Identification and Functional Characterization of Cardiac Troponin I As a Novel Disease Gene in Autosomal Dominant Dilated Cardiomyopathy

Sebastian Carballo, Paul Robinson, Robyn Otway, Diane Fatkin, Jan D.H. Jongbloed, Nicolaas de Jonge, Edward Blair, J. Peter van Tintelen, Charles Redwood, Hugh Watkins

Rationale: Idiopathic dilated cardiomyopathy (DCM) is inherited in approximately one third of cases, usually as an autosomal dominant trait. More than 30 loci have been identified, several of which encode sarcomeric proteins which can also be mutated to cause hypertrophic cardiomyopathy. One contractile protein gene well known as a hypertrophic cardiomyopathy disease gene, but with no reported mutation in autosomal dominant DCM, is TNNI3 which encodes cardiac troponin I.

Objective: To test TNNI3 as a candidate gene, a panel of 96 probands with DCM was analyzed.

Methods and Results: Genomic DNA was isolated and TNNI3 exons screened by heteroduplex analysis. Exons with aberrant profiles were sequenced and variants evaluated by segregation analysis and study of normal controls. We report 2 novel TNNI3 missense mutations, Lys36Gln and Asn185Lys, each associated with severe and early onset familial DCM. Of the 5 mutation carriers, cardiac transplantation was required in 3, at ages 6, 15, and 24 years. Analysis of Ca$^{2+}$ regulation of actin-tropomyosin–activated myosin ATPase by troponin revealed that troponin reconstituted with either mutant troponin I gave lower maximum ATPase rates and lower Ca$^{2+}$ sensitivity than wild type. Furthermore, mutant thin filaments had reduced Ca$^{2+}$ affinity compared with normal.

Conclusions: The functional alterations mirror closely a consistent phenotype found in proven DCM mutations in other thin filament proteins, thus supporting the interpretation that these mutations are disease-causing. These are the first reported autosomal dominant DCM-causing mutations in TNNI3, and so the findings expand the spectrum of disease-causing genes that lead to either hypertrophic cardiomyopathy or DCM depending on the specific mutation. (Circ Res. 2009;105:375-382.)

Key Words: Ca$^{2+}$ regulation ■ cardiomyopathy ■ contractility ■ dilated cardiomyopathy ■ mutation

Dilated cardiomyopathy (DCM) has a prevalence of 1:2500 and is characterized by ventricular chamber enlargement and systolic dysfunction. It is associated with an increased incidence of sudden death, thromboembolic risk, heart failure, and high overall mortality. Inherited genetic defects account for up to 30% of idiopathic DCM. Familial DCM is a genetically heterogeneous disease, most commonly inherited in an autosomal dominant fashion and linked to more than 30 disease loci. The identified disease genes encode contractile sarcomeric proteins, including actin (ACTC), β-myosin heavy chain (MYH7), troponin T (TNNT2), α-tropomyosin (TPM1), and cardiac myosin binding protein C (MYBPC3), as well as Z-disc proteins such as muscle LIM protein, cypher/ZASP, and titin (TTN). Mutations in the gene encoding lamin A/C, a component of the nuclear envelope, are also an important cause of familial DCM.

