Differential interactions of thin filament proteins in two cardiac troponin T mouse models of hypertrophic and dilated cardiomyopathies

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Aim Mutations in a sarcomeric protein can cause hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM), the opposite ends of a spectrum of phenotypic responses of the heart to mutations. We posit the contracting phenotypes could result from differential effects of the mutant proteins on interactions among the sarcomeric proteins. To test the hypothesis, we generated transgenic mice expressing either cardiac troponin T (cTnT)-Q92 or cTnT-W141, known to cause HCM and DCM, respectively, in the heart.

Methods and results We phenotyped the mice by echocardiography, histology and immunoblotting, and real-time polymerase chain reaction. We detected interactions between the sarcomeric proteins by co-immunoprecipitation and determined Ca2+ sensitivity of myofibrillar protein ATPase activity by Carter assay. The cTnT-W141 mice exhibited dilated hearts and decreased systolic function. In contrast, the cTnT-Q92 mice showed smaller ventricles and enhanced systolic function. Levels of cardiac troponin I, cardiac α-actin, α-tropomyosin, and cardiac troponin C co-immunoprecipitated with anti-cTnT antibodies were higher in the cTnT-W141 than in the cTnT-Q92 mice, as were levels of α-tropomyosin co-immunoprecipitated with an anti-cardiac α-actin antibody. In contrast, levels of cardiac troponin I co-immunoprecipitated with an anti-cardiac α-actin antibody were higher in the cTnT-Q92 mice. Ca2+ sensitivity of myofibrillar ATPase activity was increased in HCM but decreased in DCM mice compared with non-transgenic mice.

Conclusion Differential interactions among the sarcomeric proteins containing cTnT-Q92 or cTnT-W141 are responsible for the contrasting phenotypes of HCM or DCM, respectively.

KEYWORDS Cardiomyopathy; Genetics; Mutation; Mouse model; Pathogenesis; Fibrosis; Heart failure

1. Introduction Cardiomyopathies are primary disorders of cardiac myocytes and major causes of heart failure and sudden cardiac death. Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) represent the opposite ends of the spectrum of the phenotypic responses of the heart to genetic mutations. The molecular mechanisms governing cardiac predilection towards hypertrophy or dilatation are largely unknown.

Advances in molecular genetic studies during the past decade have led to elucidation of molecular genetic basis of HCM and DCM. Several hundred mutations in over a dozen genes encoding sarcomeric proteins have been identified in individuals with HCM (reviewed in 1). Similarly, a large number of mutations in genes encoding cytoskeletal and sarcomeric proteins have been identified in DCM patients (reviewed in 2). Recent studies have elucidated phenotypic plasticity of mutations in a single gene.3 This is best illustrated for mutations in LMNA, which are known to cause at least 13 distinct phenotypes.4 Likewise, mutations in sarcomeric proteins β-myosin heavy chain (MyHC) and cardiac troponin T (cTnT) could cause HCM or DCM.5,6 Notably, two mutations in cTnT, namely R92Q and R141W, have been identified in families with HCM and DCM, respectively.7,8 The contrasting phenotypes arising from mutations in cTnT provide for the opportunity to delineate the molecular basis for the diversity of phenotypic responses of the heart to mutations. The cTnT, a component of the thin filaments of the sarcomere, interacts with cardiac troponin C (cTnC), α-tropomyosin (α-Tm), and...
cardiac troponin I (cTnI). We hypothesized that the phenotypic plasticity of cTnT mutations could result from differential interactions among the protein constituents of the thin filaments containing mutant cTnT-Q92 or cTnT-W141 proteins. The differential protein–protein interactions could change the sensitivity of the acto-myosin complex to Ca$^{2+}$ for the generation of ATPase and force differentially. Mutations reducing Ca$^{2+}$ sensitivity of the myofibrils would be expected to reduce cardiac systolic function (DCM). The opposite would be expected for mutations that enhance Ca$^{2+}$ sensitivity of the myofibrils. To explore this hypothesis, we expressed cTnT-Q92 and cTnT-W141 in the heart of transgenic mice. We characterized the ensuing phenotypes, determined interactions between the protein constituents of the thin filaments comprising mutant cTnT proteins, by co-immunoprecipitation (Co-IP) and calcium (Ca$^{2+}$) sensitivity of myofibrillar ATPase activity.

2. Methods

An expanded version of the Methods is provided as Supplementary material online. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Institutional Animal Care and Use Committee.

