Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: September 30, 2023

Citation: Wieczorek DF, 2023. Sarcomeric Thin Filament Associated Cardiomyopathic Mouse Models, Medical Research Archives, [online] 11(9). https://doi.org/10.18103/mra.v11i9.4369

DOI
https://doi.org/10.18103/mra.v11i9.4369

ISSN: 2375-1924

RESEARCH ARTICLE

Sarcomeric Thin Filament Associated Cardiomyopathic Mouse Models

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ABSTRACT

Cardiomyopathies are diseases primarily associated with defects in the structure and physiological function of the heart. Hypertrophic and dilated cardiomyopathies are two common conditions associated with severe pathological abnormalities that often lead to heart failure. Studies in the early 1990's by the Seidman laboratories linked hypertrophic cardiomyopathy with mutations in both thick and thin sarcomeric protein genes. Since then, the development and analysis of animal models of both hypertrophic and dilated cardiomyopathy has significantly advanced our knowledge of the structural, molecular, biochemical, and physiological disease processes that lead to these cardiomyopathic conditions. The focus of this article is an examination of mouse models of hypertrophic and dilated cardiomyopathies with mutations in sarcomeric thin filament protein genes (actin, tropomyosin, troponin T, I, and C) and the information these models provide in our understanding of the disease processes. Special attention addresses the significant role that tropomyosin mutation models have contributed to this information. In addition, we address how various methods have been developed to phenotypically rescue these diseased hearts with respect to their morphological and physiological functions. By thorough analysis of these mouse models, not only can we better understand the disease processes, but there is a great potential for the development of effective therapeutics to treat these severe pathological conditions.

Introduction

The striated muscle sarcomere is composed of thick and thin filaments that slide past each other during contraction and relaxation. The principal components of the thick filament are myosin heavy and light chains, titin, and myosin binding protein C. The thin filament is primarily composed of filamentous actin, tropomyosin (Tpm) and the troponin complex (Tnn; troponin T – TnT; troponin I – TnI; and troponin C – TnC). With increases in cytosolic calcium (Ca^{2+}) concentration, TnC binds with calcium and through its association with TnT and TnI, mediates a change in the Tpm position on actin. Movement of Tpm and TnI facilitates myosin head interactions with actin, thereby stimulating muscle contraction. When cytosolic Ca^{2+} levels decrease, Ca^{2+} is released from TnC causing Tpm to assume its blocking position on actin and relaxation ensues.

Regulation of contractile activity in the vertebrate striated muscle sarcomere is dependent upon the ATP-hydrolyzing activity of myosin transducing chemical energy into mechanical movement. Hydrolysis of ATP by myosin is dramatically accelerated by actin. The Tpm-Tnn complex acts to inhibit the actin-myosin reaction, and this inhibition is reversed by an increase of Ca^{2+} in the myofilament space and binding of Ca^{2+} to TnC. Of particular interest, several types of human heart failure exhibit depressed myofibrillar ATPase activity that appear to be due to isoform changes in thin filament proteins.[1,2] Thus, alterations and mutations in Tpm and Tnn may inhibit cross-bridge activation and decrease cooperativity along the thin filament leading to cardiomyopathy via various signal transduction mechanisms.

Hypertrophic and Dilated Cardiomyopathy

Cardiomyopathies are diseases primarily associated with defects in the structure and physiological function of the heart. There are 5 different categories: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and left ventricular noncompaction cardiomyopathy (LVNC). Although there are variations in the phenotypes and etiologies, there are also similar symptoms. Common features are heart failure with reduced ejection fraction, peripheral edema, fatigue, dyspnea on exertion, syncope, and cardiac ischemia. [3,4] The focus of this article will be on hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM).

Hypertrophic Cardiomyopathy

Hypertrophic Cardiomyopathy (HCM) is defined as left and/or right ventricular hypertrophy in the absence of external load, and without chamber dilation. Interventricular septal thickening is a common feature that may contribute to obstruction of the outflow tract and mitral valve dysfunction, along with myocyte disarray, fibrosis, alterations in myofilament Ca^{2+} sensitivity, and cardiac arrhythmias. It should be noted that there is variability of these pathological clinical features depending upon the age of onset and the specific mutation contributing to the disease phenotype. It has been reported that sudden and unexpected death occurs most often in younger patients, aged from 8 – 21 years old; most heart failure-related deaths were in midlife and beyond, and death as a consequence of stroke was associated with much older patients.[5] Hypertrophic cardiomyopathy is the most common identifiable cause of sudden cardiac death in young people, with an overall population incidence of 1:200 – 1:500 individuals.[6,7]

The list of genes associated with mutations that cause both HCM and DCM is extensive.[8,9] The genes primarily associated with HCM and DCM are sarcomeric contractile protein genes associated with both thick and thin cardiac filaments, along with Z discs. There are over 1500 mutations in these sarcomeric protein genes that are associated with HCM, and 50 genes have been identified that are linked to familial DCM that encode proteins in the sarcomeres, ion channels, cytoskeleton, nuclear envelope, and mitochondria.[10,11] In 1990, John and Christine Seidman uncovered the genetic association between mutations in sarcomeric contractile proteins and HCM. Initial studies by this group found through pedigree analysis and gene mapping that HCM was associated with mutations in myosin heavy chains. Soon after, they discovered that mutations in both sarcomeric thick and thin filament protein genes could result in HCM.

