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Modulating the tension-time integral of the cardiac twitch prevents dilated cardiomyopathy in murine hearts

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Abstract

Dilated cardiomyopathy (DCM) is often associated with sarcomere protein mutations that confer reduced myofilament tension-generating capacity. We demonstrate that cardiac twitch tension-time integrals can be targeted and tuned to prevent DCM remodeling in hearts with contractile dysfunction. We employ a transgenic murine model of DCM caused by the D230N tropomyosin (Tm) mutation and design a sarcomere-based intervention specifically targeting the twitch tension-time integral of D230N-Tm hearts using multiscale computational models of intra- and inter-molecular interactions in the thin filament and cell-level contractile simulations. Our models predict that increasing the calcium-sensitivity of thin filament activation using the cardiac troponin C (cTnC) variant L48Q can sufficiently augment twitch tension-time integrals of D230N-Tm hearts. Indeed, cardiac muscle isolated from double-transgenic (DTG) hearts expressing D230N Tm and L48Q cTnC have increased calcium-sensitivity of tension development and increased twitch tension-time integrals compared to preparations from hearts with D230N Tm alone. Longitudinal echocardiographic measurements revealed that DTG hearts retain normal cardiac morphology and function, while D230N-Tm hearts develop progressive DCM. We present a computational and experimental framework for targeting molecular mechanisms governing the twitch tension of cardiomyopathic hearts to counteract putative mechanical drivers of adverse remodeling, and open new possibilities for tension-based treatments of genetic cardiomyopathies.
Introduction

Dilated cardiomyopathy (DCM) is a common and deadly genetic cardiac disorder that affects ~1/250 individuals (1) and is typically characterized by enlarged chambers and a thinning of ventricular walls that leads to systolic heart failure (2, 3). DCM is often caused by loss-of-function mutations in genes encoding sarcomere proteins (4–6) that reduce the tension-generating capacity of the myofilaments, which ultimately leads to the adverse ventricular remodeling and heart failure. Currently, treatment options for patients with DCM only delay its progression and do not address the underlying biophysical causes.

Recently, Davis and colleagues demonstrated that growth and remodeling of cardiomyopathic hearts can be predicted by the duration and magnitude of mechanical tension during cardiac twitches (7). The authors systematically perturbed the contractile performance of the sarcomere using a wide variety of genetically engineered murine models and human induced pluripotent stem cell-derived cardiomyocytes from patients with cardiomyopathies. They found that the twitch tension-time integral of genetic variants relative to controls, termed the ‘tension index’ (TI), strongly correlates with the type and severity of cardiac growth in each model. More specifically, genetic modifications to the sarcomere that decrease the twitch tension-time integral (i.e., have a TI < 0) strongly correlate with eccentric cardiac growth, whereas modifications that increase the twitch tension-time integral (i.e., have a TI > 0) strongly correlate with concentric cardiac growth (Figure 1A).

The relationship between the TI and cardiac growth (Figure 1A) suggested to us that the total net tension generated during a twitch can be rationally tuned in cardiac muscle with contractile dysfunction to engineer a tension-based intervention that prevents pathological remodeling. Because the TI inherently accounts for altered kinetics of contraction and relaxation relative to normal cardiomyocytes, modulating the TI does not inherently require targeting the peak twitch tension (or end-systolic pressure). Figure 1B shows theoretical twitch tension (T) traces (8) to illustrate how variations in twitch amplitude and kinetics compared to ‘normal’ conditions modulate the TI. Twitch variants with either increased (gray) or decreased (blue) area under the T-time trace compared to a ‘normal’ (black) twitch therefore have a positive and negative TI (respectively), which does not necessarily depend on the peak T. Thus, the multiple biophysical properties underlying the cardiac twitch that determine the TI likely represent multiple tunable
targets for modulating the twitch tension of cardiomyopathic hearts to prevent adverse growth and remodeling.

We (7) and others (9) have found that combining certain gain-of-function protein mutations (i.e., mutations that augment contractility) with the DCM-causing loss-of-function mutations can prevent or reduce the DCM phenotype in murine models. These studies inform on the potential utility of tuning myofilament contractility as a preventative option for genetic cardiomyopathies. However, with a growing number of newly identified cardiomyopathy-causing protein mutations (1), new methodology that can guide the design of treatment options for wide-ranging mutations is needed.

In this work, we combine computational and experimental approaches to target and counteract contractile dysfunction caused by a DCM-associated sarcomere protein mutation. To do so, we employ a transgenic murine model of DCM that is caused by a point mutation in tropomyosin (Tm) at the 230th residue (aspartic acid to asparagine, denoted D230N) (10, 11). This mutation has been found in at least two unrelated families with DCM (12). In-vitro studies have shown that D230N Tm decreases the calcium sensitivity of filament sliding and ATPase rates in motility assays (13, 14), decreases the calcium affinity of troponin C (12), and increases the affinity of Tm for actin by nearly 5-fold (14). Moreover, transgenic mice expressing D230N Tm have significant systolic dysfunction and eccentric hypertrophy by two months of age (13). We demonstrate here that the D230N Tm mutation also significantly decreases the twitch tension of intact cardiac muscle, thus producing a large, negative TI that correlates well with the DCM phenotype found in these mice (13). Using multiscale computational modeling as a guide, we investigate approaches to modulate the TI of sarcomeres with D230N Tm. Our models predict that targeting the calcium sensitivity of thin filament activation using the cardiac troponin C (cTnC) calcium-sensitizing variant L48Q (7, 15–20) will augment the twitch tension-generating capacity of D230N cardiomyocytes. We verify these predictions experimentally by generating a novel double-transgenic (DTG) mouse model with cardiac expression of both L48Q cTnC and D230N Tm, and show that cardiac muscle from DTG hearts have significantly increased contractility compared to D230N hearts. Lastly, longitudinal echocardiographic monitoring of DTG hearts revealed that the expression of L48Q cTnC in D230N Tm hearts not only preserves cardiac contractility but also inhibits the development of the DCM phenotype based on functional and morphological echocardiography up to 5 months of age. Thus, our work demonstrates that
molecular mechanisms governing cardiac twitch tension-time integrals can be targeted and tuned to prevent pathological ventricular growth and remodeling in hearts with sarcomeric dysfunction, opening new possibilities for other tension-based therapies.