Different mutations in many of the same genes that have been associated with DCM also cause familial hypertrophic cardiomyopathy (HCM), a disease of asymmetrical ventricular hypertrophy and myocyte disarray. In contrast, individuals affected by DCM-causing sarcomeric mutations have ventricular dilatation and contractile dysfunction that occurs in the absence of preceding myocardial hypertrophy. This implies that distinct pathways lead to the 2 phenotypes. Functional studies of HCM mutant sarcomeric proteins, in general, have shown that they are likely to cause increased activation (for example, increased Ca$^{2+}$ sensitivity or higher unloaded shortening speed) compared with wild type. In
striking contrast, studies of DCM mutations in thin filament regulatory proteins using cardiac troponin T, α-tropomyosin, and cardiac troponin C mutants have shown that these cause the opposite effect, that is, a decrease in Ca\(^{2+}\) sensitivity and maximum ATPase activity, indicating a fundamentally different effect at the level of the sarcomere.\(^{16,17}\) Furthermore, DCM mutations in β myosin heavy chain depress motor function.\(^{18,19}\) How these different alterations lead to distinct ventricular remodeling patterns is unclear. The Ca\(^{2+}\)−signaling pathways mediated by Ca\(^{2+}\)/calmodulin-dependent protein phosphatase (calcineurin) and p38 mitogen-activated protein kinase\(^{20}\) demonstrate the importance of cytosolic Ca\(^{2+}\) concentration as a potent stimulus for hypertrophy. Published data suggest a possible mechanism by which mutants in sarcomeric contractile proteins can directly lead to alterations in Ca\(^{2+}\) signaling through altered Ca\(^{2+}\) buffering.\(^{21,22}\) The elucidation of molecular cardiac remodeling pathways requires further functional investigation of mutant proteins, as well as a continued search for novel genetic determinants of both HCM and DCM. Because autosomal dominant DCM mutations have been reported in the genes encoding the troponin subunits C and T but not I, we hypothesized that TNNI3 was a strong candidate gene. As part of a systematic mutation screening of a cohort of individuals with DCM, we have identified 2 novel mutations in TNNI3 in 2 families with DCM. Using functional assays, we demonstrate that both TNNI variants lower maximum ATPase rates and decrease Ca\(^{2+}\) sensitivity. These findings accord with previously described alterations in DCM-causing thin filament mutants and provide strong support for disease causation.

**Methods**

**Subjects**

Probands and family members were ascertained through our clinical practice or through referring physicians, and evaluated by physical examination, ECG and echocardiography. DCM was diagnosed in clinically affected individuals using conventional criteria.\(^4\) Blood was collected from each affected individual and available family members and DNA was extracted using standard techniques. Informed consent for inclusion in molecular genetic studies was obtained in each case. Probands had been enrolled in an ongoing screening program of the more commonly implicated DCM genes (including MYH7, MYBPC3, TNNT2, LMAN, SCGD, DES), and no patients with known mutation were included in the TNNI3 screening, although not all genes had been screened in all families. All members of families A and B, and control DNA samples, were of white origin.

**Mutation Detection**

Oligonucleotide primers were designed for flanking intronic sequences for all coding exons of the cardiac TNNT3 gene (NM_000363). The oligonucleotides for each exon are described in the Online Data Supplement (available at http://circres.ahajournals.org). Amplifications were performed by polymerase chain reaction (PCR) using a “touch-down” protocol, high-fidelity polymerases, and standard conditions from 50 ng of genomic DNA. Annealing temperatures were optimized for each exon and touched down from 7.5° above the final annealing temperature in 0.5°C decrements. Mutation screening was undertaken using either temperature-modulated heteroduplex analysis on a semi-automated denaturing high-performance liquid chromatography instrument equipped with a DNasep column, Wave DNA Fragment Analysis System (Transgenomic Inc, San Jose, Calif) or using denaturing gradient gel electrophoresis. exonx with aberrant temperature-modulated heteroduplex analysis or denaturing gradient gel electrophoresis profiles were directly sequenced following product purification by QIAquik PCR purification (Qiagen) and variants confirmed by restriction digestion (Lys36Gln), segregation analysis, and study of normal controls. For independent confirmation of the Lys36Gln mutation, a PCR product with a restriction site for Sau96I (GGGCC) was engineered using an altered degenerate forward primer (5’-GCTTATGCCAC GAGGCGCGAGGCC-3’). Digestion of the 73-bp amplifier with Sau96I created fragments of 52 and 21 bp in presence of the mutation.

