REVIEW

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Inherited Cardiomyopathies as a Troponin Disease

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Abstract: Troponin, one of the sarcomeric proteins, plays a central role in the Ca^{2+} regulation of contraction in vertebrate skeletal and cardiac muscles. It consists of three subunits with distinct structure and function, troponin T, troponin I, and troponin C, and their accurate and complex intermolecular interaction in response to the rapid rise and fall of Ca^{2+} in cardiomyocytes plays a key role in maintaining the normal cardiac pump function. More than 200 mutations in the cardiac sarcomeric proteins, including myosin heavy and light chains, actin, troponin, tropomyosin, myosin-binding protein-C, and titin/connectin, have been found to cause various types of cardiomyopathy in human since 1990, and more than 60 mutations in human cardiac troponin subunits have been identified in dilated, hypertrophic, and restrictive forms of cardiomyopathy. In this review, we have focused on the mutations in the genes for human cardiac troponin subunits and discussed their functional consequences that might be involved in the primary mechanisms for the pathogenesis of these different types of cardiomyopathy. [The Japanese Journal of Physiology 54: 307–318, 2004]

Key words: human inherited disease, heart, cardiomyopathy, gene mutation.

Background

Cardiomyopathy, a disease of cardiac muscle, can be classified into three main forms, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and restrictive cardiomyopathy (RCM). DCM is characterized by abnormal ventricular chamber enlargement and impaired systolic function [1]; prognosis is poor, and patients often require heart transplantation. HCM, a major cause of premature sudden cardiac death, is characterized by ventricular muscle hypertrophy, especially in the interventricular septum and the diastolic dysfunction in part because of the hypertrophy itself, interstitial fibrosis, and/or myocyte disarrays [2]. RCM, the least common form of the three, is characterized by restrictive diastolic dysfunction with normal or near normal systolic function and wall thickness [3]; prognosis is poor, especially in the young, and patients often require heart transplantation, as in the case of DCM.

Recent genetic investigations of the gene in patients have in quite a short time successfully revealed a large number (>200) of mutations in the sarcomeric protein genes as a cause of various forms of cardiomyopathy. Responsible genes so far identified encode α- and β-myosin heavy chains, α-cardiac actin, cardiac troponin subunits, α-tropomyosin, cardiac myosin-binding protein-C, cardiac myosin essential light chain, cardiac myosin regulatory light chain, and cardiac titin/connectin. Interestingly, some sarcomeric protein genes, including the genes for troponin subunits, were found to be simultaneously responsible for different forms of cardiomyopathy. Mutations in the human cardiac troponin T (TnT) have been found in HCM [4] and DCM [5], and mutations in the human cardiac troponin I (TnI) have been found in HCM [6] and RCM [7] and possibly in DCM [8]. Only two missense mutations, L29Q and G159D, that have so far been reported in the human cardiac troponin C (TnC) were also found to be associated

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with HCM [9] and DCM [10, 11], respectively. The molecular mechanisms by which the mutations in these sarcomeric protein genes lead to the pathogenesis of distinct forms of cardiomyopathy, however, still have remained unclear.

A great many studies have been made to elucidate the molecular mechanisms by which troponin subunits regulate the muscle contraction since the discovery of troponin complex by Dr. S. Ebashi in 1963 [12]. Nevertheless, it is still difficult to predict the functional consequence responsible for the pathogenesis of cardiomyopathy from the position and kind of mutations found in troponin subunits. Because of the limitation of investigations using human tissues, other systems such as recombinant proteins and transgenic or gene targeting animal models have extensively been used to explore the effects of the mutations on the physiological function of troponin subunits and their contribution to the pathogenesis of cardiomyopathy. These lines of studies have also improved our understanding of the molecular mechanisms of calcium regulation in muscle contraction.

