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# The Genetic Landscape of Cardiomyopathy and Its Role in Heart Failure

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**Heart failure is highly influenced by heritability, and nearly 100 genes link to familial cardiomyopathy. Despite the marked genetic diversity that underlies these complex cardiovascular phenotypes, several key genes and pathways have emerged. Hypertrophic cardiomyopathy is characterized by increased contractility and a greater energetic cost of cardiac output. Dilated cardiomyopathy is often triggered by mutations that disrupt the giant protein titin. The energetic consequences of these mutations offer molecular targets and opportunities for new drug development and gene correction therapies.**

## Heart Failure and Cardiomyopathy

The clinical diagnosis of heart failure (HF) arises when cardiac output is insufficient to supply demand. Acute HF can occur from abrupt occlusion of a coronary artery, catastrophic valve dysfunction, malignant hypertension, or other states that provoke an urgent mismatch between supply and demand. Chronic HF is experienced as a slow decline in function, measured over years, as fatigue, breathlessness, and often evidence of end organ vascular insufficiency. Fluid overload and arrhythmias contribute to the HF spectrum. The timeline of chronic HF is punctuated by acute HF exacerbations, and the annual costs associated with HF exceed $30 billion U.S. dollars (Heidenreich et al., 2013). The major costs are calculated in repeated hospitalizations, the need for medical and device intervention, and lost productivity (Dunlay et al., 2011). Because of the chronic and progressive nature of HF, there is opportunity to intervene at early stages.

HF is frequently accompanied by cardiomyopathy, defined as a morphologically abnormal heart. In vivo, echocardiography provides critical information regarding chamber dimensions and function, while magnetic resonance imaging also provides a more in-depth visualization of myocardial tissue composition (Mahrholdt et al., 2005; Rickers et al., 2005). The major forms of cardiomyopathy include hypertrophic, dilated, restrictive, and arrhythmogenic (sometimes referred to as right ventricular) cardiomyopathy (Maron et al., 2006). Each of these forms of cardiomyopathy has a major heritable component, and genetic testing is now used in the evaluation of individuals with cardiomyopathy (Arndt and MacRae, 2014; McNally et al., 2013; Teekakirikul et al., 2013). The genes for which there is genetic testing are shown in Figure 1. Overall, there are nearly 100 genes linked to inherited forms of cardiomyopathy. More than 20 genes are implicated in hypertrophic cardiomyopathy (HCM), while fewer genes are linked to arrhythmogenic right ventricular cardiomyopathy (ARVC). Dilated cardiomyopathy (DCM) is the most genetically heterogeneous. The same gene may be implicated in multiple forms of cardiomyopathy, underscoring the importance of genomic context in the pathophysiology of disease-associated variants. In addition to genetic causes, ischemia, toxic insult, and valvular defects contribute to DCM, and more than one etiology may contribute to any form of cardiomyopathy. Despite this heterogeneity, several essential classes of genetic mutations are present around which existing and novel therapies can be applied.

## Genetic Assessment in Cardiomyopathy

The large number of genes responsible for cardiomyopathy, as well as the myriad of diverse mutations within each of these genes, produces remarkable heterogeneity for this complex disorder. Individual cardiomyopathy-associated genetic variants are infrequent in the general population (<1 in 500), and individual genetic variants associate with a range of expressivity causing mild and severe forms of disease. For example, deletion of arginine 14 in phospholamban (*PLN* gene) was described with early onset cardiomyopathy and accompanying lethal arrhythmias (Haghighi et al., 2006). In one population, this same mutation was found in 12% of ARVC and 15% of DCM subjects (van der Zwaag et al., 2012), and a follow-up retrospective evaluation of 295 gene mutations carriers confirmed an earlier age of onset of both arrhythmias and cardiomyopathy (van Rijsingen et al., 2014). Curiously, this same mutation was described in individuals with late-onset DCM without evidence of ventricular arrhythmias (DeWitt et al., 2006). The range of outcome with the same given variant is consistent with the presence of genetic and environmental factors that modify outcome (Arad et al., 2002; Marian, 2000) (Figure 2). Sex is a modifier of cardiomyopathy expression. Rare truncating mutations in *TTN*, the gene encoding the giant protein titin, are associated with more severe left ventricular (LV) dysfunction in males compared to females (Herman et al., 2012). Sex differences have also been described in HCM, where males are usually more affected, and this is recapitulated in animal models (Geisterfer-Lowrance et al., 1996; Vikstrom et al., 1996). Sex differences are attributed to a number of factors including hormone levels, gene expression differences, and basic differences in physiology, including heart size. Factors other than sex also influence the expression of genetic variants on the pathophysiology of heart disease.