2.1 Transgenic mouse models

Generation and phenotype of the cTnT-Q92 but not cTnT-W141 mice have been published.9–11 In brief, we induced the R92Q and R141W mutations in full-length human cTnT cDNA (isoform 1, NM_000364) by site-directed mutagenesis and placed the mutant cDNAs downstream to a full-length (5.5 kbp) α-MyHC promoter.12 The two constructs were identical except for the R92Q and R141W mutations (Figure 1A).

2.2 Survival and gross cardiac phenotype

We analysed survival at 12 months of age and determined ventricular weight/body weight ratios in age- and sex-matched mice.

2.3 M-mode, two-dimensional, and Doppler echocardiography

We performed echocardiography using an HP Sonos 5500 System equipped with a 15 MHz linear transducer using sodium pentobarbital for anaesthesia.11,13,14 We calculated ventricular fractional shortening, ejection fraction, ejection time, and circumferential velocity of shortening without the knowledge of the genotype, as described.11,13,14

2.4 Morphometric analysis

An investigator without the knowledge of the genotypes performed the morphometric analyses in age- and sex-matched mice in a random order.11,13,15 We determined the extent of myocyte disarray and collagen volume fraction (CVF) by quantitative automated planimetry.11,13,15

2.5 Real-time polymerase chain reaction

We determined expression levels of mRNAs for selected molecular markers of cardiac hypertrophy, namely A-type natriuretic peptide (Nppa), B-type natriuretic peptide (Nppb), skeletal α-actin (Acta1), and sarcoplasmic reticulum calcium ATPase 2a (Atp2a2) by quantitative real-time polymerase chain reaction using specific TaqMan probes and primers (n = 6 mice per group). We normalized

Figure 1 Transgene constructs and expression of the transgenes. (A) Schematic representation of the transgene constructs. The two transgenes are identical except for the Q92 (HCM) and W141 (DCM) mutations. (B) Immunoblots showing expression of the mutant and total (endogenous plus mutant) cTnT proteins in the heart of transgenic and non-transgenic mice (n = 4 per group). Proteins extracted from human hearts were used as positive control. The upper panel shows expression of the transgene proteins detected using the monoclonal anti-cTnT antibody 7G7. A 38 kDa band is present in lanes representing human heart (positive control) and transgenic mice heart protein extracts. No specific band was detected in the lane representing the non-transgenic mice. Expression levels of cTnT-W141 (responsible for DCM) and cTnT-Q92 (responsible for HCM) were equal in the lines shown. The middle panel shows expression of the total cTnT protein detected using the pan-specific anti-cTnT antibody JLT-12. As shown, a 38 kDa band is present in all lanes representing human heart (positive control) and transgenic mice heart protein extracts. No specific band was detected in the lane representing the non-transgenic mice. Expression levels of cTnT-W141 (responsible for DCM) and cTnT-Q92 (responsible for HCM) were equal in the lines shown. The lower panel shows expression levels of α-tubulin, a control for the loading condition. Quantitative data on the expression levels of transgene cTnT proteins (C) and total cTnT protein (D) as determined by spot-densitometry (n = 4 per group). MyHC, myosin heavy chain; cTnT, cardiac troponin T; NTG, non-transgenic; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; MW, molecular weight; hGH-3’UT, human growth hormone 3’ untranslated region.
the levels to those of glyceraldehyde-3-phosphate dehydrogenase (Gapdh) mRNA.11,13

### 2.6 Isolation of myofibrillar proteins

We isolated myofibrillar proteins from the hearts with treatment with Triton-X 100, as published (Supplementary material online).16,17 In brief, myocardial tissues were minced and homogenized using Polytron in a relaxing buffer. The homogenates were precipitated by centrifugation and the pellets were resuspended in a standard buffer and 2% Triton-X. Following two cycles of homogenization and centrifugation, Triton-X was removed and the final pellets were resuspended in K-60 buffer. We determined the protein concentration by Bradford protein assay.

### 2.7 Immunoblotting

We detected expression levels of the transgene proteins using a transgene-specific anti-human cTnT monoclonal antibody (7G7 clone, Research Diagnostics, Inc., Concord, MA, USA) by immunoblotting (IB), as described (n = 4 mice per group).11 We detected expression levels of the total cTnT (transgene plus endogenous) using a pan-specific anti-cTnT antibody (clone JLT12, Sigma Aldrich, St Louis, MO, USA). To re-probe, we stripped the membranes by incubating in 1% SDS and 100 mM β-mercaptoethanol in TBS for 30 min at room temperature and washing in TBS for at least six times. We then probed the membranes with an anti-α-tubulin antibody (monoclonal mouse IgG anti-α-tubulin, Santa Cruz Biotechnology).