Muscle Contraction and Role of Troponin

The contraction of vertebrate striated muscle is initiated by an increase in the cytosolic Ca^{2+}, which is sensed by a sarcomeric Ca^{2+} binding protein troponin on the thin (actin) filaments in the contractile apparatus myofibrils. Troponin is a complex of three subunits, TnC, TnI, and TnT, which have distinct structure and functions (details below). Ca^{2+} binding to TnC suppresses the inhibition exerted on the thin filaments by TnI in collaboration with TnT and another major regulatory protein tropomyosin, leading to the force generation by allowing myosin heads to interact strongly with actin in the thin filaments [13]. Each striated muscle (i.e., fast skeletal, slow skeletal, and cardiac) expresses muscle-specific isoform of sarcomeric proteins for myosin heavy and light chains, actin, troponin subunits, tropomyosin, titin, and others. In regard to troponin subunits, three isoforms of TnT, fast skeletal muscle TnT (fsTnT), slow skeletal muscle TnT (ssTnT), and cardiac muscle TnT (cTnT), are encoded in different genes, which further produce several alternatively spliced variants (review [14]). There also exist three isoforms of TnI encoded in different genes producing no spliced variants, i.e., fast skeletal muscle TnI (fsTnI), slow skeletal muscle TnI (ssTnI), and cardiac muscle TnI (cTnI) (review [15]). Because cardiac and slow skeletal muscles express the identical isoform of TnC, there are only two isoforms of TnC with no spliced variants, i.e., fast skeletal muscle TnC (sTnC) and slow skeletal/cardiac muscle TnC (cTnC). The molecular functions of these tissue-specific isoforms of each troponin subunit are essentially the same despite their considerable differences in primary structure.

Isolated TnC is a dumbbell shape molecule with its N- and C-terminal globular domains being connected by a central α-helix [16, 17]. Each globular domain has two EF-hand metal-binding motifs termed sites I to IV from the N-terminus. Sites III and IV in the C-terminal domain of both sTnC and cTnC competitively bind Ca^{2+} and Mg^{2+} with high and low affinity, respectively [18]. Sites I and II in the N-lobe of sTnC bind Ca^{2+} almost specifically with much lower affinity than sites III and IV do under physiological conditions. In contrast, site I of cTnC is inactivated by the substitutions of key residues for Ca^{2+} coordination; thus only site II binds Ca^{2+} with low affinity [19, 20]. The Ca^{2+}-binding to both sites I and II of sTnC or to only site II of cTnC is believed to be responsible for the regulation of muscle contraction, based on the Ca^{2+}-binding kinetics that can explain the fast time course of muscle contraction and relaxation *in vivo* [21]. On the other hand, the binding of Ca^{2+} or Mg^{2+} to sites III and IV has been shown to contribute to the structural stability of troponin complex rather than the Ca^{2+} regulation *per se* [21–24].

A main functional role of TnI is to inhibit the contractile interaction between actin and myosin, and a small region in the middle of TnI molecule, called the inhibitory region, involves most parts of this inhibitory action. [25–27]. The inhibitory region of TnI binds to actin in the absence of Ca^{2+} [28], and it binds to the N-terminal domain of TnC in the presence of sufficient amount of Ca^{2+}, resulting in a release of the inhibitory action of TnI [29]. cTnI has ~30 additional amino acid residues at its N-terminus being absent in sTnI and ssTnI, which play functionally and/or structurally important roles in cardiac muscle specific physiology [30]. The two adjacent serine residues in this cardiac specific N-terminal region are phosphorylated by cAMP-dependent protein kinase A (PKA) upon β-adrenergic stimulation; this leads to multiple alterations in cardiac physiology, including a decrease in the Ca^{2+} sensitivity of contraction and an acceleration of relaxation.

TnT has a structural role in anchoring other troponin subunits to the thin filament through its binding to tropomyosin. This molecule has two functionally and structurally distinct domains, T_1 and T_2, named from the experiments of chymotryptic fragments of TnT [31]. The N-terminal rod like region of TnT, T_1, strongly binds to tropomyosin, and there

is no evidence of direct interaction between T_1 and TnI or TnC [32, 33]. On the other hand, the C-terminal domain of TnT, T_2, interacts with TnI, TnC, tropomyosin, and possibly actin [33, 34]. Besides the tissue-specific isoforms, several potential "sub-isoforms" of TnT are produced by alternative splicing. This splicing occurred near the N-terminal ends of all three isoforms of TnT (most frequently in fsTnT [35]) and also near the C-terminal ends in fast and slow skeletal TnTs [36, 37]. However, their functional contribution to the muscle regulation and thus their biological significance is still not clear. It is known that there are specific expression patterns of spliced variants in chicken breast and leg fast skeletal muscles [38] and in developing cardiac muscle [39]. The expression profile of cTnT spliced variants is also known to alter in the failing heart [40]. These facts suggest that the alternative splicing of TnT may play a role in modulating the muscle contraction in response to the environmental change. The molecular function of TnT is still not clearly understood in comparison with TnC and TnI. The findings of HCM- and DCM-causing mutations in cTnT associated with malignant phenotypes, however, revealed the extreme importance of this molecule in the Ca^{2+} regulation of muscle contraction, and recent *in vitro* studies on the cardiomyopathy-linked cTnT mutations greatly help our understanding of the molecular function of TnT.