In the United States, most HCM patients have defects in the thick-filament protein genes myosin heavy chain (MYH7) and myosin binding protein C (MYBPC3). Estimates of incidence vary considerably, with studies indicating that MYH7 and MYBP3 account for 40 – 70 % of HCM cases, and thin filament protein genes (TPM1, TNNT2, TNNI3, TNNC1, ACTC1) comprise from 3-10 % of cases.[6,9,12] In Japan, the percentage of cases due to mutations in the TPM1 gene is similar to that of the US, however, the pathological symptoms of affected individuals are more severe than in the US population.[13,14] Interestingly, in Finland, TPM1 associated cases are the most prevalent of all thin

filament contractile proteins involved in causing HCM with an incidence rate ranging from 6 - 11%; this is most likely due to a "founders" effect of the Asp175Asn gene mutation. [15-17]

An interesting question that has been addressed recently is whether HCM patients with mutations in thick filament protein genes exhibit a differential phenotype from patients harboring mutations in thin filament protein genes. Coppini et al examined the clinical features of HCM in 80 patients with mutations in thin filament proteins and 150 patients with thick filament protein mutations.[18,19] Compared with thick-filament mutation HCM patients, phenotypic differences of thin-filament patients include milder left ventricular hypertrophy with less outflow tract obstruction, increased apical or concentric hypertrophy, increased fibrosis, higher prevalence of systolic dysfunction, and increased predisposition to adverse left ventricular remodeling and heart failure. Diastolic abnormalities were more common and pronounced in thin-filament HCM. The risk of sudden cardiac death is similar between both populations, and there is no difference in mortality during the 5-year follow-up period. These significant differences in cardiac morphology and function suggests that there is a distinct clinical profile that distinguishes thick and thin filament HCM patients.

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. Cardiomegaly occurs with either normal thickness or thinning of the ventricular walls and varying amounts of fibrosis. Dilated cardiomyopathy often leads to heart failure, with decreased systolic function, tachyarrhythmias, and in increased risk of sudden death. All of the cardiac chambers may be dilated with increased end-systolic volumes in both ventricles. The incidence of DCM is a relatively common disease at 1:2500 or ~ 37 out of 100,000 people.

A variety of conditions can cause DCM, including genetic (mutations in sarcomeric, cytoskeletal, and sarcolemma protein genes), idiopathic, viral and cardiotoxins. The familial type of DCM accounts for 20 – 40% of cases.[20] The causative genes in DCM are predominantly encoded by two major subgroups of proteins – cytoskeletal and sarcomeric. The most common DCM-associated cytoskeletal proteins include dystrophin, lamin A/C, desmin, and sarcoglycans.[21] Sarcomeric proteins that harbor DCM inducing-mutations include α - and β-MHC, MYBC, actin, Tpm, TnT, TnI, TnC, desmin, vinculin, and muscle LIM protein.[8] In addition, several Z-disk proteins (i.e. ZASP, muscle-LIM, actinin) and sarcoplasmic reticulum proteins (i.e. phospholamban) are also associated with being causative for DCM. The mechanisms associated with the development of DCM are thought to be force generation and transmission defects, metabolic and mechanosensory abnormalities, and disturbed Ca^{2+} homeostasis.[22,23] For the cytoskeletal proteins, defects of force transmission are thought to result in DCM, whereas defects of force generation are speculated to cause sarcomeric protein-induced DCM. As previously stated, mutations in both thick and thin filaments proteins can contribute to this disease phenotype. Mutations in myosin heavy chain may disrupt the actin-myosin interaction associated with force generation and cross-bridge movement of the myofilaments. Mutations in the Tnn complex and Tpm may alter Ca^{2+} binding or the movement of Tpm to facilitate actin-myosin interaction. Tropomyosin mutations often result in decreased Tpm flexibility, which impairs actin binding and decreases myofilament Ca^{2+} sensitivity; this impairs systolic and diastolic function.[24]

Animal Models of Hypertrophic and Dilated Cardiomyopathy

Animal models provide invaluable information on the development and pathological alterations associated with cardiomyopathies. These studies can address the molecular and biochemical changes that mutations in HCM and DCM genes impart, in addition to providing information on the disrupted molecular pathways leading to heart disease. Furthermore, the associated pathological and physiological consequences of mutations can also be determined, in addition to addressing relevant pathways that can be the target of novel therapeutic interventions, thereby providing various means of "rescuing" the animals/organ/cells from the diseased phenotype. The following section will examine various animal models of HCM and DCM, with a particular focus on mouse models associated with sarcomeric thin filament protein mutations. However, it should be noted that there are limitations in applying the knowledge obtained from animal models to humans due to inherent differences between species and varied pathological and physiological responses to gene mutations.[25]

The first animal model for HCM was developed by the Seidman lab in examining a mouse model incorporating the R403Q α-myosin heavy chain mutation in the thick filament.[26] Many of the human cardiac disease ramifications were recapitulated in this mouse model, including impaired relaxation,

myocyte disarray, progressive fibrosis and left atrial enlargement. Functional abnormalities included altered contraction kinetics, delays in left ventricular pressure relaxation and chamber filling, which are consistent with clinical findings.

