Review   · **Open Access** ·

Inherited cardiomyopathies caused by troponin mutations

Qun-Wei Lu[1], Xiao-Yan Wu[2], Sachio Morimoto[3]

[1]*Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Center for Human Genome Research, Huazhong University of Science and Technology, Wuhan 430074, China*
[2]*Department of Pediatrics, Union Hospital Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*
[3]*Department of Clinical Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*

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.

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).[1] 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 nor-

mal, 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*.,[2,8-16] 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 (TGFB3), 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 Ca^{2+} regulation of muscle contraction has extensively been investigated since Dr. Ebashi discovered troponin in striated muscle contraction system.[23,24] It is now common knowledge that troponin consists of three subunits with distinct function and structure, in which TnT anchors Tn complex to Tm, TnI inhibits the actin-myosin contractile interaction, and TnC removes the inhibitory action of TnI upon binding Ca^{2+}. Until now, there have been over 100 mutations found in three cTn 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 Mg^{2+}-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 Mg^{2+}-ATPase and inhibits actin-myosin interactions at diastolic levels of intracellular

Ca^{2+}; TnC, which is a Ca^{2+}-binding subunit that removes TnI inhibition upon Ca^{2+} binding. Tn plays a key role in the transition from diastole to systole of cardiac muscle. When the cytoplasmic Ca^{2+} 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 Ca^{2+} concentration is raised by electrochemical coupling involving a membrane controlled release of Ca^{2+} into sarcoplasm and Ca^{2+} mobilization known as calcium-induced calcium release, Ca^{2+} 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 Ca^{2+} 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, Ca^{2+}-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 Ca^{2+} regulation of muscle contraction.

Since Thierfelder, *et al*.[14] found two missense mutations (Ile79Asn and Arg92Gln) and a splice donor site (intron $16G_1 \rightarrow A$) 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
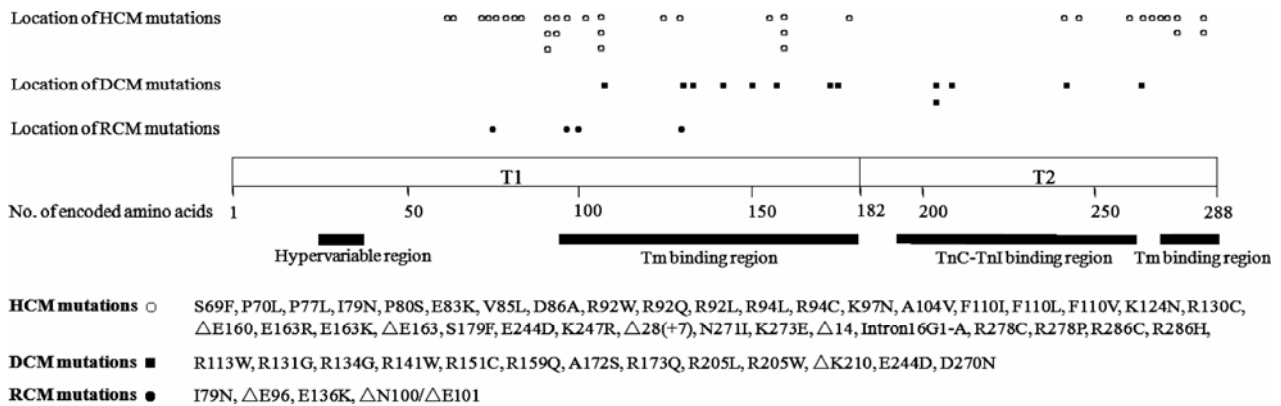
Figure 1. Distribution of mutations in human cardiac troponin T associated with HCM, DCM and RCM. DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; RCM: restrictive cardiomyopathy; Tm: tropomyosin; TnC: troponin C; TnI: troponin I.