Secondary or additional genetic variants also contribute to severity or progression of disease. In individuals with more than one mutation, there is often earlier onset and in some cases a more rapid progression of disease (Girolami et al., 2010; Golbus et al., 2014; Ingles et al., 2005; Richard et al., 1999). A recent survey used whole-genome sequencing of 11 unrelated

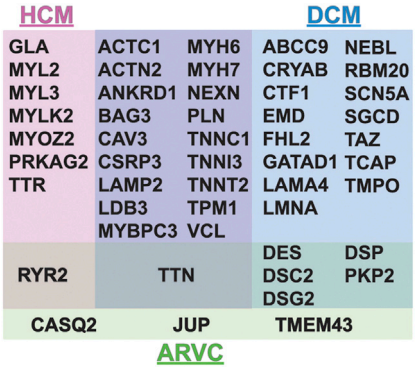


**Figure 1. Shown Are the Genes That Have Been Linked to Human Inherited Cardiomyopathy**

Those genes responsible for HCM are in pink, and for DCM in blue. There are a number of genes that cause both HCM and DCM (purple). Mutations in genes encoding desmosomal and other proteins cause arrhythmogenic right ventricular cardiomyopathy (ARVC, green), and there is overlap between mutations in these genes that leads to other forms of cardiomyopathy.

individuals to identify the spectrum of cardiomyopathy variants (Golbus et al., 2014). In 9 of 11 individuals, the primary disease-causing variant was identified, usually because it segregated with disease. However, two cases had additional, rare, potentially pathogenic variation. Closer inspection of these families revealed that these secondary variants were segregating with a more severe phenotype, indicating that multiple rare variants may contribute to altered disease course within a family. Common or higher-frequency variation also impacts the effects of underlying pathogenic variants. Recent work has revealed an association between severity of cardiomyopathy and mitochondrial DNA haplogroup (Strauss et al., 2013). Human mitochondrial DNA can be divided into groups based on shared genealogy. Strauss and colleagues studied a large Mennonite family with autosomal recessive myopathy and cardiomyopathy caused by a frameshift mutation in the gene coding for adenine nucleotide translocator-1. The authors found considerable variability in the progression and severity of the cardiac phenotype that segregated with the maternal lineage. Sequencing showed segregation of two mitochondrial haplogroups, one of which conferred more severe cardiomyopathy (Strauss et al., 2013).

These data underscore how the expressivity and penetrance of specific cardiomyopathy gene variants varies widely (Hershberger et al., 2013). In silico algorithms score pathogenicity on numeric scales, relying on conservation data and less so on structural information (Ritchie and Flicek, 2014). Based on segregation with clinical phenotype, more highly penetrant mutations have been described, but even highly penetrant mutations may require the context of specific genetic backgrounds or ethnicities to fully manifest, and this "background effect" has been modeled in mice (Semsarian et al., 2001; Suzuki et al., 2002; Wheeler et al., 2009). With the emergence of sequence data from large numbers of ethnically diverse humans, it has become clear that "pathogenic" variation is found at a higher than expected rate. Specifically, previously described pathogenic mutations are present at a frequency higher than the prevalence of cardiomyopathy (Andreasen et al., 2013; Golbus et al., 2012; Pan et al., 2012). Not all genetic variants induce the same degree of cardiac dysfunction, and for primary mutation and secondary modifiers there are "mild" and "severe" mutations. Determining the expressivity of given mutations is challenging, and computational algorithms, however imperfect, emerging now serve as an adjunct to interpreting the pathogenicity of cardiomyopathy mutations. Genetic variation remains a strong predictor of risk for developing cardiomyopathy, particularly within families where a primary gene mutation has been identified.

