Published in final edited form as:
J Mol Cell Cardiol. 2008 August ; 45(2): 281–288. doi:10.1016/j.yjmcc.2008.05.003.

Molecular and Functional Characterization of Novel Hypertrophic Cardiomyopathy Susceptibility Mutations in *TNNC1*- Encoded Troponin C

Andrew P. Landstrom[1,2,*], **Michelle S. Parvatiyar**[3,*], **Jose R. Pinto**[3,*], **Michelle L. Marquardt**[1], **J. Martijn Bos**[4], **David J. Tester**[2,4], **Steve R. Ommen**[4], **James D. Potter**[3,#], and **Michael J. Ackerman**[2,4,5,#]

1*Mayo Medical School, Mayo Clinic, Rochester, MN*

2*Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN*

3*Department of Molecular and Cellular Pharmacology, Miller School of Medicine, Miami, FL*

4*Department of Medicine/Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN*

5*Department of Pediatrics/Division of Pediatric Cardiology, Mayo Clinic, Rochester, MN*

Abstract

Hypertrophic Cardiomyopathy (HCM) is a common primary cardiac disorder defined by a hypertrophied left ventricle, is one of the main causes of sudden death in young athletes and has been associated with mutations in most sarcomeric proteins (tropomyosin, Troponin T and I, and actin, etc.). Many of these mutations appear to affect the functional properties of cardiac troponin C (cTnC), i.e., by increasing the Ca^{2+}-sensitivity of contraction, a hallmark of HCM, and surprisingly, prior to this report, cTnC had not been classified as a HCM susceptibility gene. In this study, we show that mutations occurring in the human cTnC (HcTnC) gene (*TNNC1*) have the same prevalence (~0.4%) as well established HCM-susceptibility genes that encode other sarcomeric proteins. Comprehensive open reading frame/splice site mutation analysis of *TNNC1* performed on 1025 unrelated HCM patients over the last 10 years revealed novel missense mutations in *TNNC1*: A8V, C84Y, E134D, and D145E. Functional studies with these recombinant HcTnC HCM mutations showed increased Ca^{2+} sensitivity of force development (A8V, C84Y and D145E) and force recovery (A8V and D145E). These results are consistent with the HCM functional phenotypes seen with other sarcomeric HCM mutations (E134D showed no changes in these parameters). This is the largest cohort analysis of *TNNC1* in HCM that details the discovery of at least three novel HCM-associated mutations and more strongly links *TNNC1* to HCM along with functional evidence that supports a central role for its involvement in the disease. These types of studies may help to further define *TNNC1* as an HCM-susceptibility gene that has already been established for the other members of the Troponin complex.

Correspondence and requests for materials should be addressed to either: Michael J. Ackerman, M.D., Ph.D., Windland Smith Rice Sudden Death Genomics Laboratory, Guggenheim Building; 501, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, E-mail: ackerman.michael@mayo.edu, 507-284-0101; 507-284-3757 (fax) Or James D. Potter, Ph.D., Department of Molecular and Cellular Pharmacology, University of Miami, Miller School of Medicine, Rm. 6085A RMSB, 1600 NW 10th Ave, Miami, FL, USA 33136, E-mail : jdpotter@miami.edu.

*APL, MSP, and JRP contributed equally to this work and are therefore considered co-equal first authors

#JDP and MJA are co-equal senior authors

Keywords

troponin C; TnC; hypertrophic cardiomyopathy; HCM; mutation; calcium; genetics

Introduction

The leading cause of sudden cardiac death in the young is hypertrophic cardiomyopathy (HCM) which affects approximately 1 in 500 individuals and is defined clinically as thickening of the left ventricle and septum in the absence of any identifiable cause [1–3]. Through initial linkage studies and subsequent hypotheses that HCM was a disease of the sarcomere, investigations over the past two decades have led to the identification of hundreds of HCM-associated mutations scattered among the various sarcomeric genes [4–10]. This is reflected in the commercially-available clinical genetic tests for HCM which scan for mutations in the genes encoding β-myosin heavy chain, myosin binding protein C, cardiac troponin I, cardiac troponin T, α-tropomyosin, cardiac actin, regulatory myosin light chain, and ventricular myosin light chain. Despite these tremendous advances, approximately 20% of patients with reverse curve-HCM and nearly 90% of the patients with sigmoidal-HCM are genotype negative with respect to the genetic test panel for sarcomeric/myofilament HCM [11–13]. Notably absent from this list is the *TNNC1*-encoded human cardiac troponin C (HcTnC) which has yet to be firmly associated with HCM [14,15]. To date, only one mutation in TnC has been linked to a 60 year old HCM patient [16]. In a small cohort based study, the authors did not find the L29Q TnC mutation in any other patient (the number of HCM patients screened was unreported) nor in 96 healthy volunteers and they concluded that additional studies would be necessary to elucidate whether TnC should be considered in fact a disease gene for HCM [16].