**Results**

*Modulation of the tension index of cardiomyocytes with D230N tropomyosin depends on the inotropic target.* To determine the effects of the D230N Tm mutation on tissue-level contractility, we measured twitch tension transients of electrically stimulated intact trabeculae isolated from the right ventricles of wild-type (WT) and D230N Tm transgenic murine hearts. The twitch tension ($T$) of intact trabeculae from D230N hearts is reduced overall compared to WT, with the peak twitch tension ($T_{\text{peak}}$) in D230N trabeculae significantly less than that of WT (Figure 2A). Correspondingly, the tension index ($TI$) of intact trabeculae from D230N hearts, computed as the $T$-time integral relative to WT, is $-7.0 \times 10^3$ ($T\cdot$ms). The large negative value of the $TI$ for D230N trabeculae together with the DCM phenotype observed in these hearts (13) (Figure S1) further supports the hypothesis from Davis *et al.* that the $TI$ is correlative with ventricular remodeling (7).

In normal cardiomyocytes, any inotropic modulation necessarily affects the $TI$, but the relationship between inotrope and $TI$ is likely altered by sarcomere protein mutations that dysregulate contractility. The putatively dysfunctional D230N Tm (12–14) may supersede thin filament activation altogether, rendering any inotropic modulation ineffective in augmenting the $TI$ of D230N hearts. As such, we assessed whether the $TI$ of cardiomyocytes containing dysfunctional Tm can be modulated using inotropic intervention and, if so, whether augmented calcium sensitivity of thin filament activation or augmented cross-bridge (XB) binding has a greater effect on the $TI$. To do so, we used a computational model of cardiomyocyte contraction (21), as we have done previously (7), to independently and systematically increase either the calcium affinity of cTnC or the rate of strong XB attachment in a sarcomere with dysfunctional Tm, and calculated the $TI$ for each case. Twitches of cardiomyocytes containing dysfunctional Tm were simulated by reducing the rate of transition of Tm from ‘blocked’ to ‘closed’ (21, 22) (see Table S1 and Figure S2) until $T_{\text{peak}}$ was reduced by the same amount observed experimentally in intact trabeculae from transgenic hearts containing D230N Tm (inset of Figure 2A). The resulting $TI$ of simulated D230N cardiomyocytes is $-13.4 \times 10^4$ %WT $T\cdot$ms (blue circle in Figure 2B). Progressively increasing the rate of strong XB binding increases the $TI$ of simulated D230N...
cardiomyocytes (Figure 2B, dashed line) until it eventually asymptotes at a value well below zero (the point at which the $T_I$ equals that of WT). Conversely, progressively increasing the calcium affinity of cTnC increases the $T_I$ of simulated D230N cardiomyocytes well beyond zero (Figure 2B, solid line) and does not asymptote for the range of parameters explored here.

**L48Q cTnC induces structural changes in troponin-tropomyosin-actin complexes containing D230N Tm that likely enhance thin filament activation.** Our simulated twitches suggest that the tension index of D230N cardiomyocytes can be greatly increased by augmenting calcium binding to cTnC (Figure 2B). When calcium binds to cTnC, allosteric interactions in the troponin (Tn) complex causes the inhibitory peptide region of cTnI to reduce its interaction with actin (23–25), which enables tropomyosin to move from a ‘blocked’ state to ‘closed’ and ‘open’ states that permit varying degrees of cross-bridge binding (22). The activated state of the Tn-Tm complex is stabilized by interactions between the switch peptide of cTnI and cTnC [see Ref. (24) and references therein]. Furthermore, sub-nanometer changes in intra- and inter-molecular interactions within a Tn-Tm-actin complex can have great effects on the calcium sensitivity of thin filament activation and force generation (16, 26, 27). A cTnC variant engineered to augment this process via increased calcium affinity and the cTnC-cTnI interaction is the L48Q cTnC variant (7, 15–20).

While the effects of the L48Q cTnC mutation on the molecular structure of Tn have been previously investigated (16, 19), it is not known if the L48Q cTnC variant retains its calcium-sensitizing effects on thin filament activation when coupled with the D230N Tm mutation. Thus, we employed a structural model of the cardiac thin filament (28–31) that includes atomically detailed actin, tropomyosin, and the full troponin (Tn) complex, and incorporated into the model the point mutations D230N in Tm and L48Q in cTnC (Figure S3). We then quantitatively assessed structural differences between regulatory units (RUs, defined here as Tn-Tm-actin complexes) containing D230N Tm with and without L48Q cTnC that may influence the calcium sensitivity of thin filament activation.