### 2.8 Co-immunoprecipitation

We determined linearity of the transgene-specific (7G7) and pan-specific (JLT-12) antibodies for detection of cTnT proteins in myocardial protein extracts. We loaded increasing amounts of total myocardial proteins extracts (1, 2.0, 5, 10, and 20 mg) and performed IB.

We performed Co-IP experiments on myocardial proteins extracted in 0.5% NP-40 buffer using the transgene-specific (7G7) and the pan-specific (JLT-12) anti-cTnT antibodies. Co-IP studies with 7G7 antibody afforded the opportunity to detect bindings of the mutant cTnT proteins to other thin filament protein constituents without interference by the endogenous cTnT. On the other hand, the Co-IP studies with JLT-12 represented the biology in human HCM and DCM, wherein the mutant and wild-type proteins are co-expressed.

In brief, we minced and homogenized 50 mg aliquots of ventricular myocardium in a Polytron PT 2100 homogenizer in a relaxing lysis buffer (0.5% Nonidet P-40, 120 mM, sodium chloride, 50 mM Tris-HCl, pH 7.4, 5% glycerol, and a proteinase inhibitor cocktail; Roche, Germany). Following precipitation of cell debris by centrifugation, we removed the supernatant and determined the protein concentration by Bradford assay. To co-immunoprecipitate the proteins, we gently mixed 6 μg of the primary antibody to each 500 μg aliquot of total protein extracts and incubated the reaction at 4°C overnight. Then we mixed 20 μL of Protein A/G PLUS-Agarose beads (Santa Cruz Biotechnology) into the solutions and incubated the reactions on a rocker platform at 4°C overnight. We then precipitated, washed the proteins, and resuspended the final pellets in loading buffer for IB. Detailed information on the procedure for Co-IP and antibodies is provided in the Supplementary material online.

### 2.9 Myofibrillar protein ATPase activity

We measured calcium (Ca\(^{2+}\)) sensitivity of the myofibrillar protein ATPase activity by measuring the release of inorganic phosphate (Pi) from ATP by myofibrillar proteins in triplicate sets for each Ca\(^{2+}\) concentration as described.16,17 We initiated the reactions by adding 2 mM ATP to each tube containing 100 μg of myofibrillar proteins, incubated at 30°C, and stopped the reaction by adding 250 μL of 10% ice-cold trichloroacetic acid to each tube. We measured ATPase activity by the Pi Carter assay.

### 2.10 Statistical analysis

Statistical calculations (STATA-Intercooled v.9.2 program) were as published.13,18 We tested the variables for homogeneity and normality by Bartlett’s test and compared the differences for normally distributed parametric variables among the three groups by ANOVA, followed by pairwise comparisons by Bonferroni test. We analysed the variables that violated the normality assumption and the non-parametric variables by Kurskal–Wallis test.

### 3. Results

#### 3.1 Expression of the transgene proteins

We detected expression levels of the transgene proteins in myocardial protein extracts from age- and sex-matched adult mice by IB using transgene-specific and pan-specific anti-cTnT antibodies (Figure 1). We used transgenic mice from lines expressing equal levels of cTnT-Q92 and cTnT-W141 proteins for phenotypic and molecular characterization.

#### 3.2 Survival and morphometric phenotypes

The cTnT-Q92 mice survived normally up to 2 years. In contrast, 29% (9/31) of the cTnT-W141 mice die within 1 year and almost all within 2 years of age. Hearts were enlarged in cTnT-W141 mice when compared with non-transgenic or cTnT-Q92 mice (Figure 2). The ventricular weight/body weight ratio was also significantly increased in age- and sex-matched cTnT-W141 mice when compared with non-transgenic mice (6.01 ± 0.96 vs. 4.53 ± 0.43 mg/g, respectively, n = 13 per group, P = 0.001) or cTnT-Q92 mice (4.84 ± 0.44 mg/g, n = 13, P = 0.001). In contrast, the ventricular weight/body weight ratio was not significantly different between cTnT-Q92 and non-transgenic mice.