TnT Mutations in Hypertrophic Cardiomyopathy

Three years after the Seidman group [41] first discovered a mutation in the β-myosin heavy chain associated with HCM, several mutations in other loci were found to be associated with HCM [42]. Those loci were then identified as the genes for cTnT and α-tropomyosin. Since then, more than 30 different mutations in the human cTnT gene have been found to cause HCM (Table 1 and Fig. 1). It has generally been described that HCM patients harboring cTnT mutations show no or mild cardiac hypertrophy; nevertheless, their prognosis is malignant. Indeed, several studies have demonstrated that compared to HCM associated with other genes, the incidence of sudden cardiac death is significantly higher despite a milder hypertrophy in HCM associated with the cTnT gene [43, 44]. However, these phenotypic features associated with HCM-causing cTnT mutations could not necessarily be applied to individual cases. Clinical reports from different research groups showed that the patients harboring the same mutation in cTnT had quite different clinical profiles [45–48].

TnT Mutations in Dilated Cardiomyopathy

The end stage of HCM sometimes shows DCM-like features, called a dilated phase of HCM, and this has often been seen in the HCM patients caused by cTnT mutations [49–51]. In 2000, Kamisago *et al*. reported a deletion mutation in the human cTnT gene, ΔK210, which is the first cTnT mutation responsible for familial primary DCM that is clearly distinguished from a dilated phase of HCM [5]. The same mutation has been identified in other DCM patients in a family independent from those studied by Kamisago *et al*. [52]. Since then, at least two other DCM-linked mutations in cTnT have been reported (Table 1 and Fig. 1).

TnI Mutations in Hypertrophic Cardiomyopathy

In 1997, Kimura *et al*. first reported that six mutations in the human cTnI gene were associated with HCM [6]. Although cTnI mutations are less common than cTnT mutations in HCM, currently more than 20 mutations have been found in the cTnI gene (Table 2 and Fig. 1). The prognoses of patients with cTnI-linked HCM vary from individual to individual and mutation to mutation as in the case of the cTnT-linked HCM. The ΔK183 mutation is clinically the most studied mutation in cTnI and has been shown to be involved in a malignant prognosis [6, 55–58]. In contrast, P82S and D196N mutations have been shown to exhibit a benign phenotype with late onset (>50 years old) [59].

TnI Mutation in Dilated Cardiomyopathy

An N-terminal mutation in cTnT, A2V, was recently found to cause DCM [8] (Table 2 and Fig. 1). The explanted heart from the proband showed the features of DCM, and his sister has also developed DCM. Both were homozygous for this mutation, but their parents and one remaining sibling were heterozygous and unaffected. Thus the mutation A2V in cTnI causes a rare case of DCM that is inherited in an autosomal recessive manner.

TnI Mutations in Restrictive Cardiomyopathy

In 2003, six novel missense mutations were found in the patients with idiopathic RCM, which is the first case that a specific gene has ever been shown to be responsible for RCM [7] (Table 2 and Fig. 1). A mis-

Table 1. Mutations in human cTnT and their functional consequences on the force generation of skinned cardiac muscle fibers.