Recent work has focused on reclassifying the potential pathogenicity of variants based on frequency in the population at large, with higher-frequency variants considered less pathogenic (MacArthur et al., 2014). This methodology assumes that pathogenic alleles will be found in a frequency in the population less than or equal to the disease prevalence and assumes that individual variants are sufficient to cause disease. Studies of penetrance and expressivity indicate that the entire genomic context, as well as the environment, dictate the role of particular variants (Hershberger et al., 2013). Pathogenicity of particular variants must be considered within the phenotype context, as many cardiomyopathic genetic variants are necessary but not sufficient to cause disease. Most large human genetic data sets include individuals who have not been specifically evaluated for subtle signs of cardiomyopathy and/or individuals who are too young to have yet developed disease. Similarly, it is expected that additional genetic and environmental stimuli are necessary to express the full phenotype of cardiomyopathy and HF. In addition to these secondary genetic and environmental modifiers, epigenetic influences may markedly alter the expression of mutant alleles or alternative genetic pathways that diminish or enhance pathogenicity. As sparks do not cause fire in the absence of oxygen, cardiomyopathy mutations require context to fully manifest. Variants are only pathogenic in a larger context that includes both the susceptible genetic and environmental conditions (Figure 2). Exploring and defining the genetic and environment stimuli necessary for cardiomyopathy expression is critical, as these modifiers influence outcome and are targets for intervention.

## Hypertrophic Cardiomyopathy and Thick Filament Gene Mutations

HCM is estimated at 1:500 in younger individuals and is enriched in families. This estimate derives from a population-based survey of individuals 23–35 years of age (Maron et al., 1995). Given the broad age range of HCM and the appreciation that some genetic mutations have later onset, the overall population prevalence is higher. An Olmstead County study conducted in 1985 identified a similar prevalence for HCM (19.7 per 100,000) and a higher prevalence for DCM (36.5 per 100,000). This study and the previous one rely on older methods of detection, and as such likely underestimate the prevalence of HCM. Hypertrophy of the ventricular myocardium arises in response to physiological stimuli, such as exercise, and pathological stimuli such as hypertension or aortic stenosis. In genetic HCM, autosomal dominant mutations in the *MYH7* and *MYBPC3* genes account for nearly 80% of inherited HCM (Kensler et al., 2011). These genes encode the sarcomere thick filament proteins β-myosin heavy chain (MYH7) and cardiac myosin binding protein-C (cMyBP-C). Although they are both highly associated with HCM, the mechanisms of the HCM-causing mutations in these two genes differ. The majority of pathogenic variants in *MYH7* that cause HCM result in amino

