Sarcomeric proteins and inherited cardiomyopathies

Sachio Morimoto*

Laboratory of Clinical Pharmacology, Kyushu University Graduate School of Medicine, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 8 October 2007; revised 8 November 2007; accepted 19 November 2007; online publish-ahead-of-print 4 December 2007

Time for primary review: 17 days

Over the last two decades, a large number of mutations have been identified in sarcomeric proteins as a cause of hypertrophic, dilated or restrictive cardiomyopathy. Functional analyses of mutant proteins in vitro have revealed several important functional changes in sarcomeric proteins that might be primarily involved in the pathogenesis of each cardiomyopathy. Creation of transgenic or knock-in animals expressing mutant proteins in their hearts confirmed that these mutations in genes for sarcomeric proteins induced distinct types of cardiomyopathies and provided useful animal models to explore the molecular pathogenic mechanisms and potential therapeutics of cardiomyopathy in vivo. In this review, I discuss the functional consequences of mutations in different sarcomeric proteins found in hypertrophic, dilated, and restrictive cardiomyopathies in conjunction with their effects on cardiac structure and function in vivo and their possible molecular and cellular mechanisms, which underlie the pathogenesis of these inherited cardiomyopathies.

1. Introduction

Cardiomyopathy is classified into four main forms, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C). HCM and DCM increase myocardial mass with distinct patterns of ventricular remodeling. HCM produces prominent increase in chamber volumes as well as ventricular wall thickening in the interventricular septum, with decreases in ventricular chamber volumes. DCM produces a prominent increase in ventricular wall thickness (i.e. hypertrophy), especially in the interventricular septum, with decreases in ventricular chamber volumes. DCM produces a prominent increase in chamber volumes as well as ventricular wall thickening. In HCM, systolic function is increased or at least preserved, while diastolic function is impaired in part because of the hypertrophy itself, interstitial fibrosis, and/or myocyte disarray. Diastolic dysfunction is thought to be responsible for symptoms of heart failure and premature sudden cardiac death of HCM patients. In contrast, DCM is characterized by systolic dysfunction, which often leads to heart failure requiring cardiac transplantation or sudden cardiac death. RCM is characterized by restrictive diastolic dysfunction (i.e. restrictive filling and reduced diastolic volume of either or both ventricles) with normal or near normal systolic function and wall thickness.

Since the discovery of an HCM-causing mutation in the gene for β-myosin heavy chain (β-MyHC) at 1990, >400 mutations that cause HCM, DCM, and RCM have been found in the genes for proteins constituting the sarcomere of cardiac muscle, whereas no sarcomeric protein genes have been discovered to be responsible for ARVD/C (Figure 1 and Table 1). Despite a great number of studies that have been done to elucidate the structure and function of normal sarcomeric proteins since the middle of the last century, it is still difficult to predict the exact functional consequences of mutations found in cardiomyopathies only from their nature and position. Discoveries of cardiomyopathy-causing mutations in sarcomeric proteins have led to extensive studies on their functional consequences in vitro using varieties of analyzing techniques refined over more than a half century. Furthermore, transgenic or knock-in animal models have extensively been used to explore the physiological function of mutant sarcomere proteins in vivo and its involvement into the pathogenesis of cardiomyopathies. These lines of studies are dramatically improving our views of cardiomyopathies as well as the physiological function of sarcomere proteins.

This review focuses on the mutations in sarcomeric proteins, i.e. the thin and thick filament proteins, titin, and Z-disc proteins, and their roles in the pathogenesis of cardiomyopathies. Mutations in sarcolemmal transmembrane proteins, cytoskeletal proteins and nuclear envelope proteins are the other important causes of DCM and are being discussed elsewhere.