The first animal models for HCM addressing mutations in thin filament proteins were developed by the Wieczorek lab in examining mouse models for tropomyosin (TPM1) mutations.[27,28] Since then, additional models have been developed to examine the morphological and physiological effects of mutations in most of the thin filament proteins, including actin, TnT, TnC, TnI. These model systems have provided invaluable information on the how these mutant proteins result in HCM, their functional effects, and the molecular pathways that are activated in the disease process.

There are several animal species that exhibit spontaneous HCM mutations. Canines display features of HCM which include left ventricular hypertrophy, myocardial fibrosis, disorganization of myofibers, and a high incidence of sudden cardiac death.[29,30] The Maine Coon Cat also displays features of HCM due to a mutation (Ala31Pro) in myosin binding protein C which includes an increased left ventricular wall, left atrial enlargement and papillary muscle hypertrophy.[31] Other animals that identify with spontaneous HCM are pigs and kangaroos.[32,33]

Actin Associated Cardiomyopathic Animal Models

Actin is a globular protein that assembles into filaments and plays essential roles for cellular architecture and movement, intracellular movement, muscle contractions and many other functions in striated muscle, smooth muscle, and nonmuscle cells. There are 6 actin genes: 2 striated muscle actins (skeletal and cardiac), 2 smooth muscle actins, and 2 cytoskeletal actins (β- and γ). These actin isoforms are highly conserved (>90%) at the protein level. In striated muscle, actin is a principal protein in the sarcomeric thin filament that interacts with myosin to regulate muscle contraction and relaxation. The ATP-hydrolyzing activity of myosin transduces chemical energy into mechanical movement which is dramatically accelerated in the presence of actin. The Tpm-Tnn complex which is bound to actin inhibits the actin-myosin reaction; this inhibition is reversed by an increase of Ca^{2+} in the myofilament space and binding of Ca^{2+} to TnC. In the adult heart, 80% of the striated muscle actin is the cardiac isoform, and 20% is skeletal actin. Over 70 mutations have been found in the cardiac actin gene (ACTC1), most of which are missense mutations. At least 12 different mutations in cardiac actin have been discovered in patients with HCM. These mutations have been grouped into 3 principal groups: (1) those affecting only the actin-myosin binding site; (2) those affecting only the binding site on Tpm; and (3) those affecting both binding sites on myosin and Tpm.[34] In addition, two actin mutations are linked to heritable forms of DCM: Arg312His; Glu361Gly; these mutations occur in the immobilized actin end which is attached to the Z band or intercalated disc.[35,36] In addition, the Glu361Gly substitution is within a common binding domain for actinin found in the Z bands/intercalated discs.

To examine the structural and functional effects of cardiac α-actin deficiency, Kumar et al ablated the ACTC1 gene by homologous recombination in the mouse.[37] Most of these mice do not survive to birth; the remainder die within the first 2 weeks postpartum. Mice lacking cardiac α-actin can be rescued to adulthood by the ectopic expression in the heart of enteric smooth muscle γ-actin; these rescued mice exhibit an enlarged, hypertrophied phenotype with reduced cardiac contractility. In addition, isolated cardiac myofilaments from these mice exhibit a decreased sensitivity to Ca^{2+} and an increased economy of force development.[38] The absence of cardiac α-actin leads to disorganized acto-myosin filaments in the affected cardiomyocytes. Other actin isoforms, specifically vascular smooth muscle and skeletal α-actin, increase their expression, but not enough to compensate for the absence of cardiac α-actin. This absence in cardiac α-actin induces apoptosis in the cardiomyocytes which are unable to cope with the increased workload in the perinatal period.[39] Additional studies by the Lessard laboratory demonstrated that transgenic overexpression of cardiac actin at high levels lead to atrial enlargement and cardiomyocyte hypertrophy, along with a significant decrease in contractility.[40]

Tropomyosin

Tropomyosin (Tpm) is a coiled-coil dimer that plays an important role in the regulation and contraction of striated and smooth muscle. As mentioned, the Tpm-Tnn complex inhibits the actin-myosin interaction which is reversed in the presence of increased Ca^{2+} in the myofilaments. Current data suggests that both kinetic and steric factors are involved in the regulation of myofibrillar contraction. In fact, several types of human heart failure exhibit depressed myofibrillar ATPase activity.[1,2] In nonmuscle cells, Tpm is involved in determining the architecture of the cell and modulates contractile activities through its

association with actin in the cytoskeletal microfilaments.

