Chapter

Cardiomyopathy: Getting Bigger All the Time - Lessons Learned about Heart Disease from Tropomyosin

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Abstract

In 1990, John and Christine Seidman uncovered the genetic association between mutations in sarcomeric contractile proteins and hypertrophic cardiomyopathy. Since then, the increase in knowledge and understanding of this disease has increased exponentially. Although pathologies associated with the various cardiomyopathies are vastly different, in some cases, the same proteins are causative, but with different genetic mutations. The focus of this article will be on hypertrophic and dilated cardiomyopathies, which are often caused by mutations in sarcomeric contractile proteins. Tropomyosin, a thin filament protein, serves as a paradigm to illustrate how different mutations within the same protein can generate the hypertrophic or dilated cardiomyopathic condition. As such, the significant advances in information derived from basic science investigations has led to the development of novel therapeutics in the treatment of these pathological diseases. This article will illustrate linkages which occur to bridge scientific advances to clinical treatments in cardiomyopathic patients.

Keywords: hypertrophic and dilated cardiomyopathy, tropomyosin

1. Introduction

Cardiomyopathies are diseases with primary defects associated with the structure and function of the heart. They are commonly classified into 5 different categories: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and left ventricular noncompaction (LVNC). HCM and DCM are the most common of the cardiomyopathies, with an incidence of 1:500 and 1:2500, respectively. Although there are variations in phenotypes and etiologies, there are also similar symptoms among the cardiomyopathies. For example, HCM, DCM, and RCM often present with signs and symptoms that are common in heart failure with reduced ejection fraction, including peripheral edema, fatigue, dyspnea on exertion, syncope, and cardiac ischemia [1, 2]. The focus of this article will be on HCM and DCM, the two most common cardiomyopathies.

2. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is defined as left and/or right ventricle hypertrophy in the absence of external load, and without chamber dilation. Interventricular septal thickening predominates and may cause left ventricular outflow tract obstruction and/or mitral valve dysfunction. Other common features include myocyte disarray, fibrosis, alterations in calcium sensitivity of myofilaments, and cardiac arrhythmias that may lead to premature sudden death and/or heart failure. Phenotypic expression is variable, with some genetically-identified HCM individuals dying in their late teens/early twenties, whereas others have a normal life span with minimal disability dependent upon the specific mutation within the affected gene. In addition, modifier genes and environmental factors can influence disease progression and phenotype.

The genes associated with HCM can be roughly divided into several distinct categories: (1) genes definitively established as causing HCM via large family pedigrees; (2) genes likely causing HCM via small family pedigrees; and (3) genes associated with HCM via small families and sporadic cases [3]. However, as more genetic information is obtained on incidence of diseases, these categorizations may become blurred. Usually, HCM is inherited as an autosomal dominant disease where a single missense point mutation in the affected gene is sufficient to cause the disease, although there is variability in the phenotype. This variability in phenotype is also manifest by the numerous different point mutations that occur within a specific gene; for example, the myosin heavy chain 7 (*MYH7*) R403Q mutation is associated with a severe pathological phenotype, whereas other *MYH7* mutations, such as V606M, are relatively benign [4]. Studies also show that modifier genes and their polymorphisms, such as angiotensin II type 2 receptor and calmodulin, can influence the HCM pathology [4]. In addition, mutations that occur in different HCM-causing genes have dramatically different pathologies, with some being severe and others being relatively benign.

The genes primarily associated with the HCM phenotype are sarcomeric contractile protein genes associated with both thick and thin cardiac myofilaments, along with the Z discs (**Table 1**). There are over 1500 mutations in these genes that are associated with HCM. The pioneering studies that revealed the molecular genetic basis of HCM and its association with sarcomeric protein genes were conducted by Drs. Christine and Jonathan Seidman [5]. These initial studies led to the discovery that mutations within most of the thick and thin filament sarcomeric protein genes of the heart can cause HCM (**Table 1**). In the United States, the most common genes associated with HCM are β-myosin heavy chain (*MYH7*) and myosin-binding protein C (*MYBPC3*); other thick filament protein genes which cause HCM are the regulatory light chain (*MLC2*) and the essential light chains (*MLC 1/3*). Most *MYH7* mutations occur in the globular head and hinge region of the myosin heavy chain, although mutations in the rod domain also cause HCM. Although most HCM mutations in the contractile protein genes are missense mutations, there is a bias for insertion/deletion mutations and premature truncation mutations in the *MYBPC3* gene; these insertion/deletion mutations often result in translational reading frame shifts which lead to premature stop codons with subsequent degradation of the mRNA by nonsense mediated decay mechanisms or degradation of a truncated polypeptide. Thin filament protein genes associated with HCM are α-tropomyosin (*TPM1α*), troponin T, I, and C (*TNNT2, TNNI3, TNNC1*), and cardiac actin (*ACTC1*). The muscle LIM protein CSRP3, found in the Z-disc, also is a causal gene for HCM. Interestingly, HCM mutations in cardiac troponin T often have a relatively mild pathological phenotype but can lead to sudden cardiac