2. Mutations in genes for the thin filament proteins

2.1 Troponin complex

2.1.1 Cardiac troponin T

2.1.1.1 cTnT mutations in HCM

Twenty-seven mutations in the cardiac troponin T (cTnT) gene (TNNT2) have been found to cause HCM. HCM patients
with cTnT mutations show moderate or no significant cardiac hypertrophy in spite of their malignant prognosis, i.e. high incidence of sudden cardiac death. Missense mutations I79N and R92Q, a deletion mutation ΔE160, and a splice donor site mutation intron 16G→A develop a similar malignant clinical phenotype, with the life expectancy of patients being ~35 years. Most studies have been made on the functional aspects of these cTnT mutant proteins, and functional consequences primarily involved in the molecular pathogenic mechanism have been rapidly clarified, although some controversial observations have been reported. Early studies using myocytes transected or infected with mutant cTnT cDNA consistently indicate that the mutations impair muscle contractility and thus may cause compensatory hypertrophy, which is not consistent with the current view of the pathogenic mechanism of HCM described follows.

Other types of functional studies indicate that the human cTnTs, when exchanged into the rabbit cardiac skinned muscle fibres or myofibrils, increase the Ca$^{2+}$ sensitivity, without impairing the maximum force-generating capability and ATPase activity. Szczesna et al. reported similar results concerning the mutations I79N, R92Q, R92W, and R92L. Miller et al. created transgenic mice expressing I79N human cTnT and confirmed that the skinned cardiac muscle fibres showed increased myofilament Ca$^{2+}$ sensitivity. Tardiff et al. created transgenic mice expressing R92Q human cTnT. Isolated working hearts showed hypercontractility and diastolic dysfunction. Cardiac myocytes showed increased basal sarcomeric activation, impaired relaxation, and shorter sarcomere lengths, indicative of increased Ca$^{2+}$ sensitivity. Chandra et al. demonstrated that Ca$^{2+}$ sensitivity of myofilaments was enhanced in the mice expressing R92Q, R92W, and R92L cTnTs.

Functional studies so far made strongly suggest that an increased Ca$^{2+}$ sensitivity of cardiac muscle contraction is involved in the pathogenesis of HCM associated with the cTnT mutations.

### Table 1 Numbers of mutations in sarcomeric proteins found in cardiomyopathies

<table>
<thead>
<tr>
<th>Protein (gene)</th>
<th>HCM</th>
<th>DCM</th>
<th>RCM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thin filament proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac troponin T (TNNT2)</td>
<td>27$^a$</td>
<td>6$^a$</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac troponin I (TNNT3)</td>
<td>26$^a$</td>
<td>1$^{22}$</td>
<td>6$^a$</td>
</tr>
<tr>
<td>Cardiac troponin C (TNNT1)</td>
<td>1$^a$</td>
<td>1$^a$</td>
<td>–</td>
</tr>
<tr>
<td>α-Tropomyosin (TPM1)</td>
<td>11$^a$</td>
<td>2$^a$</td>
<td>–</td>
</tr>
<tr>
<td>α-Cardiac actin (ACT1)</td>
<td>7$^a$</td>
<td>2$^a$</td>
<td>–</td>
</tr>
<tr>
<td><strong>Thick filament proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Myosin heavy chain (MYH7)</td>
<td>167$^a$</td>
<td>11$^a$</td>
<td>–</td>
</tr>
<tr>
<td>Ventricular regulatory light chain (MYL3)</td>
<td>4$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ventricular essential light chain (MYL2)</td>
<td>10$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac myosin-bining protein C (MYBPC3)</td>
<td>134$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Titin and Z-disc proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titin (TTN)</td>
<td>2$^a$</td>
<td>6$^a$</td>
<td>–</td>
</tr>
<tr>
<td>T-cap (TCAP)</td>
<td>4$^a,85$</td>
<td>2$^a,84$</td>
<td>–</td>
</tr>
<tr>
<td>MLP (CSRP3)</td>
<td>3$^a$</td>
<td>1$^a$</td>
<td>–</td>
</tr>
<tr>
<td>Myozzenin-2 (MYOZ2)</td>
<td>1$^{89}$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Actinin (ACTN2)</td>
<td>–</td>
<td>1$^91$</td>
<td>–</td>
</tr>
<tr>
<td>Obscurin (OBSCN)</td>
<td>2$^{90}$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cypher (LDB3)</td>
<td>–</td>
<td>3$^a,92$</td>
<td>–</td>
</tr>
</tbody>
</table>

*Human Gene Mutagenesis Database at the Institute of Medical Genetics in Cardiff (http://www.hgmd.cf.ac.uk/ac/index.php).*
with the previous in vitro study. Oral administration of pimobendan, a Ca$^{2+}$ sensitizer, known to directly increase the Ca$^{2+}$ sensitivity of myofilaments, was found to reduce the heart size of mutant mice and markedly improve the life expectancy, strongly suggesting that Ca$^{2+}$ sensitizers, such as pimobendan, are beneficial for the treatment of DCM patients affected by this mutation.