Four TPM genes, TPM1, TPM2, TPM3, and TPM4 (α, β, γ, and δ, respectively) have been identified, and these genes display a very high degree of conservation in species ranging from *Drosophila* to humans.[41,42] Each of these genes undergoes extensive alternative splicing to generate a tremendous amount of mRNA and protein diversity.[42,43] Studies show that human hearts contain α-Tpm protein levels of 90 – 94%, 3-5% β-Tpm isoform, and 3-% α-Tpmκ isoform.[44] In the adult mouse heart, the level of striated muscle α-Tpm expression is 98%, with 2% β-Tpm expression.[45]

Tropomyosin Associated Hypertrophic Cardiomyopathic Animal Models

In 1994, the Seidman group reported the association of Tpm with HCM, confirming that HCM was a disease of the sarcomere and not solely confined to the thick filament.[46] As mentioned, the incidence of TPM1 mutations contributing to HCM is ~ 5% in the United States, with most of these cases exhibiting benign symptoms. In Japan, the phenotype is severe, but the incidence is low.[13,14] Interestingly, TPM1-associated cases, along with MyBPC, are the most prevalent causes of HCM in Finland probably due to founder effects; most of these cases exhibit a severe pathological phenotype.[16,17] The variability in incidence and pathology in the different populations worldwide is most likely due to allelic variants, modifier genes, founder effects, and environmental influences.

Mutations in the TPM1 gene are known to cause both HCM and DCM. There are at least 17 mutations found to cause HCM and 11 mutations that can give rise to DCM.[8] No mutations have been identified in the TPM2, TPM3, or TPM4 genes that result in HCM or DCM. The mutations that cause HCM are scattered throughout the gene/protein with a significant number located in the TnT-binding regions, around Tpm amino acids 170 – 190 and 270 – 284. Many of these mutations lead to a change in the amino acid charge which disrupts the dimerization of Tpm with itself, or Tpm's interactions with actin and/or troponins.[47] Also, HCM mutations occurring in the thin filaments often lead to increased calcium sensitivity of the myofilaments and decreased systolic and diastolic cardiac function which may be causative for the development of HCM and DCM.

Our laboratory developed two mouse models for TPM1 mutations that lead to HCM: Asp175Asn and Glu180Gly).[27,28,48] These were the first *in vivo* transgenic mouse model systems to examine mutations in thin filament proteins that cause HCM. Since there is a 100% amino acid identity between mouse and humans, the mutations used in these transgenic mice reflect mutations and expression found in HCM patients. A consistent feature with transgenic expression of Tpm is that as exogenous Tpm expression is increased, there is a reciprocal decrease in the endogenous Tpm protein expression so that the total amount of Tpm protein remains unchanged in the heart.[49,50] A similar situation exists in these transgenic HCM mouse hearts. Initially we investigated the Asp175Asn HCM mouse model (Table I). Histological analyses show these hearts exhibit a mild hypertrophic response with diminished contractile and relaxation rates in mice expressing 60% mutant protein, but not in those with expression levels < 40%.[27] In contrast, the Glu180Gly mice demonstrate a severe cardiac hypertrophy with significant fibrosis and atrial enlargement.[27,28,48] Physiological analyses show significant impairment of both contractility and relaxation in the hearts and enhanced myofilament Ca^{2+} sensitivity (Table I). These severe pathological changes in the Glu180Gly hearts result in death between 4.5 – 6 months of age. Also, isolated cardiomyocytes from these hearts exhibit an increase in myofilament Ca^{2+} sensitivity of force production that collectively cause the aberrant function of the entire heart leading to HCM.

Table I: Tropomyosin Cardiomyopathic Models, Phenotypes, and Select Physiological Parameters

Mouse Model	**Phenotype**	**Contraction/Relaxation Function**	**Myofilament Ca^{2+} Sensitivity**
α-Tpm	Wildtype	Wildtype Control	Wildtype Control
Tpm Glu180Gly	HCM	↓ ↓ ↓	↑ ↑ ↑
Tpm Asp175Asn	HCM	↓ ↓	↑ ↑
Tpm Glu54Lys	DCM	↓ ↓ ↓	↓ ↓ ↓
Tpm S283D	DCM	↓ ↓ ↓	Wildtype levels
β-Tpm (High expression)	DCM	↓ ↓ ↓	------
α-Tpmκ	DCM	↓ ↓ ↓	↓ ↓ ↓

To determine whether it is possible to rescue HCM mice from their lethality and cardiac hypertrophic phenotype, we and others have taken several approaches.[51-53] Our initial approach was to target the cardiac myofilaments themselves through incorporation of proteins that counteract the properties exhibited by the HCM Tpm 180 mutant mice. As mentioned, myofilaments from these HCM hearts exhibit an increased sensitivity to Ca^{2+}. To address whether normalizing the myofilament Ca^{2+} sensitivity could rescue the HCM phenotype, we tested the hypothesis that attenuation of myofilament Ca^{2+} sensitivity would modulate the severe physiological and pathological consequences of the HCM mutation. We generated a transgenic mouse expressing a chimeric Tpm protein containing the α-Tpm amino terminus and the carboxyl terminus of β-Tpm protein;[52] the myofilaments from these α-Tpm/β-Tpm chimera mice exhibit a decreased sensitivity to Ca^{2+}.[54,55] Additional work shows double-transgenic mice (HCM Tpm180 mice crossbred with the α-Tpm/β-Tpm chimera mice) display a normal morphology with no pathological abnormalities, improved cardiac function, and normal myofilament Ca^{2+} sensitivity (Table II).[52,55] These results demonstrate that Ca^{2+} desensitization in myofibrils is a therapeutic option for the treatment of this disease.