Mutation	Type of mutation[†]	Type of cardiomyopathy	Ca^{2+}-dependent force generation of skinned cardiac fibers with cTnT mutations[‡]		
			Ca^{2+}-sensitivity	Maximum force	Ref.
A28V	M	HCM[a]	–	–	–
S69F	M	HCM[b]	–	–	–
F70L	M	HCM[c]	–	–	–
P77L	M	HCM[b]	–	–	–
I79N	M	HCM[d]	↑	↔	[64]
E83K	M	HCM[e]	–	–	–
D86A	M	HCM[f]	–	–	–
R92L	M	HCM[g]	↑	↔	[75]
R92Q	M	HCM[d]	↑	↔	[64]
R92W	M	HCM[h]	↑	↔	[75]
R94C	M	HCM[i]	–	–	–
R94L	M	HCM[j]	↑	↔	[73, 75]
K97N	M	HCM[k]	–	–	–
A104V	M	HCM[l]	↑	↔	[75]
F110I	M	HCM[m]	↔	↑	[69]
F110L	M	HCM[n]	–	–	–
F110V	M	HCM[c]	–	–	–
R130C	M	HCM[o]	↑	↔	[75]
R141W	M	DCM[p]	↓	↔	[73]
ΔE160	D	HCM[m]	↑	↔	[70]
E163K	M	HCM[m]	–	–	–
E163R	M	HCM[o]	↑	↔	[75]
A172S	M	DCM[q]	–	–	–
S179F	M	HCM[r]	↑	↔	[75]
ΔK210	D	DCM[s]	↓	↔	[70]
E244D	M	HCM[m]	↔ or ↑	↑	[69, 75]
K247E	M	HCM[t]	–	–	–
N271I	M	HCM[c]	–	–	–
K273E	M	HCM[u]	↑	↔	[72]
Int 16[*]	IM	HCM[m]	↑	↔ or ↓	[80]
R278C	M	HCM[m]	↑	↓	[95]
R278P	M	HCM[f]	–	–	–
R286C	M	HCM[c]	–	–	–
R286H	M	HCM[f]	–	–	–
W287ter	T	HCM[c]	–	–	–

[†]M: missense mutation; D: deletion mutation; IM: intronic mutation; T: termination. *G to A mutation occurs at the splicing donor site of intron 16. That may result in an early termination (truncation of last 14 residues) or exon skipping, which causes a replacement of the last 28 residues with novel 7 residues. [‡]The functional consequences of cTnT mutations we determined are summarized. We incorporated recombinant human cardiac cTnT mutants into skinned cardiac muscle fibers by using the troponin exchange technique. Ca^{2+}-dependent isometric force generation of skinned fibers was evaluated in terms of Ca^{2+} sensitivity and the maximum level of force generation [96]. Ref: [a][97], [b][44], [c][98], [d][4], [e][99], [f][45], [g][48], [h][47], [i][100], [j][101], [k][102], [l][103], [m][43], [n][104], [o][49], [p][53], [q][54], [r][105], [s][5], [t][106], [u][51].

sense mutation D190G was found in 12 affected patients in the same family, but two mutations, K178E and R192H, were *de novo*, and three others, L144Q, R145W, and A171T, were identified only in the probands. Patients with D190G mutation in the family showed clinical phenotypes with a mixed appearance of RCM and HCM, suggesting a common molecular mechanism for the pathogeneses of RCM and HCM associated with the cTnI mutations.

TnC Mutations in Hypertrophic and Dilated Cardiomyopathies

Two missense mutations in the human cTnC gene have so far been found in patients affected by HCM and DCM (Table 3 and Fig. 1). In 2001, a missense mutation L29Q in the human cTnC gene was found to be a cause of HCM [9]. Although the family history was not available, the proband showed typical features of