2.1.2 Cardiac troponin I

2.1.2.1 cTnI mutations in HCM

Kimura et al. reported six mutations in the cardiac troponin I (cTnI) gene (TNNI3) that were associated with HCM. Although the prevalence of cTnI mutations is less than that of cTnT mutations, currently 26 mutations have been identified (Table 1). Ca$^{2+}$-sensitizing effect on cardiac muscle contraction has commonly been observed for most mutations. Transgenic mice expressing R145G showed the pathological features of typical HCM, including myocyte disarray and interstitial fibrosis, with no significant cardiac hypertrophy. Working heart preparations showed enhanced systolic function and impaired diastolic function. Skinned cardiac muscle fibres showed a significant increase in Ca$^{2+}$ sensitivity with maximum force depression. This mouse model showed a very early death at 13–17 days after birth in mice with mutant cTnI being incorporated into myofilament by ~50%.

2.1.2.2 cTnI mutation in DCM

An N-terminal mutation in cTnI, A2V, has been found to cause a rare case of DCM inherited in an autosomal ‘recessive’ manner and this mutation impairs the interaction of cTnI with cTnT but not cardiac troponin C (cTnC) as demonstrated in a mammalian two-hybrid assay system. Six missense mutations have been found in the patients with idiopathic RCM. A mixed appearance of RCM and HCM in a family with D190G suggested that there might be a common molecular mechanism for the pathogenesis of RCM and HCM. Functional studies indicate that the six mutations increase the Ca$^{2+}$ sensitivity of force generation in skinned cardiac muscle fibres, with their effects being much greater than those of HCM-causing mutations in cTnI. NMR studies show that dramatic Ca$^{2+}$ sensitizing effects are caused by an unexpectedly subtle structural perturbation in a small region within cTnI molecule.

2.1.3 Cardiac TnC

2.1.3.1 cTnC mutation in HCM

A missense mutation L29Q in the cTnC gene (TNNC1) was reported in an HCM patient with late onset of the disease. An N-terminal small region containing the L29 residue interacts with an N-terminal small region containing Ser22/23 of cTnI, and this interaction is abolished upon phosphorylation of cTnI Ser22/23 by protein kinase A (PKA), leading to a decrease in the myofilament Ca$^{2+}$ sensitivity. Schmidtmann et al. found that this mutation abolished the N-terminal interaction between cTnI and cTnC, leading to a loss of change in myofilament Ca$^{2+}$ sensitivity upon phosphorylation of cTnI by PKA.

2.1.3.2 cTnC mutation in DCM

A missense mutation, G159D, in TNNC1 was found in a DCM family with a malignant phenotype. Mirza et al. reported that this mutation reduced the Ca$^{2+}$ sensitivity in actomyosin ATPase and in vitro motility assays. However, this mutation had no effects on the Ca$^{2+}$ sensitivity of force generation when mutant cTnC was exchanged into skinned fast skeletal or cardiac muscle fibres. Alternatively, this mutation has been shown to blunt the Ca$^{2+}$ desensitization of force generation induced by phosphorylation of cTnT Thr203 by PKC or cTnI Ser22/23 by PKA.

2.2. Tropomyosin

Skinned cardiac muscle fibres showed a very early death at 13–17 days after birth in mice with mutant cTnI being incorporated into myofilament by ~50%.

2.2.1 αTM mutations in HCM

A total of 11 missense mutations have been found in the αTM gene (TPM1) as a cause of HCM (Table 1). TPM1 is an uncommon cause of familial HCM (~5%), except for the Finnish population (~25%). A Ca$^{2+}$-sensitizing effect on skinned cardiac muscle contraction has commonly been observed for most mutations in αTM, as are the cases with cTnT and cTnI mutations.