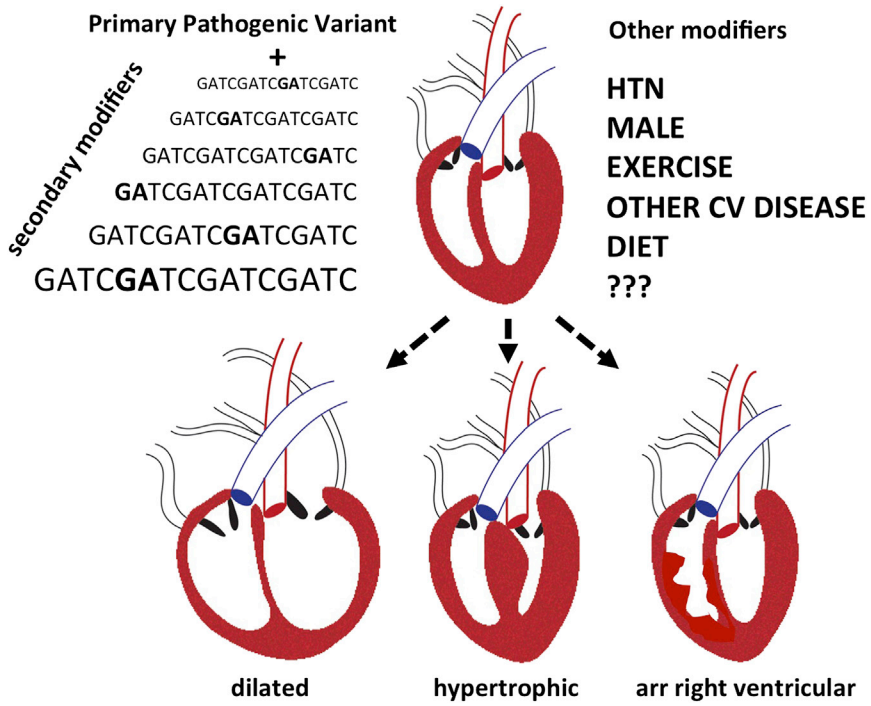


**Figure 2. Familial Studies for Inherited Cardiomyopathy Often Demonstrate a Primary Pathogenic Variant, and Pathogenic Variants Differ in Their Effect on Phenotypic Outcome**

Each genome contains many additional variants that serve to modify the expression of the primary pathogenic variant. These secondary modifers may be common or rare in the population. In addition to these genetic modifiers, comorbidities, environmental factors, and sex modulate the expression of cardiomyopathy. The manifestation of cardiomyopathy varies over the lifetime of the individual. Those mutations, or combinations of mutations, with the most potent effect on phenotype manifest earlier in life. Milder mutations may not express until later in life or may remain subclinical throughout the lifetime of the individual. (Dilated, DCM; hypertrophic HCM; arr right ventricular, ARVC.)

acid substitutions in critical residues and domains that adversely affect function. In contrast, the majority of pathogenic HCM-causing *MYBPC3* variants are premature stop codons or frame-shifting mutations, frequently resulting in absence of protein. *MYBPC3* variants are thought to have a milder disease course with later onset then mutations in *MYH7* (Charron et al., 1998; Maron et al., 2001), which may be attributed to the difference in pathogenic mechanisms between mutations in these genes.

*MYH7* encodes β myosin heavy chain (βMHC), the thick filament protein responsible for hydrolyzing ATP to produce force. Myosin can be divided into the globular head domain and its coiled-coil rod domain. The myosin head is attached to an arm that articulates away from the rod region on a flexible hinge to extend into the interfilament space (Figure 3). The rod domain mediates the formation of the thick filament with its characteristic periodicity (Moore et al., 2012). Mutations in *MYH7* have been identified in all regions of the protein, with more mutations concentrating in the ATPase domain, actin binding domain, and domains responsible for force transmission (Walsh et al., 2010). Although occurring at lower frequency, mutations in the rod domain have also been linked to HCM (Blair et al., 2002). Modeling *MYH7* mutations has been achieved using materials from human tissues, in vitro or cell-based expression, or genetic engineering in mice. Each of these methods has limitations, and the results from distinct approaches have not always produced consistent findings. Mice, like other small mammals, express α-MHC as the major cardiac myosin (encoded by *MYH6*), rather than βMHC, like the larger human heart. αMHC, while similar in overall structure to βMHC, has an intrinsically faster rate of ATP hydrolysis and contractile kinetics (Korte et al., 2005). The intrinsic capabilities of α-MHC versus β-MHC can lead to the same mutation demonstrating different biophysical characteristics (Lowey et al., 2008).