Cardiac troponin is a heterotrimeric complex comprised of a Ca^{2+}-binding subunit TnC, an inhibitory subunit troponin I (TnI) encoded by *TNNI3*, and an elongated troponin T (TnT) encoded by *TNNT2*. TnC acts as a cytosolic Ca^{2+} sensor which, when bound to the divalent cation at the single Ca^{2+}-specific binding site, strengthens its interaction with TnI and transversely weakens the inhibitory function of TnI causing its release from actin. The troponin-tropomyosin complex then shifts deeper into the actin groove thereby exposing the myosin binding sites on actin making them available for contraction (for review see [17]). cTnC belongs to the EF-hand superfamily of Ca^{2+} binding proteins and consists of N and C terminal globular domains that are connected through a flexible linker. Each globular domain has a pair of EF-hand helix-loop helix Ca^{2+} binding motifs [18,19]. The C-terminus (also called as structural domain) contains two high affinity Ca^{2+} binding sites III and IV (~10^7 M^{-1}) that also binds to Mg^{2+} competitively with low affinity (~10^3 M^{-1}) and the N-terminus contains only one functional low affinity "Ca^{2+}-specific" regulatory Ca^{2+} binding site II (~10^5 M^{-1}) [20–22]. The N-terminus is considered the regulatory domain since Ca^{2+} binding initiates muscle contraction [23,24]. In this manner, TnC represents a critical molecular switch through which defects in the primary sequence of the protein may disrupt the TnC-Ca^{2+} regulation process.

At least 90% of HCM Tn mutations (TnT and TnI), that have been investigated *in situ* cause an increase in the Ca^{2+} sensitivity of force development that would result in increased force at sub-maximal Ca^{2+} concentrations [25–27]. The same functional phenotype has also been observed in transgenic mice containing Tn mutations related to HCM when compared to the WT. Also there seems to be a correlation between the change in Ca^{2+} sensitivity of force development and time of onset of disease and prognosis [25–27]. Only one HCM-associated TnC mutation (L29Q) has been functionally studied by two different groups. Schmidtmann et al, showed, using a reconstituted fast skeletal system containing cardiac troponin complex, a decrease in the Ca^{2+} sensitivity measured by ATPase activity and *in vitro* motility assays

[28]. However, Liang et al have shown an increase in the Ca^{2+} sensitivity of force development measured in TnC depleted mouse skinned cardiac myocytes reconstituted with recombinant mouse cardiac TnC [29].

Since a seven year span has elapsed since the first report of an HCM-associated mutation in cTnC and no subsequent reports have shown any linkage between HCM and cTnC, we sought to determine whether genetic perturbations in *TNNC1* may play a role in the pathogenesis of HCM in a large cohort-based study. We report four novel missense mutations in HcTnC – A8V, C84Y, E134D, and D145E – which at least three alter the Ca^{2+} sensitivity of contraction when reconstituted into TnC-depleted porcine cardiac muscle fibers. Furthermore, these results suggest that a role for calcium mishandling in the pathogenesis of sarcomeric-HCM and warrants further scrutiny.

Methods

Study Population

Between April 1997 and April 2007, 1025 unrelated patients, evaluated in the Hypertrophic Cardiomyopathy Clinic at Mayo Clinic, Rochester, Minnesota, consented to genetic testing. Following receipt of written consent for this Mayo Foundation Institutional Review Board-approved protocol, DNA was extracted from peripheral blood lymphocytes using the Purgene DNA extraction kit (Gentra, Inc, Minneapolis, MN). Clinical data was collected on all patients including physical examination, pertinent personal and family history, 12-lead electrocardiogram (ECG) analysis, and echocardiographic testing to determine maximum left ventricular wall thickness (MLVWT) and maximum left ventricular outflow tract gradient (MLVOT). Each of the subjects met the clinical diagnostic criteria for HCM of a MLVWT greater than 13 mm in the absence of other confounding diagnoses.

Troponin C Mutational Analysis

All six *TNNC1* exons, with flanking intronic regions and splice junction, were amplified by PCR using oligonucleotide primers. Each amplicon was evaluated for mutations using denaturing high performance liquid chromatography (DHPLC, Transgenomic, Omaha, Nebraska), and samples with an abnormal elution profile were directly sequenced (ABI Prism 377, Applied Biosystem, Foster City, CA) to characterize the difference between the wild type and variant alleles. Primer sequences, PCR, and DHPLC conditions are available upon request. Using previously published conditions, *TNNC1*-positive subjects were analyzed for mutations in 15 established HCM-susceptibility genes including the eight genes that comprise the commercially available genetic test for sarcomeric-HCM.

Site-Directed Mutagenesis, Expression, and Protein Purification of Human Cardiac Troponin C

The cDNA for human cardiac TnC (HcTnC) was cloned previously in our laboratory by RT-PCR using human heart total RNA (Stratagene), sequence specific primers and a Omniscript RT Kit (Qiagen) [30]. The HcTnC-cDNA was used as a template for PCR using primers designed to produce the specific mutants: A8V, C84Y, E134D and D145E. All subcloned DNA sequences were inserted into the pET3d expression plasmid and sequenced to verify that sequences were correct prior to expression and purification. *Escherichia coli* BL21 (DE3) Codon plus bacterial cells were transformed with pET-3d constructs containing HcTnC. After sonication (Heat Systems XL 2020), the cell lysate containing the HcTnC was centrifuged at 39,000 g for 45 m at 4°C and first applied onto a pre-equilibrated fast flow Q-Sepharose column and eluted in buffer 50 mM Tris-HCl pH 7.8, 6 M Urea, 1 mM EDTA and 1 mM DTT using a 0 – 0.6 M KCl gradient. The cleanest fractions were then dialyzed against 50 mM Tris-HCl pH 7.5, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 50 mM NaCl and 1 mM DTT. After HcTnC was extensively

dialyzed, ammonium sulfate was added to a final concentration of 0.5M and the protein was loaded onto a pre-equilibrated Phenyl Sepharose column. Pure HcTnC was directly eluted using a buffer containing 50 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.5. Fractions of > 98% purity as determined by SDS-PAGE were pooled, dialyzed extensively against 5 mM ammonium bicarbonate and then lyophilized.