D175N has been found in three families with a favourable prognosis of near normal life expectancy as well as in a family with a malignant prognosis associated with frequent sudden deaths. Biopsy samples from slow skeletal muscles of two patients showed an increased Ca$^{2+}$ sensitivity of force generation. Transgenic mice expressing D175N mouse αTM showed a normal life expectancy. Skinned cardiac muscle fibres had increased Ca$^{2+}$ sensitivity, which could account for the diastolic dysfunction in working heart preparations.

Mice expressing E180G ‘mouse’ αTM showed a very severe and lethal phenotype with no mice surviving beyond 6 months of age. Working heart preparations showed significant diastolic dysfunction and skinned cardiac muscle fibres had increased Ca$^{2+}$ sensitivity. However, transgenic mice expressing E180G ‘human’ αTM showed a very mild phenotype with apparent normal longevity similar to the clinical phenotype of human patients. Nevertheless, skinned single cardiac myocytes showed increased Ca$^{2+}$ sensitivity, which manifested as a significant diastolic dysfunction in vivo.

2.2.2 αTM mutations in DCM

Two missense mutations, E40K and E54K, have been identified in the TPM1 gene as a cause of DCM with a relatively malignant phenotype. Both mutations change the acidic residue into a basic residue. Interestingly, no missense mutations that cause an electrical charge reversal has not been reported in any HCM mutations in αTM. Both mutations decreased the Ca$^{2+}$ sensitivity as demonstrated in studies employing reconstituted actomyosin or myofibrillar ATPase activity and the in vitro motility assay.

Transgenic mice expressing E54K mouse αTM showed variable phenotype depending on the copy number of transgene. The mice with high-copy number all died within 1.5 months of age. In contrast, mice with moderate-copy number showed a relatively mild phenotype with a tendency...
of developing DCM after 2 months of age and starting dying by 4–6 months of age. In skinned cardiac muscle preparations, both moderate- and high-copy transgenic mice demonstrated significant decreases in the Ca\(^{2+}\) sensitivity, consistent with the in vitro studies, as well as a marked depression in maximum force/cross-sectional area.

### 2.3. Mammalian Actin

Mammalian cells express six isoforms encoded in different gene which can be classified into three main groups: α-, β-, and γ-actins. Sarcomeric actins, α-cardiac and α-skeletal, are known to be co-expressed in myocardium.\(^{35,56}\)

#### 2.3.1 α-Cardiac actin mutations in HCM

The α-cardiac actin gene (ACTC1) is a rare cause of HCM (1.5%) and seven missense mutations have been found (Table 1). These mutations decreased the thermal stability of actin monomer and impaired the filament formation, suggesting that the inability to form myofilaments and/or the accumulation of aggregates could be one of the cellular pathological effects of ACTC1 mutations.\(^{57–59}\) E99K reduced the sliding velocity and averaged force in an in vitro motility assay and decreased the affinity of actin for myosin,\(^{59}\) suggesting that impaired actomyosin interaction is the primary defect at molecular level leading to HCM associated with this mutation.

#### 2.3.2 α-Cardiac actin mutations in DCM

Two missense mutations, R312H and E361G, have been identified in ACTC1 as a cause of DCM with apparently favourable prognosis.\(^{60}\) R312H occurs at the residue next to D311 in subdomain 3 that forms an attractive electrostatic interaction with TM in the high Ca\(^{2+}\) state.\(^{61}\) E361G occurs in a binding domain for α-actinin in subdomain 1, which anchors thin filaments to Z-discs and intercalated discs.\(^{62}\) Wong et al.\(^{57}\) reported that this mutation had no effects on actin polymerization, rigor binding, actomyosin ATPase, and in vitro motility, but slightly reduced the affinity of actin filament for α-actinin, suggesting that impaired force transmission via Z-discs and intercalated discs might be responsible for the pathogenesis. Vang et al.\(^{58}\) reported that these two mutations impaired the protein-folding pathway and filament formation, as were the cases with HCM-causing mutations.

