Inherited cardiomyopathies caused by troponin mutations

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Abstract

Genetic investigations of cardiomyopathy in the recent two decades have revealed a large number of mutations in the genes encoding sarcomeric proteins as a cause of inherited hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), or restrictive cardiomyopathy (RCM). Most functional analyses of the effects of mutations on cardiac muscle contraction have revealed significant changes in the Ca$^{2+}$-regulatory mechanism, in which cardiac troponin (cTn) plays important structural and functional roles as a key regulatory protein. Over a hundred mutations have been identified in all three subunits of cTn, i.e., cardiac troponins T, I, and C. Recent studies on cTn mutations have provided plenty of evidence that HCM- and RCM-linked mutations increase cardiac myofilament Ca$^{2+}$ sensitivity, while DCM-linked mutations decrease it. This review focuses on the functional consequences of mutations found in cTn in terms of cardiac myofilament Ca$^{2+}$ sensitivity, ATPase activity, force generation, and cardiac troponin I phosphorylation, to understand potential molecular and cellular pathogenic mechanisms of the three types of inherited cardiomyopathy.

Keywords: Troponin; Cardiomyopathy; Calcium sensitivity; Muscle contraction

1 Introduction

Cardiomyopathies are difficult and complicated diseases of heart muscle associated with heart failure and/or sudden cardiac death, which are classified by the World Health Organization (WHO) in 1995 into four main forms: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). HCM is characterized by ventricular muscle hypertrophy, which especially involves the increased thickness of interventricular septum, leading to a marked decrease in left ventricular (LV) chamber volume. HCM has impaired LV diastolic function probably because of hypertrophy itself, interstitial fibrosis and/or myocyte disarray, while it usually has normal, or slightly attenuated LV systolic function.[2,3] The prevalence of HCM is about 1/500, in which more than 70% are familial cases. DCM is characterized by abnormal enlargement of LV or both ventricular chambers with impaired systolic function, affecting about 36.5 in 100,000 people, of whom about 25%–30% are familial cases. Its prognosis is poor due to high frequency of arrhythmias and sudden death, and there are no effective therapeutic drugs at end stage and heart transplantation is the most effective treatment for survival.[4] RCM, which is sometimes familial, is a rare form of cardiomyopathy, characterized by impaired ventricular filling with normal or decreased diastolic volume of either, or both ventricles and normal, or near normal, systolic function and wall thickness.[5,6] ARVC is characterized by progressive fibrofatty replacement of right ventricular myocardium, with palpitations, syncope, and sudden death. Its prevalence is between 1/1000 and 1/5000, with 10% of deaths occurring before age 19 and 50% before age 35.[1,7]

Since Geisterfer-Lowrance, et al.[8] reported the first HCM-causing mutation of β-myosin heavy chain (β-MyHC) gene in 1990, a large number of mutations in sarcomeric protein genes that encode β-MyHC, cardiac troponin T (cTnT), cardiac troponin I (cTnI), cardiac troponin C...
(cTnC), α-tropomyosin (α-Tm), cardiac myosin binding protein C (MyBP-C), ventricular myosin light chains 1 and 2 (LC1, LC2), actin and titin/connection, etc. [28-30] as well as cytoskeletal and nuclear membrane protein genes that encode dystrophin, desmin, tafazzin, δ-sarcoglycan, lamin A/C, etc. [17-20] have been identified as a cause of HCM, DCM and RCM. In contrast, no sarcomeric protein genes have been shown to be responsible for ARVC. Molecular genetic testing has so far indicated that ARVC is associated with more than eight genes which encode the transforming growth factor β-3 gene (TGFβ3), ryanodine receptor 2, desmoplakin, transmembrane protein 43, junction plakoglobin (also known as gamma catenin), etc. [21,22] Many studies have been made on the structural and/or functional consequences of mutations, but the molecular mechanisms by which mutations found in the above genes lead to the pathogenesis of various forms of cardiomyopathy are not entirely clear.