**Single Nucleotide Polymorphism Haplotyping**

Identification of a T-to-A polymorphism at position 8 in intron 3 (5’ of the Lys36Gln mutation) enabled haplotype analysis in family A. Genomic DNA (20 ng) was amplified using 5’-AGATTCCTGGA AGAGCCAGGG-3’ and 5’-ATCCAGATCCGCCAGCTCC-3’ to generate a 144 bp PCR product; this was ligated into TA cloning vector (Invitrogen) and individual recombinant plasmids sequenced.

**Proteins**

Bacterial expression constructs in pMW172 encoding Lys36Gln or Asn185Lys human cardiac troponin I were created by 2-step PCR site-directed mutagenesis. These, along with wild-type troponin I, troponin T, troponin C, and Ala-Ser-α-tropomyosin were overexpressed in BL21(DES)pLysS Escherichia coli cells and subsequently purified according to our established protocols.\(^23,24\) Actin and myosin subfragment-1 (S-1) were obtained from rabbit skeletal muscle by standard procedures.\(^25,26\)

Wild-type and mutant troponin complexes were reconstituted from individual subunits using stepwise dialysis and gel filtration as previously described.\(^17,27\) Thin filaments were reconstituted at an actin, tropomyosin, and troponin ratio of 7:1:1 respectively. Levels of free troponin were ascertained by centrifugation of thin filaments at 325 000 g for 10 minutes and analysis of the total, supernatant, and pellet fractions on 12.5% sodium dodecyl sulfate polyacrylamide gels.

**Actin-Tropomyosin–Activated Myosin ATPase Assay**

Assays were carried out as previously described using 0.5 μmol/L myosin S-1 and thin filaments reconstituted using 3.5 μmol/L actin, 0.5 μmol/L tropomyosin, and 0.5 μmol/L troponin in 5 mmol/L PIPES, 3.87 mmol/L MgCl\(_2\), 1 mmol/L dithiothreitol, pH 7.0, at 37°C.\(^23\) The free Ca\(^{2+}\) concentration was set using 1 mmol/L EGTA and the appropriate concentration of CaCl\(_2\) as previously described.\(^17,24\) Phosphate release was determined colorimetrically by our standard protocol.

**Measurement of Ca\(^{2+}\) Affinity Using IAANS Troponin**

Our recently described method was used to measure thin filament Ca\(^{2+}\) affinity using 2-[4,7-(iodoacetamido)aniline]-naphthalene-6-sulfonate (IAANS) label bound to Cys35 of recombinant human troponin C.\(^22\) Thin filaments were reconstituted with 21 μmol/L actin, 3 μmol/L Ala-Ser-α-tropomyosin and 3 μmol/L IAANS.
troponin. The final buffer concentration of EGTA was 1 mmol/L, and the free Ca$^{2+}$/H$^{1001}$ concentration was set using the appropriate concentration of CaCl$_2$ as calculated by WINMAXC version 2.0. Steady state fluorescence measurements (excitation 325 nm, emission 455 nm) were made using a RF-1501 spectrofluorometer (Shimadzu) at 22°C. The change in fluorescence was monitored as the Ca$^{2+}$/H$^{1001}$ was titrated with final F$^{values adjusted for the difference in assay mix volume following each incremental addition of 10 mmol/L CaCl$_2$. The adjusted and normalized F$^{values were plotted as a function of Ca$^{2+}$/H$^{1001}$ concentration and the resultant curves fitted to the Hill equation.

Results

Identification of Lys36Gln and Asn185Lys Mutations in TNNI3

Mutation screening was carried out on a panel of 94 probands with DCM, the majority of whom (n=55) had clinical histories suggesting familial disease. Screening of all coding exons of TNNI3 enabled the identification of an A-to-C substitution at position 106, encoding the novel Lys36Gln missense mutation, in individual III:2 from family A (Figures 1 and 2). In the index patient from family B (II-1), a C-to-G substitution at position 555 (Asn185Lys) was found (Figures 1 and 2). These variants were not found in more than 280 chromosomes tested from a normal ethnically matched control population and have not been reported in previous large-scale screens of TNNI3 in HCM (see, for example, http://cardiogenomics.med.harvard.edu/home); they are therefore not common polymorphisms. Both mutated residues show high levels of evolutionary conservation (Online Figure I).