We first investigated the interactions of the $\text{Ca}^{2+}$ ion in the Site II calcium-binding region of cTnC, and found that there are no differences in the distances between the $\text{Ca}^{2+}$ ion and the $\text{Ca}^{2+}$-coordinating atoms in Site II of cTnC when comparing the WT RU (Figure 3A, green bars) to RUs containing D230N Tm with or without L48Q cTnC (Figure 3A, red and blue bars, respectively). However, when calcium is bound to Site II of cTnC, there are notable differences in the cardiac
troponin I (cTnI) subunit structure between RU types. Compared to the WT RU, the inhibitory peptide of cTnI is closer to its neighboring actin monomer in the RU containing D230N Tm; whereas, it is shifted away from actin in the RU containing both D230N Tm and L48Q cTnC (Figure 3B). The interactions between the cTn and cTnC subunits were also different for each RU type. Figures 3C and D show the cTnC (gray) and cTnI subunits for the WT RU (green cTnI) and RUs containing D230N Tm without L48Q cTnC (blue cTnI in Figure 3C) and with L48Q cTnC (red cTnI in Figure 3D). In the RU with D230N Tm and WT cTnC, the inhibitory and switch peptides of cTnI are generally shifted away from cTnC compared to the WT RU (Figure 3E). Conversely, in the RU containing both D230N Tm and L48Q cTnC, the inhibitory peptide is shifted closer to cTnC while the switch peptide is closer to some cTnC residues and farther away from others compared to the WT RU (Figure 3F). We also note that, in a RU containing D230N Tm, the H1 helix in the I-T arm of cTnI is slightly shifted away from cTnC compared to WT, while the H1 helix of cTnI in a DTG RU is shifted slightly closer to cTnC compared to WT (Figure S4).

The magnitude of these changes in interaction distances, however, is much smaller (≤ 0.8 Å) compared to the changes observed in the inhibitory and switch peptides (Figure 3). Together, these results suggest that, when combined with D230N Tm, L48Q cTnC does not significantly affect the affinity of cTnC for calcium, but rather strengthens the cTnC-cTnI interaction when calcium is bound to Site II. Thus, because interactions between the cTnC and cTnI subunits regulate contraction (26, 32–34), our structural model predicts that L48Q cTnC variant likely augments the calcium sensitivity of activation of thin filaments containing D230N Tm by allosterically enhancing the strength of the cTnC-cTnI interaction.

The reduced contractility of cardiac muscle containing D230N tropomyosin is prevented by the expression of L48Q cTnC. To demonstrate that the predicted structural effects of L48Q cTnC on RUs containing D230N Tm (Figure 3) translate into heightened thin filament activation in cardiac muscle preparations, we measured the steady-state T generated in membrane-permeabilized cardiac muscle strips (see Methods) isolated from WT, D230N, and D230N + L48Q double-transgenic (DTG) mouse hearts for a range of calcium concentrations (pCa 9.0 to 4.0). The data were converted to a percentage of the maximum steady-state T value (at pCa 4.0) within each group and fit to the Hill equation. As shown in Figure 4A, the steady-state T-pCa curve of cardiac muscle from D230N hearts (blue) is right-shifted compared to WT (green), demonstrating a
decrease in calcium-sensitivity of $T$ in D230N hearts. Conversely, the $T$-pCa curve of cardiac muscle from L48Q hearts was left-shifted compared to WT, which is in good agreement with previous studies on the L48Q cTnC variant (17). Most notably, the steady-state $T$-pCa curve of cardiac muscle from DTG hearts is nearly superimposed with that of WT. Correspondingly, the calcium concentration at 50% maximum $T$ (the pCa$_{50}$) is significantly reduced in cardiac muscle from D230N hearts compared to all other groups (Figure 4B), while the pCa$_{50}$ of cardiac muscle from DTG hearts is not different from WT. Neither the Hill coefficient ($n_H$) nor the maximum steady-state $T$ (at pCa 4.0) had statistically significant differences between any of the groups (Table S2).

Next, we assessed whether the improved calcium sensitivity of steady-state tension of cardiac muscle from DTG hearts compared to D230N hearts translates to increased twitch tension of intact trabeculae. Figure 4C shows the average twitch $T$-time traces of intact trabeculae isolated from hearts of each genotype, which are shown as a percentage of WT $T_{\text{peak}}$. The WT $T$-time trace is shown as a dashed green trace against each variant twitch for comparison. We found that the $T_{\text{peak}}$ of trabeculae from DTG hearts is significantly greater than that of D230N hearts, while it is not different from that of WT (Table S2). The kinetics of the twitches were not different between groups, with the exception of significantly decreased relaxation kinetics in trabeculae from L48Q hearts (Table S2). We then calculated the area under each twitch $T$-trace and compared it to WT to determine the $TI$ for each variant (Figure 4D). The $TI$ of trabeculae from DTG hearts is $-2.7 \times 10^3$ (%WT $T$-ms), which is $>2.5$-fold smaller in magnitude than the $TI$ of trabeculae from D230N hearts ($-7 \times 10^3$ %WT $T$-ms; see also Table S2). These results confirm the combined predictions of our computational models and demonstrate that the expression of L48Q cTnC in hearts expressing D230N Tm prevents reductions in both the calcium sensitivity of tension and the twitch tension-generating capacity caused by the D230N Tm mutation.