Gene	Protein	Protein function	Cardiomyopathy
MYH7	β-Myosin heavy chain	ATPase activity and Force generation in sarcomere	HCM, DCM
MYH6	α-Myosin Heavy Chain	Principle protein of the thick filament with low expression in adult human ventricles	HCM, DCM
MYL2	Regulatory myosin light chain	Binds myosin heavy chain	HCM
MYL1/3	Essential myosin light chain	Binds myosin heavy chain	HCM
ACTC1	Cardiac α-actin	Principle component of the thin filament	HCM, DCM
TPM1	α-Tropomyosin	Blocks myosin interaction with actin in sarcomere	HCM, DCM
TNNT2	Cardiac troponin T	Holds troponin complex on tropomyosin	HCM, DCM
TNNI3	Cardiac troponin I	Inhibits actomyosin interaction	HCM, DCM
TNNC1	Cardiac troponin C	Binds calcium to regulate sarcomeric contraction	HCM, DCM
MYBPC3	Myosin binding protein C	Structure & Contraction in the sarcomere	HCM, DCM
TTN	TItin	Structural component of the sarcomere	HCM, DCM
CSRP3	Cysteine-and glycine-rich protein 3	Muscle LIM protein located in the Z disc	HCM, DCM
ACTN2	Actinin	Attaches actin filaments to the Z lines in muscle	HCM, DCM
TCAP	Tcap (telethonin)	Capping protein for titin	HCM, DCM
MYOZ2	Myozenin 2 (calsarcin 1)	Tethers calcineurin to the Z disc via actinin	HCM
PLN	Phospholamban	Regulates calcium entry into the sarcoplasmic reticulum	HCM, DCM
LDB3	Lim domain binding 3	Stabilizes the sarcomere during muscle contraction	HCM, DCM
FHL1	Four-and-a-half LIM domains 1	Muscle development and cardiac hypertrophy	HCM
MYLK2	Myosin light chain kinase 2	Phosphorylates myosin light chain 2	HCM
NEXN	Nexilin	Actin binding protein, part of T-tubule complex and Z discs	HCM, DCM
JPH2	Junctophilin-2	Structural protein linking the plasma membrane with the sarcoplasmic membrane.	HCM
CASQ2	Calsequestrin 2	Calcium binding protein	HCM
VCL	Vinculin	Cytoskeletal protein	HCM, DCM
ANKRD1	Ankyrin repeat domain 1	Transcriptional repressor of cardiac genes	HCM, DCM
TRM63	Muscle ring finger protein	Involved in proteasome-ubiquitin system for protein degradation	HCM,

Table 1.
Genes found to cause cardiomyopathies.

death. Over the years, the identification and verification of many of these sarcomeric genes with HCM has been primarily through large family pedigrees, and often confirmed through experimental animal systems.

In addition to those genes mentioned that have a strong association with causing HCM, there are other cardiac muscle protein genes that when mutated are likely candidates for HCM. These genes include four-and-a-half LIM domains 1 (*FHL1*), myozenin 2 (*MYOZ2*), phospholamban (*PLN*), titin (*TTN*), titin capping protein (*TCAP*), and muscle ring finger protein 1 (*TRM63*) (**Table 1**) [3, 6, 7]. Although some of these associated proteins are located in the sarcomere (titin, titin capping protein), others are found peripherally, such as phospholamban which is in the sarcoplasmic reticulum membrane, and myozenin 2, located in the Z disc. There are also genes that are associated with HCM but occur more sporadically [3, 6, 7]. Some of these proteins are associated with cardiac muscle, such as troponin C, myosin light chain kinase 2, actinin 2, vinculin, nexilin, α-myosin heavy chain, and Lim domain binding 3 protein; other proteins are found globally, such as caveolin, junctophilin-2, and calsequestrin (**Table 1**). The fact that a vast array of different genes encoding proteins with diverse functions can all trigger the HCM pathological response demonstrates a common end point in the development of cardiovascular disease. However, we must also consider HCM is a large phenotypic category and that with more detailed pathological and physiological analyses, an improved diagnostic system might be developed. This has already been demonstrated by the addition of other cardiomyopathic classifications, such as restrictive cardiomyopathy, storage and metabolic cardiomyopathies, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and mitochondrial cardiomyopathy.

3. Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is defined as dilation of the left or both ventricles that is not explained by coronary artery disease or abnormal loading of the heart. The cardiac enlargement occurs with either normal thickness or thinning of the ventricular walls and varying amounts of fibrosis. Oftentimes, DCM leads to heart failure with reduced ejection fraction, tachyarrhythmias, and increased risk of sudden death. All four cardiac chambers may be dilated with increased end-systolic volumes in both ventricles. The incidence of DCM is less well defined, with numbers varying between 1/250 individuals to 1/2500 individuals [6, 7]. Some of this variability can be attributed to the increased number of causes associated with DCM which include familial, viral myocarditis, cardiac toxins (i.e. alcohol, cocaine, amphetamine, cancer chemotherapeutic agents), peripartum cardiomyopathy, and prolonged tachycardia-related cardiomyopathy.