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.[13,14,44,45] The functional consequences of these mutations have been studied by many groups.[44,46-53] We studied the functional effects of these HCM mutations using rabbit left ventricular skinned fibers and myofibrils with a cTnT-exchange technique.[54,55] We found most of these HCM-linked cTnT mutations, such as I79N, R92Q, R92L, R92W, R94L, A104V, R130C, ΔE160, E163R, 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, $TnT_{\Delta 28(+7)}$ and $TnT_{\Delta 14}$ decreased Ca^{2+}-cooperativity in force generation in skinned fibers in addition to a Ca^{2+}-sensitizing effect.[56-61] Almost all HCM-causing mutations occur in the Tm-anchoring region (residues 79-170 and 272-288) in cTnT,[62] 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.*[63] 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.[64] 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.[65] Other groups also reported similar results.[66-68] 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.[65,68-70] 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.[69] 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).[71,72] Pinto, *et al.*,[73] 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.*,[74] 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.
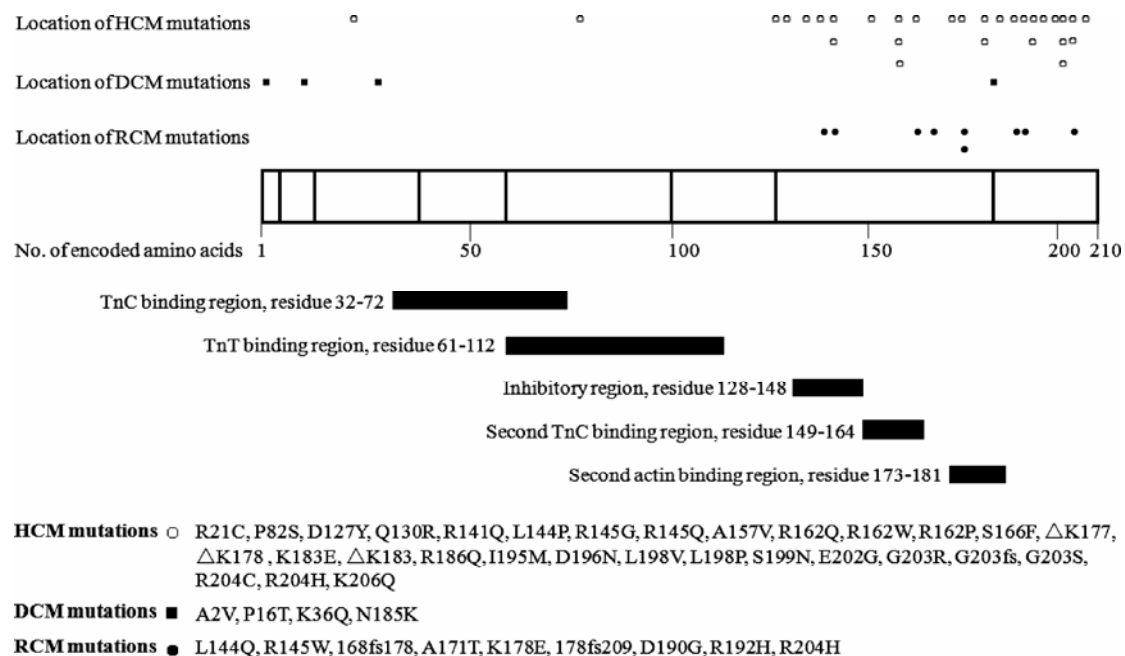

Figure 2. Distribution of mutations in human cardiac troponin I associated with HCM, DCM and RCM. DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; RCM: restrictive cardiomyopathy; Tm: tropomyosin; TnC: troponin C; TnI: troponin I.

Until 2009, only one mutation A2V was reported to cause DCM in an autosomal recessive mannar, 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 dose not bind Ca^{2+} under physiological conditions. Analysis of equilibrium Ca^{2+} binding of cTnC demonstrates that the dissociation constant (*K*d) of site II is approximate 1.2×10^6 M^{-1}, and plays an important Ca^{2+}-regulatory role in cardiac muscle contraction. On the other hand, the dissociation constant (*K*d) of sites III and IV are approximate 7.4×10^7 M^{-1}. Sites III and IV also bind Mg^{2+} with *K*d at 0.9×10^3 M^{-1},[34] 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 cTnT and cTnI, 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.[68] Interestingly, Biesiadecki, *et al*.[89] 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.[105] 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*.[106] 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 I148V.