Fiber Preparation and Determination of the Ca^{2+} Dependence of Force Development

Cardiac tissue from newly slaughtered pigs was obtained from a nearby slaughterhouse. Strips of muscle, 3–5 mm in diameter and ~5 mm in length were dissected from the papillary muscle of the left ventricle and skinned overnight in a 50% glycerol relaxing solution containing low Ca^{2+} concentration (10^{-8} M $[Ca^{2+}]_{free}$, 1 mM $[Mg^{2+}]_{free}$, 7 mM EGTA, 2.5 mM $MgATP^{2-}$, 20 mM MOPS (pH 7.0), 20 mM creatine phosphate, and 15 units/ml creatine phosphokinase, I = 150 mM) and 1% Triton X-100 at − 20°C. Fibers were then transferred to a similar solution without Triton X-100 and kept at − 20°C. A small skinned fiber bundle with the diameter of ~ 75 – 100 μm was mounted using stainless steel clips to a force transducer and immersed in a pCa 8.0 relaxation solution (described above). The contraction solution (pCa 4.0) had the same composition as the pCa 8.0 solution except that the Ca^{2+} concentration was $10^{-4}{}_{M}$ and was used to measure the initial force. To analyze the Ca^{2+} dependence of force development, the skinned fiber tension was tested in intermediate Ca^{2+} solutions ranging from pCa 8.0 to 4.0 that were calculated using the pCa calculator program developed in our laboratory [31]. Data was analyzed using the following equation: % Change in Force = 100 X $[Ca^{2+}]^n$ / ($[Ca^{2+}]^n$ + $[Ca^{2+}{}_{50}]^n$) where "$[Ca^{2+}{}_{50}]$" is the free $[Ca^{2+}]$ that produces 50% force and "n_{Hill}" is the Hill coefficient. All fiber experiments were carried out at room temperature (22° C).

The native cardiac TnC was depleted by incubating the fiber in a CDTA extracting solution (5 mM CDTA and 25 mM Tris-HCl, pH 8.4) for ~ 1.5h. The efficiency of TnC extraction the residual tension was assessed by activating the fiber in pCa 4.0 solution. Residual tensions of 15% or below from the initial maximal force were considered satisfactory for experimentation. After this, fibers were successively incubated for 20 min with 28 μM of mutant or WT HcTnC diluted in pCa 8.0 solution. To ensure exogenous cTnC reconstitution the force recovery was verified using pCa 4.0 solution. We only considered the fiber fully reconstituted when there was no additional increase in the force recovery values obtained using pCa 4.0 after successive TnC incubations. The average time for full recovery of force for all the proteins was ~ 1 – 1.3h (incubation of three to four times 20 min with recombinant protein).

Statistical analysis and three-dimensional modeling

For the clinical studies ANOVA analysis was performed to establish differences between experimental groups. HcTnC mutations were modeled in each functional domain of PDB (1AJ4) using PyMol software. Student's *t* test was used to determine the significance of skinned fiber Ca^{2+} sensitivity and force recovery. P-values less than 0.05 were considered statistically significant.

Results

The demographics for one of the largest ever assembled cohorts of unrelated patients with HCM (N=1025) are shown in the first column of Table 1. The mean age at diagnosis was 49.2 ± 18 years with a maximal left ventricle wall thickness (MLVWT) of 22.3 ± 7 mm. Four novel missense mutations in *TNNC1* were discovered in four unrelated, Caucasian patients within this cohort: Ala8Val (nucleotide 23, C>T), Cys84Tyr (nucleotide 251, G>A), Glu134Asp (nucleotide 402, G>T), and Asp145Glu (nucleotide 435, C>A). The topographical location for each mutation is shown in Figure 1A as well as the three dimensional position of each residue on the amino- and carboxy-termini of HcTnC Figure 1B. The missense mutations involved

residues that were completely conserved across all species queried (Figure 2). No other mutations were detected in these four patients following comprehensive open reading frame/splice site mutational analysis of known HCM-susceptibility genes including the eight genes comprising the commercially available genetic test for sarcomeric HCM. As a control, a panel of 1000 reference alleles derived from 100 African American and 200 Caucasian Coriell Repository (Camden, NJ) DNA samples and 200 Caucasian subjects with normal screening electro- and echo-cardiograms were comprehensively genotyped for *TNNC1*. Absence of these variants in 1000 reference alleles demonstrates with 95% confidence that the true allelic frequency of these variants is less than 0.003 – statistically excluding the possibility that the mutations are genetic polymorphisms. Furthermore, with the exception of a single synonymous variant (G70G) found in a single reference allele, no sequence variants of any type were observed throughout the coding region and splice sites of all control alleles (data not shown).