The genes associated with familial DCM are numerous and varied in their function (**Table 1**); however, the most common mode of inheritance is autosomal dominant. Over 50 genes have been identified that are linked to familial DCM, which encode proteins in the sarcomeres, ion channels, cytoskeleton, nuclear envelope, and mitochondria [6, 7]. Familial DCM comprises 30–50% of the DCM population. There is also allelic heterogeneity with mutations occurring in multiple regions within a specific gene. In fact, many genes are associated with causing both DCM and HCM, dependent upon the specific mutation (**Table 1**).

Titin, lamin A/C, and β-myosin heavy chain account for >25% of genetically-inherited DCM [7, 8]. Titin, the longest human protein, is composed of 34,350 amino acids with a mass of 3,816,030 Da. This sarcomeric protein functions as a scaffold for both thick and thin filaments in striated muscle. Many of the DCM-associated mutations in titin encode premature stop codons, resulting in truncated

forms of the protein. These truncations often map to the A band of the sarcomere, rather than the I band, and are associated with phenotypically mild DCM. Other titin mutations result in sarcomere instability, decreased binding to its cap-binding protein (TCAP), decreased binding to the Z-disc, and a decreased stretch response during sarcomere contraction.

Mutations in the *lamin A* gene account for approximately 6% of all DCM mutations, and is oftentimes associated with a high incidence of sudden cardiac death [8]. Lamin proteins are associated with intermediate filaments which support the nuclear membrane, along with a role in chromatin structure and possibly gene transcription. Mutations in the lamin gene often lead to nuclear membrane damage and/or chromatin disorganization and impaired gene transcription. Because of lamin's diverse function, mutations lead to a wide variety of disease conditions, including premature aging and various myopathies, including DCM. In the heart, *LMN A* mutations often lead to dysrhythmias including sinus node and AV node dysfunction, atrial and ventricular fibrillation, and sudden cardiac death [8].

Mutations in genes involved in calcium/sodium handling are also associated with the onset of DCM. Phospholamban (PLN), a regulator of the sarcoplasmic reticulum Ca^{2+}-ATPase pump, has several autosomal dominant mutations that result in DCM. In fact, the R14del mutation in PLN is associated with a founder effect in the Netherlands which results in a severe phenotype [9]. However, a milder DCM phenotype may also occur with the R14del mutation which demonstrates that modifying genes may play a role in the disease pathology. Another ion channel gene associated with DCM is *SCN5A*, a major sodium channel expressed in the heart. DCM mutations in this gene increase the risk for arrhythmias, whereas other *SCN5A* mutations result in channelopathies [6].

An examination of the genes associated with DCM and HCM clearly demonstrates commonality in causing cardiomyopathies (**Table 1**). A clear example of this are the numerous sarcomeric protein genes, including titin, α- and β-myosin heavy chains, troponin T, I, and C, α-tropomyosin, α-actin, and titin capping protein, vinculin, desmin, and nexin (**Table 1**). There are also genes which appear more specific in causing only a single phenotype; genes associated with HCM are myosin light chain 2 and 1/3, myosin light chain kinase, and myozenin, whereas genes associated with only DCM include laminin α4, presenilin 1 and 2, and numerous others [6, 7]. The multitude of mutated genes that can result in DCM and HCM would infer that a continuum of phenotypes may exist for these cardiomyopathies dependent upon when the diseased heart is examined, which gene is mutated, where the mutation occurs, the type of mutation, associated modifying genes, and environmental influences. In fact, there are many cases where HCM hearts transition to DCM and heart failure as the disease progresses.

4. Tropomyosin and HCM

Tropomyosin (TPM) is an essential component of the sarcomeric thin filament that regulates muscle contraction and relaxation through its interactions with actin and the troponin complex. More specifically, striated muscle TPM, along with the troponin complex, regulates Ca^{2+}-mediated actin-myosin crossbridges. As stated previously, the Seidman laboratory discovered through pedigree analysis and gene mapping that HCM was associated with mutations in myosin heavy chains [5]. The association of TPM with HCM was also reported by the Seidman laboratory in 1994 which confirmed that HCM was a disease of the sarcomere and not solely confined to the thick filament [10]. In the United States, the percentage of HCM attributed to mutations in TPM is ~5%, with most of these cases exhibiting benign symptoms,

oftentimes first displayed in later years in life. However, in Japan, the phenotype is severe, but the incidence is low [11, 12]. Interestingly, TPM-associated cases are the most prevalent of all contractile proteins in causing HCM in Finland, with a severe pathological phenotype [13, 14]. The variability in incidence and pathology in the different populations is most likely due to allelic variants, modifier genes, founder effects, and environmental influences.