The DCM phenotype in hearts with D230N Tm is prevented by expression of L48Q cTnC. Because the net tension generated during a twitch of intact trabeculae from double-transgenic (DTG) hearts is significantly greater than that of hearts containing D230N Tm (Figure 4), we hypothesized that the degree of ventricular hypertrophy and dysfunction of DTG hearts will be reduced compared to that of D230N hearts. To test this, we used echocardiography to monitor the progression of the DCM phenotype (see Methods) in D230N hearts compared to WT, L48Q, and
DTG hearts over a 4-month span (from ages 2 to 5 months). Compared to WT hearts, the left ventricular inner diameters during diastole (LVID_D, Figure 5A) and systole (LVID_S, Figure 5B) of D230N hearts progressively increase between 2–5 months of age, while they are unchanged in DTG hearts. Notably, the LVID_D and LVID_S of DTG hearts are not significantly different from WT hearts at any time-point investigated here (2–5 months). Consistent with this, the LV mass and anterior wall thickness [measured from echocardiogram images, as previously described (35, 36)] of D230N hearts is significantly increased and decreased (respectively) compared WT by 4–5 months of age, while neither the LV mass nor anterior wall thickness of of DTG hearts is different from WT (Figure S5). Furthermore, the preserved ventricular dimensions in DTG hearts compared to D230N hearts are accompanied by preserved function. The fractional shortening (FS) and ejection fraction (EF) of D230N hearts progressively worsens from 2 to 5 months of age, while the FS and EF of DTG hearts is approximately constant with age and does not differ significantly from WT hearts at any age (Figures 5C & D). (See Table S3 for numerical values of all echocardiography measurements.) These results demonstrate that hearts containing D230N Tm progressively develop DCM while the expression of L48Q cTnC in these hearts prevents the development of the DCM phenotype.

**Discussion**

The goal of this work was to demonstrate that the total tension generated during a cardiac twitch is not only an important predictor of myocardial growth and remodeling (7) but also an effective guide for designing approaches to prevent pathological remodeling in hearts with sarcomere dysfunction. To demonstrate this, we used a combined experimental and computational approach to identify tunable molecular interactions in the sarcomere that would enable the modulation of the twitch tension-time integral of hearts containing the DCM-causing D230N Tm mutation. Experimentally executing the approach to tune the contractility of D230N Tm hearts developed through our computational methods, we were able to suppress DCM pathogenesis in murine hearts containing D230N Tm. Our work demonstrates the ability to prevent pathological growth and remodeling of cardiomyopathic hearts by rationally engineering molecular determinants of the cardiac twitch, opening new possibilities for the development of tension-based treatments of DCM.
A limitation in the $TI$ metric as a predictor for cardiac growth is that the absolute value of the $TI$ may depend on the experimental or computational conditions in which it is calculated. For example, absolute value of $TI$s calculated from unloaded single-myocyte shortening [as in Ref. (7)] may vary from $TI$s calculated from isometric tissue-level tension (this work), which may also vary from $TI$s calculated from ventricular-level pressures. Moreover, $TI$s determined from simulated versus experimentally measured twitches may also attribute to variation in $TI$ absolute values. Importantly, however, the $TI$ is by definition relative to WT regardless of the experimental or computational conditions in which it is calculated, and we therefore hypothesize that it will be predictive of ventricular and/or cardiomyocyte growth within any given set of conditions. Future work will confirm the scalability and translatability of the $TI$ as a global predictor of hypertrophy and remodeling.

Modulation of the $TI$ can be achieved through a number of different inotropic interventions, and methods to target the molecular mechanisms of cardiac contractility in the treatment of heart failure have been studied for decades. Small molecules targeting the thick filament that are in human clinical trials [e.g., omecamtiv mecarbil (37–40)], or are being heavily investigated for translation to the clinic [e.g., 2-deoxy-ATP (41–46)], show great promise in treating systolic heart failure. Genetic approaches to engineer the molecular mechanisms of thin filament activation have provided new insights into targeting specific regulators of cardiac contraction for therapeutic applications, including the use of L48Q cTnC as a treatment for systolic heart failure in rat and mouse models of myocardial infarction (15, 17, 18). In DTG mice, the L48Q cTnC variant may not fully correct structural defects in thin filament regulatory units caused by the D230N mutation in Tm, but instead provides beneficial alterations to intramolecular interactions in troponin that are sufficient to overcome putative D230N-associated dysregulation. Thus, our work and others’ (15, 17, 18) highlight the importance of understanding the molecular interactions in the sarcomere that underlie cardiac twitch properties when developing myofilament-based treatments for contractile dysfunction.

Rationally designed approaches to target contractile dysfunction at the myofilament level require a significant level of prior knowledge of: (i) the consequences of a given mutation on the protein structure and function, (ii) the molecular structure-functional effects of the therapeutic under normal circumstances, and (iii) potential combinatorial structure-function effects of the mutation and the therapeutic. As the molecular properties of cardiomyopathy-associated protein
mutations continue to be characterized, it will therefore be important to catalog mutations based on their effects on cardiac function—particularly how they affect molecular mechanisms that determine twitch tension and kinetics. Additionally, we note that very few studies of potential treatments for contractile dysfunction to date have investigated effects on twitch kinetics. However, a recent study by Chen et al. revealed that suppressing detyrosination of cardiac microtubules increases the rate of contraction and relaxation without affecting calcium transients in failing human hearts (47), which may augment the TI of patients with heart failure. Their study, along with the work we present here, highlights the importance of assessing twitch kinetics in potential therapies for heart failure.