The clinical characteristics of the patients with *TNNC1*-HCM are summarized in Table 1 and 2. The *TNNC1* genotype-positive subjects were diagnosed with HCM at 29.4 ± 10.6 years with a MLVWT of 24.3 ± 2.7 mm. As summarized in Table 2, Cases 1, 2, and 3 had no apparent family history of HCM among either first- or second-degree relatives suggesting the possibility of a sporadic *de novo* mutation or incomplete penetrance. Case 4 has a positive family history consistent with autosomal dominant, familial HCM involving a brother, a maternal grandmother, two maternal uncles, and two daughters of the noted maternal uncles. Lastly, Case 3, a female diagnosed in childhood with HCM died at 22 years of age of an unspecified cause. She had undergone extended surgical myectomy several years previously for management of significant left ventricular outflow tract obstruction (LVOTO) that was refractory to pharmacotherapy. Unfortunately, relatives of all four families have declined participation precluding a molecular determination of co-segregation or sporadicity.

To determine whether these *TNNC1* mutations functionally perturb myofilament Ca^{2+} regulation, the Ca^{2+} sensitivity of force development and force recovery were evaluated using cTnC-depleted, porcine cardiac skinned fibers reconstituted with each HcTnC mutant. A8V produced the largest leftward shift (~0.4 log units) from wild type, followed by D145E and C84Y (~0.3 log units), while E134D showed no significant difference compared to the wild type (Figure 3A). Furthermore, the force recovery under the same conditions of fiber incubation, i.e., same HcTnC concentration and dissolving buffer (see methods for additional details) was shown to be significantly increased in fibers reconstituted with A8V and D145E (Figure 3B). The pCa_{50} (defined as the $[Ca^{2+}]$ to reach 50% of the maximal tension), n_{Hill}, and the relative force recovery values are summarized in Table 3.

Discussion

Since the identification of the first mutation in *MYH7*, HCM has been viewed conceptually as a disease of the sarcomere. This is reflected in the commercially-available clinical genetic test which is comprised of a Panel A (β-myosin heavy chain, myosin binding protein C, cardiac troponin I, cardiac troponin T, and α-tropomyosin) and a Panel B (cardiac actin, regulatory myosin light chain, ventricular myosin light chain). Absent from this is cardiac troponin C which has yet to be established as a sarcomeric-HCM susceptibility gene in a cohort-based study. To this end, we report the novel discovery of 4 missense mutations, A8V, C84Y, E134D, and D145E, in a cohort of 1025 patients with HCM. In addition, our cohort demonstrates that the prevalence of *TNNC1* mutations among unrelated patients with HCM is approximately 0.4% (4/1025) which is comparable in frequency to both α-tropomyosin-HCM (~0.5%) and actin-HCM (~0.3%), previously elucidated in our original Mayo cohort that comprised 388 unrelated patients with HCM [11]. Accordingly, it seems reasonable for *TNNC1* to be added to the panel of HCM-susceptibility genes.

In the functional studies, surprisingly, the A8V and D145E mutations, located in two different functional regions of HcTnC, demonstrated a nearly equivalent increase in Ca^{2+} sensitivity of force development and force recovery. The A8V mutation is located in the N-Helix of the amino-terminal domain of HcTnC, a region known to affect the Ca^{2+} affinity of the regulatory EF-hand domain. Indeed, deletion of residues 1–14 in skeletal TnC, corresponding to 1–11 in HcTnC, produced alterations in the Ca^{2+} sensitivity of force development and maximal force [32,33]. Conversely, the D145E mutation may disrupt or lessen the affinity of Ca^{2+}/Mg^{2+} site IV for its divalent cation (Figure 1). This possibility is supported by prior work demonstrating that Asp to Ala mutations in HcTnC at the Z-coordinated position of the Ca^{2+} binding loop disrupt Ca^{2+} binding to sites III and/or IV resulting in an increase in the Ca^{2+} sensitivity of force development [34]. Importantly, the effect of this mutation on Ca^{2+}-sensitivity would likely be indirect, as the binding of Ca^{2+} at site IV plays a structural role in HcTnC. It is possible that the effects seen on force recovery with the A8V and D145E mutations may be due to their intrinsic properties in modulating the ratio between strong and weak crossbridges or due to alterations in their affinity for the thin filament consequently intervening in the protein incorporation. In any event, the altered force recovery seen with these mutants may be an important new phenotype not observed previously.

In case 2 (C84Y), the cysteine 84 is situated at the beginning of the central helix and has been shown to affect Ca^{2+} regulation and maximal force generation, as this position is involved with changes in the orientation between the central helix and the N-terminal domain [35,36]. Ca^{2+} binding to the N-terminal portion of HcTnC induces separation of the C-Helix from the central helix allowing greater exposure of Cys 84 to solvent [37]. The substitution of a bulky tyrosine at this position could modify the angle between these two domains mimicking an intermediate HcTnC Ca^{2+} open state and consequently cause an increase in the Ca^{2+} sensitivity of force development. C84Y was the only mutation that showed a slight but significant decrease in the cooperativity of force development. However, it is known that variation in active TnC content or TnC Ca^{2+} affinity can modify cooperativity [38,39].