Mutations in the *TPM1α* gene are known to cause both HCM and DCM. There are at least 17 mutations that have been found to cause HCM and 11 mutations that can give rise to DCM (**Table 2**) [15, 16]. The striated muscle α-tropomyosin protein encodes 284 amino acids; this TPM isoform is the predominant TPM found in the adult human heart. The mutations that cause HCM are scattered throughout the

Amino Acid Mutation	**Phenotype**
Met8Arg	DCM
Lys15Asn	DCM
Arg21His	HCM
Ala22Ser	HCM
Glu23Gln	DCM
Glus40Lys	DCM
Glu54Lys	DCM
Asp58His	HCM
Glu62Gln	HCM
Ala63Val	HCM
Lys70Thr	HCM
Asp84Asn	DCM
Ile92Thr	DCM
Val95Ala	HCM
Ala107Thr	HCM
Ile172Thr	HCM
Asp175Asn	HCM
Glu180Gly	HCM
Glu180Val	HCM
Leu185Arg	HCM
Glu192Lys	HCM
Thr201Met	DCM
Ser215Leu	HCM
Asp230Asn	DCM
Ala239Thr	DCM
Ala277Val	DCM
Met281Thr	HCM
Ile284Val	HCM

Adapted from [15, 16].

Table 2.
TPM1α mutations that cause cardiomyopathy.

gene/protein with a significant number located in the troponin-T binding regions, around amino acids 170–190 (Ile172Thr; Asp175Asn; Glu180Gly; Glu180Val; Leu185Arg; Glu192Lys) and amino acids 270–284 (Met281Thr; Ile184Val). A number of these mutations lead to a change in amino acid charge which may disrupt the dimerization of TPM with itself, or TPM's interactions with actin and/or troponin T [17]. Also, most, if not all, of the HCM mutations occurring in thin filament sarcomeric proteins lead to increased calcium sensitivity of the myofilaments, coupled with decreased systolic and diastolic cardiac function which may be causative for the development of this cardiomyopathy.

To understand the role of TPM in the development of HCM, our laboratory generated animal models of HCM. We produced the first *in vivo* transgenic mouse models expressing TPM with known HCM human mutations (Asp175Asn; Glu180Gln) [18–20]. Since there is a 100% amino acid sequence identity and comparable expression in the heart between mouse and human TPM, the mutations used in these transgenic mice reflect mutations and expression found in HCM patients. In addition, the exogenous cardiac-specific TPM transgene expression leads to a reciprocal decrease in endogenous TPM levels so that the total amount of TPM protein expression is unchanged in the hearts of these transgenic mice. Histological analyses demonstrate that the Asp175Asn transgenic mouse hearts show a moderate hypertrophic response; in contrast, the Glu180Gln mice demonstrate a severe cardiac hypertrophy with significant fibrosis and atrial enlargement (**Figure 1**) [18–20]. Physiologically, mice from both models display significant systolic and diastolic dysfunction, coupled with increased sensitivity to Ca^{2+} in the myofilaments. The pathological and physiological disease state progresses rapidly in the HCM Glu180Gln mice, with the mice dying between 4 and 6 months postpartum.