CVF comprised 5.3 ± 0.9% and 4.9 ± 1.3% of the myocardium in 1-year-old cTnT-Q92 and cTnT-W141 mice, respectively, compared with 1.1 ± 0.6% in non-transgenic mice (n = 8 per group, P < 0.001) (Figure 2). The findings were in accord with the previous data in the cTnT-Q92 mice.11,15,19 Likewise, significant myocyte disarray (11.4 ± 3.3%) was detected in the cTnT-Q92 mice11,15,19 but not in the cTnT-W141 mice (Figure 2).

#### 3.3 Echocardiographic phenotype

The results are summarized in Table 1. Representative M-mode echocardiograms are shown in Figure 3. The most noteworthy findings were increased left ventricular end-systolic diameter (LVEDD) and end-diastolic diameter (LVEDD) and decreased left ventricular systolic function in the cTnT-W141 mice. In contrast, LVEDD was smaller and systolic function was enhanced in the cTnT-Q92 when compared with non-transgenic or cTnT-W141 mice. As shown in Table 1, there were no significant differences in the mean age, sex, and body weight among the groups. Nonetheless, since body weight could affect the LVEDD and left ventricular mass (LVM), we compared the LVEDD and LVM indexed to body weight among the three groups. The indexed LVEDD and LVM were also significantly increased in the cTnT-W141 mice when compared with cTnT-Q92 or non-transgenic mice.
mice. They were not significantly different between the cTnT-Q92 and non-transgenic mice.

3.4 Expression levels of molecular markers of cardiac hypertrophy

Expression levels of Nppa and Nppb mRNAs were increased by 9.7 ± 1.9 and 2.6 ± 1.1-fold, respectively, in the cTnT-W141 when compared with non-transgenic mice (Figure 4). In contrast, expression levels of Atp2a2 and Acta1 mRNAs were reduced by 4.1 ± 0.11 and 2.3 ± 0.27-fold, respectively, in the cTnT-W141 mice. Changes in the expression levels of the markers were less remarkable in the cTnT-Q92 mice. Nonetheless, the differences between the cTnT-Q92 and cTnT-W141 mice as well as between cTnT-Q92 and non-transgenic mice were also significant (Figure 4).

3.5 Differential bindings of thin filaments’ protein constituents in cTnT-W141 and cTnT-Q92 mice

The results of IB showing linearity of the transgene-specific and pan-specific anti-cTnT antibodies to detect transgene and total cTnT proteins in DCM and HCM mice are shown in Figure 5. These results illustrate the linearity of the detection at total myocardial protein concentrations ranging from 1 to 20 μg for both antibodies. The results of Co-IP experiments with the transgene-specific anti-cTnT antibody are shown in Figure 6A. As shown, levels of co-immunoprecipitated cTnl, α-cardiac actin, cTnC, and α-Tm were higher in the cTnT-W141 when compared with cTnT-Q92 mice.

The results of the Co-IP experiments with the pan-specific anti-cTnT antibody JLT-12 corroborated those observed with the transgene-specific antibody (Figure 6B). In addition, the results showed that both mutant proteins bind weaker to cTnl, cardiac α-actin, and cTnC in DCM and HCM when compared with non-transgenic mice. In contrast, the binding of cTnT-W141 (DCM) to α-Tm was stronger than the binding of non-transgenic cTnT to α-Tm, and the binding of cTnT-Q92 to α-Tm was weaker (Figure 6B).

Finally, we analysed Co-IP of cTnl and cardiac α-actin as well as cardiac α-actin and α-Tm. The results (Figure 6C) showed that in both mouse models, the binding of cTnl to cardiac α-actin was reduced compared with that in the non-transgenic mice. However, the reduction was greater in the cTnT-W141 than in the cTnT-Q92 mice. The opposite was the case for the binding of α-actin to α-Tm, which was reduced in both models when compared with that in the non-transgenic. However, the reduction was greater in the cTnT-Q92 when compared with the cTnT-W141 mice.