### 3. Mutations in genes for the thick filament proteins

#### 3.1. Myosin

Cardiac muscle expresses two isoforms, α- and β-cardiac MyHCs. The α-MyHC is abundant in both atria and ventricles during mammalian embryogenesis. In small mammals, including mouse and rat, α-MyHC remains a predominant isoform expressed in both atria and ventricles during adulthood.\(^{63}\) In contrast, β-MyHC is expressed predominantly in the ventricles and α-MyHC in the atria during adulthood in large mammals, including rabbit and human. The β-cardiac MyHC is also known to be expressed in slow skeletal muscle, type I fibres.

#### 3.1.1 β-MyHC mutations in HCM

Mutations in the β-MyHC gene (MYH7) are the most frequent causes of HCM, with at least 167 mutations identified in both exon and intron, the majority of which are missense mutations (Table 1). R403Q and R453C are associated with a malignant phenotype characterized by early onset, a 100% disease penetrance in adults, and a high incidence of premature sudden death.\(^{64,65}\)

Geisterfer-Lowrance et al.\(^{66}\) created a knock-in mouse model in which R403Q was introduced into the endogenous α-MyHC gene (MYH6). Heterozygous mice were viable, reproduced normally, and lacked overt symptoms. Hearts of young mice showed delayed left ventricular pressure relaxation and chamber filling without gross morphologic or histologic abnormalities, demonstrating that diastolic dysfunction is the primary response to the mutation.\(^{66,67}\) Gao et al.\(^{58}\) studied calcium cycling in intact cardiac muscle fibres and reported that mutant myofilaments were more sensitive to Ca\(^{2+}\) below half-maximal [Ca\(^{2+}\)], and lead to a diastolic dysfunction. Warshaw and colleagues\(^{69,70}\) investigated cardiac myosin isolated from homozygous mice and reported that R403Q enhanced the force-generating capacity and actomyosin ATPase activity as well as the velocity of actin filament sliding, whereas R453C enhanced only the force-generating capacity. Although the reason for the discrepancy from the previous studies remains unclear,\(^{71}\) they proposed that the HCM-causing mutations should augment the power output of the hearts beyond the mechanical stress tolerance of a normal cardiac sarcomere, which might be a primary stimulus for the hypertrophic response.\(^{59}\)

Marian et al.\(^{72}\) created a transgenic rabbit model expressing human β-MyHC R403Q mutant. These rabbits exhibited the phenotype virtually identical to that of human HCM, i.e. premature death, cardiac hypertrophy, myocyte disarray, interstitial fibrosis, and normal systolic function, so that this model promises to be an important resource for pathogenic and therapeutic studies of human HCM.

#### 3.1.3 β-MyHC mutations in DCM

A total of 11 missense mutations have been found in MYH7 as a cause of DCM (Table 1). Kamisago et al.\(^{20}\) reported two mutations S532P and F746L in families with early-onset phenotype. Schmitt et al.\(^{73}\) created knock-in mouse models with S532P and F746L being engineered into endogenous genes. Heterozygous and homozygous mice were fully viable and fertile, and they survived >1 years. Systolic function was impaired in homozygous but not in heterozygous mice. Cardiac myocytes isolated from heterozygous mice showed impaired contractility. They investigated the cardiac myosin isolated from homozygous mice and reported that S532P reduced the force-generating capacity and the velocity of actin filament sliding, whereas F746L reduced only the actin-activated ATPase activity.\(^{70,73}\) Based on these findings, they proposed that the compromised enzymatic and/or mechanical activities in cardiac myosin may trigger the cascade of events that lead to DCM.

