myosin heads project out laterally. Within the thick filament, myosin polypeptides form homodimers.

During relaxation, the heads of paired myosin molecules undergo conformational changes through residues within the IHM (3, 6), which leads to inhibition of ATPase activity (**Figure 4*a***). The IHM is an evolutionarily conserved motif that is found in all muscle myosins, implying its importance in conserving energy consumption (63). Relaxed myosin exhibits two conformations that are denoted disordered relaxation (DRX) and super relaxation (SRX; **Figure 4*b*,*c***). DRX occurs when one of the two paired myosin heads adopts a folded state (blocked head) along the thick filament, causing steric hindrance of its ATP-binding pocket, while the other myosin head maintains ATPase activity and potential for actin interactions (free head). In the DRX state, myosin activity is further regulated by light-chain phosphorylation (6, 26, 55) and mechanosensing processes (30, 102). The SRX state occurs when both heads are folded back along the thick-filament backbone and both myosin ATPase domains are inhibited (49, 79, 90) (**Figure 4*c***). Myosins in SRX conserve energy, as their ATP consumption is one-fifth that of myosins in the DRX conformation (49, 79). Recent single-particle negatively stained electron microscopy images of purified human β-cardiac myosin have confirmed these dynamic structural changes and associated ATPase activities (8). In cardiac muscle, myosin blocked heads may remain in SRX in the active muscle during contraction while free heads generate force (**Figure 4*f***). By modulating the proportion of myosin heads within DRX or SRX conformations, muscles match energy consumption with functional demands and have additional reserve heads that can be activated in response to increased mechanical requirements (49, 79).

There are no atomic structures available for human β-myosins, but cryo-electron microscopy of the tarantula striated thick filament has defined myosin residues that participate in the IHM and in molecular interactions between myosins and the regulatory and essential light chains (3, 4). Fitting this structure with the human β-myosin sequence enabled human interacting residues to be defined. Analyses of pathogenic and likely pathogenic *MYH7* variants identified in more than 6,000 HCM patients demonstrated that these variants are highly enriched in IHM interacting residues (5). Approximately 78% of HCM variants in *MYH7*, *MYL2*, and *MYL3* alter residues that participate in these interactions, and most changed the charge of the encoded amino acid, indicating that HCM variants destabilize protein–protein interactions. Variants reported in population databases and *MYH7* variants that cause DCM are not enriched in interacting residues. As variants more frequently alter residues that support the SRX conformation, the net effect of HCM variants is predicted to increase the proportion of myosin heads in DRX (5, 80). A shift in the balance of myosin heads in DRX and SRX would increase the proportion of myosins that are