3.6 Calcium sensitivity of myofibrillar protein ATPase activity

To determine whether differential bindings of cTnT-Q92 and cTnT-W141 to other constituents of thin filaments affected myofibrillar protein ATPase activity, we measured myofibrillar protein ATPase activity for a range of Ca2+ concentration (n = 8 mice per group). The quality of myofibrillar protein extracts was analysed by PAGE and identification of the major sarcromer proteins according to their molecular weights on Coomassie blue staining gels (Figure 7A). The results are notable for increased Ca2+ sensitivity of myofibrillar protein ATPase activity in the cTnT-W141 mice and decreased sensitivity in the cTnT-Q92 mice (Figure 7B). Notably, the differences in the Ca2+ sensitivity of the
myofibrillar ATPase activity between the cTnT-W141 and non-transgenic mice was more pronounced at the higher Ca\(^{2+}\) concentrations (pCa < 6.0). At lower Ca\(^{2+}\) concentrations (pCa > 6.5), there were no statistically significant differences between the cTnT-W141 and non-transgenic mice. In contrast, Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity in the cTnT-Q92 mice was significantly enhanced throughout the ranges of Ca\(^{2+}\) concentrations.

### 4. Discussion

We generated two transgenic mouse models with cardiac-restricted expression of either mutant cTnT-W141 or cTnT-Q92 protein, known to cause DCM and HCM in humans.\(^7,8\) The cTnT-W141 and cTnT-Q92 mice recapitulate the phenotype of DCM and HCM, respectively. Co-IP studies showed differential bindings of the thin filaments protein constituents containing cTnT-W141 and cTnT-Q92 proteins. The Q92 and W141 mutations, which are not located in the known binding domains to cTnI, likely, impacted their effects on bindings to other protein components of the thin filaments by altering the secondary structure of the cTnT protein. Expression of the mutant cTnT proteins also affected interactions among other thin filament protein constituents containing cTnT-W141 and cTnT-Q92 proteins. The findings suggest that the primary structural defects in the cTnT imparted by the mutations convey non-covalent changes not only in the cTnT itself but also in the protein constituents of the thin filaments.

### Table 1: Echocardiographic findings in cTnT-W141 and cTnT-Q92 transgenic mice

<table>
<thead>
<tr>
<th></th>
<th>Non-transgenic</th>
<th>cTnT-W141</th>
<th>cTnT-Q92</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>M/F</td>
<td>5/3</td>
<td>5/3</td>
<td>7/1</td>
<td>0.411</td>
</tr>
<tr>
<td>Age (months)</td>
<td>12.3 ± 2.7</td>
<td>11.8 ± 2.9</td>
<td>9.8 ± 3.8</td>
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<tr>
<td>BW (g)</td>
<td>31.5 ± 1.9</td>
<td>29.3 ± 3.4</td>
<td>28.2 ± 3.4</td>
<td>0.098</td>
</tr>
<tr>
<td>HR (b.p.m.)</td>
<td>597.0 ± 77.4</td>
<td>587.5 ± 62.7</td>
<td>575.6 ± 67.1</td>
<td>0.828</td>
</tr>
<tr>
<td>ST (mm)</td>
<td>1.00 ± 0.01</td>
<td>1.07 ± 0.01</td>
<td>0.993 ± 0.01</td>
<td>0.418</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>2.97 ± 0.22</td>
<td>3.94 ± 0.39**</td>
<td>2.45 ± 0.27***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDD/BW (mm/g)</td>
<td>0.94 ± 0.08</td>
<td>1.33 ± 0.11***</td>
<td>0.88 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>1.13 ± 0.19</td>
<td>2.47 ± 0.50***</td>
<td>0.75 ± 0.12</td>
<td>&lt;0.001a</td>
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<tr>
<td>LVM (mg)</td>
<td>96 ± 22</td>
<td>150 ± 31**</td>
<td>76 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVM/BW (mg/g)</td>
<td>3.1 ± 0.76</td>
<td>5.0 ± 0.86**</td>
<td>2.7 ± 0.57</td>
<td>&lt;0.001</td>
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<td>FS (%)</td>
<td>61.7 ± 6.3</td>
<td>37.8 ± 8.0**</td>
<td>69.6 ± 2.5**</td>
<td>&lt;0.001a</td>
</tr>
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<td>Vcf (c.p.s.)</td>
<td>1068.4 ± 410.7</td>
<td>659.5 ± 166.3**</td>
<td>1140.7 ± 410.7</td>
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<tr>
<td>E (cm/s)</td>
<td>1.89 ± 0.73</td>
<td>1.26 ± 0.35</td>
<td>1.94 ± 0.46</td>
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<tr>
<td>Ao Vmax (cm/s)</td>
<td>1.54 ± 0.41</td>
<td>1.62 ± 0.40</td>
<td>1.74 ± 0.54</td>
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<tr>
<td>ET (ms)</td>
<td>56 ± 9</td>
<td>58 ± 7</td>
<td>59 ± 6</td>
<td>0.710</td>
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*P-value represents comparisons of differences among the three groups by ANOVA or Kruskal–Wallis because of unequal variances.