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.[107] 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.[64,108] 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+} 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.*[109] 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.[110,111]

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.[88] 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.[112] 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 myo-

filament 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.*,[113] 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.*,[114] recently created a mouse model expressing HCM-related mutation R92L cTnT and β-MHC by crossing 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 cariomyopathies 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.[115,116] However, it should be noted that almost all mutations in cTn shift the Ca^{2+}-sensitivity of cardiac muscle contraction. Recently, Liu, *et al.*,[117] 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.[53,90,117,118] To fully understand the pathogenic

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 *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.

Acknowledgements

References

1 Richardson P, McKenna W, Bristow M, *et al*. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 1996; 93: 841–842.

2 Morimoto S. Sarcomeric proteins and inherited cardiomyopathies. *Cardiovasc Res* 2008; 77: 659–666.

3 Seidman CE, Seidman JG. Identifying sarcomere gene mutations in hypertrophic cardiomyopathy: a personal history. *Circ Res* 2011; 108: 743–750.

4 Dec GW, Fuster V. Idiopathic dilated cardiomyopathy. *N Engl J Med* 1994; 331: 1564–1575.

5 Rivenes SM, Kearney DL, Smith EO, *et al*. Sudden death and cardiovascular collapse in children with restrictive cardiomyopathy. *Circulation* 2000; 102: 876–882.

6 Siegel RJ, Shah PK, Fishbein MC. Idiopathic restrictive cardiomyopathy. *Circulation* 1984; 70: 165–169.

7 Hamilton RM. Arrhythmogenic right ventricular cardiomyopathy. *Pacing Clin Electrophysiol* 2009; 32 Suppl 2: S44–S51.

8 Geisterfer-Lowrance AA, Kass S, Tanigawa G, *et al*. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell* 1990; 62: 999–1006.

9 Poetter K, Jiang H, Hassanzadeh S, *et al*. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* 1996; 13: 63–69.

10 Bonne G, Carrier L, Bercovici J, *et al*. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. *Nat Genet* 1995; 11: 438–440.

11 Satoh M, Takahashi M, Sakamoto T, *et al*. Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. *Biochem Biophys Res Commun* 1999; 262: 411–417.

12 Mogensen J, Klausen IC, Pedersen AK, *et al*. Alpha-cardiac actin is a novel disease gene in familial hypertrophic cardiomyopathy. *J Clin Invest* 1999; 103: R39–R43.

13 Watkins H, McKenna WJ, Thierfelder L, *et al*. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N Engl J Med* 1995; 332: 1058–1064.

14 Thierfelder L, Watkins H, MacRae C, *et al*. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell* 1994; 77: 701–712.

15 Kimura A, Harada H, Park JE, *et al*. Mutations in the cardiac troponin I gene associated with hypertrophic cardiomyopathy. *Nat Genet* 1997; 16: 379–382.

16 Olson TM, Michels VV, Thibodeau SN, *et al*. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. *Science* 1998; 280: 750–752.

17 Towbin JA, Hejtmancik JF, Brink P, *et al*. X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. *Circulation* 1993; 87: 1854–1865.

18 Li D, Tapscoft T, Gonzalez O, *et al*. Desmin mutation responsible for idiopathic dilated cardiomyopathy. *Circulation* 1999; 100: 461–464.

19 Sakamoto A, Ono K, Abe M, *et al*. Both hypertrophic and dilated cardiomyopathies are caused by mutation of the same gene, delta-sarcoglycan, in hamster: an animal model of disrupted dystrophin-associated glycoprotein complex. *Proc Natl Acad Sci USA* 1997; 94: 13873–13878.

20 Tsubata S, Bowles KR, Vatta M, *et al*. Mutations in the human delta-sarcoglycan gene in familial and sporadic dilated cardiomyopathy. *J Clin Invest* 2000; 106: 655–662.

21 McNally E, MacLeod H, Dellefave L. Arrhythmogenic Right

Ventricular Dysplasia/Cardiomyopathy, Autosomal Dominant. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online), 2008; initial posting, 2005. Available at: http://www.genetests.org.

22 Moric-Janiszewska E, Markiewicz-Loskot G. Review on the genetics of arrhythmogenic right ventricular dysplasia. *Europace* 2007; 9: 259–266.