Computational models such as those we present here will likely be important tools for predicting and sorting the effects of different sarcomere mutations on twitch tension and kinetics, as well as facilitate the design of new treatments for genetic cardiomyopathies based on combinatorial effects of the mutation and the treatment. Towards that end, Davis and colleagues found that by classifying mutations that activate or deactivate the thin filament as weak, moderate, or strong, they could construct a model to predict cell behavior for potentially thousands of combinations of known mutations in thin filament proteins (48). Some combinatorial effects between activating and deactivating mutations have already been shown to result in neutralized cardiac function (7, 48), similar to what we present here. Moreover, the ability of computational models to accurately predict structural and functional consequences of pathological protein mutations in the heart enables a completely non-invasive approach to designing therapies. While we rely heavily on such computational predictions in this work, many of the metrics we use to experimentally confirm those predictions require access to cardiac muscle (e.g., the calcium sensitivity measurements and intact trabeculae mechanics). Thus, an important next step in the advancement of computational models is to scale atomistic models to ventricular function such that predictions can be made and corroborated with non-invasive measurements (e.g., echocardiography, MRI) of patients with mutation-causing cardiomyopathies to guide the development of new therapies.

While significant progress has been made towards using gene therapy to treat heart failure (49–52), current gene delivery approaches can result in low cellular penetrance of the therapy. In the mouse models we present here, the expression of L48Q cTnC is ~30% (Figure S6). Consequently, when combined with the D230N transgenic mice that express ~57% D230N (13)
Tm, < 20% of thin filament regulatory units include both L48Q cTnC and D230N Tm. Despite this, we still see a robust enhancement of cardiac contractility in our double-transgenic mice compared to D230N hearts that is sufficient to prevent the development of the DCM phenotype. Therefore, future work investigating myofilament-targeted treatments for heart failure may not necessarily require high penetrance of the treatment to see a robust enhancement in function (or prevention of dysfunction). However, we note that the (roughly) linear relationship between the tension index and calcium sensitivity modulation predicted by our computational model (solid line of Figure 2B) suggests a dose-dependent response to genetic modification of the myofilaments. Experimental observations support this, as a ~15% incorporation of L48Q cTnC has been shown to cause little alteration to twitch relaxation kinetics (18), whereas 30% L48Q cTnC incorporation reduces relaxation (Figure 4C and Table S2). Furthermore, ~45% L48Q cTnC replacement is predicted to significantly increase twitch force and further decrease relaxation kinetics compared to what we report here (7). Thus, the possibility of modulating the tension index beyond a point of correction and inducing unwanted hypertrophy warrants further investigation. Lastly, an important next step towards the advancement of therapies targeting cardiac contractility is to elucidate the preventative versus restorative outcomes. Here, we show that inhibiting dysfunction caused by the DCM-associated D230N Tm mutation by expressing L48Q cTnC can prevent the development of DCM, but whether the same approach can be used to reverse pathological remodeling remains entirely unknown. Thus, the timing of intervention likely plays a role in determining the outcome of the treatment, and future studies will aid our understanding of when versus how to treat heart failure.

In conclusion, our study presents a framework for employing computational and experimental techniques to rationally tune the molecular mechanisms governing the twitch tension of cardiomyopathic hearts to counteract mechanical drivers of adverse remodeling, and it has the potential to inform on new tension-based therapeutics for genetic cardiomyopathies.

Methods

Animal use & ethics

All animal experiments were done in accordance with protocols approved by the University of Washington Institutional Animal Care and Use Committee and followed The Guide for the Care and use of Laboratory Animals (53). Adult male and female mice between 4 and 6 months of age
were euthanized following the procedures approved by the Institutional Animal Care and Use Committee for the University of Washington. Mice were sedated by inhalation of isoflurane and an intraperitoneal (IP) injection of 0.1 mL of heparin was administered to minimize blood clotting in the ventricles. Approximately four minutes after the injection of heparin, an IP injection of a lethal dose (0.1 mL) of pentobarbital (Beuthanasia-D) was administered.

Excision of murine hearts

Hearts were rapidly excised via thoracotomy, and immediately immersed in oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}), room-temperature Krebs buffer containing (in mM) 118.5 NaCl, 5 KCl, 1.2 MgSO\textsubscript{4}, 2 NaH\textsubscript{2}PO\textsubscript{4}, 25 NaHCO\textsubscript{3}, 1.8 CaCl\textsubscript{2}, and 10 glucose. Hearts were then rinsed via aortic retrograde perfusion with Krebs buffer containing low calcium (0.1 mM CaCl\textsubscript{2}) and 20 mM 2,3-Butanedione 2-monoxime (BDM) to minimize contraction and subsequent damage during dissection.

Intact trabecula mechanics

Thin, unbranched, and intact trabeculae were carefully dissected from the right ventricular wall and mounted between a force transduce (Cambridge Technology, Inc., Model 400A) and a length-controlling motor (Aurora Scientific, Model 300C). Each end of the trabecula was sutured to custom arms attached to the motor and force transducer made from 22-gauge needles. The trabecula was then submerged in a custom experimental chamber that was continuously perfused with modified Krebs buffer (1.8 mM CaCl\textsubscript{2}) at 30° C. Twitches were elicited by field stimulation with custom platinum plate electrodes at 1 Hz with oscillating polarity. Sarcomere length (SL) was set to 2.0 µm using an inverted stereomicroscope with a 40x dry objective lens and a 10x eyepiece. If sarcomeres could not be seen for direct measurement (e.g., if the trabecula was too thick), a SL of 2.0 µm was assumed to be at trabecula slack length (the length of the trabecula at the onset of passive tension development). Trabeculae were allowed to pace at 1 Hz for ~20 minutes at SL 2.0 µm (and 30°C), and then stretched to SL 2.3 µm for data acquisition.