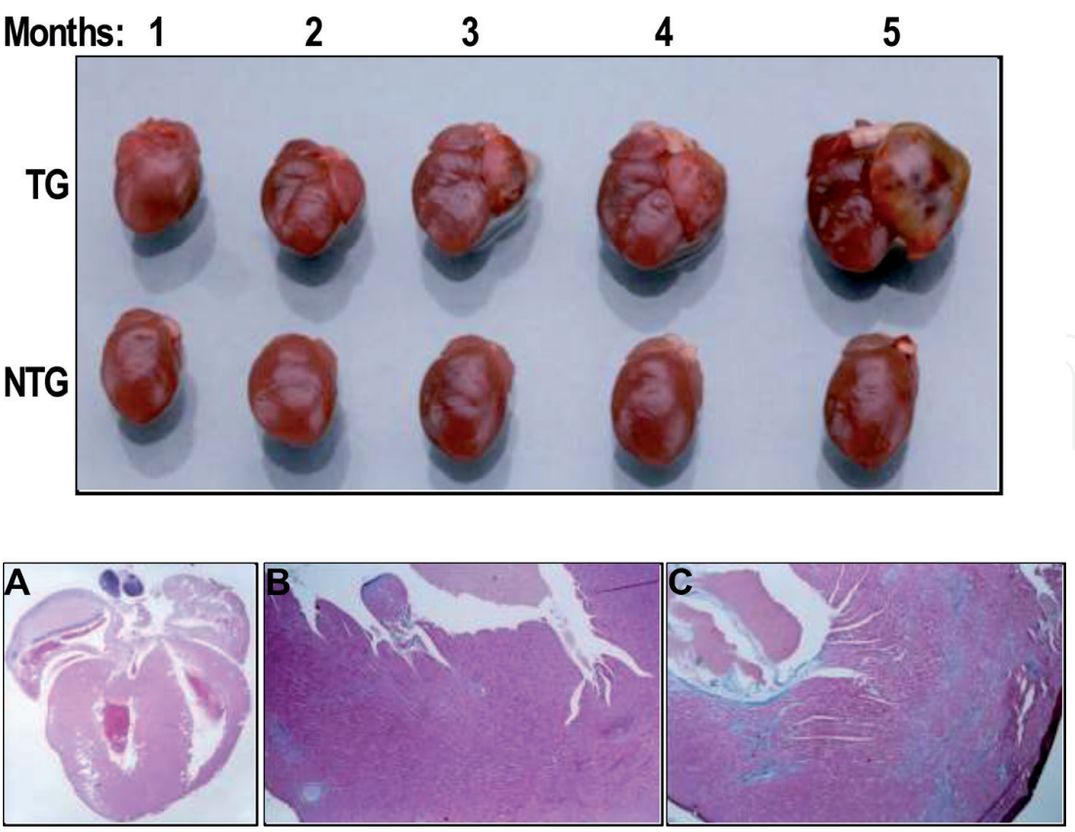

Figure 1.
HCM TPMα180 and NTG control hearts at the designated 1-month time intervals. (A) Cross-section of a three-month-old TPMα180 heart. (B and C) trichrome stain of left ventricular wall from control and TPMα180 hearts. B NTG control. C TPMα180. Note, blue fibrous staining in panel C.

To understand the molecular mechanisms associated with the development of cardiomyopathy, we conducted a detailed comparative microarray analyses of hearts obtained from mild and severe HCM mice [21]. Ventricular tissue was obtained from 2.5-month-old TPMα175 and TPMα180 hearts, along with control (NTG) samples. Results show 754 genes (from a total of 22,600) were differentially expressed between the NTG and HCM hearts; 178 between NTG and TPMα175, and 388 between NTG and TPMα180. There are 266 differentially expressed genes between HCM TPMα175 and TPMα180. The genes that exhibit the largest increase in expression are associated with "secreted/extracellular matrix" category, and the most significant decrease in expression are in the "metabolic enzyme" category. This work illustrates the diverse array of genes that are activated and repressed during the early signaling processes of mild and severe cardiac hypertrophy.

5. Rescue of HCM TPMα180 mice

The development of mouse models that mimic human HCM physiological and pathological conditions afford researchers the opportunity to examine various methods for rescuing these mice from cardiomyopathy. Studies demonstrate cardiac thin filaments with HCM mutations exhibit an increased sensitivity to calcium. As calcium is a prime regulator of muscle contraction, we hypothesized that by normalizing myofilament calcium sensitivity, we could phenotypically rescue the HCM phenotype in our TPMα180 mice. Previously, we generated transgenic mice that exchanged the carboxyl terminal region of TPMα with that of TPMβ (Chi 1) [22]; these mice exhibit a decreased myofilament calcium sensitivity. By mating mice from the HCM TPMα180 with the Chi 1 mice, we tested the hypothesis that attenuation of myofilament calcium sensitivity would modulate the severe physiological and pathological consequences of the HCM mutation. Results show the double-transgenic mice "rescue" the hypertrophic phenotype by exhibiting a normal morphology with no pathological abnormalities, improved cardiac function, and normal myofilament calcium sensitivity [23, 24]. These results demonstrate that alterations in calcium response by modification of contractile proteins can prevent the pathological and physiological effects of this disease.

To extend our studies on rescuing HCM TPMα180 mice by modulation of cytosolic calcium, we crossbred the TPMα180 mice with phospholamban knockout mice (PLNKO) [25]. PLN is a Ca^{2+}-handling protein that regulates calcium uptake into the sarcoplasmic reticulum. Previous studies show that PLNKO mice exhibit hypercontractility with no change in morphology or heart rate, no alterations in myofilament Ca^{2+} sensitivity, and myosin ATPase activity [26]. Results show that PLN ablation in the TPMα180 mice rescues cardiac function and morphological abnormalities for up to one year [25]. There was a reversal of the cardiac hypertrophy, fibrosis, and abnormal physiological function in these rescued mice. This work shows that by modulating sarcoplasmic reticulum calcium cycling, many of the deleterious aspects of HCM caused by a mutation in the thin filament protein TPM can be reversed.

We investigated whether oxidative myofilament modifications can reverse the diastolic dysfunction associated with HCM. The TPMα180 hearts display early signs of oxidative stress in the form of increased oxidative modifications of myosin binding protein C and activation of the MAPK signaling cascade. We hypothesized that treatment with the glutathione precursor N-acetylcysteine (NAC) may reverse the oxidative stress in the TPMα180 mice and improve the cardiomyopathic condition and diastolic dysfunction. To address this, NAC was administered for 30 days to control and TPMα180 mice. After NAC administration, the morphology, diastolic

dysfunction, and myofilament Ca^{2+} sensitivity of the TPMα180 mice was similar to controls, indicating that NAC had reversed the abnormal pathology and physiology associated with HCM [27]. These studies indicate that oxidative myofilament modifications are an important mediator in diastolic function which can be of potential use in the treatment of HCM.