23 Ebashi S. Third Component Participating in the Superprecipitation of 'Natural Actomyosin'. *Nature* 1963; 200: 1010.

24 Ebashi S, Ebashi F. A New Protein Component Participating in the Superprecipitation of Myosin B. *J Biochem* 1964; 55: 604–613.

25 Craig R, Lehman W. Crossbridge and tropomyosin positions observed in native, interacting thick and thin filaments. *J Mol Biol* 2001; 311: 1027–1036.

26 Solaro RJ, Van Eyk J. Altered interactions among thin filament proteins modulate cardiac function. *J Mol Cell Cardiol* 1996; 28: 217–230.

27 Farah CS, Reinach FC. The troponin complex and regulation of muscle contraction. *FASEB J* 1995; 9: 755–767.

28 Ohtsuki I, Morimoto S. Troponin: regulatory function and disorders. *Biochem Biophys Res Commun* 2008; 369: 62–73.

29 Bers DM. Calcium fluxes involved in control of cardiac myocyte contraction. *Circ Res* 2000; 87: 275–281.

30 Geeves MA, Holmes KC. The molecular mechanism of muscle contraction. *Adv Protein Chem* 2005; 71: 161–93.

31 Geeves MA, Holmes KC. Structural mechanism of muscle contraction. *Annu Rev Biochem* 1999; 68: 687–728.

32 Solaro RJ. Sarcomere control mechanisms and the dynamics of the cardiac cycle. *J Biomed Biotechnol* 2010*;*2010: 105648.

33 Ohtsuki I. Molecular arrangement of troponin-T in the thin filament. *J Biochem* 1979; 86: 491–497.

34 Kobayashi T, Solaro RJ. Calcium, thin filaments, and the integrative biology of cardiac contractility. *Annu Rev Physiol* 2005 67: 39–67.

35 Tanokura M, Tawada Y, Onoyama Y, *et al*. Primary structure of chymotryptic subfragments from rabbit skeletal troponin T. *J Biochem* 1981; 90: 263–265.

36 Tanokura M, Tawada Y, Ono A, *et al*. Chymotryptic subfragments of troponin T from rabbit skeletal muscle. Interaction with tropomyosin, troponin I and troponin C. *J Biochem* 1983; 93: 331–337.

37 Ohtsuki I, Yamamoto K, Hashimoto K. Effect of two C-terminal side chymotryptic troponin T subfragments on the Ca^{2+} sensitivity of superprecipitation and ATPase activities of actomyosin. *J Biochem* 1981; 90: 259–261.

38 Kobayashi T, Jin L, de Tombe PP. Cardiac thin filament regulation. *Pflugers Arch* 2008; 457: 37–46.

39 Franklin AJ, Baxley T, Kobayashi T, *et al*. The C-terminus of troponin T is essential for maintaining the inactive state of regulated actin. *Biophys J* 2012; 102: 2536–2544.

40 Gomes AV, Guzman G, Zhao J, *et al*. Cardiac troponin T isoforms affect the Ca^{2+} sensitivity and inhibition of force de-

velopment. Insights into the role of troponin T isoforms in the heart. *J Biol Chem* 2002; 277: 35341–35349.

41 VanBuren P, Alix SL, Gorga JA, *et al*. Cardiac troponin T isoforms demonstrate similar effects on mechanical performance in a regulated contractile system. *Am J Physiol Heart Circ Physiol* 2002; 282: H1665–H1671.

42 Sumandea MP, Pyle WG, Kobayashi T, *et al*. Identification of a functionally critical protein kinase C phosphorylation residue of cardiac troponin T. *J Biol Chem* 2003; 278: 35135–35144.

43 Otsuka H, Arimura T, Abe T, *et al*. Prevalence and distribution of sarcomeric gene mutations in Japanese patients with familial hypertrophic cardiomyopathy. *Circ J* 2012; 76: 453–461.

44 Chandra M, Rundell VL, Tardiff JC, *et al*. Ca^{2+} activation of myofilaments from transgenic mouse hearts expressing R92Q mutant cardiac troponin T. *Am J Physiol Heart Circ Physiol* 2001; 280: H705–713.

45 Pasquale F, Syrris P, Kaski JP, *et al*. Long-term outcomes in hypertrophic cardiomyopathy caused by mutations in the cardiac troponin T gene. *Circ Cardiovasc Genet* 2012; 5: 10–17.