There are hundreds of distinct missense *MYH7* mutations responsible for HCM, and a clear unifying hypothesis has been elusive. Clinically, HCM is often characterized by a hyperdynamic state in which there is an increase in LV ejection fraction from 60% to 70% or more. For example, the R453C *MYH7* HCM mutation displays an impaired catalytic cycle of ATP hydrolysis despite few biochemical alterations in the ATPase domain, and this mutation counterintuitively results in increased contractility (Bloemink et al., 2014; Sommese et al., 2013). Increasing evidence suggests that HCM mutations in *MYH7* cause increased energy usage due to a less efficient myosin motor and that this energetic mismatch results in perturbed metabolic state (Crilley et al., 2003). As one indication of this energetic mismatch, reduced phosphocreatine levels (PCr) have been observed using $^{31}$P NMR spectroscopy of animal models of HCM and materials from human HCM patients (Ingwall, 2014; Witjas-Paalberends et al., 2014a, 2014b). Human hearts expressing the *MYH7* R403Q mutation generate increased tension and faster actin sliding velocities, but at a higher energetic cost (Alpert et al., 2005). Mice engineered with the R403Q HCM-associated mutation had reduced rate of relaxation and increased end diastolic pressure after inotropic stimulation (Tyska et al., 2000). A similar decrease in PCr and increased ADP was seen in these hearts, consistent with a higher energetic cost of contraction (Spindler et al., 1998).

In addition to *MYH7* missense mutations in HCM, *MYH7* missense variants are also found in DCM. DCM-associated *MYH7* missense mutations have been modeled in mice, albeit in the context of *Myh6*. However, these mutations show an increased tension cost, with more ATP required for a given amount of shortening, depressed actin sliding velocities, and gross dilation (Schmitt et al., 2006). In contrast to the hypercontractile HCM mutations, DCM mutations, when modeled in expressed myosin, cause a hypocontractile state and quickly lead to HF (Bloemink et al., 2014; Sommese et al., 2013).

The second major thick filament protein implicated in HCM is *MYBPC3*, which encodes cardiac myosin binding protein C

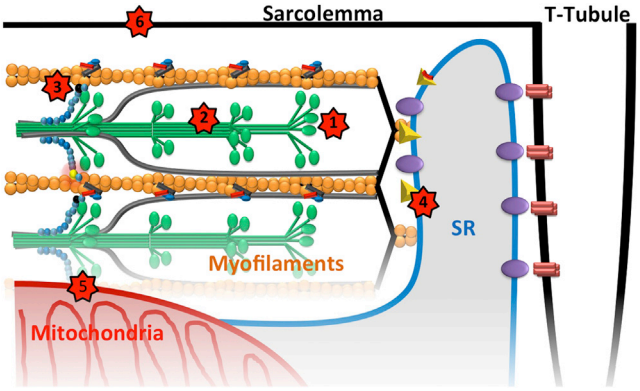


**Figure 3. Myosin Heads Protrude from the Thick Filaments, in Green,to Interact with Actin Containing Thin Filaments, in Yellow**
Multiple sites throughout the sarcomere and cardiomyocyte are now the targets for new drug development for heart failure (red stars). (1) Small molecules like omecamtiv are aimed at myosin ATPase activity to increase or decrease contractility. (2) Antisense or RNAi approaches are being tested to silence mutant alleles, but not normal alleles. (3) cMyBP-C phosphorylation can be modified through kinase/phosphatases to modulate its "brake effect" on crossbridge cycling. (4) Calcium handing in the sarcoplasmic reticulum is a target in development. (5) Palmitoyltransferase-1 can be altered using perhexiline to shift metabolic substrate usage from fatty acid oxidation to glycolysis. (6) The regulation of nitric oxide synthase can be used to change cellular redox state and prevent glutathionylation and dysregulation of myofilament proteins.