TPM is phosphorylated at a single site in the protein, located at the penultimate amino acid, serine 283. To address the significance of TPM phosphorylation, we generated transgenic mice where this serine residue is exchanged for alanine [28, 29]. These transgenic mice (S283A) exhibit a compensated hypertrophic response with significant increases in SERCA2a expression and phosphorylation of PLN. Having obtained these results, we postulated that decreasing TPMα phosphorylation may be beneficial in the context of a chronic, intrinsic stressor, such as HCM. To test this hypothesis, we generated mice expressing both the TPMα180 and S283A mutations and found the HCM phenotype was rescued [29, 30]. The double mutant transgenic mice exhibit no signs of HCM, displayed improved cardiac function, and have normal myofilament Ca^{2+} sensitivity. Changes in Ca^{2+} handling proteins may be responsible for the improved functional performance found in the double transgenic hearts. Also, changes in local flexibility of the TPM molecule conferred by the replacement of the Serine residue with an Ala residue in the S283A mice, and the significant loss of phosphorylation, may be responsible for the restoration of TPM to proper flexibility. Structural alterations in actin-TnT-TPM protein interactions could play a vital role, however, the precise mechanism whereby decreased TPM phosphorylation rescues the HCM phenotype remains to be elucidated.

6. TPM and DCM

DCM, a disease often associated with heart failure, is characterized by depressed systolic function, cardiomegaly, and ventricular dilation. As mentioned previously, DCM is caused by a variety of conditions, including idiopathic, viral and cardiotoxins. Mutations in genes associated with DCM include sarcomeric proteins, the cytoskeleton, and the sarcolemma. Sarcomeric protein genes that harbor DCM mutations include α- and β-myosin heavy chain, myosin binding protein C, actin, TPM, troponin T, I, and C, desmin, vinculin, and muscle LIM protein (**Table 1**).

TPM mutations known to cause DCM are located throughout the *TPM1* gene, from the 5′ to 3′ end of the associated transcript (**Table 2**). Some of the corresponding amino acid changes are positioned in the inner regions of the TPM coiled-coil dimer where electrostatic charge interactions between specific amino acids may alter the TPM dimerization and/or binding to actin [31]. These non-conserved amino acid substitutions are thought to disrupt force transmission through the sarcomere leading to DCM.

To investigate the structural and physiological consequences of known DCM mutations in TPM with cardiac morphology and performance, we generated the first mouse model of a sarcomeric thin filament protein that leads to DCM (TPMαGlu54Lys) [32]. As with the transgenic HCM mice that were generated, the increase in transgenic TPM protein expression led to a reciprocal decrease in endogenous wildtype TPMα levels, with the total myofilament TPM levels remaining unchanged. Also, since there is 100% amino acid identity between human and mouse TPM, the Glu54Lys DCM mutation is the same manifest in human DCM patients. Histological and morphological analyses of these transgenic mice revealed development of DCM with progression to heart failure, and death often ensuing by 6 months (**Figure 2**) [32]. Echocardiographic analyses confirmed the dilated phenotype of the heart with significant decreases in left ventricular fractional

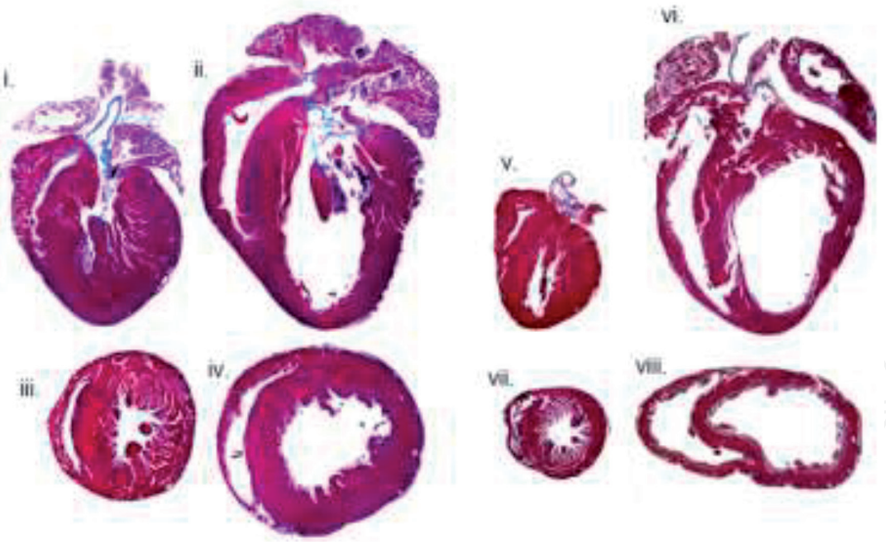

Figure 2.
Histopathology of DCM TPMα54 hearts. Masson trichrome staining of whole-heart longitudinal sections (i and ii) and cross sections (iii and iv) from 5-month-old NTG and moderate-copy TPMα54 mice; longitudinal sections (v and vi) and cross sections (vii and viii) from 1-month-old NTG and high-copy TPMα54 mice. Note the severe dilation of right and left ventricles in both the moderate and high-copy mice. Images in i through viii are all enlarged at the same magnification.