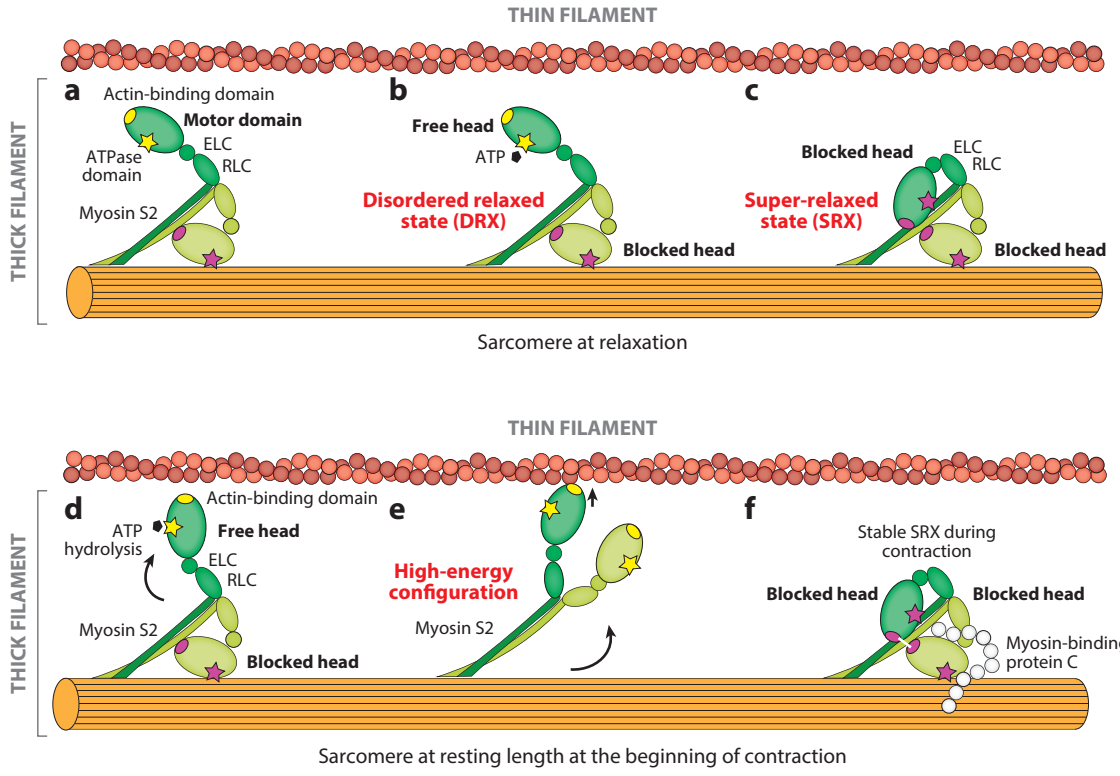

Figure 4

The interacting-heads motif paradigm and consequences for sarcomere mechanical properties and energetics. (*a*,*b*) Relaxed cardiac muscle contains a proportion of myosin heads in a state of disordered relaxation (DRX), in which only one of the two paired heads is sequestered in a folded-back configuration that results in inhibition of ATPase (*pink stars*) and actin-binding domains (*pink ellipses*). (*c*) The remaining proportion of relaxed myosins are in a state of super relaxation (SRX), in which both heads are in a folded-back configuration and there is dual inhibition of ATPase (*pink stars*) and actin-binding domains (*pink ellipses*). (*d*,*e*) At the beginning of contraction, ATP hydrolysis changes the configuration of the available myosin heads (high-energy configuration) (panel *d*) and promotes actomyosin interactions (panel *e*). (*f*) A population of cardiac myosin heads can remain in SRX in the active muscle during contraction. Myosin-binding protein C (*white molecule*) may participate in stabilizing SRX.

available for actin interactions, resulting in hypercontractility, impairment of full relaxation, and increased energy consumption, the three major pathophysiologic features of HCM (**Figure 5**).

The mechanism by which *MYBPC3* variants cause HCM has also been linked to changes in the proportion of myosin heads in DRX and SRX. Mice lacking cMyBP-C (81) and human HCM hearts with *MYBPC3* variants (80) have greater proportions of myosin heads in DRX than in SRX. cMyBP-C is thought to position myosin heads to stabilize the IHM, while reduced levels or phosphorylation of cMyBP-C attenuates IHM interactions and increases free heads (131). Consistent with this, human cardiomyocytes derived from induced pluripotent stem cells carrying HCM variants in *MYBPC3* show hypercontractility and impaired relaxation, abnormalities that can be normalized by attenuating myosin's motor properties (discussed in Section 4.1.2). Together, these data indicate that *MYBPC3* variants cause HCM by directly modulating myosin properties.

4.1.2. Protein phosphorylation in hypertrophic cardiomyopathy. Protein kinase A (PKA) mediates the physiologic response to β-adrenergic stimulation in the heart, which enhances