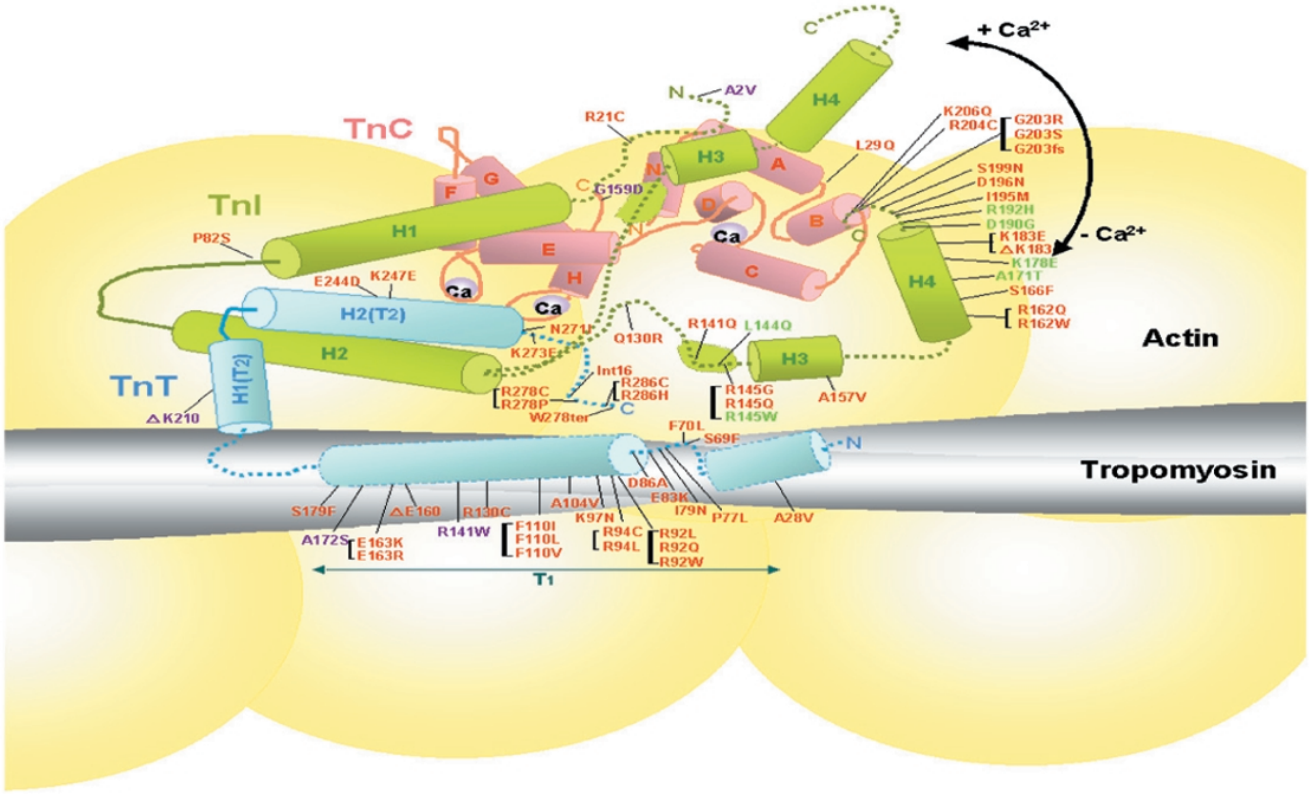

Fig. 1. Diagram of the tertially structure of troponin complex on the thin filament and localization of troponin mutations. cTnT, cTnI, and cTnC are colored in light blue, light green, and pink, respectively, with cylinder-represented α-helices and line-represented coils (solid line and cylinder: based on the X-ray crystal structure of partial troponin complex solved by Takeda *et al.* [94]; dashed line and cylinder: estimated structure). The localization of mutations causing HCM (red), DCM (magenta), and RCM (green) are approximately represented in each troponin subunit.

HCM, including abnormal ECG and hypertrophy of left ventricle, with late onset of the disease (at age 59). A DCM-causing missense mutation G159D was also found in the human cTnC gene very recently, but a detailed clinical status has not been reported [10, 11].

Functional Consequences of Troponin Mutations

TnT mutations

In 1996, Lin *et al*. first showed that an HCM-causing cTnT mutation, I79N, could alter the molecular function of cTnT [60]. They introduced a corresponding mutation into rat cTnT and found that the mutation did not affect the affinity of cTnT for tropomyosin, troponin-induced tropomyosin binding to actin, or Ca^{2+}-sensitive myosin subfragment-1 ATPase activity, but it enhanced the thin filament movement in an in vitro motility assay. Palm *et al*., when using a cTnT fragment containing residues 70–170, found that this mutation did not change the affinity of the fragment for tropomyosin and troponin-induced tropomyosin binding to actin [61]. The functional consequences of this mutation were also studied by two independent groups using a similar experimental system in which cTnT mutant was overexpressed in quail skeletal myotubes [62] or in rat cardiac myocytes [63]. Both groups showed that I79N mutation decreased the Ca^{2+} sensitivity of force generation in a permeabilized cell. In contrast, we showed that the I79N mutation increased the Ca^{2+} sensitivity of force generation when the mutant human cTnT was directly incorporated into rabbit skinned cardiac muscle fibers [64] (Table 1). Another group also reported a similar result and confirmed our finding by using almost the same system [65]. Miller *et al*. created a transgenic (Tg) mouse model expressing this mutation and found that the skinned cardiac muscle fibers prepared from Tg mice did show an increase in the Ca^{2+} sensitivity of force generation and ATPase activity [66]. We have also found that many other mutations in cTnT associated with HCM, i.e., R92L, R92Q, R92W, R94L, A104V, R130C, ΔE160, E163R, S179F, E244D, K273E, R278C, and Intron16$G_1 \rightarrow$ A, increase the Ca^{2+} sensitivity of force generation (Table I). A Ca^{2+}-sensitization of cardiac myofibrillar ATPase activity has also been con-

Table 2. Mutations in human cTnI and their functional consequences on the force generation of skinned cardiac muscle fibers.