shortening. There was also impaired systolic and diastolic function, coupled with a decreased Ca^{2+} sensitivity and tension generation in cardiac myofilaments. Results indicate the Glu54Lys mutation decreases TPM flexibility, which may influence actin binding and myofilament Ca^{2+} sensitivity. In summary, the pathological and physiological phenotypes exhibited by these mice are consistent with those seen in human DCM and heart failure patients.

Phosphorylation of cardiac sarcomeric and non-sarcomeric proteins play a major role in the regulation of the physiological performance of the heart. Phosphorylation of the thin filament proteins, such as troponin T and I, dramatically affect myofilament Ca^{2+} sensitivity, along with systolic and diastolic function. Less is known about the physiological effect of TPM phosphorylation on cardiac performance. To address this issue, we generated transgenic mice having a phosphorylation mimetic substitution in the phosphorylation site of TPM (Ser283Asp) [33]. Previous work in our laboratory demonstrated that ablating the ability of TPM phosphorylation in transgenic mice (TPMαS283A) leads to a compensated physiological hypertrophy [28]. Our results show that high expression of the TPM Ser283Asp transgene leads to an increased heart:body weight ratio, coupled with a severe dilated cardiomyopathic phenotype resulting in death within 1 month of birth [33]. Moderate TPM Ser283Asp expression mice causes a mild myocyte hypertrophy and fibrosis, without affecting lifespan; physiological analysis revealed diastolic dysfunction, without changes in systolic performance. Surprisingly, there were no alterations in Ca^{2+} sensitivity of the myofibers, cooperativity, or calcium-ATPase activity in the myofibers. This work revealed for the first time that constitutive phosphorylation of TPM could result in a DCM phenotype with its severity dependent upon the extent of the posttranslational phosphorylation modification.

Studies demonstrate that during embryonic and fetal cardiogenesis, the murine heart expresses both TPMα and TPMβ isoforms, with the TPMα isoform being predominant in the adult heart [34–36]. During developmental, the ratio of TPMα:TPMβ changes from 5:1 to 60:1 in the embryonic to adult transition in the murine heart [36]. To address whether the TPMβ isoform could substitute for the TPMα protein, we generated transgenic mice that overexpressed TPMβ in the heart. Results show that

with 60% TPMβ expression, there were no morphological changes in the heart [37]. However, there were physiological differences; although there were no systolic alterations, diastole was impaired in both the time and rate of relaxation, coupled with an increase in myofilament Ca^{2+} sensitivity. Additional studies demonstrated that when the TPMβ transgene was expressed at high levels (80% TPMβ, 20% TPMα) in the heart, the mice developed a severe DCM phenotype and die with 14 days postpartum [38]. In these high expression TPMβ hearts, there is significant chamber dilation, thrombus formation in the atria and ventricles, and diastolic dysfunction.

An extension of the research on the high expression TPMβ mice entailed treatment with cyclosporin and FK506, inhibitors of calcineurin. Calcineurin is a calcium-regulated phosphatase, which can initiate cardiac hypertrophy in hearts of transgenic mice that overexpress calcineurin [39]. Results show that treatment with cyclosporin or FK506 in various mouse models of cardiac hypertrophy, including the high expression TPMβ DCM mice, led to phenotypically rescued hearts [40]. This work suggests that in certain cases, inhibitors of calcineurin may play a potential therapeutic role in the treatment of heart disease.

There are 4 distinct tropomyosin genes, each one subject to alternative splicing which generates multiple isoforms of TPM. Our investigation into striated muscle TPM isoform content in the adult human heart found there is 92% TPMα1, 4% TPMβ, and 4% TPMα1κ [41]. TPMα1k is a unique human cardiac-specific TPM isoform which is normally not expressed in rodents [41, 42]. Additional studies show the associated protein is expressed and incorporated into organized myofibrils and that its level is increased in human dilated cardiomyopathy and heart failure patients [41]. To investigate the role of TPMα1κ in sarcomeric function, we generated transgenic mice overexpressing this cardiac-specific isoform. Incorporation of increased levels of TPMα1κ protein in myofilaments leads to DCM, coupled with systolic and diastolic dysfunction and decreased myofilament Ca^{2+} sensitivity [41, 43]. Additional biophysical studies demonstrate less structural stability and weaker actin-binding affinity of TPMα1κ protein compared with TPMα1. This functional analysis of TPMα1κ provides a possible mechanism for the consequences of the TPM isoform switch observed in DCM and heart failure patients.