Family A

The proband from family A (Table and Figure 2A) was diagnosed with severe DCM at the age of 15, when he presented with a short history of symptoms of congestive heart failure. He experienced a rapid deterioration and required cardiac transplantation 2 months after his first presentation. The explanted heart was described as globular with biventricular dilatation. LV histology revealed moderate anisocytosis with occasional markedly enlarged brick-shaped nuclei, prominent myocyte loss, patchy interstitial fibrosis centered on a thickened endocardium, with occasional trapped inflammatory cells. Staining for amyloid and iron was negative. The younger son, IV:2, of the proband presented with severe DCM at age 6, again with a rapidly progressive course requiring LV assist device (LVAD) support and early cardiac transplantation. On screening, the older son, IV:1, was found to have mild DCM with LV dilatation and mildly reduced systolic contractility (Table). Both IV:1 and IV:2 were shown to carry the Lys36Gln mutation.

The father, II:1, of the proband died at 50 years following a motor vehicle accident, with no previous history of cardiac disease. The mother, II:2, studied at age 51, is asymptomatic with normal ECG and echocardiography. The siblings of the proband (all brothers; III:1, III:3, III:4; aged 29, 26, and 24 years, respectively) were all asymptomatic. ECG and echocardiography performed in individual III:4 confirmed normal cardiac function. Haplotype analysis was performed to investigate the parental origin of the Lys36Gln mutation. Cloning and sequencing of the DNA of the proband demonstrated that an −8T-to-A variant in intron 3 in cis with the Lys36Gln mutation. This haplotype is only present in the proband (Online Figure II), indicating that this chromosome was inherited from the father of the proband.
Table. Patient and Family Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at Diagnosis (y)</th>
<th>LV End Diastolic Diameter (mm)</th>
<th>LV End Systolic Diameter (mm)</th>
<th>LV Ejection Fraction (%)</th>
<th>Symptoms</th>
<th>Intervention</th>
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</thead>
<tbody>
<tr>
<td>Family A (Lys36Gln)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>A, III:2</td>
<td>M</td>
<td>15</td>
<td>70</td>
<td>57</td>
<td>15–20</td>
<td>CHF</td>
<td>Cardiac transplant</td>
</tr>
<tr>
<td>A, IV:1</td>
<td>M</td>
<td>7</td>
<td>49 (NR 35–47)</td>
<td>37</td>
<td>47</td>
<td>CHF</td>
<td>Cardiac transplant</td>
</tr>
<tr>
<td>A, IV:2</td>
<td>M</td>
<td>6</td>
<td>64</td>
<td>56</td>
<td>19</td>
<td>CHF</td>
<td>Cardiac transplant</td>
</tr>
<tr>
<td>Family B (Asn185Lys)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>B, II:1</td>
<td>M</td>
<td>24</td>
<td>89</td>
<td>82</td>
<td>&lt;15</td>
<td>CHF</td>
<td>Cardiac transplant</td>
</tr>
<tr>
<td>B, I:1</td>
<td>M</td>
<td>50</td>
<td>98</td>
<td>92</td>
<td>15</td>
<td>CHF</td>
<td>LV pacing; died</td>
</tr>
</tbody>
</table>

CHF indicates congestive heart failure; M, male; NR, normal range for age.
\( n_H \) was 1.87±0.16 \((n=5)\) compared with values of 1.19±0.09 \((n=5)\) and 1.49±0.02 \((n=5)\) for Lys36Gln and Asn185Lys respectively. One-to-one wild-type/mutant mixtures also gave lowered \( n_H \) values of 1.16±0.07 \((n=5)\) and 1.66±0.01 for Lys36Gln and Asn185Lys, respectively. The observed changes were highly significant in each case \((P<0.001)\).