Continuous twitch tension traces were recorded using custom LabView software at a sampling rate of 1 kHz and were analyzed with custom code written using MATLAB software (version 2018a, The MathWorks, Natick MA).
**Permeabilized trabecula mechanics**

Hearts were permeabilized in ‘skinning’ solution containing 100 mM KCl, 10 mM MOPS, 5 mM EGTA, 9 mM MgCl₂ and either 1 mM dATP or 4 mM ATP (adjusted to pH = 7 with KOH), 1% (by volume) Triton X-100, 1% protease inhibitor (sigma P8340), and glycerol at 4°C overnight. Permeabilized trabeculae were then dissected from the right and left ventricles and mounted between a force transducer and motor using custom aluminum T-clips. Sarcomere length (SL) was measured using a Fourier transform of a digitized image of the sarcomeres using an IonOptix camera connected to a 40x dry objective lens. SL was set to 2.3 µm for the experiments. Trabeculae were submerged in physiological solution at 15°C containing a range of pCa (=-log[Ca²⁺]) from 9.0 to 4.0, and allowed to reach steady-state tension (T_SS) at each pCa. T_SS-pCa curves for each genotype were collected and analyzed with custom code using LabView software, and were fit to the Hill equation, as
\[ T_{SS} = T_{SS,\text{Max}} \cdot [1 + 10^{n_H \cdot (pCa_{50} - pCa)}]^{-1}, \]
where T_SS,Max is the maximum steady-state tension (at pCa 4.0), pCa_{50} is the pCa at half-maximal tension, and n_H is the Hill coefficient (the slope of the T_SS-pCa relation and a measure of the cooperativity of tension).

**Echocardiography**

Male and female mice of each genotype from 2–5 months of age were used for echocardiographic measurements, as previously described (44). Briefly, animals were lightly anesthetized and held under anesthesia via inhalation of 1% isoflurane in 95% oxygen. Transthoracic echocardiography was performed using Vevo 2100 high-frequency, high-resolution imaging system (VisualSonics) equipped with MS400 MicroScan Transducer. The parasternal short axis view at the mid-papillary level was used to obtain M-mode images for measurements of left ventricular inner diameters at the end of diastole and end of systole, fractional shortening, and ejection fraction. The fractional shortening was calculated from these data using the relationship
\[ 100 \times (\text{LVID}_d - \text{LVID}_s)/\text{LVID}_d, \]
where LVID_d and LVID_s are the left ventricular inner diameters at the end of diastole and systole, respectively.

**Calculation of the tension indexes**

Similar to Davis *et al.* (7), the tension index was calculated based on the tension-time integral from either intact trabecula mechanics or the computationally simulated cardiac twitches. Twitch tension-time traces were normalized to the maximum WT value for each genotype. The
time integral of the twitch tension was then calculated using a point-by-point integration method
based on cumulative trapezoidal approximations using MATLAB software (2018a version, The
MathWorks, Inc., Natick, MA). The tension index was then calculated as the difference between
the time integral of either parameter for each genotype relative to WT. (The tension index for WT
is therefore 0 by definition.)

Computational simulations of cardiac twitches

Similar to what we have done previously (7), we simulated twitches of cardiomyocytes
from hearts with DCM-causing mutations in the sarcomere by modifying the parameters in the
Negroni-Lascano model (21) to fit our experimental measurements of cardiac twitches. As shown
in Figure S2, the model consists of 6 actomyosin states: no Ca\(^{2+}\) bound to troponin (TS), Ca\(^{2+}\)
bound to troponin with no cross-bridges (TSCa\(_3\)), Ca\(^{2+}\) bound to troponin with weak cross-bridge
attachment (TSCa\(_3\)~), Ca\(^{2+}\) bound to troponin with strong (tension-generating) cross-bridge
attachment (TSCa\(_3\)^*), no Ca\(^{2+}\) bound to troponin with strong (tension-generating) cross-bridge
attachment (TS^*), and no Ca\(^{2+}\) bound with weak cross-bridge attachment (TS~).

Original parameter values and initial conditions from the Negroni-Lascano model (4) were
used to simulate ‘wild-type’ (WT) twitches. To simulate D230N Tm twitches, we reduced the
transition rate from TSCa\(_3\) to TSCa\(_3\)~ (parameter f, see Table S1) by 43.5\% such that the peak
twitch tension was decreased by the same amount compared to WT (~50\%) as observed
experimentally. Y\(_b\), Z\(_b\), Y\(_r\), and Z\(_r\), which represent Ca\(^{2+}\) association and dissociation from
troponin, were varied to assess the effects of Ca\(^{2+}\) modulation on sarcomeres with the simulated
D230N Tm. Z\(_p\), and Y\(_p\), the transition rates between TSCa\(_3\)~ and TSCa\(_3\)^*, and g, the transition rate
from TSCa\(_3\)~ to TSCa\(_3\), were varied to assess the effects of crossbridge modulation on
cardiomyocytes with simulated D230N Tm. Forward rates (Y\(_b\), Z\(_r\), and Y\(_p\)) were progressively
increased by factors of 2-10 (with the exception of Y\(_b\), which was increased by a maximum of 9
fold to allow for model convergence), and reverse rates (Z\(_b\), Y\(_r\), Z\(_p\), and g) were simultaneously
decreased by factors of 0.9-0.5 to assess a range of Ca\(^{2+}\) and cross-bridge modulation (Table S1).
Sarcomere length was set to 1.8 \(\mu\)m. Model equations were implemented using MATLAB software
(2018b version, The MathWorks, Inc., Natick, MA) and solved using a forward Euler method for
1000 ms under isometric conditions.
Molecular simulations of cardiac thin filament structure