7. Gene therapy approaches for repair of cardiomyopathies

Calcium plays a pivotal role in the regulation of muscle contraction and relaxation. As seen in the TPM mouse models, the HCM and DCM phenotypes all exhibit abnormalities in myofilament Ca^{2+} sensitivity and Ca^{2+} handling. As mentioned, when mice harboring a phospholamban (PLN) knockout are crossed with the HCM TPMα180 mice, the pathological phenotype is rescued from their offspring [25]. To extend our work, studies were conducted to more fully examine the role of calcium and calcium-handling proteins in the development of HCM. To test whether improvements in the hypertrophic phenotype can be achieved through increased Serca2 expression, the HCM TPMα180 mice were treated with exogenous Serca2a, the protein involved in sequestering calcium from the cytoplasmic space into the sarcoplasmic reticulum [44, 45]. We implemented a gene transfer approach using an adenoviral vector to express Serca2a in HCM TPMα180 hearts. Results showed that injection of a single dose improved heart morphology and cardiac function. As the mice aged, there was a significant decrease in heart:body weight ratio, and a decrease in fibrosis when compared with controls. Additional work demonstrated that parvalbumin, a calcium buffer, may also play a role in ameliorating HCM; when parvalbumin transgenic mice were crossed with HCM TPM1α180 mice, there was improvement in cardiovascular performance [45, 46].

With improvements in cardiac morphology and performance in the HCM and DCM mouse models that occur with modification in calcium handling proteins, therapeutic gene therapy trials in patients utilizing Serca2a expression as a potential treatment for cardiac disease were initiated. Using adeno-associated viruses to drive extended Serca2a expression, Phase 2 studies were conducted in patients with advanced heart failure [47, 48]. Results show there was a striking reduction in cardiovascular events that persisted through the 36 months of follow-up compared to patients who received the placebo. Additional work in this area is in progress.

Recently, investigators have examined the potential of the C-terminal end peptide of troponin I as a novel reagent to selectively facilitate cardiac muscle relaxation [49]. This is a highly conserved protein fragment across numerous vertebrate species. Protein binding studies found that this terminal fragment retains its binding affinity for TPM similar to intact cardiac troponin I. Addition of this fragment to skinned cardiac muscle preparations reduces myofibril Ca^{2+} sensitivity without decreasing maximum force production. Using this short peptide, studies were initiated to address whether it would be of therapeutic value in the treatment of HCM [50]. Recent work demonstrates that myofilament Ca^{2+} sensitivity isolated from TPMα180 hearts exhibit a more normalized decrease in myofilament Ca^{2+} sensitivity when treated with the C-terminal troponin I fragment. This demonstrates the C-terminal peptide of troponin I as a potential therapeutic reagent for the treatment of diastolic dysfunction in the heart.

There is no scientific doubt that CRISPR-Cas9-base targeting has revolutionized how research is being conducted. With the ability to modify genomes, there is the potential to conduct precise gene-editing in animal models along with correcting human disease mutations. With respect to cardiomyopathic diseases, Ma et al. corrected a human heterozygous germline HCM mutation in the myosin binding protein C gene using the CRISPR-Cas9 system [51]. This targeting strategy was employed on preimplantation human embryos; following targeting, the embryos were genetically analyzed for correctly targeted nucleotide changes and then allowed to develop to the 8-cell stage. Results show that over 50% of blastomeres were correctly targeted, but used the wildtype allele as the correcting genetic template. This work demonstrates that CRISPR has the potential for usage as a corrective therapeutic system of heritable mutations; however, additional research needs to be conducted and ethical considerations need to be addressed.

8. Lessons learned from TPM

Many lessons have been learned about HCM and DCM in the examination and usage of TPM and associated mouse model systems [52, 53]. Multiple mutations within the TPM1α gene lead to HCM and DCM. Surprisingly, for both disease conditions, the mutations are scattered throughout the gene, and are not confined to one or two specific regions or domains. The severity of the disease phenotype appears dependent upon the specific mutation, modifying genes, and environmental factors. The genetic animal models of HCM and DCM TPM mutations accurately reflect the disease process with respect to structural and functional abnormalities as they occur in humans. For HCM, the thickening of the left ventricular wall and interventricular septum with significant fibrosis is pronounced in these animal models. For DCM, the thinning of the ventricular walls and dilation of the ventricular cavities reflect the pathological features observed in patients. For both HCM and DCM, the functional abnormalities in systole and diastole are similar to those experienced by patients. More importantly, these basic research studies have been translated into potential therapeutic modalities, especially for investigations into

the usage and modification of calcium-handling proteins as treatments of cardiovascular disease. Expansion of potential treatments utilizing phosphatases and kinases, along with sarcomeric protein peptides, may also prove beneficial for the treatment of specific cardiovascular conditions. An area of future expansion will be to focus on the identification and modification of protein expression for genes which are signaling agents for the development of cardiac HCM, DCM, and heart failure.

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Conflict of interest

There are no conflicts of interest to report.

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