**Effects of the Lys36Gln and Asn185Lys Mutations in Troponin I on Thin Filament Ca\(^{2+}\) Affinity**

We have recently developed a fluorescent cardiac troponin C specifically labeled by IAANS at cys35, which acts as a reporter of Ca\(^{2+}\) binding to its low affinity site (site II).\(^{22}\) This allows the determination of whether an observed decrease in Ca\(^{2+}\) sensitivity is attributable to actual change in Ca\(^{2+}\) affinity as opposed to an apparent change caused by altered troponin/tropomyosin switching. Thin filaments were reconstituted rabbit skeletal muscle actin, recombinant human α-tropomyosin and recombinant human cardiac troponin, 7:1:1, respectively, using IAANS-labeled troponin C and wild-type, Lys36Gln, Asn185Lys, 1:1 wild-type/Lys36Gln, or 1:1 wild-type/Asn185Lys mixture. The fluorescence decrease was titrated with Ca\(^{2+}\) and the data fitted to the Hill

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Functional properties of the Lys36Gln troponin I mutant. A and B, The Ca\(^{2+}\) sensitivity of thin filament regulation of actin-tropomyosin-activated myosin ATPase activity by wild-type, mutant troponin I, and 1:1 mixture of wild-type and mutant troponin I. Experiments were carried out at 37°C, as described in Methods and the rate of phosphate per myosin calculated. Experiments were carried out at 37°C as described in Methods. Data were fitted to the Hill equation using Kaleidograph (Synergy Software). C and D, The Ca\(^{2+}\) affinity of reconstituted thin filaments containing either wild-type, Lys36Gln (C), or Asn185Lys (D) troponin I or 1:1 mixture of wild-type and mutant troponin I. Experiments were carried out at 22°C, as described in Methods, and the data were fitted to the Hill equation using Kaleidograph (Synergy Software).
equation. Wild-type thin filaments bound Ca$^{2+}$ with a $pC_{a_{50}}$ of 6.24 with $n_H$ of 1.87 (Figure 3B). The affinity of filaments containing either Lys36Gln ($pC_{a_{50}}=5.65$) or Asn185Lys ($pC_{a_{50}}=5.63$) were significantly decreased as was the affinity of filaments containing 1:1 wild-type/mutant mixture. Lys36Gln ($pC_{a_{50}}=5.81$) and Asn185Lys ($pC_{a_{50}}=5.99$). The Hill coefficient was not significantly altered by the mutation (Figure 3C and 3D).

Discussion

We have detected the first reported mutations in TNNI3 to cause autosomal dominant DCM and thereby expanded the spectrum of disease genes that lead to either HCM or DCM dependent on the specific mutation. The in vitro functional characterization of the mutant proteins provides strong evidence for disease-causation and suggests that both the Lys36Gln and Asn185Lys variants are likely to act via a similar pathogenic mechanism to the DCM mutations reported in other thin filament proteins.16,17,22

The novel Lys36Gln mutation in TNNI3 was detected in a proband who presented with severe idiopathic DCM in adolescence and who transmitted the mutation to his 2 children who then presented with childhood-onset DCM. Haplotype analysis of the pedigree shows that the proband did not inherit the mutation from his mother; it therefore has either arisen as a de novo mutation on a paternal chromosome (in keeping with the lack of known disease in the father despite the severe phenotype in the affected members of the family) or it was present, but nonpenetrant, in the father. In either case, it is present in the proband as a germline heterozygous mutation that then causes autosomal dominant disease in his descendants. In family B where the Asn185Lys mutation is present in the 2 individuals with DCM and absent in the unaffected sibling, thus also providing some evidence of cosegregation. Although the amount of clinical data are limited, it is striking that the 5 individuals known to carry TNNI3 mutations show particularly severe features of DCM. Four showed massive LV dilatation and rapid progression from the time of presentation, going on to death or transplantation within months or a few years. The wide range in age of symptomatic onset (6, 15, 24, and 50 years of age) mirrors that seen with other contractile protein DCM disease genes.6