#### 3.1.4 Essential myosin light chain mutations in HCM

Mutations in the ventricular essential myosin light chain (ELC) gene (MYL3) is a rare cause of HCM (<1%), with only four missense mutations being identified (Table 1). M149V was found in a large family, of which six had a rare
phenotype, as a familial condition, involving mid-left ventricular chamber thickening. Low mortality rate in this family suggests M149V is associated with a benign prognosis. Transgenic mice expressing human ventricular ELC with M149V faithfully recapitulated the cardiac disease of the patients with this mutation at old age (≥1 year) (i.e. an unusual phenotype of mid-cavitary obstruction). Transgenic mice expressing 'mouse' ventricular ELC with M158V, corresponding to M149V in human, developed no hypertrophy, even in senescent animals (1.5 years). Transgenic rabbits expressing rabbit ventricular ELC with M154V, corresponding to M149V in human, again showed no discernible pattern of disease at the structural or functional levels except for a very subtle increase in the myofilament Ca²⁺ sensitivity, suggesting that this mutation is not causative for HCM at least in rabbit, although it remains possible that a phenotype might present in older rabbits.

3.1.5 Regulatory myosin light chain mutations in HCM

Eight missense mutations and two splice site mutations have been found in the ventricular regulatory myosin light chain (RLC) gene (MYL2) as a cause of HCM (Table 1). The MYL2 gene has been shown to be common in HCM families with a malignant prognosis.

Szczesna-Cordary et al. created transgenic mice expressing E22K, N47K, and R58Q human ventricular RLC. These mice showed no cardiac hypertrophy. Nevertheless, cardiac myofibrils or skinned fibres prepared from E22K and N47K, but not from R58Q mice showed increased Ca²⁺ sensitivity. Dumka et al. studied mechanical properties of myosin cross-bridges during single-turnover contraction in cardiac myofibrils from mutant mice and reported that E22K had no effect on the mechanical properties of cross-bridges.

3.2. Myosin-binding protein C

Mutations in the cardiac myosin-binding protein C (cMyBP-C) gene (MYBPC3) are one of the most frequent genetic causes of HCM, with at least 134 different mutations identified in both exon and intron of the gene (Table 1). Missense mutations constitute only about half of the mutations and the remaining half include insertions, deletions, and splice donor/acceptor site mutations that are predicted to cause a C-terminal truncation of cMyBP-C molecule. MYBPC3 mutations are associated with later onset, less hypertrophy, lower penetrance, and a better prognosis compared with mutations in MYH7 or TNNT2.

Transgenic mice expressing a mutant cMyBP-C lacking its C-terminal half, which mimicked the truncation mutations in HCM, showed no significant cardiac hypertrophy and no significantly increased morbidity or mortality. The truncated protein was not correctly incorporated into the A-band of the sarcomere, suggesting that haplo-insufficiency might play a role in the pathogenic process. Homozygous knockout mice were also viable and displayed well-developed sarcomeres, but exhibited significant cardiac hypertrophy with reduced diastolic function. These data demonstrated that cMyBP-C is not essential for forming and maintaining sarcomere ultrastructure, but that the absence of cMyBP-C results in cardiac hypertrophy and dysfunction.

4. Mutations in genes for titin and Z-disc proteins

4.1. Titin

In the titin gene (TTN), two missense mutations have been identified as a cause of HCM, and five missense mutations and one frameshift mutation have been identified as a cause of DCM (Table 1). Titin has multiple important roles in muscle physiology as a determinant of passive muscle stiffness and myofilament Ca²⁺ sensitivity as well as a biochemical sensor controlling gene expression. Titin is encoded by a single gene TTN and differential splicing of exons encoding the central I-band region produces muscle type-specific isoforms.

The affinity of titin Z1-Z2 domains for T-cap (or telethonin) is decreased by DCM-causing mutation V154M. The affinity of the titin Z-repeat region for α-actinin is increased by HCM-causing R740L while decreased by DCM-causing A743V, suggesting that opposite effects on the integrity of titin in the Z-disc might explain the distinct phenotypes caused by these mutations. DCM-causing S3799Y increases the affinity of the titin N2B region for four and half LIM protein 2, known to bind metabolic enzymes, suggesting that altered recruitment of metabolic enzymes to the sarcomere may play a role in the pathogenesis of cardiomyopathies.