Table II: Tropomyosin Cardiomyopathic Mice and Rescue Treatment

Mouse Model	Phenotype	Rescue Treatment	Action of Rescue
HCM Tpm Glu180Gly	Rescued - Normal	α-TPM/β-TPM chimera	Ca^{2+} Desensitization
HCM Tpm Glu180Gly	Rescued - Normal	NAC Treatment	Modifier of oxidative stress
HCM Tpm Glu180Gly	Rescued - Normal	Ser283Ala mutation mouse	Decreased Tpm Phosphorylation
HCM Tpm Glu180Gly	Rescued - Normal	PLN Knock-out	Modification of Ca^{2+} Cycling
HCM Tpm Glu180Gly	Rescued - Normal	SERCA2a AAV vector expression	Increased Ca^{2+} uptake in SR
HCM Tpm Glu180Gly	Rescued - Normal	C-terminus of TnI	Myofilament Ca^{2+} Desensitizer
DCM β-Tpm (High expression)	Rescued - Normal	Cyclosporin	Inhibitor of Calcineurin

Changes in myofilament oxidative stress occur in the Tpm Glu180Gly hearts associated with modifications in myosin binding protein C and activation of the MAPK signaling cascade.[56] We hypothesized that treatment with the glutathione precursor N-acetylcysteine (NAC) may reverse the oxidative stress in the Tpm Glu180Gly mice and improve their cardiac morphology and function. Results show that NAC treatment reversed the pathological abnormalities and cardiac dysfunction; myofilament Ca^{2+} sensitivity was also normalized (Table II).[56] These studies indicate that myofilament oxidative stress modifications are an important mediator in diastolic function and may be a potential therapeutic method in the treatment of HCM.

Tropomyosin 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 we substituted an alanine for the serine which prevents phosphorylation.[57,58] These transgenic mice (Ser283Ala) exhibit a compensated hypertrophic response with significant increases in SERCA2A expression and phosphorylation of PLN. Having obtained these results, we tested the hypothesis that decreasing Tpm phosphorylation may be beneficial in the presence of a constant intrinsic stressor, such as HCM. To test this, we generated transgenic mice that expressed both the Tpm Glu180Gly and Ser283Ala mutations. Results show the HCM phenotype was rescued in these double-mutation mice (Table II).[58,59] There were no signs of cardiac hypertrophy, showed improved cardiac function, and normal myofilament Ca^{2+} sensitivity. Changes in local flexibility of the Tpm protein conferred by the replacement of serine with alanine in the Tpm Glu180Gly / Ser283Ala double-mutation mice (coupled with the significant decrease in phosphorylation) may be responsible for the restoration of Tpm to proper flexibility.

To extend our investigations on rescuing mice exhibiting the HCM phenotype through modulation of cytosolic Ca^{2+}, we crossbred the Tpm Glu180Gly mice with phospholamban knockout mice.[60] Phospholamban (PLN) regulates Ca^{2+} uptake into the sarcoplasmic reticulum through its interaction with SERCA2a. In the absence of PLN, Ca^{2+} uptake

into the sarcoplasmic reticulum (SR) is significantly increased leading to cardiac hypercontractility with no change in morphology or myofilament Ca^{2+} sensitivity.[61] Results show that PLN ablation in the Tpm Glu180Gly mice rescues the morphological and functional abnormalities of the heart; there is a reversal of cardiac hypertrophy, fibrosis, and systolic and diastolic dysfunction (Table II). This work shows that through modulation of calcium cycling, many of the deleterious aspects of HCM caused by mutations in the TPM1 gene can be reversed.

As previously mentioned, PLN acts to inhibit SERCA2a activity of re-sequestering Ca^{2+} into the SR. Since a knockout of PLN could result in rescuing the HCM phenotype in the Tpm Glu180Gly mice,[60] we extended this work by using an adenoviral vector to increase exogenous SERCA2a expression in the HCM Glu180Gly hearts.[53,62] Results showed that injection of a single dose improved heart morphology, cardiac function, fibrosis, and cardiac hypertrophy (Table II). In an extension of this work in humans, percutaneous administration using an adeno-associated virus expressing SERCA2a was conducted in phase 1 and 2 clinical studies in human heart failure patients.[63,64] Results showed most patients exhibited marked improvements within 6 months, which included improved ejection fraction and end-systolic volume measurements. Thus, the success of these human trials demonstrates a potential treatment modality for heart failure patients.

Studies show that the C-terminal peptide of troponin I (TnI), which acts as a myofilament Ca^{2+} desensitizer, is highly conserved among species.[65] Protein binding studies found that this terminal fragment retains its binding affinity for Tpm similar to intact cardiac TnI. Addition of this fragment to 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 mice.[66] Results show a normalized decrease in Ca^{2+} sensitivity in myofilaments isolated from the Tpm Glu180Gly hearts, thereby demonstrating a potential therapeutic potential for the treatment of diastolic dysfunction in the heart (Table II).