(cMyBP-C). *MYPBC3* and *MYH7* mutations are at nearly equal frequency in HCM cohorts, each representing approximately 40% of identified mutations. In contrast to *MYH7* HCM mutations, which are mainly nonsynonymous single nucleotide polymorphisms (nsSNPs), *MYBPC3* mutations more commonly disrupt the reading frame. By disrupting the carboxyl terminus of cMyBP-C, these mutations alter the myosin and titin binding sites and disallow mutant cMyBP-C incorporation in the sarcomere. cMyBP-C is incorporated into the thick filament through direct binding of its carboxyl terminus to the myosin rod and also to the giant protein titin (Freiburg and Gautel, 1996; Gilbert et al., 1996). cMyBP-C includes eight immunoglobulin (Ig) domains along its length, with three fibronectin (Fn) domains toward its carboxyl terminus. cMyBP-C extends transversely into the interfilament space where its amino-terminal region interacts with the myosin S2 region and actin in a phosphorylation-dependent manner (Barefield and Sadayappan, 2010; Bezold et al., 2013; Kunst et al., 2000). The phosphorylation state of three serine residues mediates cMyBP-C's ability to regulate crossbridge cycling, and dysregulation of these residues results in cardiomyopathy (Sadayappan et al., 2005, 2006). Recent work has clearly defined the role of cMyBP-C as a "molecular brake" on myosin crossbridge cycling, resulting in slowing of actin sliding velocities while in a dephosphorylated state (Previs et al., 2012). However, phosphorylation of cMyBP-C by various upstream pathways, notably PKA, reduces the inhibitory effect of cMyBP-C in a graded manner, with phosphorylation of additional sites providing additional relief of myosin inhibition, allowing throttling of this effect (Weith et al., 2012a, 2012b). In addition, it has been recently shown that cMyBP-C's interaction with actin displaces α-tropomyosin, thus modifying thin-filament activation (Mun et al., 2014).

Oxidative modifications, often accompanying pathological remodeling of the heart, have been reported having detrimental effects on cMyBP-C and sarcomere function. Using a hypertensive mouse model demonstrating diastolic dysfunction prior to hypertrophic remodeling, cMyBP-C was determined to be glutathionylated at three cysteine residues (Patel et al., 2013). This oxidative modification resulted in increased myofilament calcium sensitivity in isolated myofilaments. Treatment with tetrahydrobiopterin prevented cMyBP-C glutathionylation and improved relaxation kinetics and diastolic dysfunction (Jeong et al., 2013). While additional work is required to determine whether these channels are wholly mediated by cMyBP-C oxidation, the possibility of treatment prior to the onset of hypertrophy with antioxidant agents warrants further investigation.

With these data, it is now clear that cMyBP-C regulates contractility and alters sarcomere energetics. HCM-associated *MYBPC3* mutations, especially those that reduce the amount of cMyBP-C, promote a loss of crossbridge cycling inhibition. The loss of cMyBP-C regulation has been shown to decrease maximal force development in samples of human tissue from mutation carriers (van Dijk et al., 2014) and in mouse models of disease (Barefield et al., 2014; Harris et al., 2002). However, these *MYBPC3* mutations have been shown to increase the energetic cost of contraction in a manner similar to that of *MYH7* mutations (Witjas-Paalberends et al., 2014b). Whether *MYBPC3* mutations act by haploinsufficiency or dominant-negative activity has been examined, and evidence for truncated cMyBP-C protein has been lacking (Marston et al., 2009). Thus, *MYBPC3* mutations appear to act mainly by reducing protein content (Barefield et al., 2015; van Dijk et al., 2009). Treatment has therefore focused on restoring protein levels through gene therapy, with some notable recent success in a mouse model (Mearini et al., 2014).

In South Asian populations, it is estimated that 4% of the population carries a 25 bp deletion in intron 32 of *MYBPC3* (Dhandapany et al., 2009). This variant increases the risk for HF and cardiomyopathy. This deletion induces skipping of the downstream exon near the 3′ end of *MYBPC3*. The true prevalence throughout South Asia has been estimated to be lower, but larger population samples are likely needed (Simonson et al., 2010). This deletion has also been linked to increased LV dysfunction after myocardial infarction (Srivastava et al., 2011), consistent with this variant increasing susceptibility to HF, especially in combination with other cardiac insults. Rare instances of individuals with two mutant alleles of *MYBPC3* and early onset lethal disease have been described and often associate with the feature of noncompaction (Dellefave et al., 2009; Lekanne Deprez et al., 2006; Schaefer et al., 2014; Wessels et al., 2014). There is an emerging view that HCM can be subdivided into "sarcomere" versus "nonsarcomere," and that pathophysiology and outcome are different between these groups (Olivotto et al., 2008, 2011).