4.2. Z-disc proteins

T-cap binds to the N-terminus of titin at the Z-disc. T-cap interaction with titin is stabilized by another Z-disc protein, muscle LIM protein (MLP), and MLP/T-cap/titin complex are thought to serve as a mechanical stress sensor. Four missense mutations have been identified in the T-cap gene (TCAP) as a cause of HCM. Yeast two-hybrid (Y2H) assays show that HCM-causing mutations, T137I and R153H, enhance the interaction of T-cap with MLP. Y2H assays show that both mutations impair the complex formation of T-cap with MLP while E132Q also impairs the interaction with titin and myozin-2, strongly suggesting that altered interactions among the Z-disc mechanical sensor complex are responsible for the disease.

Several missense or frameshift mutations have been found in the MLP gene (CSRP3) as a cause of HCM. Y2H assays show that C58G leads to a decreased binding activity of MLP to α-actinin. Only one missense mutation, K69R, in CSRP3 has been identified as a cause of familial DCM. This mutation is in a nuclear localization signal adjacent to the LIM1 domain of MLP.

A missense mutation, S48P, in the myozin-2 gene (MYOZ2) has been found in a large family with HCM characterized by early onset of symptoms, pronounced cardiac hypertrophy, and cardiac arrhythmias.

A missense mutation, Q9R, in the α-actinin gene (ACTN2) has been found in a patient with DCM and reported to disturb the interaction of α-actinin with MLP.

Two missense mutations, R4344Q and A4448T, in the obscurin gene (OBSCN) have been found in a patient with HCM and reported to decrease the affinity of obscurin for the titin Z-disc domain.
Three missense mutations, D117N, K136M, and D626N, in the cypher gene (LDB3) have been found in patients with DCM. 95,96 2YH assays and pull-down assays show that D626N decreases the affinity of the C-terminal LIM domain of cypher for PKC, suggesting that an abnormality in the anchoring-protein of PKC in the Z-disc may play a role in the pathogenesis of DCM.

5. Conclusion

Functional studies that have so far been made on sarcomeric regulatory proteins shows that HCM- or RCM-causing mutations increase the Ca\textsuperscript{2+} sensitivity of cardiac myofilaments, whereas DCM-causing mutations decrease it. Increased Ca\textsuperscript{2+} sensitivity of cardiac myofilaments is also caused by HCM-causing mutations in the thick filament proteins; this is not surprising because it is well known that there exists a positive feedback mechanism between troponin Ca\textsuperscript{2+} binding and myosin crossbridge attachment. Increased myofilament Ca\textsuperscript{2+} sensitivity is expected to increase the ATP utilization by actomyosin at submaximal Ca\textsuperscript{2+} concentrations, which might cause an imbalance in energy supply and demand in the heart under severe stress. NMR studies have revealed that myocardial energetics is compromised in mouse models of HCM caused by R92Q mutation in cTnT and R403Q mutation in α-MyHC,\textsuperscript{97,98} as is the case with human patients affected by mutations in sarcomeric proteins, including cTnT.\textsuperscript{99} Chandra et al.\textsuperscript{19} reported that the skinned cardiac muscle fibres from transgenic mice with HCM-causing ΔE160 mutation in cTnT showed an increased ATP consumption of force maintenance (i.e. increased tension cost). Based on the similarity of the clinical phenotypes of diseases that limit myocardial energy production to those of HCM, Ashrafian et al.\textsuperscript{100} have proposed that the increased energy demand owing to inefficient sarcomeric ATP utilization in HCM compromises the contraction and homeostatic functions of the cardiac myocyte, leading to myocyte hypertrophy.

The increase and decrease in the myofilament Ca\textsuperscript{2+} sensitivity well account for the diastolic and systolic dysfunction of model animals as well as human patients of HCM and DCM, respectively. Diastolic and systolic dysfunction should increase and decrease the ventricular wall stress, which could be transmitted to the biomechanical sensor in Z-discs or intercalated discs controlling gene expression and lead to HCM (or RCM) and DCM, respectively. Further studies on the detailed pathogenic mechanisms involving the huge numbers of mutations of sarcomeric proteins found in cardiomyopathies will contribute to clarifying the entire feature of the physiological mechanisms controlling cardiac function and structure.

Conflict of Interest: none declared.

Funding

This work was supported by a grant from the Vehicle Racing Commemorative Foundation.

References