While the E134D HcTnC mutant had no effect on the parameters measured, the mutation may indirectly induce misregulation of another physiological system. For example, the only reported HCM HcTnC mutation to date, L29Q, appears to diminish the effects of PKA phosphorylation of cardiac troponin I [28]. Additionally, despite its rarity and species conservation, it is possible that E134D is not a pathogenic, HCM-causing mutation but simply a functionally/clinically insignificant rare variant.

Due to a lack of studies relating TnC to HCM, not much information was available in the literature about the possible functional consequences of such a mutation. However, two groups have been investigating the possible molecular mechanism of the only mutation described in cTnC that is linked to HCM (L29Q). In contrast to our results, Schmidtmann, et al. using reconstituted fast skeletal muscle myosin, actin and tropomyosin combined with cardiac troponin, reported a decrease in the Ca^{2+} sensitivity measured by ATPase activity and *in vitro* motility studies [28]. The contractile machinery has a great deal of cooperativity between proteins and the presence of proteins from different muscle systems may mask effects of the mutations on the cardiac system. The paper from Liang, et al. corresponded with our results and showed a leftward shift in the Ca^{2+} sensitivity of force development in a more physiological system, i. e. mouse skinned fibers reconstituted with mouse cTnC [29]. The disparate results from the two groups demonstrate that results from functional studies can differ, possibly due to the dissimilar systems in which the experiments were conducted.

The effect of the HcTnC mutations on Ca^{2+} sensitivity and force recovery may also be caused by alterations in myosin crossbridge interactions with the thin filament. An explanation of this phenomenon is that the ratio between strongly and weakly bound crossbridges affects the

Ca^{2+} sensitivity of force development. In addition, changes in TnC conformation may occur depending on the number and type of attached crossbridges and conversely TnC may control the kinetic parameters of crossbridges in skinned fibers [40,41]. These reports indicate the existence of a bi-directional mechanism of TnC and crossbridges that could explain some of the results. More structural and functional studies need to be performed to elucidate how these mutations alter thin filament regulation.

Importantly, these observations warrant increased scrutiny of calcium mishandling as a novel pathogenic mechanism of disease in HCM based on the functional results that demonstrate that changes in Ca^{2+} sensitivity of contraction can also be caused by mutations in cTnC. Changes in the Ca^{2+} sensitivity of contraction may lead to an altered state of activation/relaxation of muscle contraction which is phenotypically manifested by thickening of the ventricular walls and eventually leads to diastolic dysfunction [42]. Mutations in genes encoding calcium-handling or calcium-sensitive proteins are a newly established pathogenic mechanism for HCM. Indeed, mutations in the promoter and coding regions of phospholamban, a regulator of the sarcoplasmic reticulum calcium ATPase (*SERCA2a*) and a modulator of calcium flux within the cell, have been identified in HCM and dilated cardiomyopathy (DCM) respectively [43–45]. Recently, mutations in junctophilin-2 (*JPH2*), a putative structural cardiac protein, confer susceptibility for HCM through disruption of the cardiac dyad and perturbed calcium-induced calcium release of the contracting cardiocyte [46]. Despite all the investigations, it is still poorly understood how these mutations in proteins related to Ca^{2+} cell homeostasis could lead to drastic cardiac remodeling.

We are reporting for the first time the relationship between newly discovered HcTnC mutations and the substantial effects they have on key functional parameters that may lead to an explanation of the mechanism of disease in these HCM patients. TnC is a major intracellular Ca^{2+} buffer and the sarcomeric protein responsible for triggering muscle contraction. There is also growing evidence that many of the sarcomeric HCM mutations (tropomyosin, Troponin T and I, and actin, etc.) ultimately work through TnC as these mutations affect the Ca^{2+} affinity of cTnC, making it a good target for the development of new therapeutics. To this end, we have detailed the discovery of 4 novel missense mutations in *TNNC1*: A8V, C84Y, E134D, and D145E, in a cohort of 1025 patients with HCM. Derived from the largest assembled cohort of unrelated patients with HCM, we provide molecular and functional evidence suggesting that mutations in *TNNC1* may be a novel pathogenic basis for HCM. In conclusion, this report, shows that *TNNC1*-HCM occurs at a similar frequency to two of the eight genes that currently comprise the commercially available genetic test for sarcomeric-HCM and indicate that the *TNNC1* should be routinely included in the genetic tests that screen for HCM mutations.

Acknowledgements

Supported by NIH Grants HL-67415 (JDP) and HL-42325 (JDP) and the Windland Smith Rice Comprehensive Sudden Cardiac Death Program (MJA)