Tropomyosin Associated Dilated Cardiomyopathic Animal Models

Dilated cardiomyopathy (DCM) is associated with 11 distinct mutations in TPM1. As is the case with HCM, mutations in Tpm that cause DCM are found throughout the TPM1 gene. Some of the mutations correspond to amino acid substitutions located in the inner regions of the Tpm coiled-coil dimer where electrostatic charge interactions between specific residues may alter dimerization and/or binding to actin.[67] These non-conserved amino acid substitutions are thought to disrupt force transmission through the sarcomere leading to the development of DCM.

To investigate the structural and functional 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, a substitution of a lysine for a glutamic acid at amino acid 54 (Glu54Lys) (Table I).[24] As with the transgenic HCM mice, the increase in exogenous Tpm protein expression led to a reciprocal decrease in endogenous wildtype α-Tpm levels, with the total myofibrillar Tpm levels remaining unchanged. Histological and morphological analyses of these hearts revealed development of DCM with progression to heart failure, and death often ensuing by 6 months.[24] The dilated phenotype was confirmed by echocardiography, as was a significant decrease in left ventricular fractional shortening and impaired systolic and diastolic function. There was also decreased sensitivity and tension generation in cardiac myofilaments. The response of Ca^{2+} sensitivity in the myofilaments is opposite that observed with HCM in thin filament mutations. This Glu54Lys amino acid change decreases Tpm flexibility which influences actin binding and myofilament Ca^{2+}. In summary, the pathological and physiological alterations exhibited by these mice are consistent with those observed in human DCM and heart failure patients. Phosphorylation of cardiac proteins plays a major role in the regulation of the physiological performance of the heart. Phosphorylation of thin filament proteins dramatically affect myofilament Ca^{2+} sensitivity, along with systolic and diastolic function. To address how phosphorylation of Tpm affects cardiac function, we generated transgenic mice that express a phosphorylation mimetic at the sole phosphorylation site: Ser283Asp (Table I). Our results show that high expression of the Tpm Ser283Asp transgene leads to a severe dilated cardiomyopathic phenotype resulting in death within 1 month of birth.[68] Moderate transgene expression causes a mild myocyte hypertrophy and fibrosis, along with diastolic dysfunction but without affecting lifespan. Surprisingly, there are no alterations in Ca^{2+} sensitivity of the myofilaments, cooperativity, or Ca-ATPase activity of the myofilaments.

Previous studies demonstrated that during embryonic and fetal cardiogenesis, the murine heart

expresses both α-Tpm and β-Tpm isoforms; α-Tpm is the predominant isoform in the adult heart (98% protein expression). To address whether β-Tpm could substitute for α-Tpm, we generated transgenic mice that overexpressed β-Tpm in the heart.[49] With moderate transgene expression (60% β-Tpm), there were changes in diastole and increased myofilament Ca^{2+} sensitivity, but no morphological alterations. With high expression (80% β-Tpm), a severe DCM phenotype developed, with death occurring within 14 days postpartum (Table I).[69] There is significant chamber dilation, thrombus formation in both atria and ventricles, and diastolic dysfunction.

We extended our studies on high expression β-Tpm mice by treating them with cyclosporin, an inhibitor of calcineurin. Calcineurin is a Ca^{2+}-regulated phosphatase which can initiate cardiac hypertrophy in hearts of transgenic mice that overexpress calcineurin.[70] Results show that cyclosporin or FK605 treatment in various mouse models of cardiac hypertrophy, including the high expression β-Tpm mice, can rescue the mice from a cardiomyopathic phenotype (Table II).[71] This work demonstrates that inhibitors of calcineurin may play a potential therapeutic role in the treatment of heart disease.

As mentioned, there are 4 TPM genes, each one subject to alternative splicing to generate multiple isoforms of Tpm. Our investigations revealed that in the adult human heart, a unique cardiac specific α-Tpm isoform is expressed: α-Tpmκ.[44] Additional work demonstrated that the level of this isoform is increased in human patients with DCM and heart failure.[44] To explore the role of the α-Tpmκ isoform in the sarcomere, we generated transgenic mice that express the α-Tpmκ isoform in the heart (Table I). Our results show that incorporation of increased levels of α-Tpmκ in myofilaments leads to DCM, coupled with systolic and diastolic dysfunction and decreased myofilament Ca^{2+} sensitivity.[44,72] Biophysical studies demonstrate less structural stability and weaker actin-binding affinity of α-Tpmκ compared with wildtype α-Tpm protein, thus providing a possible mechanism for the consequences of the Tpm isoform switch observed in DCM and heart failure patients.

Troponin T and Cardiomyopathic Animal Models

Troponin T (TnT) is an essential component of the sarcomeric thin filament with an essential role of linking the troponin complex to tropomyosin. Troponin acts by modulating the availability of actin to the myosin head via the movement of Tpm. Troponin T interacts with the middle and 3'ends of Tpm thereby anchoring the TnT-Tpm complex to the actin filament. In the presence of low Ca^{2+} levels (relaxation/diastole), TnT binding to TnI-TnC inhibits the myofibrillar actinomyosin MgATPase. With increases in cytosolic Ca^{2+} (contraction/systole), Ca^{2+} binds to TnC, shifts the position of TnI-TnC and Tpm which allows Tpm to shift its position on actin facilitating the interaction of myosin heads with actin binding sites.