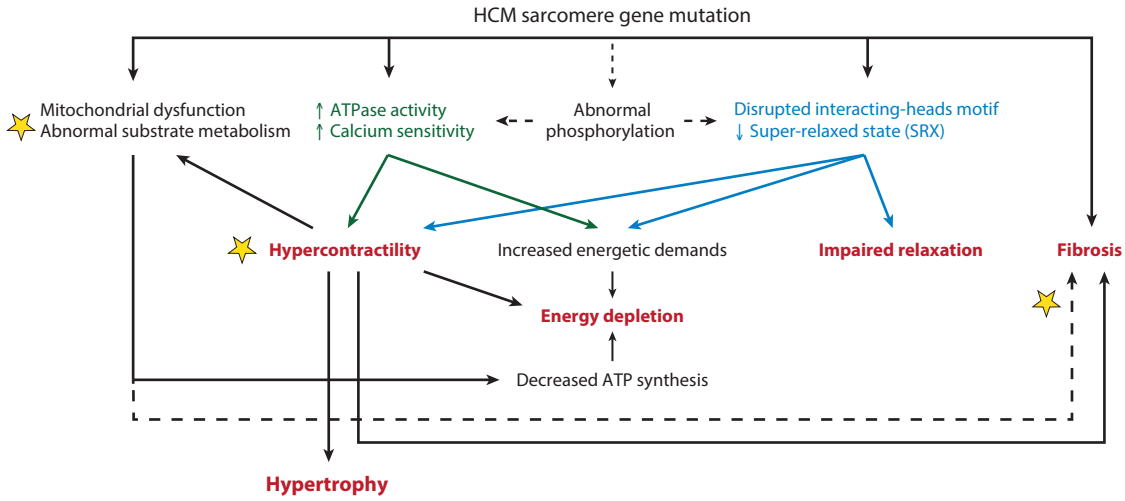

Figure 5

Schematic representation of the principal molecular mechanisms of hypertrophic cardiomyopathy (HCM). Yellow stars designate pharmacological targets that are currently being explored.

contractility and relaxation (lusitropy) by phosphorylating key sarcomere proteins, including cMyBP-C, troponin I, and titin. Troponin I phosphorylation reduces its affinity for troponin C, resulting in diminished calcium sensitivity and enhanced cardiac relaxation (17). Pathogenic HCM variants in genes encoding troponins (*TNNI3* and *TNNT2*) and tropomyosin (*TPM1*) alter residues within domains of the troponin I and troponin C interface and responses to β-adrenergic signaling. These variants decrease PKA-mediated phosphorylation of troponin I to increase calcium sensitivity, which enhances contractility but impairs relaxation (**Figure 5**).

cMyBP-C phosphorylation is a dynamic regulator of sarcomere function (12, 118, 145) that is mediated by PKA, protein kinase C, Ca^{2+}-calmodulin-activated kinase II, and other kinases. cMyBP-C can limit cross-bridge interactions through the biophysical interactions of its amino and carboxyl termini with both myosin and actin (95), while cMyBP-C phosphorylation fosters cross-bridge formation (58), possibly by increasing the number of available myosin heads or increasing heads in DRX (63).

Tissues from patients with *MYBPC3* mutations show profound dephosphorylation of troponin I and normalization of calcium sensitivity by PKA, suggesting that activation of thin-filament proteins is a compensatory response (139) to offset hypercontractility and poor relaxation in HCM. By contrast, dramatic changes in cMyBP-C phosphorylation do not occur in HCM (52), but other posttranslational modifications may influence calcium sensitivity. Integrated analyses by Kumar et al. (61) showed that, independent of disease, modulation of myofilament length-dependent activation depended on the additive effect of cMyBP-C and troponin I phosphorylation. Phosphorylated cMyBP-C appears to have its most profound effect on contractility at low calcium levels (during relaxation) and has much less effect at peak contraction, when calcium levels are high (98).

4.1.3. The metabolic consequences of hypertrophic cardiomyopathy. Several different mechanisms have been proposed to explain the energy deficits (**Figure 5**) that are recognized in HCM patients (11). Energy consumption would be increased by a direct increase in ATPase activity (85, 133), decreased contraction efficiency with a higher ATP utilization (16, 120, 150), shifts

in the proportion of myosin heads in SRX and DRX (5), reduced ATP synthesis due to mitochondrial dysfunction (71), and abnormal substrate metabolism (53, 70). A reduced phosphocreatine/ATP ratio in the hearts of preclinical carriers of pathogenic HCM variants (128) supports the conclusion that impaired energetics is a primary component of HCM pathophysiology. The imbalance between energetic demands and supply can cause an energy-starved state (135), and with the emergence of hypertrophy and associated microvascular dysfunction, these defects in cardiac energetics can propel the HCM heart to failure (39).