Sarcomere, a structural unit of the contractile apparatus myofibril in cardiac muscle, has thick and thin filaments, which are composed of myosin and actin/tropomyosin/troponin complex, respectively. The molecular mechanism of which are composed of myosin and actin/tropomyosin/troponin in cardiac muscle, has thick and thin filaments, actomyosin Mg2+-ATPase. Until now, there have been over 100 mutations found in three cTnT subunits causing HCM, DCM or RCM. This article focuses on the molecular mechanisms underlying the pathogenesis of cardiomyopathies caused by these mutations.

2 Troponin complex

Cardiac muscle contraction is generated by the interaction between myosin head and thin filament, upon activated actomyosin Mg2+-ATPase. [25,26] Thin filament is composed of actin, Tm and Tn. Actin molecules polymerize into a double helical filament, which forms the backbone of the thin filament. Tm is an α-helical coiled-coil fibrous protein interacting with adjacent Tm molecules in a head to tail manner and is located along polymerized actin together with Tn complex at a actin: Tm: Tn ratio of 7:1:1. [27,28] Tn consists of three subunits: TnT, which anchors Tn complex to Tm, also interacts directly with actin; TnI, which is involved in the inhibition of actomyosin Mg2+-ATPase and inhibits actin-myosin interactions at diastolic levels of intracellular Ca2+; TnC, which is a Ca2+-binding subunit that removes TnI inhibition upon Ca2+ binding. Tn plays a key role in the transition from diastole to systole of cardiac muscle. When the cytoplasmic Ca2+ concentration of cardiac myocytes is low around 2 × 10−7 mol/L, the contractile interaction of actin with myosin head is inhibited by Tn-Tm. When cytoplasmic Ca2+ concentration is raised by electrochemical coupling involving a membrane controlled release of Ca2+ into sarcoplasm and Ca2+ mobilization known as calcium-induced calcium release, Ca2+ binds to TnC and triggers a series of conformational changes of protein-protein interactions in the thin filament, which relieves the inhibition by Tn-Tm and thus promotes the contractile interaction between actin and myosin. [29-32]

2.1 cTnT mutations in HCM, DCM and RCM

TnT has two functionally and structurally distinct domains named T1 and T2. [33,34] T1 (N-terminal region of TnT) anchors Tn complex to the thin filament through its strongly binding to Tm. On the other hand, T2 (C-terminal region of TnT) interacts with other Tn subunits (TnI and TnC) and Tm/actin. [35,39] The molecular weight of TnT is 31–36 ku, with 250–300 amino acid residues. cTnT is a peptide of 288 amino acid residues with approximate 35 ku of molecular weight. In human heart, there are four cTnT isoforms (cTnT1-cTnT4) produced by alternative splicing of cTnT transcripts at exon 4 and exon 5. [40,41] It is suggested that the alternative splicing of cTnT may contribute to altered myofilament response to Ca2+ in cardiac muscle contraction. [40] cTnT is a substrate for protein kinase C (PKC) containing up to four potential phosphorylation sites. Sumandea, et al. [42] reported that phosphorylation at Thr-206 significantly reduces maximum force generation, Ca2+-sensitivity and cross-bridge cycling rate, suggesting that PKC-mediated phosphorylation of cTnT may play a role in the regulation of cardiac contraction. Studies on inherited cardiomyopathies caused by cTnT mutations have revealed an important function of this protein in the Ca2+ regulation of muscle contraction.

Since Thierfelder, et al. [14] found two missense mutations (Ile79Asn and Arg92Gln) and a splice donor site (intron 16G→A) mutation in cTnT in inherited HCM patients in 1994 and until only recently, a new mutation P80S was reported in a Japanese family, [43] 36 HCM-linked mutations, 13 DCM-linked mutations and 4 RCM-linked mutations have been reported in cTnT (Figure 1).