**P-value < 0.05 by Bonferroni pairwise comparison between non-transgenic and cTnT-W141.

***P-value < 0.05 by Bonferroni pairwise comparison between cTnT-W141 and Q92.

### Figure 3: Echocardiographic phenotype. Representative M-mode echocardiograms of the left ventricle from non-transgenic, cTnT-W141, and cTnT-Q92 mice (1 s strips) are shown. Compared with non-transgenic mice (the upper panel), LVEDD and LVESD were increased and systolic function was reduced in cTnT-W141 mice (middle panel). In contrast, LVEDD was normal and systolic function was enhanced in cTnT-Q92 mice (lower panel). IVST and PWT are also labelled. LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVM, left ventricular mass; FS, fractional shortening; Vcf, velocity of circumferential shortening; c.p.s., circumference per second; E, early mitral inflow velocities; Ao Vmax, maximum aortic outflow velocity; ET, ejection time.
myofibrillar proteins, which is more likely to model the in vivo events than the in vitro studies with isolated proteins. The availability of transgene-specific antibody was essential for the detection of specific interactions between the transgene proteins and other myofibrillar proteins. It not only mitigated the potential confounding effect of the endogenous cTnT but also eliminated the need for epitope-tagging the transgenes, which might have compounded the
Figure 6  Immunoblots showing co-immunoprecipitations of the protein constituents of the thin filaments in the presence of cTnT-W141 or cTnT-Q92 transgene proteins.  (A) The blots show the results of co-immunoprecipitation experiments with the transgene-specific cTnT antibody (n = 3 per protein). (B) The blots show the results of co-immunoprecipitation experiments with the pan-specific anti-cTnT JLT-12 antibody (n = 2 per protein). (C) The blots show the results of Co-IP experiments for additional protein components of the thin filaments (n = 3 per protein). cTnI, cardiac Troponin I; cTnC, cardiac Troponin C; α-Tm, alpha tropomyosin; others as in Figure 1.

Figure 7  Ca\(^{2+}\) sensitivity of the myofibrillar protein ATPase activity.  (A) A Coomassie blue-stained polyacrylamide gel electrophorogram of myofibrillar protein extracts from the experimental groups. Selected major myofibrillar proteins are identified according to their expected molecular weights. (B) Ca\(^{2+}\) sensitivity of myofibrillar proteins ATPase activity. ATPase activities were determined by measuring the release of inorganic phosphate (Pi) from ATP by myofibrillar proteins in the presence of different concentration of Ca\(^{2+}\). Per cent increase in the levels of inorganic phosphate (Pi), a measure of ATPase activity of the acto-myosin complex in the reaction plotted against Ca\(^{2+}\) concentrations (pCa). As shown, Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity was reduced in the cTnT-W141 mice compared with non-transgenic mice. In contrast, it was increased in the cTnT-Q92 mice. MyHC, myosin heavy chain; MyBP-C, myosin-binding protein C; others as in Figure 1.
experiments. We documented the biological significance of the differential interactions by measuring myofibrillar proteins ATPase activity, which paralleled the main findings. The differential effects of mutant cTnT-W141 and cTnT-Q92 on protein–protein interactions, which are novel findings, also provide a basis for the differential effects of the mutations on Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity. The later finding is also in accord with the data showing differential effects of cTnT mutations on Ca\(^{2+}\) sensitivity of myofibrillar force generation and ATPase activity.10,20–27 Moreover, the mouse models utilized in the present studies largely recapitulate the phenotype of human HCM and DCM. The cTnT-W141 mice exhibit severe cardiac dilatation and impaired cardiac systolic function, the defining phenotype of human DCM. The cTnT-W141 mice also exhibit ventricular arrhythmias (data not shown) and premature death, which are also observed in human DCM. In contrast, the cTnT-Q92 mice have a normal left ventricular size with enhanced systolic function, increased myocyte disarray and interstitial fibrosis, and enhanced Ca\(^{2+}\) sensitivity of myofibrillar force generation.11,15,19 The cTnT-Q92 mice do not show discernible cardiac hypertrophy, as has been observed in other mouse models of cTnT mutations.13,19,28 Notably, humans with HCM caused by the cTnT-Q92 generally exhibit minimal to mild hypertrophy.29 Finally, we have previously shown that cardiac-restricted expression of the wild-type human cTnT protein in the mouse heart does not impart a discernible phenotype.19 Therefore, the observed differential protein–protein interactions and the ensuing molecular, physiological, and morphological phenotypes are unlikely to be the consequence of expression of human cTnT protein in the mouse heart. The findings likely reflect the effects of two transgene cTnT proteins which are identical except for the point mutations.