In the mitochondria, enhanced ATPase activity and ATP utilization increase ADP levels, which in turn reduce NADH and NADPH and increase oxidative stress with the accumulation of reactive oxygen species. In addition, the enhanced myofilament calcium sensitivity in HCM reduces the amount of calcium available for activating the Krebs cycle, impairing NADH regeneration (71, 135). Data from mice (69) and humans (134) support the idea that both mitochondrial disorganization and dysfunction occur in HCM. While disruption of mitochondrial antioxidant defense mechanisms is not HCM specific, this disruption may be influenced by genotype (136).

Substrate metabolism in HCM is characterized by a shift from a preferential utilization of fatty acid oxidation as the main source of ATP to glucose metabolism. This change is initially adaptive, because glucose metabolism produces a higher amount of ATP that is advantageous in a situation characterized by energy depletion. However, the persistence of this metabolic shift may be deleterious due to the accumulation of fatty acids, as well as of pyruvate and lactate resulting from glycolysis (138). The reduction of fatty acid translocase (CD36), which is the main protein involved in the transport of fatty acids into the cardiomyocyte (73, 124), provides evidence for abnormal cardiac lipid metabolism in HCM. Human metabolomics studies demonstrate higher concentrations of branched-chain amino acids, triglycerides, and ether phospholipids that correlate with remodeling and dysfunction of the left ventricle (53). The activity of AMP-activated protein kinase (AMPK), a cellular sensor of the energetic state and a regulator of metabolic processes (44), seems to be paradoxically decreased despite an energy-deprived state in HCM (73) and may promote cardiac fibrosis by modulating TGF signaling (44).

4.2. Dilated Cardiomyopathy and Titin-Truncating Variants

DCM is characterized by the depletion of mechanical force generation. Deficits in force production by the sarcomere can be incited by changes in multiple events. Pathogenic variants in *MYH7* (54) reduce its fundamental motor properties by impairing actin-activated ATPase activity and sliding velocity along actin filaments (113). Pathogenic variants in thin-filament proteins decrease the calcium sensitivity of the myofibrils, resulting in reduced tension and promoting a faster relaxation for the same amount of calcium (32, 40, 106). Detailed mechanisms by which pathogenic variants in these sarcomere proteins and in nonsarcomere proteins cause DCM have been recently reviewed (13, 15, 78, 89, 148). Below, we review experimental and clinical findings that support the conclusion that TTNtvs are the most common genetic cause of DCM and define the mechanisms by which these mutations cause disease.

Titin is a massive protein composed of 35,000 amino acids that span one half of the sarcomere. The protein comprises repeating immunoglobulin and fibronectin III modules that are interspersed with nonrepetitive sequences with phosphorylation sites, PEVK motifs, and a terminal kinase (59). The amino terminus of titin is anchored in the sarcomere Z-disk, where it participates in myofibril assembly and maintenance (38). Titin contains an elastic I-band that restores the resting length of sarcomeres after contraction and provides passive tension by limiting sarcomere stretch during early relaxation (65) and an inextensible A-band that binds myosin heavy chain and cMyBP-C, with functions in biomechanical sensing and signaling. The carboxyl M-band region

contains a strain-sensitive kinase that may evoke transcriptional responses to altered sarcomere mechanics (99).

The extraordinary size and complexity of the titin gene (*TTN*) impeded early analyses of *TTN* sequence variants, but the advent of next-generation sequencing has provided considerable insights. Contemporary studies focus on variants that alter gene structure, while the impact of *TTN* missense variants is largely unexplored. Despite this limitation, TTNtvs are now recognized to account for 15–25% of idiopathic DCM and approximately 10% of DCM that occurs in the setting of another condition.

4.2.1. The position-dependent consequences of titin-truncating variants. Integrated analyses of sequencing and transcriptional data from large human cohorts have shown that the effect of a TTNtv depends on its position in the protein (105). *TTN* undergoes extensive alterative splicing of 364 exons to yield multiple isoforms that are expressed in cardiac and skeletal muscle. This process is facilitated by its gene structure, including considerable (85%) exon symmetry (i.e., multiples of three base pairs), so that exon exclusion can occur while maintaining the translational frame (105). The inclusion of each *TTN* exon in the human left ventricle has been measured by RNA sequencing and quantified as the proportion of transcripts that incorporate that particular exon [the proportion spliced in (PSI)] (105). Clinical data demonstrate that TTNtvs within exons with very low expression (PSI < 15%) are not associated with DCM (112), whereas TTNtvs located in constitutive exons (PSI > 90%) cause DCM (105).