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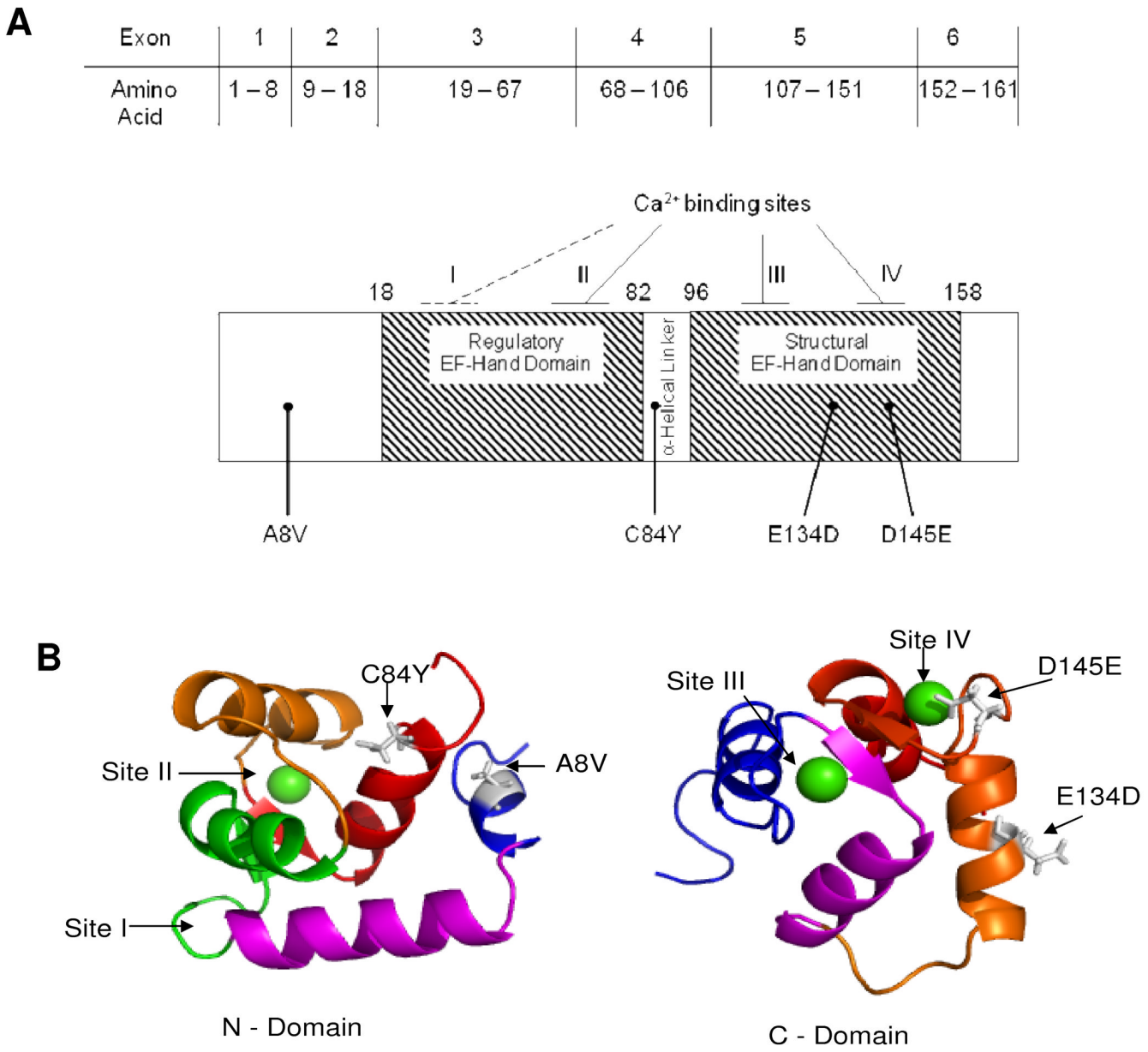

Figure 1. Mapping and modeling of HCM-susceptibility mutations in *TNNC1*-encoded cardiac troponin C (HcTnC)

A) The gene and protein linear topology of HcTnC, including exon, splice junction, functional domains, and calcium binding site locations. The location of each of the four mutations is noted respectively. **B**) N-terminus (left) depicting the location of A8V and C84Y mutations in relationship to the Ca^{2+} binding sites and HcTnC helices. Ca^{2+} binding site I is defunct and Ca^{2+} binding site II shows Ca^{2+} bound (green sphere); N-helix (blue), A-helix (pink), B-helix (green), C-helix (orange), and D-helix (red). The Ala8 is located in the first helix of TnC in the beginning of the flexible linker connecting the two domains. C-terminus (right) depicting the location of E134D and D145E mutations in relationship to Ca^{2+} binding sites and helices. Ca^{2+} binding site III and IV pictured with Ca^{2+} bound (green spheres); E-helix (blue), F-helix (pink), G-helix (orange), H-helix (red). Glu134 is located in the G-helix between Ca^{2+} binding

sites III and IV and Asp145 is situated at the Z position of Ca^{2+} coordinating residues of site IV.