There are 3 TnT encoding genes: cardiac (TNNT2), slow (TNNT1), and fast (TNNT3) that produce multiple striated muscle TnT isoforms.[73,74] Expression of the genes in adult cardiac and skeletal muscle is controlled in a muscle fiber type-specific manner. These 3 isoforms appear to play non-redundant roles in contraction of different muscle types. The primary structural diversity of the 3 muscle fiber type-specific TnT isoforms is mainly in the N-terminal region due to alternative splicing of exons.[75]

There are 36 confirmed mutations in the TNNT2 gene associated with HCM which account for 15-30 % of this cardiac disease.[76] These mutations in TnT also increase the Ca^{2+} sensitivity of the myofilaments, similar to the Tpm mutations causing HCM. Interestingly, mouse models encoding HCM TnT mutations generally exhibit less cardiac hypertrophy, but have a higher incidence of sudden cardiac death, as seen in humans.[29,77] The mutations in the TNNT2 gene that lead to HCM are usually point mutations leading to changes in the encoded amino acids, along with premature stop codons that lead to truncated TnT proteins. One TnT model that has been extensively studied is the TnT Arg92Gln mutation located in one of the Tpm binding domains.[29,78] The phenotypes in several TnT mouse models that have been developed generally exhibit impaired systolic and diastolic function coupled with Ca^{2+} hypersensitivity in the absence of ventricular hypertrophy.[29] These mouse models generally recapitulate various aspects of the human disorder including varying degrees of myocellular disarray, fibrosis and little ventricular hypertrophy.[12]

Mutations that lead to DCM have also been identified in the TNNT2 gene. There have been 13 DCM mutations linked to TnT.[76] In 2000, a deletion mutation (del Lys210 of cardiac TNNT2) was reported as the first DCM-causing mutation of TnT.[79] The mechanism associated with the development of this cardiac pathology might result from the decreased Ca^{2+} sensitivity in the myofilaments found with most TnT DCM mutations;[76] this is a similar finding to mutations in Tpm that cause DCM. These findings suggest that disruption of TnT-Tpm

interactions impair the systolic/diastolic process which result in the DCM phenotype.

Troponin I Associated Cardiomyopathic Animal Models

Troponin I (TnI) is the subunit of troponin which is responsible for inhibition of actomyosin ATPase activity. In the absence of Ca^{2+}, cardiac TnI (cTnI) inhibits contraction through its interactions with Tpm and actin; this inhibition is relieved during muscle contraction upon Ca^{2+} binding to TnC. In addition, phosphorylation of cTnI plays an important regulatory role in muscle contraction. Protein kinase A (PKA)-mediated phosphorylation of cTnI reduces myofilament Ca^{2+} sensitivity, increases the rate of Ca^{2+} dissociation from cTnC, and increases crossbridge cycling rate. Protein Kinase C (PKC) also phosphorylates cTnC, but decreases maximum Ca^{2+}-activated force generation and maximal sliding velocity in motility assays. This PKC phosphorylation also depresses cooperative activation of the thin filament.

There are 30 defined cTnI mutations that lead to HCM, most of which are located in the carboxyl region of the protein.[76] Many of these mutations reveal an increase in Ca^{2+} sensitivity, similar to the finding with HCM mutations in Tpm and TnT. Also, cTnI HCM mutations often lead to increases in the basal level of ATPase activity and increases in Ca^{2+} binding affinity of the regulatory site of cTnC in the thin filament. In an HCM mouse model of cTnI (Glu203Ser), cardiac hypertrophy did not develop until 21 weeks with mutant protein expression of 48%.[80] The hearts in these mice develop left ventricular hypertrophy, fibrosis, myocyte disarray, and altered Ca^{2+} handling. A knock-in HCM model of TnI Arg21Cys was generated which is located in a region associated with TnI phosphorylation.[29] Cardiac TnI phosphorylation was reduced in this knock-in model in their skinned myofibers, along with impaired cardiac relaxation, cardiac hypertrophy, and fibrosis.

Relatively few mutations (4 in number) in cTnI have been reported that lead to DCM with one mutation (Ala2Val) exhibiting an autosomal recessive mode of inheritance. Two mutations (Lys35Gln, Asn185Lys) are autosomal dominant mutations and the inheritance pattern of one mutation (Pro16Thr) has not been reported.[76,81] These DCM mutations in cTnI appear to decrease the maximum activity and Ca^{2+} sensitivity of actin-myosin S1 ATPase and reduce the binding affinity of the regulatory site of cTnC in the thin filament.