Mutations in cTnT account for approximately 15%-30% of all HCM case. They cause only mild cardiac hypertrophy in an autosomal dominant manner. However, they usually lead to poor prognosis and high incidence of sudden cardiac
death; e.g., I79N, R92W, R92Q and ΔE160, etc., induce a malignant clinical phenotype with the average life of patients being no more than middle age. The functional consequences of these mutations have been studied by many groups. We studied the functional effects of these HCM mutations using rabbit left ventricular skinned fibers and myofibrils with a cTnT-exchange technique. We found most of these HCM-linked cTnT mutations, such as I79N, R92Q, R92L, R92W, R94L, A104V, R130C, ΔE160, E163K, and E244D, etc., increased the Ca\(^{2+}\) sensitivity of force generation in skinned fibers and shifted the force-pCa relationship leftwards, but did not significantly affect the maximum force generating capability or ATPase activity and Ca\(^{2+}\)-cooperativity. On the other hand, R278C, R278P, TnTΔ28(+7) and TnTΔ14 decreased Ca\(^{2+}\)-cooperativity in force generation in skinned fibers in addition to a Ca\(^{2+}\)-sensitizing effect. Almost all HCM-causing mutations occur in the Tm-anchoring region (residues 79-170 and 272-288) in cTnT, strongly suggesting that these mutations impair the interaction of cTnT and Tm leading to an increase in the Ca\(^{2+}\)-sensitivity by decreasing the inhibitory action of cTnI on the thin filament. Recently, Manning, et al. suggested that the cTnT HCM-related mutations in, or flanking, the N-tail T1 domain (residues 79-170) directly interacting with overlapping Tm, may alter the flexibility of T1, which is inversely proportional to Ca\(^{2+}\)-cooperativity.

In 2000, a deletion mutation ΔK210 of cTnT was reported as the first DCM-causing mutation of sarcomeric proteins. Since then, this mutation has extensively been studied both in vitro and in vivo. With the cTnT-exchange technique, we have shown that this mutation confers a lower Ca\(^{2+}\)-sensitivity on the force generation of skinned cardiac muscle fibers and ATPase activity of cardiac myofibrils compared with wild-type, while having no effects on the maximum force, or ATPase activity, and Ca\(^{2+}\)-cooperativity. Other groups also reported similar results. Almost all DCM-causing mutations reported in cTnT to date, e.g., R131W, R141W, R205, ΔK210, R205L, and D270N, etc., have demonstrated a Ca\(^{2+}\)-desensitizing effect on skinned fiber force generation and myofibrillar or actomyosin ATPase activity. These results strongly suggest that Ca\(^{2+}\)-desensitization of cardiac muscle contraction is a primary mechanism for the pathogenesis of DCM caused by cTnT mutation, in contrast to HCM where Ca\(^{2+}\)-sensitization is a primary mechanism for the pathogenesis. However, it is still not completely clarified how one amino acid mutation can increase or decrease the Ca\(^{2+}\)-sensitivity of force generation of cardiac muscle in the respective form of inherited cardiomyopathy. We demonstrated that the DCM-causing mutation of cTnT R141W increased the affinity of cTnT for Tm, by using a quartz-crystal microbalance. This result strongly suggests that R141W mutation in the strong Tm-binding region in cTnT strengthens the integrity of cTnI in the thin filament by stabilizing the interaction between cTnT and Tm, which might allow cTnI to inhibit the thin filament more effectively, leading to Ca\(^{2+}\)-desensitization.

RCM-causing mutations, unlike HCM- and DCM-causing mutations, are rare and recently found in cTnT (I79N, ΔE96, and E136K). Pinto, et al., reported that ΔE96 showed a large increase in the Ca\(^{2+}\)-sensitivity and impaired abilities to inhibit ATPase and to relax skinned fibers, which could contribute to the severe diastolic dysfunction in RCM. More recently, Pinto, et al., found a novel double deletion in cTnT of two highly conserved amino acids (N100 and E101) in a RCM pediatric patient, which also conferred a large increase in the Ca\(^{2+}\)-sensitivity. In addition, this double deletion mutation decreased the Ca\(^{2+}\)-cooperativity of force development, suggesting alterations in intra-filament protein-protein interactions.
2.2 cTnI mutations in HCM, DCM and RCM