Mutation	Type of mutation†	Type of cardiomyopathy	Ca^{2+}-dependent force generation of skinned cardiac fibers with cTnI mutations‡			
			Ca^{2+} sensitivity	Minimum force	Maximum force	Ref.
A2V	M	DCM[a]	–	–	–	–
R21C	M	HCM[b]	↑	↔	↔	[107]
P82S	M	HCM[c]	–	–	–	–
Q130R	M	HCM[d]	–	–	–	–
R141Q	M	HCM[e]	–	–	–	–
L144Q	M	RCM[f]	–	–	–	–
R145G	M	HCM[g]	↑	↑	↔	[77]
R145Q	M	HCM[g]	↑	↑	↔	[77]
R145W	M	RCM[f]	–	–	–	–
A157V	M	HCM[h]	–	–	–	–
R162Q	M	HCM[e]	–	–	–	–
R162W	M	HCM[g]	↑	↑	↔	[77]
S166F	M	HCM[e]	–	–	–	–
A171T	M	RCM[f]	–	–	–	–
K178E	M	RCM[f]	–	–	–	–
K183E	M	HCM[f]	–	–	–	–
ΔK183	D	HCM[g]	↑	↔	↔	[77]
D190G	M	RCM[f]	–	–	–	–
R192H	M	RCM[f]	–	–	–	–
I195M	M	HCM[b]	–	–	–	–
D196N	M	HCM[c]	–	–	–	–
S199N	M	HCM[f]	–	–	–	–
G203R	M	HCM[f]	–	–	–	–
G203S	M	HCM[g]	↔	↑	↔	[77]
G203fs	N	HCM[i]	–	–	–	–
R204C	M	HCM[b]	–	–	–	–
K206Q	M	HCM[g]	↑	↔	↔	[77]

†M: missense mutation; D: deletion mutation; N: nonsense mutation. ‡The functional consequences of cTnI mutations we determined are summarized. We incorporated recombinant human cardiac TnI mutants into skinned cardiac muscle preparation by using the troponin exchange technique. The Ca^{2+}-dependent isometric force generation of skinned fibers was evaluated in terms of Ca^{2+} sensitivity and the minimum and maximum levels of force generation [96]. Ref: [a][8], [b][102], [c][59], [d][97], [e][45], [f][7], [g][6], [h][99], [i][108].

Table 3. Mutations in human cTnC.

Mutation	Type of mutation†	Type of cardiomyopathy	Ref.
L29Q	M	HCM	[9]
G159D	M	DCM	[10]

†M; missense mutation.

firmed in most of these mutations [67, 68, 70]. Several mutations in cTnT, however, had a potentiation effect on the steady-state maximal force with or without increasing the Ca^{2+} sensitivity. F110I mutation increased the maximum force without affecting the Ca^{2+} sensitivity, and E244D mutation increased both the Ca^{2+} sensitivity and the maximum force [69].

The functional consequences of the mutations in cTnT associated with DCM are clearly different from those of the mutations associated with HCM. In a study we made, the deletion mutation ΔK210, the first mutation in cTnT found in DCM, had a Ca^{2+}-desensitizing effect on the force generation in skinned cardiac muscle fibers and the ATPase activity in isolated cardiac myofibrils [70]. Basically the same finding has been reported by other groups [71, 72]. We have also found that the missense mutation R141W in cTnT associated with DCM decreases the Ca^{2+} sensitivity of force generation in skinned cardiac muscle fibers [73]. Venkatraman *et al*. reported that this mutation did not significantly decrease the Ca^{2+} sensitivity of force generation in an experimental system similar to ours and that it reduced the maximum actomyosin ATPase activity [72]. However, they also found that a Ca^{2+}-desensitization of force generation does occur when this mutation is introduced into the

fetal isoform of human cTnT [74]. On the other hand, three mutations, R92W, E163R, and K273E, which have been shown to cause a dilated form of HCM (i.e., secondary DCM), have functional consequences similar to those of the other mutations in cTnT associated with HCM, that is, Ca^{2+}-sensitizing effects on the cardiac muscle contraction [72, 75].