46 Szczesna D, Zhang R, Zhao J, *et al*. Altered regulation of cardiac muscle contraction by troponin T mutations that cause familial hypertrophic cardiomyopathy. *J Biol Chem* 2000; 275: 624–630.

47 Miller T, Szczesna D, Housmans PR, *et al*. Abnormal contractile function in transgenic mice expressing a familial hypertrophic cardiomyopathy-linked troponin T (I79N) mutation. *J Biol Chem* 2001; 276: 3743–3755.

48 Knollmann BC, Blatt SA, Horton K, *et al*. Inotropic stimulation induces cardiac dysfunction in transgenic mice expressing a troponin T (I79N) mutation linked to familial hypertrophic cardiomyopathy. *J Biol Chem* 2001; 276: 10039–10048.

49 Knollmann BC, Potter JD. Altered regulation of cardiac muscle contraction by troponin T mutations that cause familial hypertrophic cardiomyopathy. *Trends Cardiovasc Med* 2001; 11: 206–212.

50 Harada K, Potter JD. Familial hypertrophic cardiomyopathy mutations from different functional regions of troponin T result in different effects on the pH and Ca^{2+} sensitivity of cardiac muscle contraction. *J Biol Chem* 2004; 279: 14488–14495.

51 Tardiff JC. Sarcomeric proteins and familial hypertrophic cardiomyopathy: linking mutations in structural proteins to complex cardiovascular phenotypes. *Heart Fail Rev* 2005; 10: 237–248.

52 Redwood C, Lohmann K, Bing W, *et al*. Investigation of a truncated cardiac troponin T that causes familial hypertrophic cardiomyopathy: Ca^{2+} regulatory properties of reconstituted thin filaments depend on the ratio of mutant to wild-type protein. *Circ Res* 2000; 86: 1146–1152.

53 Manning EP, Guinto PJ, Tardiff JC. Correlation of molecular and functional effects of mutations in cardiac troponin T

linked to familial hypertrophic cardiomyopathy: an integrative in silico/in vitro approach. *J Biol Chem* 2012; 287: 14515–14523.

54 Hatakenaka M, Ohtsuki I. Replacement of three troponin components with cardiac troponin components within single glycerinated skeletal muscle fibers. *Biochem Biophys Res Commun* 1991; 181: 1022–1027.

55 Hatakenaka M, Ohtsuki I. Effect of removal and reconstitution of troponins C and I on the Ca^{2+}-activated tension development of single glycerinated rabbit skeletal muscle fibers. *Eur J Biochem* 1992; 205: 985–993.

56 Morimoto S, Nakaura H, Yanaga F, *et al*. Functional consequences of a carboxyl terminal missense mutation Arg278Cys in human cardiac troponin T. *Biochem Biophys Res Commun* 1999; 261: 79–82.

57 Nakaura H, Yanaga F, Ohtsuki I, *et al*. Effects of missense mutations Phe110Ile and Glu244Asp in human cardiac troponin T on force generation in skinned cardiac muscle fibers. *J Biochem* 1999; 126: 457–460.

58 Harada K, Takahashi-Yanaga F, Minakami R, *et al*. Functional consequences of the deletion mutation deltaGlu160 in human cardiac troponin T. *J Biochem* 2000; 127: 263–268.

59 Takahashi-Yanaga F, Ohtsuki I, Morimoto S. Effects of troponin T mutations in familial hypertrophic cardiomyopathy on regulatory functions of other troponin subunits. *J Biochem* 2001; 130: 127–131.

60 Yanaga F, Morimoto S, Ohtsuki I. Ca^{2+} sensitization and potentiation of the maximum level of myofibrillar ATPase activity caused by mutations of troponin T found in familial hypertrophic cardiomyopathy. *J Biol Chem* 1999; 274: 8806–8812.

61 Morimoto S, Yanaga F, Minakami R, *et al*. Ca^{2+}-sensitizing effects of the mutations at Ile-79 and Arg-92 of troponin T in hypertrophic cardiomyopathy. *Am J Physiol* 1998; 275: C200–C207.