cTnI binds to a specific region of each Tm molecule with a 38 nm periodicity along the Tm/actin filament.[75,76] cTnI is the inhibitory subunit of cTn complex, which is responsible for inhibition of actomyosin ATPase activity. In the absence of Ca\(^{2+}\), cTnI inhibits contraction through interacting with actin; the inhibitory effect of cTnI is relieved during muscle contraction through interacting with cTnC upon Ca\(^{2+}\) binding to cTnC. Hence, cTnI is a key regulatory protein in cardiac muscle contraction and relaxation cycle. cTnI consists of several functional domains, a cardiac specific N-terminal extension region (residues 1-32), an IT-arm region, the inhibitory region (residues 128-147), the switch region (residues 147-163), and the C-terminal mobile domain.[77,78] Phosphorylation of cTnI by several different kinases plays important roles in the regulation of cardiac muscle contraction under physiological or pathological conditions. Protein kinase A (PKA)-mediated phosphorylation of cTnI at Ser-23/Ser-24 reduces myofilament Ca\(^{2+}\)-sensitivity, increases the rate of Ca\(^{2+}\) dissociation from cTnC, increases crossbridge cycling rate, and enhances unloaded shortening velocity.[79-83] PKC may phosphorylate cTnI at Ser-23/Ser-24, Ser-43/Ser-45 and Thr-144; phosphorylation at Ser-43/Ser-45 decreases maximum Ca\(^{2+}\)-activated force generation in skinned fibers and maximal sliding velocity in motility assays.[84,85] We have recently found that cTnI is phosphorylated by PKC at Ser-23/Ser-24 and Thr-144, which leads to the depressed cooperative activation of the thin filament.[86] Phosphorylation of cTnI at different sites by different protein kinases may have distinct effects in vivo, although they are poorly understood.[87-90] The effects of cTnI phosphorylation could be affected by some mutations that cause inherited cardiomyopathies.

Thirty, four, and nine mutations of cTnI have so far been found in HCM, DCM, and RCM, respectively.[70,74,91] most of which are located in the C-terminal region (Figure 2). In 1997, Kimura, et al.[15] reported six mutations in cTnI (R145G, R145Q, R162W, ΔK183, G203S, and K206Q) associated with HCM in an autosomal dominant manner. Studies on the functional consequences of these mutations at the level of skinned cardiac muscle force generation and myofibrillar ATPase activity revealed that they have a Ca\(^{2+}\)-sensitizing effect on cardiac muscle contraction as in the HCM-linked mutations in cTnT.[92] Studies of the Ca\(^{2+}\) affinity of reconstituted cardiac thin filament with a fluorescence (IAANS) labeling technique revealed that R145G, which is located in the inhibitory region of cTnI, increases the basal level of ATPase activity and increases the Ca\(^{2+}\) binding affinity of the regulatory site of cTnC in the thin filament.[93] Mutations of R145G, R145Q, R162W, and G203S show increased minimum force in skinned cardiac muscle fibers with no significant changes in the maximum force. A deletion mutation ΔK183 increases the Ca\(^{2+}\)-sensitivity with no effects on either maximum or minimum force generation of skinned cardiac muscle.

Figure 2. Distribution of mutations in human cardiac troponin I associated with HCM, DCM and RCM. DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; RCM: restrictive cardiomyopathy; Tm: tropomyosin; TnC: troponin C; TnI: troponin I.
Until 2009, only one mutation A2V was reported to cause DCM in an autosomal recessive manner, which impairs the interaction of cTnI with cTnT.[94] In 2009, Carballo, et al.[91] reported that the mutations of K36Q and N185K of cTnI cause DCM in an autosomal dominant manner. K36Q has been shown to mediate the movement of the N-terminal region in cTnI upon phosphorylation of S22/23 by cAMP-dependent protein kinase.[95] Functional studies of K36Q and N185K have revealed that these DCM mutations of cTnI decrease the maximum activity and Ca\(^2+\)-sensitivity of actin-myosin S1 ATPase and significantly reduce the Ca\(^2+\)-binding affinity of the regulatory site of cTnC in the thin filament. More recently, Murakami, et al.[96] reported a new missense mutation P16T in DCM patients.