The majority of DCM patients carry TTNtvs within the A-band (2, 41, 105, 112). While initially the evidence that supports the pathogenicity of some TTNtvs in the I-band, Z-disk, or M-band was more difficult to establish, a meta-analysis of 2,495 DCM cases and 61,834 controls demonstrated that, regardless of the affected domain, TTNtvs in constitutive exons (PSI > 90%) are significantly associated with DCM (112).

Approximately 1% of subjects from the general population carry a TTNtv, and approximately half of these variants are located in high-PSI exons (64, 105, 112). These estimates suggest that, worldwide, approximately 35 million people carry a TTNtv in a high-PSI exon (112). As detailed above, high-resolution cardiac MRI of healthy individuals with such TTNtvs demonstrates significantly larger left ventricular volumes and subtle depression of contractility (112). While not all of these individuals are expected to develop DCM, the penetrance (or fraction of mutation-carrying individuals with disease) is significant. Within families, approximately 50% of men over age 50 and women over age 65 with a TTNtv exhibit DCM, and among participants in the Framingham Heart Study, individuals with TTNtvs compared to those without had higher rates of heart failure (relative risk = 16, $p = 0.008$) (74). How the background genotype and environment affect the expression of TTNtvs remains an area of active research.

4.2.2. Titin-truncating variants and susceptibility to mechanical stress and cardiotoxicity. Genetic analyses of DCM that occurs concurrently with another condition led to the hypothesis that the cardiac effects of TTNtvs can be unmasked by increased mechanical stress and cardiotoxicities. Three observations support this conclusion. Pregnancy, a paradigm for physiologic hemodynamic overload, can incite peripartum cardiomyopathy, a devastating disorder associated with high rates of cardiac transplantation and death (23). TTNtvs were identified in 10% of women with peripartum cardiomyopathy ($p = 1 \times 10^{-7}$ versus the general population) (147). Analyses of patients with alcoholic cardiomyopathy identified TTNtvs in a similar proportion of affected patients (10%) and demonstrated that, among DCM patients with TTNtvs, those with excessive alcohol consumption had an 8% lower ejection fraction (146). TTNtvs also contribute to the pathogenesis of cancer-therapy-induced cardiomyopathy (33, 66), which occurs in approximately

10% of these patients. These findings imply that environmental factors and exposures interact with the TTNtv genotype to influence the development of DCM. Importantly, these observations also indicate that recognition of at-risk genetics may help to stratify individuals who would benefit from cardioprotective strategies.

4.2.3. Mechanisms by which titin-truncating variants cause dilated cardiomyopathy. Although initial studies suggested that TTNtvs cause DCM via a dominant negative mechanism (105), the cumulative evidence from analyses in rodents, human induced pluripotent stem cell–derived cardiomyocytes, and human cardiac tissue (proteomics and transcriptomics) supports a mechanism of haploinsufficiency (45, 112). Presumably, premature truncations in *TTN* transcripts trigger nonsense-mediated decay and rapid turnover of the mutant protein (112). Consistent with this mechanism, truncated titin peptides are absent from DCM hearts that harbor TTNtvs (105). Whether depletion of titin alters the stoichiometry of other sarcomere proteins is unknown (105, 112, 141).

Experimental data demonstrate that TTNtvs result in quantitative and qualitative impairment of sarcomerogenesis (45). During development, titin serves as a scaffold for sarcomere formation (88), but recent studies have demonstrated that titin also functions in sarcomere maintenance. Analyses of human induced pluripotent stem cell–derived cardiomyocytes using live microscopy showed that the re-formation of sarcomeres after stress requires titin molecules to transmit diastolic tension (18). Sarcomere formation initiates at protocostameres, and through protein linkages with α-actinin-2, titin, and myosins, these mechanical forces are centripetally transmitted to direct fiber assembly and sarcomere formation. A corollary to these observations is that insufficient levels of titin reduce tension and diminish sarcomere formation. Based on human genetic data that TTNtvs are associated with stress-induced DCM (e.g., peripartum or alcohol cardiomyopathy and cancer-therapy-induced cardiotoxicity), this model predicts that sufficient levels of titin are required to maintain and restore damaged sarcomeres.