The molecular model of the cardiac thin filament was constructed using the previously described initial low-temperature structure (28–31) that includes actin, tropomyosin, and the full troponin complex (Figure S3). Point mutations were integrated into each respective protein using the CHARMM42 program (54) with CHARMM36 parameters, the latest version of the CHARMM force field (55). This substitution is performed by initially deleting the side chain atoms of the respective residue and constructing in the new atoms using the parameters.

The complete structures, including the point mutations, were then explicitly solvated in a waterbox allowing at least a 15 Å barrier from all edges of the protein with TIP3P waters using the SOLVATE plugin in VMD1.9.3 (56). The system was ionized to a concentration of 0.15 mol/L using potassium and chloride ions with the AUTOIONIZE plugin in VMD1.9.3. All simulations were performed with NAMD2.12 (57) using CHARMM36 parameters with the SHAKE algorithm. The Particle-Mesh Ewald method was used with a cutoff of 12 Å to calculate all non-bonded interactions between atoms. Each individual system underwent 5000 steps of minimization, slow heating to a temperature of 300 K and at rate of 1 K/ps, and then equilibration in an isobaric-isothermal ensemble at 1 atm and 300 K for 690 ps. With the equilibrated structure, three separate 10 ns production runs were performed with randomly generated velocities from a Boltzmann distribution. The average coordinates of each atom within each respective structure were calculated and these average structures were used to calculate all statistics.

The distance between the inhibitory peptide of cTnI and actin was calculated by averaging the distance between the alpha carbons of cTnI residues 137-147 and the center of mass of the closest actin monomer. The cTnI–cTnC residue contact maps were created by extracting distances between each cTnC–cTnI residue using VMD1.9.3 for each structure. Residues that were ≤ 10 Å apart in the WT regulatory unit were used to analyze the change in those distances upon introducing the D230N Tm mutation and the L48Q cTnC mutation. The distances between each of the cTnC–cTnI residue pairs from the WT structure were subtracted from that of each mutated
structure (D230N and D230N+L48Q) and then plotted using MATLAB (Version 2018a, The MathWorks, Natick MA).

Data analysis & statistics

All experimental data were collected using custom data acquisition software developed using LabView and were analyzed using MATLAB (Version 2018a, The MathWorks, Natick MA) software and built-in statistical packages. Unless stated otherwise, error bars represent the standard error of the mean. A one-way ANOVA with a Tukey post-hoc test of significance was used to compare values across multiple genotype groups, unless stated otherwise.

Author Contributions

JDP, MR, JD, and FM-H designed the study. JDP, KBK, ABM, AET, and GVF performed the experiments. JDP, KBK, ABM, AET, GVF, JD, and FM-H analyzed the data. All authors helped interpret the results. JCT provided the D230N transgenic murine model. MR, JD, and FM-H developed the double-transgenic murine model. JDP wrote the manuscript.

Acknowledgements

The authors are grateful for helpful scientific discussions with Professor Thomas L. Daniel (UW Biology) during the initial stages of this project. This work was supported by NIH Grants T32-HL007312, T32-HL007444, and F32-HL152573 (to JDP), NIH Grant T32-HL105373 (to AET), NIH Grant 5R01HL075619 to (JCT), NIH Grant 5R01HL107046 (to SDS and JCT), NIH Grants U01 HL122199 and R01 HL137100 (to ADM), NIH Grants P30 AR074900, R01 HL128368, RM1 GM131981, and Grant Horizon 2020 #777204 (to MR), NIH Grant K08 HL128826 (to FM-H). FM-H and KBK were also supported in part by the Locke Charitable Trust. Disclosure: ADM is a co-founder of and has an equity interest in Insilicomed Inc. and an equity interest in Vektor Medical, Inc. He serves on the scientific advisory board of Insilicomed, and as scientific advisor to both companies. Some of his research grants have been identified for conflict of interest management based on the overall scope of the project and its potential benefit to these companies. The author is required to disclose this relationship in publications acknowledging the grant support; however, the research subject and findings reported in this study did not involve the companies in any way and have no relationship with the business activities or scientific interests.
of either company. The terms of this arrangement have been reviewed and approved by the
University of California San Diego in accordance with its conflict of interest policies.