Our prior work on DCM mutants of thin filament proteins has shown that they almost without exception decrease maximum ATP turnover and lower Ca$^{2+}$ sensitivity of actin-tropomyosin–activated myosin S-1 ATPase and decrease thin filament Ca$^{2+}$ binding.16,17,22 We were able to test the novel Lys36Gln and Asn185Lys troponin I mutants in our established assays to examine whether this protein exhibited these stereotypic abnormalities and to confirm the likelihood that both the mutations are disease-causing. Both the mutant proteins did indeed display each of the characteristic abnormalities (Figure 3), with the deviation from wild type within the range of that previously seen with other DCM mutants.16,17,22 We conclude that these novel variants are highly likely to be disease-causing in a similar functional manner to one another, as well as previously studied DCM-causing thin filament regulatory protein mutations.

The majority of published mutations in TNNI3 lead to HCM, with a subset of alleles showing a predilection for a restrictive cardiomyopathy phenotype. Available data suggest that these are manifestations of the same disease spectrum because patients with restrictive cardiomyopathy and mutations in TNNI3 tend to have myocyte disarray and relatives with typical HCM29; functional studies of restrictive TNNI3 alleles have shown an extreme increase in Ca$^{2+}$ sensitivity (ie, in keeping with an exaggerated HCM-like phenotype).28 One TNNI3 variant, a Ala2Val missense mutation, has been reported to cause recessive DCM in one family.29 Most cases of DCM have an autosomal dominant mode of inheritance, and in the aforementioned report, 85% of the studied families fell into this category, with 15% being compatible with autosomal recessive inheritance. The Ala2Val mutation was identified in an individual who underwent heart transplantation. The proband had one affected homozygous sister, but both heterozygous parents and a heterozygous sibling were asymptomatic. The suggested mechanism of action of this mutant is decreased interaction with troponin T, ultimately leading to diminished contractility. Our analyses of the effect of the Ala2Val mutation on ATPase regulation have found

charged groups shown in red, positively charged groups in blue. Amino acids 33 to 40 of troponin I are shown in ball-and-stick format, with Lys36 in yellow. Note the proximity of its terminal amine group (blue) with acidic residues (red) at the entrance to the Ca$^{2+}$-binding pocket. All structures were drawn using iMol software (http://www.pirx.com/iMol).

Figure 4. Location of Lys36 of troponin I in the structural models of unphosphorylated and phosphorylated cardiac troponin. A and B, Ribbon diagrams showing the structure of troponin C (red), troponin I (blue), and troponin T (yellow) in the composite models of cardiac troponin in both the unphosphorylated (A) and phosphorylated (B) states.30 Lys36 of troponin I is shown in space-fill form in green. Note the phosphorylation-dependent movement of the N-terminal extension (residues 1 to 32; light blue), with residues 35 to 42 acting as a hinge.30 C, Interaction of Lys36 troponin I with the regulatory Ca$^{2+}$-binding site of cardiac troponin C. From the unphosphorylated model,30 the Ca$^{2+}$-binding site of cardiac troponin C (residues 65 to 77) is shown in space-fill format with negatively charged groups shown in red, positively charged groups in blue, and phosphorylated (A) and phosphorylated (B) states.30 Lys36 of troponin I is shown in space-fill form in green. Note the phosphorylation-dependent movement of the N-terminal extension (residues 1 to 32; light blue), with residues 35 to 42 acting as a hinge.30
that troponin function is not significantly altered, in contrast to the dominant negative mutations described in this report (data not shown).