Rodent models carrying TTNtvs show abnormal cardiac metabolism (112). These models demonstrate shifts in substrate usage from fatty acids toward glycolysis, similar to the metabolic abnormalities observed in other forms of heart failure. In contrast to HCM, these metabolic changes are not accompanied by energy deprivation (112). Enhanced glucose metabolism may initially be an adaptive mechanism to maintain the heart in a compensated state, but this metabolic shift may also reduce cardiac reserves in response to stress.

5. FROM MOLECULAR MECHANISMS TO PHARMACOLOGICAL TARGETS: NEW DRUGS FOR HYPERTROPHIC AND DILATED CARDIOMYOPATHY

Genetic analyses and mechanistic studies position the sarcomere as a central mediator of cardiac pathophysiology. Pathogenic variants that cause HCM increase sarcomere power, impair relaxation, and promote energy depletion, which can activate remodeling pathways and increase fibrosis. Pathogenic variants that cause DCM impair sarcomere force generation without major effects on relaxation but incite remodeling pathways to maintain circulatory demands (the Frank–Starling mechanism), with associated increased energy consumption. Given these pathogenic mechanisms, specific therapies for both cardiomyopathies would be molecules that either enhance (DCM) or reduce (HCM) sarcomere function. High-throughput screens have successfully identified small allosteric molecules that modulate the sarcomere, and two are currently in human clinical trials: CK-1827452 (omecamtiv mecarbil), which selectively activates cardiac myosin ATPase (74, 86), and MYK-461 (mavacamten), which selectively reduces cardiac myosin ATPase activity (37).

Omecamtiv mecarbil binds the myosin catalytic domain, resulting in reversible conformational changes that increase ATP hydrolysis (74, 86). Recent functional studies indicate that the action of omecamtiv results from its binding and stabilization of the myosin pre-power-stroke conformation (97), as well as from modulation of the IHM so as to increase the availability of myosin heads for contraction (56). Initial preclinical models showed that omecamtiv increased cardiac contractility (67) without modifying myocardial oxygen consumption (116). Human clinical trials (phase I and phase II) have demonstrated safety, tolerability, and appropriate pharmacokinetics and pharmacodynamics of omecamtiv mecarbil, including an adverse event profile that was comparable to placebo. However, intravenous administration of this drug to approximately 600 patients with acute heart failure [in the Acute Treatment with Omecamtiv Mecarbil to Increase Contractility in Acute Heart Failure (ATOMIC-AHF) study] did not meet the primary endpoint of dyspnea improvement, although decreased left ventricular end-systolic volumes and increased systolic ejection times were observed (127). In patients with chronic stable heart failure, a phase II clinical trial that assessed a pharmacokinetic-based dose-titration strategy [the Chronic Oral Study of Myosin Activation to Increase Contractility in Heart Failure (COSMIC-HF) study] also showed improved cardiac function and reduced ventricular diameters compared with placebo (126). An ongoing phase III clinical trial involving 8,000 patients will evaluate whether omecamtiv reduces the risk of cardiovascular death or heart failure in patients with a reduced ejection fraction receiving standard of care for chronic heart failure [the Global Approach to Lowering Adverse Cardiac Outcomes Through Improving Contractility in Heart Failure (GALACTIC-HF) study, ClinicalTrials.gov identifier NCT02929329].

Mavacamten decreases contractility by inducing allosteric inhibition of cardiac myosin ATPase (37). Experimental studies show that mavacamten decreases steady-state ATPase activity by inhibiting the rate of phosphate release of the myosin motor domain and increases the proportion of myosin heads in the SRX state, thereby decreasing the proportion of myosin heads that are available for actin thin-filament interactions (57, 129) (**Figure 4**). These effects are predicted to conserve energy. Analyses of mavacamten-treated human HCM heart tissues showed normalization of the proportions of myosins in SRX and DRX (80, 81, 129).