TnI mutations

The Ca^{2+}-sensitizing effect on cardiac muscle contraction has commonly been observed for most mutations in cTnI associated with HCM, as with the mutations in cTnT, although the mutations occur in quite different functional regions of cTnI molecule (Table II, Fig. 1). Two mutations R145G and R145Q in the so-called inhibitory region of cTnI have almost the same functional consequence on cardiac muscle contractile properties [76, 77]; i.e., an increased Ca^{2+} sensitivity of myofibrillar ATPase activity and force generation in cardiac muscle contraction with an impaired inhibition (or relaxation) and a decrease in the maximum level of ATPase activity. The R162W mutation in the so-called second TnC binding region adjacent to the inhibitory region has a similar but smaller effect. The deletion mutation ΔK183 near the second actin-tropomyosin binding region reduces the apparent affinity of cTnI for actin, but it does not alter the maximal inhibitory action of cTnI on the myofibrillar ATPase activity and force generation in skinned cardiac fibers (i.e., the minimum ATPase activity and force generation were not changed). This mutation also shows quite a large Ca^{2+}-sensitizing effect on both myofibrillar ATPase and force generation in skinned fibers. Two other mutations, G203S and K206Q in the C-terminal end of cTnI, have a marginal Ca^{2+}-sensitizing effect on both myofibrillar ATPase and force generation in skinned fibers.

As described above, the cardiac isoform of TnI can be phosphorylated by PKA, and this event plays an important physiological role in modulating the cardiac function *in vivo*. β-adrenergic stimulation on cariomyocytes activates PKA in cytoplasm and causes a decrease in the Ca^{2+} sensitivity of force generation through the phosphorylation of cTnI by PKA, which is thought to play a cardioprotective role in preventing an overstimulation of cardiac myofilaments by a simultaneous dramatic increase in cytosolic Ca^{2+} induced by β-adrenergic stimulation [78]. It has been shown that the mutations in cTnI associated with HCM modify the effect of cTnI phosphorylation by PKA. Deng *et al*. have shown that R145Q mutation increases the Ca^{2+} sensitivity of myosin subfragment-1 ATPase activity and the *in vitro* filament motility independently of the phosphorylation state of cTnI [79], suggesting that this mutation impairs the mechanism involving cardioprotection through the phosphorylation of cTnI, as with a cTnT mutation [80]. The same group has also reported that the C-terminal mutation K206Q abolishes the sensitivity of myofilaments to the phosphorylation of cTnI by PKA [81]. Besides the phosphorylation of cTnI by PKA, HCM-linked mutations have been shown to affect the regulatory mechanism involving the phosphorylation of cTnI by protein kinase C (PKC) [82]. cTnT is also known to be phosphorylated by PKC[83], but the effect of HCM-linked mutations on its potential regulatory mechanism has not yet been studied.

TnC mutations

Although any functional study of the mutations in cTnC associated with HCM has not yet been reported, the mutations in cTnC could be expected to alter the contractile properties of cardiac muscle in the light of results from the functional studies on cTnT and cTnI mutations. The L29Q mutation found in HCM occurs in the first Ca^{2+}-binding EF hand motif (site I) of cTnC, which has lost the ability to bind Ca^{2+} in contrast to sTnC. Coincidently, the residue corresponding to Leu^{29} of human cTnC is Gln in trout cTnC, and the N-lobe of trout cTnC has been shown to have a greater affinity for Ca^{2+} than that of mouse cTnC with Leu residue at position 29, like human cTnC [84]. It is interesting that the substitution of Leu for Gln at the corresponding position in trout cTnC reduced the affinity for Ca^{2+} to a level of mouse cTnC, suggesting that the residue Gln^{29} is a key residue for trout cTnC to maintain its high affinity for Ca^{2+}. Therefore the L29Q mutation in human cTnC associated with HCM could be expected to affect the Ca^{2+} affinity of cTnC, leading to an alteration of the contractile response to Ca^{2+}.