62 Palm T, Graboski S, Hitchcock-DeGregori SE, *et al*. Disease-causing mutations in cardiac troponin T: identification of a critical tropomyosin-binding region. *Biophys J* 2001; 81: 2827–2837.

63 Manning EP, Tardiff JC, Schwartz SD. Molecular effects of familial hypertrophic cardiomyopathy-related mutations in the TNT1 domain of cTnT. *J Mol Biol* 2012; 421: 54–66.

64 Kamisago M, Sharma SD, DePalma SR, *et al*. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med* 2000; 343: 1688–1696.

65 Morimoto S, Lu QW, Harada K, *et al*. Ca^{2+}-desensitizing effect of a deletion mutation DK210 in cardiac troponin T that causes familial dilated cardiomyopathy. *Proc Natl Acad Sci USA* 2002; 99: 913–918.

66 Robinson P, Mirza M, Knott A, *et al*. Alterations in thin filament regulation induced by a human cardiac troponin T mutant that causes dilated cardiomyopathy are distinct from those induced by troponin T mutants that cause hypertrophic car-

diomyopathy. *J Biol Chem* 2002; 277: 40710–40716.

67 Venkatraman G, Harada K, Gomes AV, *et al*. Different functional properties of troponin T mutants that cause dilated cardiomyopathy. *J Biol Chem* 2003; 278: 41670–41676.

68 Mirza M, Marston S, Willott R, *et al*. Dilated cardiomyopathy mutations in three thin filament regulatory proteins result in a common functional phenotype. *J Biol Chem* 2005; 280: 28498–28506.

69 Lu QW, Morimoto S, Harada K, *et al*. Cardiac troponin T mutation R141W found in dilated cardiomyopathy stabilizes the troponin T-tropomyosin interaction and causes a Ca^{2+} desensitization. *J Mol Cell Cardiol* 2003; 35: 1421–1427.

70 Willott RH, Gomes AV, Chang AN, *et al*. Mutations in Troponin that cause HCM, DCM AND RCM: what can we learn about thin filament function? *J Mol Cell Cardiol* 2010; 48: 882–892.

71 Peddy SB, Vricella LA, Crosson JE, *et al*. Infantile restrictive cardiomyopathy resulting from a mutation in the cardiac troponin T gene. *Pediatrics* 2006; 117: 1830–1833.

72 Kaski JP, Syrris P, Burch M, *et al*. Idiopathic restrictive cardiomyopathy in children is caused by mutations in cardiac sarcomere protein genes. *Heart* 2008; 94: 1478–1484.

73 Pinto JR, Parvatiyar MS, Jones MA, *et al*. A troponin T mutation that causes infantile restrictive cardiomyopathy increases Ca^{2+} sensitivity of force development and impairs the inhibitory properties of troponin. *J Biol Chem* 2008; 283: 2156–2166.

74 Pinto JR, Yang SW, Hitz MP, *et al*. Fetal cardiac troponin isoforms rescue the increased Ca^{2+} sensitivity produced by a novel double deletion in cardiac troponin T linked to restrictive cardiomyopathy: a clinical, genetic, and functional approach. *J Biol Chem* 2011; 286: 20901–20912.

75 Zhou X, Morris EP, Lehrer SS. Binding of troponin I and the troponin I-troponin C complex to actin-tropomyosin. Dissociation by myosin subfragment 1. *Biochemistry* 2000; 39: 1128–1132.

76 Ohtsuki I, Shiraishi F. Periodic binding of troponin C·I and troponin I to tropomyosin-actin filaments. *J Biochem* 2002; 131: 739–743.

77 Solaro RJ, Rosevear P, Kobayashi T. The unique functions of cardiac troponin I in the control of cardiac muscle contraction and relaxation. *Biochem Biophys Res Commun* 2008; 369: 82–87.

78 Baryshnikova OK, Li MX, Sykes BD. Modulation of cardiac troponin C function by the cardiac-specific N-terminus of troponin I: influence of PKA phosphorylation and involvement in cardiomyopathies. *J Mol Biol* 2008; 375: 735–751.

79 Metzger JM, Westfall MV. Covalent and noncovalent modification of thin filament action: the essential role of troponin in cardiac muscle regulation. *Circ Res* 2004; 94: 146–158.