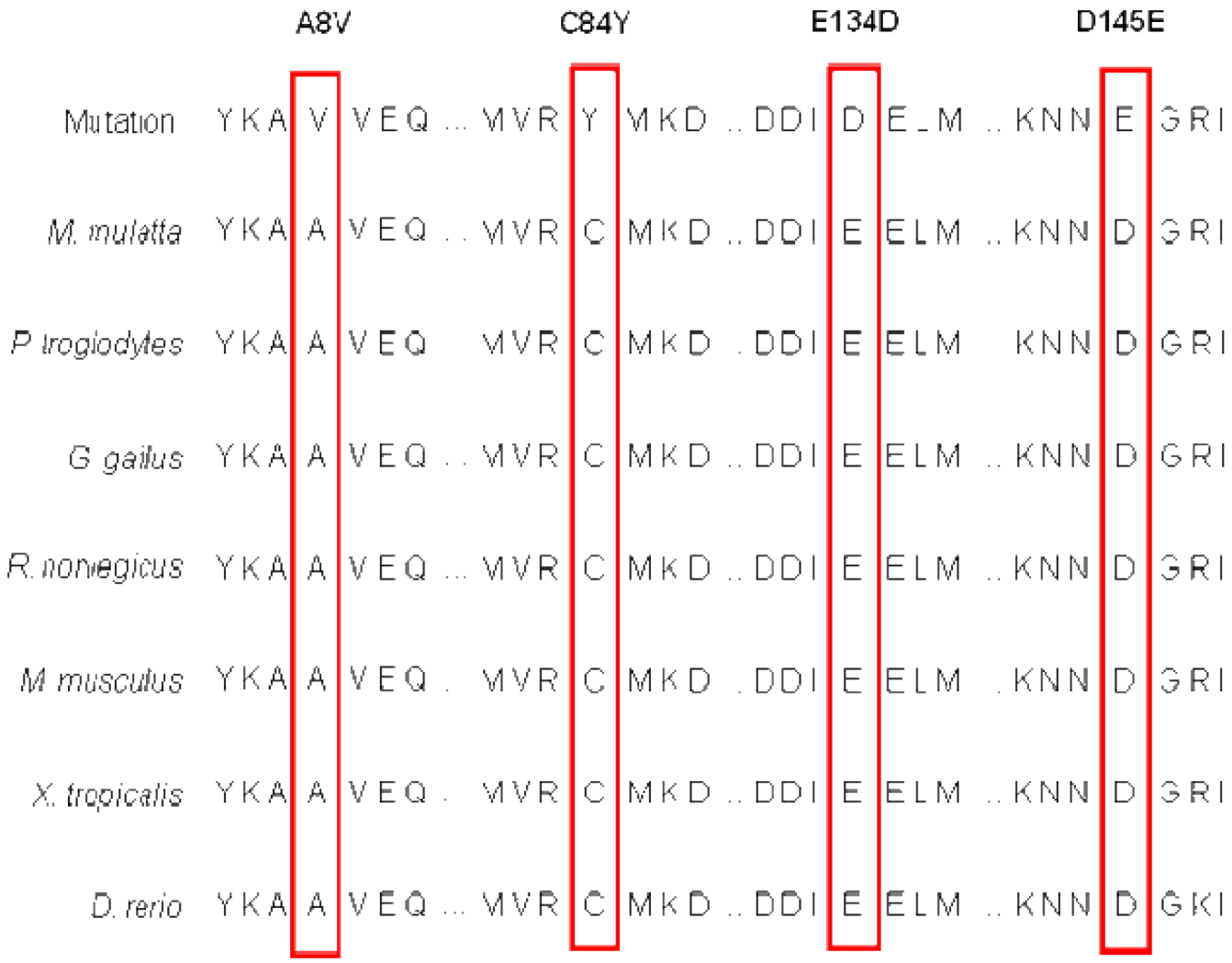

Figure 2. Sequence conservation

The identified mutations in *TNNC1* localize to residues completely conserved across all species queried.