Lys36 of human cardiac troponin I is one of a triplet of lysine residues and is highly conserved in vertebrate sequences (Online Figure I). Composite models of the structure of cardiac troponin in both unphosphorylated and phosphorylated (Ser23/Ser24) forms have recently been produced by Rosevear and coworkers,30 combining the x-ray crystal structure of the core domain31 with novel NMR data on the N-terminal extension of cardiac troponin I.30 Using these structures, it has been postulated that residues 33 to 42 of cardiac troponin I form a hinge that mediates the movement of the N terminus in response to phosphorylation (Figure 4A and 4B).30 The position of the Lys36 residue in unphosphorylated troponin, is striking (Figure 4): the lysine chain protrudes from the troponin I to interact via its terminal amine group with the acidic residues at the mouth of the regulatory Ca2+-binding site II of troponin C (Figure 4C). As such, the side chain might have been predicted to have a blocking effect on the entry of Ca2+. However, in the phosphorylated form in which the Ca2+ affinity is known to be lower,32 Lys36 is shifted from this position and no longer interacts with troponin C.30 Moreover, the substitution to Gln as encoded by the mutation, would generate a shorter, uncharged (although polar) side chain, that also would be predicted to reduce the interaction of troponin I with the Ca2+-binding site of troponin C. We speculate that the Lys36 interaction may stabilize site II and when this interaction is removed, either by mutation of the residue or by movement of the N terminus of troponin I induced by phosphorylation, Ca2+ affinity is reduced. We propose that this, at least in part, accounts for the effect of the mutation on Ca2+ affinity.

AASN185 is also a highly conserved residue in vertebrate sequences (Online Figure I). The crystal structure of human cardiac troponin shows that amino acids 164 to 188 form an amphipathic α-helix (referred to as H4) that does not have interactions with other troponin subunits.33 Biochemical work has found that the equivalent portion of skeletal muscle troponin I binds to actin-tropomyosin33; this interaction is reflected in recent 3D reconstructions models of whole thin filaments that suggest this region is involved in keeping the tropomyosin in the blocked position at low Ca2+.34,35 We note that HCM-causing missense mutations within this helix, which are likely to cause increased Ca2+ sensitivity,24 replace basic side chains at Lys178, Lys183, and Arg186 (http://cardigenomics.med.harvard.edu/home), whereas the DCM-causing mutation reported here, which has the opposite functional effect, introduces a basic residue. This suggests that ionic interactions of this helix with actin-tropomyosin are involved in transducing the Ca2+ signal.

The precise pathways that link decreased Ca2+ sensitivity of contractility to disease pathogenesis remain unclear. A gene-targeted mouse model of a DCM troponin T mutation has recently been described in which the dilated phenotype was recapitulated.36 It was found that developed force in intact fibers was similar to wild type because of an enhanced Ca2+ transient; the latter suggested to be the result of compensatory adaptations to the reduced myofilament Ca2+ responsiveness. Chronic elevation of the Ca2+ transient may contribute to the disease progression by alteration of Ca2+-dependent signaling; in addition, the direct effect of the mutation on troponin Ca2+ buffering may also contribute to altered signaling.22 Further experimentation is necessary to dissect these pathways and to understand how the opposite changes to the Ca2+-sensitivity of contractility caused by the DCM and HCM mutations lead to the distinct cardiac phenotypes.

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Disclosures

None.

References


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Online Figure I

Evolutionary conservation of human cardiac troponin I Lys36 and Asn 185 residues and flanking sequences

The human cardiac troponin I sequence (residues 29-46 and 178-195) aligned with the equivalent regions of the mouse (amino acids 30-47 and 178-195; NP_033432), rat (30-47 and 179-196; NP_058840), dog (30-47 and 179-196; NP_001003041), Rhesus macaque (29-46 and 179-196; XP_001085820), bovine (31-48 and 180-197; XP_588363.2), frog (55-72 and 230-247; NP_001011410), nematode (39-56 and 207-224; NP_509906), opossum (81-98 and 179-194; XP_001381826), fruit fly (24-41 and 179-194; NP_728141) and mosquito (24-41 and 197-214; DAA05511) sequences.
Haplotypes: (upper) -8 intronic variant; 1=A and 2=T (wild type); (lower) codon 36; 1=Gln36 and 2=Lys36 (wild type). Haplotype analysis shows that the father of the proband (II:1) must either have carried the Lys36Gln mutation or the mutation arose \textit{de novo} in the proband. Black filled symbol= affected; bold arrow=proband.