80 Saeki Y, Shiozawa K, Yanagisawa K, *et al*. Adrenaline increases the rate of cross-bridge cycling in rat cardiac muscle. *J Mol Cell Cardiol* 1990; 22: 453–460.

81 Zhang R, Zhao J, Mandveno A, *et al*. Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation. *Circ Res* 1995; 76: 1028–1035.

82 Zhang R, Zhao J, Potter JD. Phosphorylation of both serine residues in cardiac troponin I is required to decrease the Ca^{2+} affinity of cardiac troponin C. *J Biol Chem* 1995; 270: 30773–30780.

83 Kentish JC, McCloskey DT, Layland J, *et al*. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. *Circ Res* 2001; 88: 1059–1065.

84 Burkart EM, Sumandea MP, Kobayashi T, *et al*. Phosphorylation or glutamic acid substitution at protein kinase C sites on cardiac troponin I differentially depress myofilament tension and shortening velocity. *J Biol Chem* 2003; 278: 11265–11272.

85 Pyle WG, Sumandea MP, Solaro RJ, *et al*. Troponin I serines 43/45 and regulation of cardiac myofilament function. *Am J Physiol Heart Circ Physiol* 2002; 283: H1215–H1224.

86 Lu QW, Hinken AC, Patrick SE, *et al*. Phosphorylation of cardiac troponin I at protein kinase C site threonine 144 depresses cooperative activation of thin filaments. *J Biol Chem* 2010; 285: 11810–11817.

87 Albury AN, Swindle N, Swartz DR, *et al*. Effect of hypertrophic cardiomyopathy-linked troponin C mutations on the response of reconstituted thin filaments to calcium upon troponin I phosphorylation. *Biochemistry* 2012; 51: 3614–3621.

88 Wang Y, Pinto JR, Solis RS, *et al*. Generation and functional characterization of knock-in mice harboring the cardiac troponin I-R21C mutation associated with hypertrophic cardiomyopathy. *J Biol Chem* 2012; 287: 2156–2167.

89 Biesiadecki BJ, Kobayashi T, Walker JS, *et al*. The troponin C G159D mutation blunts myofilament desensitization induced by troponin I Ser23/24 phosphorylation. *Circ Res* 2007; 100: 1486–1493.

90 Marston SB. How do mutations in contractile proteins cause the primary familial cardiomyopathies? *J Cardiovasc Transl Res* 2011; 4: 245–255.

91 Carballo S, Robinson P, Otway R, *et al*. Identification and functional characterization of cardiac troponin I as a novel disease gene in autosomal dominant dilated cardiomyopathy. *Circ Res* 2009; 105: 375–382.

92 Takahashi-Yanaga F, Morimoto S, Harada K, *et al*. Functional consequences of the mutations in human cardiac troponin I gene found in familial hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 2001; 33: 2095–2107.

93 Kobayashi T, Solaro RJ. Increased Ca^{2+} affinity of cardiac thin filaments reconstituted with cardiomyopathy-related mutant cardiac troponin I. *J Biol Chem* 2006; 281: 13471–13477.

94 Murphy RT, Mogensen J, Shaw A, *et al*. Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy. *Lancet* 2004; 363: 371–372.

95 Howarth JW, Meller J, Solaro RJ, *et al*. Phosphoryla-

tion-dependent conformational transition of the cardiac specific N-extension of troponin I in cardiac troponin. *J Mol Biol* 2007; 373: 706–722.

96 Murakami C, Nakamura S, Kobayashi M, *et al*. Analysis of the sarcomere protein gene mutation on cardiomyopathy - Mutations in the cardiac troponin I gene. *Leg Med* 2010; 12: 280–283.

97 Mogensen J, Kubo T, Duque M, *et al*. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. *J Clin Invest* 2003; 111: 209–216.

98 Yumoto F, Lu QW, Morimoto S, *et al*. Drastic Ca^{2+} sensitization of myofilament associated with a small structural change in troponin I in inherited restrictive cardiomyopathy. *Biochem Biophys Res Commun* 2005; 338: 1519–1526.

99 Sundaralingam M, Bergstrom R, Strasburg G, *et al*. Molecular structure of troponin C from chicken skeletal muscle at 3-angstrom resolution. *Science* 1985; 227: 945–948.