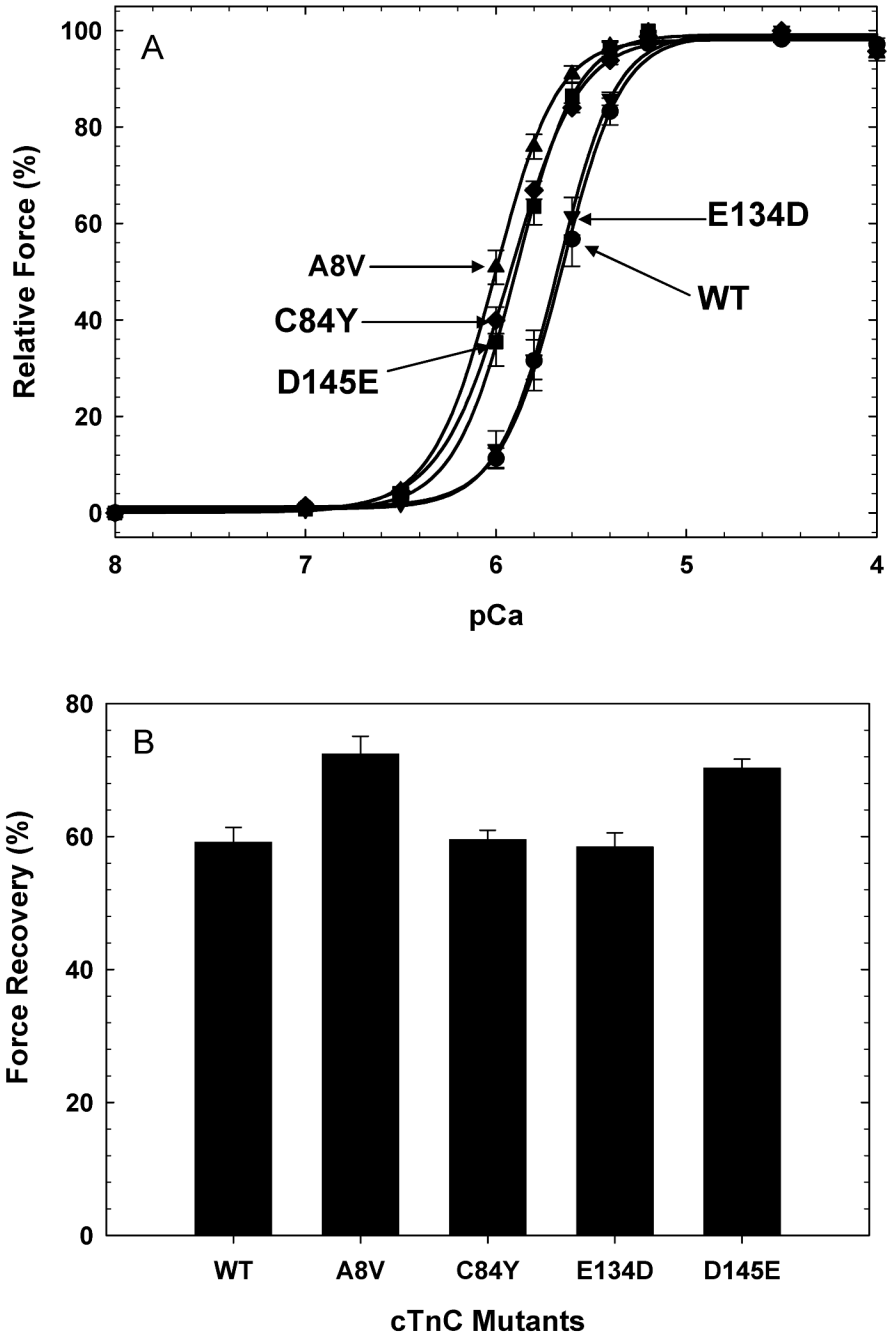

Figure 3. The Ca^{2+} dependence of force development and maximal relative force in reconstituted muscle fibers

A) Ca^{2+} dependence of force development in (●) WT, (▲) A8V, (♦) C84Y, (▼) E134D, and (■) D145E. **B**) Relative force recovery measured after HcTnC reconstitution normalized to the initial force. Data in each experiment are the average of 7–9 experiments and are expressed as mean ± S.E. in Table 3.

Table 1

Clinical Characteristics of HCM Cohort

Clinical Characteristic	HCM Cohort	*TNNC1* Positive
No. of Individuals	1025	4
Male/Female	621/404	3/1
Age at Diagnosis (years)	49.2 ± 18	29.4 ± 10.6
Cardiac Symptoms	55%	75%
Max LVWT (mm)	22.3 ± 7	24.3 ± 2.7
Resting LVOTO (mmHg)	43.9 ± 44	77.3 ± 19.2
Pos. FH for HCM	277 (27%)	1 (25%)
Pos. FH for SCD	185 (18%)	0 (0%)
Surgical Myectomy	376 (37%)	3 (75%)
Pacemaker	176 (17%)	0 (0%)
ICD	167 (16%)	1 (25%)

Values are mean ± SD or n (%). FH, family history; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; LVOTO, left ventricular outflow tract obstruction; LVWT, left ventricular wall thickness.

Table 2

Clinical Phenotype of *TNNC1*-Positive Patients with HCM

tation	Age (y)/ Sex	Age at Dx (y)	Race	Symptoms at Presentation	Subsequent symptoms	AF	Max LVWT (mm)	Resting LVOTO (mmHg)	FH of HCM	FH of SCD	Treatment
8V	37/M	33.9	C	Dyspnea	Dyspnea, chest pain	No	18	117	No	No	β-blockade, myectomy
34Y	17/M	8.4	C	Syncope on exertion	Pre-syncope	No	19	32	No	No	β-blockade
34D	22[a]/F	16	C	Chest pain	Dyspnea, chest pain	No	26	60[b]	No	No	β-blockade, Ca2+-channel blockade, extended myectomy
45E	58/M	57.3	C	Chest pain	Dyspnea, chest pain	No	22	100	Yes	No	β-blockade, myectomy

phic cardiomyopathy; M, male; F, female; Dx, diagnosis; C, Caucasian; SBE, subacute bacterial endocarditis; AF, atrial fibrillation; LVWT, left ventricular wall thickness; LVOTO, left low tract obstruction; FH, family history; SCD, sudden cardiac death; ICD, implantable cardioverter-defibrillator; NA; not available

tricular gradient with a 5 mmHg gradient at rest and provocation across outflow tract.

Table 3

Summary of Skinned Cardiac Fibers Reconstituted with Mutant Cardiac Troponin C

HcTnC	pCa_{50}	ΔpCa_{50}	n_{Hill}	Force Recovery (%)	# of experiments
WT	5.657 ± 0.012	0	2.74 ± 0.19	59.1 ± 2.3	8
A8V	6.017 ± 0.011*	+ 0.36	2.68 ± 0.18	72.4 ± 2.7*	9
C84Y	5.929 ± 0.011*	+ 0.272	2.42 ± 0.15*	59.5 ± 3.7	8
E134D	5.679 ± 0.009	+ 0.022	2.82 ± 0.16	58.4 ± 2.1	7
D145E	5.898 ± 0.008*	+ 0.241	2.73 ± 0.17	70.3 ± 1.4*	8

[a] ΔpCa_{50} = WT pCa_{50} − HCM TnC pCa_{50}

* $P < 0.05$ comparing HCM TnC with WT TnC values.