In 2003, Mogensen, et al.[97] published the first report of cTnI mutations in RCM patients (L144Q, R145W, A171T, K178E, D190G, and R192H). We examined the functional and structural consequences of these six mutations by using in vitro assays of skinned cardiac muscle fibers and NMR and revealed that they cause dramatic Ca\(^2+\)-sensitization of force generation in cardiac muscle with a subtle structural perturbation within cTnI molecule.[98] Ca\(^2+\)-sensitizing effects of these RCM mutations are much greater than those of HCM mutations in cTnI. However, D190G mutation with the smallest Ca\(^2+\)-sensitizing effect was reported to cause both RCM and HCM in a single large family, suggesting that RCM and HCM are caused by the same mechanism through Ca\(^2+\)-sensitization with greater sensitization leading to RCM. In addition, all six mutations elevate the resting force of skinned cardiac muscle fibers at low Ca\(^2+\) concentrations, and four mutations, L144Q, R145W, A171T, and R192H decrease the maximum level of force generation at high Ca\(^2+\) concentrations.

2.3 cTnC mutations in HCM, DCM and RCM

Troponin C is a member of the EF-hand family of Ca\(^2+\)-binding proteins and consists of two globular lobes at N- and C-terminus, which are connected with an α-helix.[99] Each globular lobe contains a pair of Ca\(^2+\)-binding sites, hence there are four Ca\(^2+\)-binding sites named I through IV from the N-terminal of vertebrate skeletal TnC (sTnC). In contrast to sTnC, site I of cTnC does not bind Ca\(^2+\) under physiological conditions. Analysis of equilibrium Ca\(^2+\)-binding of cTnC demonstrates that the dissociation constant (Kd) of site II is approximate 1.2 × 10\(^8\) M\(^{-1}\), and plays an important Ca\(^2+\)-regulatory role in cardiac muscle contraction. On the other hand, the dissociation constant (Kd) of sites III and IV are approximate 7.4 × 10\(^7\) M\(^{-1}\). Sites III and IV also bind Mg\(^2+\) with Kd at 0.9 × 10\(^7\) M\(^{-1}\).[134] play a structural role to keep the C lobe tightly bound to cTnI and to stabilize the interaction between cTnC and cTnT.[100]

Compared with cTnI and cTnT, much fewer mutations have so far been found in cTnC, with only six mutations both in HCM and DCM. Functional analyses suggested that the HCM-linked mutations A8V, C84Y and D145E increase the Ca\(^2+\)-sensitivity of force generation in skinned cardiac muscle fibers.[101] A8V and D145E also increase the force recovery. In contrast to these mutations, E134D shows no changes in Ca\(^2+\)-sensitivity and force recovery. Fluorescence studies using IAANS-labeled cTnC suggested that L29Q increases the Ca\(^2+\)-binding affinity of site II of cTnC.[102] Most recently, a new mutation A31S in cTnC was identified to cause HCM.[103] Functional studies indicated that this mutation increased the Ca\(^2+\)-sensitivity with no effect on the maximal force generation, and increased the Ca\(^2+\)-affinity of cTnC isolated or incorporated into troponin complex and thin filament.