Restrictive cardiomyopathy (RCM) is a human cardiomyopathic condition characterized by bi-atrial dilation, restricted left ventricle, and sudden cardiac death. A transgenic mouse model was generated (cTnI Arg193His) that exhibits many of the pathological features of RCM, including restrictive ventricles, bi-atrial enlargement and sudden cardiac death.[53,82] These mice were crossbred with another transgenic mouse line that expresses a cTnI truncated N-terminal fragment.[57,83] These double transgenic mice rescued the lethal RCM phenotype of the TnI Arg193His mice; cardiac function was significantly improved and the myofilaments increased sensitivity to Ca^{2+} was reversed. These results are similar to those found with the HCM Tpm Glu180Gly rescued mice which suggests that Ca^{2+} desensitization in myofilaments is a therapeutic option for the treatment of diastolic dysfunction.[66]

Troponin C Associated Cardiomyopathic Animal Models

Troponin C (TnC) is a key regulatory protein in striated muscle contraction where its function is to bind Ca^{2+} to trigger actomyosin interactions and initiate sarcomeric contraction. The TNNC1 gene expresses TnC in both cardiac and slow skeletal muscle isoforms. Mutations in TnC that cause HCM and DCM are rare in humans. However, some mutations in TnC that cause cardiomyopathies can possibly alter its function by changing its binding affinity for Ca^{2+}, or by altering its interaction with its principal binding proteins – actin, Tpm, and TnI. There are six mutations in TNNC1 that are associated with HCM: Ala8Val, Lys29Gln, Ala31Ser, Cys84Tyr, Glu134Asp, Asp145Glu.[84,85] Most of the mutations in TnC occur at the interface with actin and Tpm. Recent studies find that pathogenic mutations in the Ca^{2+}-binding TnC N-lobe which controls contraction are few; the reason for this paucity of HCM mutations in this region is unknown.[86] The physiological alterations caused by other HCM mutations results in a significantly increased Ca^{2+} sensitivity of force development. A comprehensive analysis was conducted on TnC transgenic mice that overexpress the Leu48Gln mutation, the hearts from these mice display some HCM properties.[87,88] This HCM mutation decreases the rate of Ca^{2+} release, along with a slower trabeculae relaxation. There was also a significantly increased cellular fractional shortening in myocyte contractile assays. Interestingly, although the transgenic mice had hyperfunctional hearts, there was no change in cardiac growth or chamber dimensions up to 1 year.[87]

There are also 6 TNNC1 mutations associated with DCM: Tyr5His, Gln50Arg, Glu59Asp-Asp75Tyr, Met103Ile, Ile148Val, Glu159Asp.[85] Currently, no known DCM-inducing loss-of-function alleles have been detected in the TnC N-lobe. One possibility for this is that missense mutations in this region are poorly tolerated and cause a lethal phenotype.[86] Interestingly, unlike most HCM mutations in thin filament proteins, the DCM mutations in TnC do not always exhibit a decreased myofilament Ca^{2+} sensitivity, and sometimes have no effect on this property.[76,85] However, the double mutant Glu59Asp and Asp75Tyr seen in a DCM patient, did show a decrease in myofilament Ca^{2+} sensitivity and Ca^{2+} binding affinity in the force-pCa relationship.[76] Other DCM mutations also show decreases in Ca^{2+} sensitivity of force generation.[89] A DCM transgenic mouse has been developed that harbors the Ile61Gln TnC mutation has reduced myofilament tension, reduced cardiac function, increased diastolic left ventricular chamber size, and decreased septal wall thickness.[87]

Conclusions

Much has been learned about the HCM and DCM disease processes through the examination of animal model systems. For most of the mutations that cause these cardiomyopathies, the mutations are not confined to a single region, but are scattered throughout the gene. The severity of the disease phenotype appears dependent upon the specific mutation, modifying genes, and environmental factors. The genetic animal model systems reflect many of the disease processes 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 often 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 are being translated into potential therapeutic modalities. For example, usage of calcium handling proteins as treatments of HCM are being explored,[63,64] with a potential for expansion into other cardiovascular abnormalities. Various drugs, such as mavacamten and omecamtiv mecarbil which modulate myosin function, were tested in animal model trials and have advanced to human clinical trials for treating HCM.[90] Recently, the usage of human pluripotent stem cells (hPSC) and induced pluripotent stem cells (iPSC) have been employed to address abnormalities associated with cardiovascular disease.[91] Coupling these pluripotent stem cells with CRISPR/Cas9 has allowed investigators to specifically engineer HCM mutations and assess their physiological effect. For example, Wang et al examined HCM mutations in cardiac TnT in hPSC-cardiomyocytes and these recapitulated associated phenotypes, such as hypercontractility, impaired relaxation, increased Ca^{2+} sensitivity.[92] A similar approach was taken by Smith et al, investigating HCM mutations in actin from patient-derived hPSCs.[93] With the multitude of experimental systems and techniques which are available (animal models, iPSC, hPSC, organoids, viral vectors, and CRISPR), potential areas of future investigations into gene therapy should include a focus on targeting molecules which trigger the development of HCM, DCM and heart failure.

Acknowledgments

I would like to thank and acknowledge all of the investigators who contributed to the investigations presented in this article, especially Drs. Emily Schulz, Sudarsan Rajan, Ganapathy Jagatheesan, Mariappan Muthuchamy, and Hajer Sheikh.

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