100 Takeda S, Yamashita A, Maeda K, *et al*. Structure of the core domain of human cardiac troponin in the Ca^{2+}-saturated form. 2003; 424: 35–41.

101 Landstrom AP, Parvatiyar MS, Pinto JR, *et al*. Molecular and functional characterization of novel hypertrophic cardiomyopathy susceptibility mutations in TNNC1-encoded troponin C. *J Mol Cell Cardiol* 2008; 45: 281–288.

102 Dweck D, Hus N, Potter JD. Challenging current paradigms related to cardiomyopathies. Are changes in the Ca^{2+} sensitivity of myofilaments containing cardiac troponin C mutations (G159D and L29Q) good predictors of the phenotypic outcomes? *J Biol Chem* 2008; 283: 33119–33128.

103 Parvatiyar MS, Landstrom AP, Figueiredo-Freitas C, *et al*. A mutation in TNNC1-encoded cardiac troponin C, TNNC1-A31S, predisposes to hypertrophic cardiomyopathy and ventricular fibrillation. *J Biol Chem* 2012; 287: 31845–31855.

104 Mogensen J, Murphy RT, Shaw T, *et al*. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 2004; 44: 2033–2040.

105 Lim CC, Yang H, Yang M, *et al*. A novel mutant cardiac troponin C disrupts molecular motions critical for calcium binding affinity and cardiomyocyte contractility. *Biophys J* 2008; 94: 3577–3589.

106 Pinto JR, Siegfried JD, Parvatiyar MS, *et al*. Functional characterization of TNNC1 rare variants identified in dilated cardiomyopathy. *J Biol Chem* 2011; 286: 34404–34412.

107 Du CK, Morimoto S, Nishii K, *et al*. Knock-in mouse model of dilated cardiomyopathy caused by troponin mutation. *Circ Res* 2007; 101: 185–194.

108 Hanson EL, Jakobs PM, Keegan H, *et al*. Cardiac troponin T lysine 210 deletion in a family with dilated cardiomyopathy. *J Card Fail* 2002; 8: 28–32.

109 Hernandez OM, Szczesna-Cordary D, Knollmann BC, *et al*. F110I and R278C troponin T mutations that cause familial hypertrophic cardiomyopathy affect muscle contraction in

transgenic mice and reconstituted human cardiac fibers. *J Biol Chem* 2005; 280: 37183–37194.

110 Gomes AV, Potter JD. Molecular and cellular aspects of troponin cardiomyopathies. *Ann N Y Acad Sci* 2004; 1015: 214–224.

111 Chang AN, Parvatiyar MS, Potter JD. Troponin and cardiomyopathy. Biochem *Biophys Res Commun* 2008; 369: 74–81.

112 Gomes AV, Harada K, Potter JD. A mutation in the N-terminus of troponin I that is associated with hypertrophic cardiomyopathy affects the Ca^{2+}-sensitivity, phosphorylation kinetics and proteolytic susceptibility of troponin. *J Mol Cell Cardiol* 2005; 39: 754–765.

113 Sanbe A, James J, Tuzcu V, *et al*. Transgenic rabbit model for human troponin I-based hypertrophic cardiomyopathy. *Circulation* 2005; 111: 2330–2338.

114 Ford SJ, Mamidi R, Jimenez J, *et al*. Effects of R92 mutations in mouse cardiac troponin T are influenced by changes in myosin heavy chain isoform. *J Mol Cell Cardiol* 2012; 53: 542–551.

115 Watkins H, Ashrafian H, Redwood C. Inherited cardiomyopathies. *N Engl J Med* 2011; 364: 1643–1656.

116 Chalovich JM. Disease causing mutations of troponin alter regulated actin state distributions. *J Muscle Res Cell Motil* 2012; 33: 493–499

117 Liu B, Tikunova SB, Kline KP, *et al*. Ca^{2+} association and dissociation rates. *PLoS One* 2012; 7: e38259.

118 Schober T, Huke S, Venkataraman R, *et al*. Myofilament Ca sensitization increases cytosolic Ca binding affinity, alters intracellular Ca homeostasis, and causes pause-dependent Ca-triggered arrhythmia *Circ Res* 2012; 111: 170–179.