The first DCM-linked mutation found in cTnC is G159D.[104] In contrast to the DCM-linked mutations found in cTnT and cTnI, G159D mutation in cTnC does not significantly decrease the Ca\(^2+\)-sensitivity of force generation of skinned fibers, but it induces a decrease in ATPase activity of reconstituted myofilaments as well as filament sliding velocity.[105] Interestingly, Biesiadecki, et al.[106] provide evidence that G159D may alter myocardial functional responses to β-adrenergic stimulation by blunting the Ca\(^2+\)-desensitizing effect of cTnI phosphorylation at Ser-23/Ser-24. Afterward, two novel missense mutations localized in the regulatory Ca\(^2+\)-binding site II of cTnC, E59D and D75Y, were identified in a DCM patient.[107] These mutations showed a decrease in the myofilament Ca\(^2+\)-sensitivity and Ca\(^2+\)-binding affinity in the force-pCa relationship measurements and fluorescence spectroscopy, respectively. D75Y disrupts molecular motions critical for Ca\(^2+\) binding and cardiomyocyte contractility, whereas functional defect caused by E59D is benign. Most recently, Pinto, et al.[108] reported Y5H, M103I, and I148V mutations in cTnC in DCM patients. Functional studies showed that Y5H and M103I decrease the Ca\(^2+\)-sensitivity of force generation and that the effects of PKA phosphorylation of cTnI on the myofilament Ca\(^2+\)-sensitivity is abolished by M103I but diminished by Y5H and I1148V.

3 Animal models of cardiomyopathies caused by troponin mutations

In order to determine the significance of the in vitro findings concerning inherited cardiomyopathies, especially to validate whether the shift in cardiac myofilament Ca\(^2+\)-sensitivity is the most important and a common cause of...
cardiomyopathies associated with Tn mutations in vivo, it is necessary and important to produce animal models. We have succeeded to create a knock-in mouse model in which a deletion of three base pairs coding for K210 in cTnT found in DCM patients was introduced into mouse endogenous cTnT gene using gene-targeting technology. This mouse model showed a particularly high incidence of sudden death in their growth periods from one to three months old, and enlarged hearts and heart failure, closely recapitulating the phenotypes of human DCM patients. Surface ECG showed that mutant mice commonly had an electrophysiological abnormality in the heart with long QT, which might be involved in their frequent sudden death. Telemetric ECG recordings showed that mice died by abruptly developing repetitive Torsade de Pointes several hours before death, which ultimately degenerated into ventricular fibrillation. Consistent with the results of in vitro studies, skinned cardiac muscle fibers prepared from mutant mice showed significantly lower Ca\(^{2+}\)-sensitivity in force generation. Ca\(^{2+}\)-sensitive transient analysis using fura-2 in cardiomyocytes showed a significant increase in the peak amplitude in mutant mice, suggesting that Ca\(^{2+}\) transient is augmented to compensate for decreased myofilament Ca\(^{2+}\)-sensitivity. An increased intracellular cAMP level might be responsible for the augmented Ca\(^{2+}\) transient in mutant mice.

Hernandez, et al. created HCM-causing F110I- and R278C-cTnT transgenic mice. Skinned fibers prepared from F110I-cTnT transgenic mice showed an increased Ca\(^{2+}\)-sensitivity of force and ATPase activity, and a much increased energy cost. In contrast, no changes in force or the ATPase-pCa dependencies were observed in transgenic R278C-cTnT skinned fibers. The increased Ca\(^{2+}\)-sensitivity and higher energy cost in F110I-cTnT hearts may be responsible for the severe phenotype compared with R278C-cTnT. This result supports the hypothesis that a greater shift in Ca\(^{2+}\)-sensitivity of force development results in more severe clinical phenotype and prognosis.

A knock-in mouse model of HCM caused by cTnI mutation of R21C also showed an increased Ca\(^{2+}\)-sensitivity of force development, consistent with the findings in mouse models of HCM caused by cTnT mutations. Heterozygous and homozygous mutant mice both developed a remarkable degree of cardiac hypertrophy and fibrosis. R21C is the only mutation identified within the unique N-terminus of cTnI and is located in the region close to β-adrenergic-activated PKA-mediated phosphorylation sites. Heterozygous mice reduced and homozygous mice abolished the well-known decrease in the Ca\(^{2+}\)-sensitivity of force generation by the phosphorylation of cTnI at Ser23/Ser24 with PKA, suggesting that the impaired regulation of myofilament Ca\(^{2+}\) sensitivity by PKA phosphorylation in cTnI may also play a role in pathogenesis of HCM caused by this mutation.