## The Thin Filament

Crossbridge formation between myosin heads and actin filaments is largely regulated by the proteins of the thin filament. The thin filament is composed of actin, tropomyosin, the troponin complex including troponin T (the tropomyosin binding subunit), troponin I (the inhibitory subunit), and troponin C (the

$Ca^{2+}$ binding subunit). Titin and other Z-disk-related proteins also contribute to this regulation. Regulation of crossbridge formation depends on the $Ca^{2+}$ and ATP availability and the conformation of the troponin-tropomyosin complex on the actin filament (Lehman et al., 2000). The complex exists in three states, each of which determines the extent of actin and myosin interaction (McKillop and Geeves, 1993). HCM-causing mutations have been identified in each of these components, and the possibility that these proteins exert their effect through changing sarcomere energetics and the cost of contraction has been suggested (Tardiff, 2011).

Recently, Moore and colleagues examined the tropomyosin-binding region of cardiac troponin T, a region that harbors severe and phenotypically diverse HCM mutations (Moore et al., 2014). In vitro motility assays showed that specific mutations in cardiac troponin T disrupted weak electrostatic interactions between the thin filament and myosin. Complementary in vivo data indicate that these same mutations cause cardiac remodeling and disarray of the myofiber, suggesting that the weak crossbridge formation causes destabilization of the myofilament structure, ultimately resulting in disease (Moore et al., 2014).

## Titin, the Third Filament, a Gene for DCM

The giant protein titin is necessary for the passive forces that maintain sarcomere integrity, and these passive forces play a critical role in LV mechanics, especially filling during diastole. Titin is the largest known protein and spans half the sarcomere from Z-disk to M-line (Fürst et al., 1988). Adjacent to the M-line titin contains a titin kinase domain (TK) (Gautel, 2011). The A-band portion of titin is composed of both Ig and fibronectin domains (Labeit et al., 1992). The I-band portion is composed of two "spring-like" domains in cardiac muscle: the N2B, and PEVK along with tandem immunoglobulin (Ig) segment. These "spring-like" domains are responsible for passive force during sarcomere stretch (reviewed in Anderson and Granzier, 2012).

Several mouse models have been created to dissect titin's role in the sarcomere. Lee and colleagues deleted the region of *TTN* encoding its N2B region and found that passive tension was elevated, triggering an increase in calcium sensitivity at long sarcomere length (Lee et al., 2010). Increases in calcium sensitivity result in increased length-dependent activation, indicating that passive tension induced by titin is a factor in the Frank-Starling mechanism of the heart (Katz, 2002). In further support of the idea that increased titin-induced passive tension results in increased length-dependent activation, N2B-deleted mice have higher LV diastolic stiffness and diastolic dysfunction (Lee et al., 2010). Truncation of the PEVK region, another spring-like element, also exhibited increased passive tension along with diastolic dysfunction (Granzier et al., 2009). A mouse lacking the tandem Ig segment showed similar physiology (Chung et al., 2013). Disrupting the I-band/A-band junction of titin also increased strain of the spring regions of the I-band and caused diastolic dysfunction in the mice (Granzier et al., 2014). Currently, mouse models specifically lacking the A-band region of titin are lacking. However, a recent study has shown that the A-band of titin is not required for thick filament assembly in zebrafish (Myhre et al., 2014). These zebrafish have a truncated *TTN*, lacking the C-terminal, A-band-associated rod domain. The zebrafish form normal muscle with grossly normal thick and thin filament assembly, and only after embryonic development does the sarcomere break down, consistent with *TTN* having a role in sarcomere maintenance (Myhre et al., 2014). The zebrafish also display reduced heartbeat and cardiac edema, consistent with cardiac dysfunction.

*TTN* has recently been identified as a major cardiomyopathy gene in humans (Herman et al., 2012). Herman et al. captured and sequenced the 360 exons of *TTN* in large cohorts of more than 300 DCM and 200 HCM subjects as well as a control group with normal heart function. They identified a large number of missense mutations in *TTN*, even in the control population, making these variants difficult to interpret. Instead, they focused on truncating variants that created frameshifts, stops, and splice site alterations. Approximately 25% of DCM patients had a truncating variant in *TTN*, while only 1% of HCM and 3% of the control population had truncating variants, making *TTN* mutations the most common genetic source of DCM to date. Truncating variants identified in the DCM cohort disproportionately occurred in the A-band region of *TTN* (Herman et al., 2012). A recent study supports that DCM-associated *TTN* truncating variants fall into the A band of titin (Roberts et al., 2015). Furthermore, the truncating variants in the general population fall into *TTN* isoforms expressed at much lower levels in the heart.