The eccentric hypertrophic growth, evidenced by a 50% increase in the left ventricular weight/heart weight ratio and a 40% increase in left ventricular size in the cTnT-W141, is induced only by a single amino acid change in the cTnT protein. The molecular events that link the mutation to the induction of cardiac growth and dilatation are unknown. Our data suggest that the initial phenotype incited by the single amino acid change is altered by protein–protein bindings, which could instigate a series of functional phenotypes, including altered gene expression and Ca\(^{2+}\) sensitivity of the myofibrillar ATPase activity and force generation. The differential binding strengths of the mutant cTnT proteins to α-Tm could reflect the changes in the Ca\(^{2+}\) sensitivity of myofibrillar protein ATPase activities. Increased binding of cTnT-W141 to α-Tm was associated with reduced Ca\(^{2+}\) sensitivity of acto-myosin ATPase activity. The opposite was observed for the cTnT-Q92, which showed a lower binding strength for α-Tm and enhanced Ca\(^{2+}\) sensitivity. It is also noteworthy that Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity in the cTnT-Q92 and cTnT-W141 mice showed contrasting patterns at different Ca\(^{2+}\) concentrations. The sensitivity was enhanced for all Ca\(^{2+}\) concentrations tested in the cTnT-Q92 mice, particularly at low and mid Ca\(^{2+}\) concentrations. In contrast, reduced Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity in the cTnT-W141 mice was only remarkable at high but not low Ca\(^{2+}\) concentrations. The latter finding suggests that the myofilaments containing the cTnT-W141 protein may not generate adequate contraction force upon the influx of Ca\(^{2+}\) following opening of the ryanodine receptors during cardiac systole. We suggest that the differences in the Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity provide a basis for enhanced and reduced myocardial contractile function in HCM and DCM, respectively. Collectively, the changes lead to gross cardiac phenotypes, clinically recognized as DCM or HCM. The cTnT-W141 and cTnT-Q92 mouse models provide the opportunities to delineate the molecular events that link the mutations to cardiac growth and dilatation and to identify potential therapeutic targets.

Our findings for the pathogenesis of the contrasting phenotypes of HCM and DCM are restricted to the cTnT mutations. The exact prevalence of the cTnT-Q92 or cTnT-W141 in cardiomyopathies is unknown but they are not the sole or main causes of HCM and DCM. There is considerable genetic heterogeneity and there is no single common mutation. Thus, the observed mechanism may not apply to the pathogenesis of HCM and DCM caused by other mutations including those in other sarcomeric proteins, such as the β-MyHC.6 Notwithstanding, the findings may have implications for the pathogenesis of genetic cardiomyopathies in general. We posit that mutations by changing the amino acid composition of the proteins alter the secondary and tertiary structures of the proteins and possibly even the interacting partners. The change in turn could affect not only protein–protein interactions but also enzymatic or mechanical functions of the protein complexes. The ensuing morphological phenotype is likely to reflect the composite effect of the structural and functional changes in the mutant proteins and their interacting partners. Whether the initial structural and functional defects converge into common mechanisms to induce either HCM or DCM remains to be established. Likewise, the potential clinical implications of the findings and the possibility of developing specific therapies targeted at the differential protein–protein interactions and Ca\(^{2+}\) sensitivity of myofilaments could only be speculated. Accordingly, one would expect that interventions that increase Ca\(^{2+}\) sensitivity to prevent, attenuate, or reverse the evolving phenotype in cardiomyopathies caused by Ca\(^{2+}\) desensitizing mutations. In contrast, pharmacological and non-pharmacological interventions that reduce the sensitivity of the myofilaments to Ca\(^{2+}\) could prove beneficial in the prevention and treatment of cardiomyopathies caused by the Ca\(^{2+}\)-sensitizing mutations.

In conclusion, we show that differential bindings between the protein components of the thin filaments containing two different mutant cTnT proteins provide a mechanism for the contrasting effects on myofibrillar ATPase activity, myocardial systolic function, and the phenotypes of HCM and DCM.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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References