Although studies using knock-in and transgenic mouse models have provided much important and useful information, we have to note that mouse models are different from human in Ca\(^{2+}\) handling during contraction/relaxation and in alterations in Ca\(^{2+}\) flux during heart failure. Sanbe, et al., made a HCM-causing R146G-cTnI transgenic rabbit model that reflects the human system more accurately. This rabbit model also showed an increased Ca\(^{2+}\)-sensitivity of skinned fibers prepared from ventricular papillary muscle, as well as cardiomyocyte disarray, fibrosis, and altered connexin43 organization, which recapitulate the human HCM phenotype. Human patients should have different contractile dynamics in cardiac muscle compared with mouse models, because mouse heart mainly expresses the α-myosin heavy chain (MHC) isoform, whereas human heart mainly expresses the β-MHC isoform with much lower ATPase activity. Ford, et al., recently created a mouse model expressing HCM-related mutation R92L cTnT and β-MHC by crossbreeding the transgenic mouse strain expressing R92L cTnT with the transgenic mouse strain predominantly expressing the β-MHC isoform. They found that the length-dependence of contractile activation and myofilament Ca\(^{2+}\)-sensitivity was blunted in these mice, suggesting that the MHC isoform has an important effect on the outcome of cTnT mutations.

4 Conclusion

Many functional consequences of cTn mutations in inherited cardiomyopathies have been revealed, such as changes in regulatory protein-protein interaction, alterations of crossbridge cycling rate or unloaded shortening velocity, and alterations of ATPase activity or phosphorylation level of key regulatory proteins like cTnI. However, it should be noted that almost all mutations in cTn shift the Ca\(^{2+}\)-sensitivity of cardiac muscle contraction. Recently, Liu, et al., demonstrated by fluorescence studies that cardiomyopathy-related cardiac Tn mutations alter the Ca\(^{2+}\) association and dissociation rates of cTnC in the thin filament, also implying that the alteration of steady state Ca\(^{2+}\)-sensitivity of the thin filament by the mutations in cTn may be a primary cause for cardiomyopathies. Changes in Ca\(^{2+}\)-sensitivity and subsequent alteration of Ca\(^{2+}\) homeostasis could be a common, and the most important underlying mechanisms that trigger the arrhythmias leading to cardiac sudden death or the development of congestive heart failure. To fully understand the pathogenic effect of these mutations, it is necessary to examine the effects of mutations on calcium ions and their regulatory proteins.
mechanism of inherited cardiomyopathies and their therapies, it is important to generate animal models that closely recapitulate human pathological features. We attempted to treat the DCM-causing knock-in ΔK210-cTnT mice with a Ca\(^{2+}\) sensitizer pimobendan, which was expected to be beneficial for the mice with decreased cardiac myofilament Ca\(^{2+}\)-sensitivity. Pimobendan significantly improve the life span and cardiac function of this DCM mice model.\(^{[107]}\) This result strongly supports the hypothesis that Ca\(^{2+}\) desensitization of cardiac muscle contraction is a primary mechanism for the pathogenesis of DCM caused by this cTnT mutation, which has been led by our \textit{in vitro} studies, and suggests that Ca\(^{2+}\) sensitizers might be beneficial for the treatment of DCM patients associated with the sarcomeric regulatory protein mutations that cause myofilament Ca\(^{2+}\) desensitization, including ΔK210 cTnT mutation. On the other hand, Ca\(^{2+}\) desensitizers might be beneficial for the treatment of HCM, in which increased Ca\(^{2+}\)-sensitivity could be a primary pathogenic mechanism. Further studies using animal models closely recapitulating human disease phenotypes are important to develop therapeutic drugs for inherited cardiomyopathy patients.

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