## Implications for Gene-Based Therapy and Drug Development

Gene-based correction is now possible by targeting RNA using reduction strategies and other methods that "bypass" mutations, creating internally truncated proteins. For dominant diseases, especially in *MYH7*, targeting the mutant allele with RNAi is possible in a mutation-specific manner (Jiang et al., 2013). Developing individualized treatment plans with mutation-specific sequences may be cumbersome and require unique validation methods. As an alternative, it is possible to target more common variation, present on the mutant but not the normal allele. In this manner, sequences could be developed to treat larger numbers of patients and providing a more feasible regulatory approval pathway. The degree to which the mutant allele must be reduced can be guided by data from human hearts, where the expression of mutant proteins is often less than 50% (Helms et al., 2014). Genetic "bypass" methods are also being developed, and these methods manipulate RNA using antisense sequences to induce alternative splicing to avoid the mutation. This approach, referred to as exon skipping, takes advantage of naturally occurring splice forms or creates newly engineered, internally truncated proteins (Veltrop and Aartsma-Rus, 2014). As long as there is physiological evidence that such internally truncated proteins can compensate, these approaches may be useful for some genes linked to cardiomyopathy. Complete functional restoration may not be needed, as even partial improvement may be sufficient to improve phenotype. Given the structural data available on βMHC, it is unlikely that internal truncation methods are suitable for *MYH7* gene mutations. However, for proteins such as titin that are composed of repetitive domains, internal truncations may be a viable alternative (Freiburg et al., 2000). Newer DNA-based gene editing methods are an active area of research, currently being tested in cell-based models (Li et al., 2014; Xie et al., 2014). These methods have been employed to correct muscle disease in the mdx

mouse model (Long et al., 2014), and are currently being tested in cardiomyopathy models.

The energetic and metabolic deficits in HF offer opportunities for small-molecule-based therapy. Modulating contractility can occur by either blunting hypercontractile states or by using positive inotropic agents to improve deficits in contractility. A recent potential therapeutic for reduced cardiac performance is omecamtiv mecarbil, which directly activates MHC through enhancement of contractility, and with a corresponding improvement in cardiac output (Cleland et al., 2011; Malik et al., 2011). Identifying targets that regulate the cardiomyocyte metabolic state has also shown some promising preliminary results. Modest improvements in the HCM phenotype were observed in a mouse model carrying a *MYBPC3* mutation following the application of perhexiline, a molecule that targets mitochondrial palmitoyltransferase-1, altering metabolic substrate usage from fatty acid oxidation to glycolysis (Gehmlich et al., 2015). However, more work remains to be done to evaluate the efficacy of altering metabolic substrate usage. The differences between the mouse and human hearts, reflected by their distinct MHC usage, will likely mandate that these approaches be validated in larger mammalian hearts.

## Conclusions

The genetic complexity underlying cardiomyopathy is challenging the concept of "single gene disorders." The number of genes and individual mutations is greater than had been expected, and it has only been through the availability of next-generation sequencing that such genetic diversity has been appreciated. While this genetic landscape is daunting in scope and breadth, key themes have emerged. HCM, with its distinct phenotype, is largely linked to two key thick filament proteins. A wealth of data supports an energetically inefficient myocardium in HCM. DCM, while more genetically heterogeneous than HCM, has one major gene, *TTN*, which now begins to focus the etiology of a sizable subset of disease. With improved DNA sequencing, it is now possible to identify combinations of genetic mutations that contribute to cardiomyopathy. HCM can now be subdivided into sarcomere and nonsarcomere HCM with clinically meaningful differences in physiology, and the same classification will develop for DCM. A better understanding of the molecular subtypes of cardiomyopathy will help more precisely apply existing and evolving therapies.

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