The Gly to Asp substitution at position 159 in cTnC has been found to cause DCM [10]. Preston *et al*. have examined this mutation in the human cTnC-exchanged rabbit skinned fast skeletal muscle fibers and found that the mutation had no effects on the Ca^{2+} sensitivity, the maximum force level, or the cooperativity, but it slowed the rate of force development by 50% [11].

Conclusion: Implication for Pathophysiological Mechanisms

Functional studies have been made to elucidate the molecular mechanisms by which HCM is developed in patients harboring mutations in sarcomeric proteins

since the discovery of a mutation in the gene for β-myosin heavy chain in 1990 [41]. Especially, the functional consequences of cTnT mutations were intensively examined at different levels from molecules to animal models, revealing a great heterogeneity in the functional consequences of a variety of mutations spreading over the entire molecule (reviews [85–87]). For instance, Palm *et al*. have demonstrated that mutations within a region including residues 92–110 (R92L/Q/W, R94L, A104V, and F110I) impair the tropomyosin-dependent function of cTnT, but mutations outside the region (I79N, ΔE160, and E163K) do not [61]. Nevertheless, a wide variety of mutations including these mutations are responsible for a similar heart disease diagnosed as HCM, implying that there may be a common mechanism for the pathogenesis of HCM associated with cTnT mutations. In 1998, we demonstrated that the two HCM-linked cTnT mutations, I79N and R92Q, increased the Ca^{2+} sensitivity of force generation in cardiac muscle [64]. Since then it has been shown that most mutations of cTnT and cTnI associated with HCM have a Ca^{2+}-sensitizing effect on the force generation of cardiac muscle and the ATPase activity of cardiac myofibrils. Moreover, it has been demonstrated that the skinned cardiac muscle fibers from transgenic mice expressing cTnT or cTnI mutants show an increased Ca^{2+} sensitivity of force generation [66, 88–90]. These studies strongly suggest that Ca^{2+}-sensitization is a common gateway to the development of HCM with mutations in the gene for cTnT and cTnI. An increased Ca^{2+} sensitivity would involve an increase in the utilization of ATP by actomyosin ATPase at submaximal intracellular Ca^{2+} concentrations, which facilitates the rapid exhaustion of the intracellular ATP under severe stress and leads to an imbalance in energy supply and demand in the heart that might be responsible for sudden death. A recent study has revealed that cardiac energetics is altered in Tg mice harboring the cTnT mutation R92Q [91], as seen in HCM patients with sarcomeric protein mutations, including cTnT [92].

On the other hand, troponin mutations so far identified in DCM occur much less often than those identified in HCM, and their functional consequences have just begun to be investigated. In a few studies, however, two cTnT mutations found in DCM have been shown to have an effect directly opposite to that seen in the HCM-causing mutations, i.e., Ca^{2+}-desensitization of the force generation and/or myofibrillar ATPase activity in cardiac muscle. Because intact cardiac muscle is known to never be activated beyond a half-maximal level [78], a decrease in Ca^{2+}-sensitivity is expected to cause a significant reduction in the force generation of cardiac muscle and thus in the systolic function of the heart, leading to a ventricular dilation as a compensatory mechanism for the decrease in stroke volume.

An ultimate goal of our studies is to cure heart disease including cardiomyopathies, and one of the most probable ways to attain this goal is to discover effective drug(s). Normal cardiac function is maintained by complicated regulatory systems, and any deflect in these systems could potentially be a cause for cardiac problems. It could therefore be possible to find drugs targeting the intermediate process of development of the disease. Elevated levels of myocardial aldosterone and aldosterone synthase mRNA have been found in patients with HCM, and phenotypic features of HCM such as hypertrophy, fibrosis, myocardial disarray, and diastolic dysfunction was attenuated by spironolactone, an antagonist of mineralocorticoid receptor (MR), in a Tg mouse model, suggesting the application of MR blockade in human HCM [93]. However, it has been clarified in HCM and DCM associated with troponin mutations that the development of disease just starts from the alteration of troponin function by mutations leading to an increased or decreased Ca^{2+} sensitivity of cardiac muscle contraction. Therefore the design or discovery of a drug that acts directly on troponin and corrects its response to Ca^{2+} signaling might be the key to a radical treatment for cardiomyopathies caused by troponin mutations.

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