Preclinical studies in wild-type and HCM mice treated with mavacamten reduced cardiac contractility in a dose-dependent manner without significant impairment of skeletal muscle function (37). Early and chronic oral administration of mavacamten in young mice harboring pathogenic mutations in *MYH7* prevented the development of left ventricular hypertrophy, significantly reduced fibrosis, and normalized the expression of mitochondrial proteins (37). In HCM mice with overt disease, mavacamten treatment promoted partial regression of hypertrophy and improved profibrotic gene expression but had little impact on focal fibrosis. These data suggest that the link between increased sarcomere power output and fibrotic response in HCM is an early phenomenon that may be best targeted in prehypertrophic stages.

In a feline model for HCM with left ventricular outflow tract obstruction, acute administration of intravenous mavacamten reduced contractility, eliminated systolic anterior motion of the mitral valve, and relieved left ventricular outflow tract pressure gradients (123). Early reports of a phase II clinical trial with mavacamten in obstructive HCM [A Phase 2 Open-Label Pilot Study Evaluating MYK-461 in Subjects With Symptomatic Hypertrophic Cardiomyopathy and Left Ventricular Outflow Tract Obstruction (PIONEER-HCM), ClinicalTrials.gov identifier NCT02842242] showed significantly reduced diastolic filling pressures, increased ventricular volumes, and improved biomarkers.

While myosin ATPase activators and inhibitors are first-generation drugs for the treatment of cardiomyopathy, the cardiomyopathy community expects that additional multiple targets within sarcomere domains will be identified that can be effectively modulated by small molecules. Not

only would these benefit cardiomyopathy patients, but these small molecules also provide exquisite probes for studying sarcomere physiology in health and disease.

6. CONCLUSIONS AND FUTURE DIRECTIONS

Technical and conceptual progress in molecular and cellular biology, genetic engineering, biophysics, and human genetics has led to great advances in the discovery of novel mechanisms by which sarcomere mutations cause cardiomyopathies. In HCM, understanding the mechanisms that promote myocardial hypercontractility and impaired relaxation has propelled new pharmacological targets that may revolutionize strategies to limit disease progression and adverse outcomes. Similarly, establishing haploinsufficiency and sarcomere insufficiency as the mechanisms that underlie impaired contractility in many cases of DCM provided new opportunities to improve contractile performance and limit patient symptoms.

Despite this progress, many important questions remain to be answered. First, the causes of HCM and DCM in many patients remain unknown, and efforts to identify new genetic or environmental causes should be reinforced. Clinical collaborative research networks together with next-generation sequencing platforms will advance this purpose. Second, translation of well-characterized cellular phenotypes and myocardial functional properties (i.e., contractility, relaxation, and passive stiffness) to the clinical setting is not always straightforward, especially in the early stages of disease expression or in incompletely penetrant cardiomyopathy variants. Integrating advanced cardiac imaging and, in particular subgroups, analysis of intracardiac pressure–volume data has the potential to provide valuable information on the consequences of pathogenic variants at the organ level in the intact circulation. Third, efforts must continue to understand the molecular mechanisms of well-established pathogenic sarcomere mutations. Truncating variants in *MYPBC3* and *TTN* are particularly interesting, because mechanistic advances may promote future gene therapy strategies to correct or modulate haploinsufficiency and may limit or prevent the development of HCM or DCM. Finally, while the primary causes of inherited cardiomyopathies are now being recognized, these discoveries have so far done little to indicate how changes in the sarcomere cause myocytes to change size and shape or to induce fibrosis in neighboring cells.

DISCLOSURE STATEMENT

C.E.S. and J.G.S. are founders and own shares in Myokardia Inc., a startup company that is developing therapeutics that target the sarcomere. Myokardia had no involvement in this review.

ACKNOWLEDGMENTS

We apologize to colleagues whose publications were not cited due to space limitations; please see referenced articles for additional citations. This work was supported in part by grants from the Instituto de Salud Carlos III to R.Y. (PI15/02229, BA16/00032, and CIBERCV), from the National Institutes of Health (5HL084553 and 5HL080494) and the Leducq Foundation to C.E.S. and J.G.S., and from the Howard Hughes Medical Institute to C.E.S.

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