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Figure 1. A ‘Tension Index’ based on cardiac twitch tension-time integrals correlates with the type and severity of myocardial growth. (A) Depiction of the relationship between the tension index (TI) and the degree and severity of cardiac growth [modified from Ref. (7)]. The TI is determined by subtracting the area under a twitch tension curve of a cardiomyocyte with perturbed contractility from that of a ‘normal’ or ‘wild-type’ cardiomyocyte. A positive TI correlates with concentric hypertrophy while a negative TI correlates with eccentric hypertrophy (7). (B) Top panel: Theoretical 300-ms twitch tension (T) traces of a ‘wild-type’ (WT) cardiomyocyte (black) and four potential variants with altered twitch T magnitude and kinetics. All twitches are represented as a percentage of the peak T of the WT twitch, which is shown as a dashed black trace with each variant twitch for comparison. Twitches were generated using a simple exponential model, as in Ref. (8). Bottom panel: The area under the T-time trace (left ordinate) for each corresponding twitch above and the resulting TI (right ordinate) calculated as the difference in the area under the twitch tension-time curve between WT and each variant twitch. Blue and gray correspond to twitches with decreased and increased (respectively) twitch-time integrals compared to WT (black). We note that, because the TI encompasses contraction and relaxation kinetics, the absolute value of the TI does not depend on the peak twitch tension.
Figure 2. Measuring and modulating the tension index of D230N hearts. (A) Average twitch tension (T) traces of intact trabeculae from WT and D230N hearts as a percentage of WT T. The peak twitch tension (T_peak) of trabeculae from D230N hearts is ~half that of WT (inset). Sample sizes of n = 6 and 7 for WT and D230N trabeculae, respectively. The error bars of the inset represent standard deviation and *p < 0.01 using an unpaired student’s t-test. (B) Dependence of the tension index of simulated D230N twitches on modulation of cross-bridge (XB) or calcium (Ca²⁺) binding. The simulated tension index for D230N twitches without any modulation is indicated by the blue circle. The rate of XB transition from a weak to a strong (tension-generating) state was independently increased to simulate D230N twitches with augmented XB binding (dashed line). The affinity of Ca²⁺ for cTnC was also independently increased to simulate twitches of D230N cardiomyocytes with augmented Ca²⁺ sensitivity (solid line).
Figure 3. Computational structural analysis of an atomically detailed thin filament with regulatory units containing D230N Tm and L48Q cTnC. (A) Distances (in Å) between the Ca\(^{2+}\) ion and each Ca\(^{2+}\)-coordinating oxygen atom in Site II of cTnC for a WT RU (green), a RU with D230N Tm (blue) and a RU with both D230N Tm and L48Q cTnC (red). (B) Distances (in Å) between the inhibitory peptide of cTnI and the center of mass of the closest actin monomer for each RU. The color scheme is the same as described for panel A. (C & D) Structural analysis of cTnC and cTnI subunits in the three different RUs. The cTnI subunit is shown as green when in

\[
\begin{align*}
\text{Distance between Ca}^{2+} & \text{ and cTnC Site II atoms} \\
\text{Distances (in Å) between the inhibitory peptide and actin} \\
\text{Switch peptide} & \text{Inhibitory peptide} \\
\text{Inhibitory Switch} & \text{Inhibitory Switch}
\end{align*}
\]
the WT RU, blue when in the RU with D230N Tm, and red when in the RU with both D230N Tm and L48Q cTnC. cTnC is shown in gray, and Ca$^{2+}$ ions are indicated by the yellow spheres. The switch and inhibitory peptides of cTnI are indicated by the arrows in the close-up insets. (E & F) Changes in the interactions between cTnC and the inhibitory and switch peptides of cTnI, relative to the WT RU, for the RU containing D230N Tm (E) and the RU containing both D230N Tm and L48Q cTnC (F). The colorbar denotes the change in distances (in Å) between cTnC and cTnI residues in each variant RU relative to those in the WT RU. Thus, magenta indicates movement of cTnC-cTnI residues away from one another and black indicates movement of cTnC-cTnI residues towards one another (relative to WT). Residues corresponding to the inhibitory and switch peptides of cTnI are on either side of the vertical dashed line.
Figure 4. L48Q cTnC prevents contractile abnormalities in cardiac tissue isolated from hearts containing D230N tropomyosin. (A) Steady-state tension \( T \) as a percentage of the maximum value (at pCa 4.0) of demembranated cardiac muscle measured over a range of extracellular calcium concentrations (pCa = −log([Ca\(^{2+}\)])). The data are fit with the Hill equation (see SI Methods) shown by the solid lines. (B) The pCa at half-maximal \( T \) (pCa\(_{50}\)) of cardiac preparations from D230N hearts is significantly less than all other groups, while the pCa\(_{50}\) of preparations from L48Q + D230N double-transgenic (DTG) hearts is not different from WT. Error bars represent standard deviation. Black lines above the bars indicate \( p < 0.05 \) between groups using a one-way ANOVA and a Tukey post-hoc test of significance. (C) Average twitch \( T \)-time traces (in % WT \( T \_\text{peak} \)) of intact trabeculae for each genotype (same color scheme as panel A). The WT \( T \)-time trace is shown as a dashed green trace against each variant twitch for comparison. (D) The area under the \( T \)-time trace (left ordinate) for each genotype and the resulting TI (right ordinate). See Table S2 in the Supplemental Material for numerical values.
Figure 5. Ventricular remodeling and dysfunction in D230N hearts is prevented by expression of L48Q cTnC. Echocardiographic measurements from mice of 2 to 5 months of age reveal that the diastolic (A) and systolic (B) left-ventricular inner diameter (LVID_D and LVID_S, respectively) of D230N hearts (blue) progressively increase with age, while that of DTG (red) do not change with age and are not significantly different from WT (green) at any age. The fractional shortening (C) and ejection fraction (D) also progressively worsen with age in D230N hearts, while DTG hearts remain approximately constant and do not significantly differ from WT. (*) indicates p < 0.05 for D230N versus WT and (+) indicates p < 0.05 for D230N vs. DTG using a one-way ANOVA and a Tukey post-hoc test of significance. Error bars represent the SEM. See Table S3 in the Supplemental Material for all values and sample sizes.