Clinical and functional characterization of *TNNT2* mutations identified in patients with dilated cardiomyopathy

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Abstract

Background—A key issue for cardiovascular genetic medicine is ascertaining if a putative mutation indeed causes dilated cardiomyopathy (DCM). This is critically important as genetic DCM, usually presenting with advanced, life-threatening disease, may be preventable with early intervention in relatives known to carry the mutation.

Methods and Results—We recently undertook bidirectional resequencing of *TNNT2*, the cardiac troponin T gene, in 313 probands with DCM. We identified six *TNNT2* protein-altering variants in nine probands, all who had early onset, aggressive disease. Additional family members of mutation carriers were then studied when available. Four of the nine probands had DCM without a family history, and five had familial DCM. Only one mutation (Lys210del) could be attributed as definitively causative from prior reports. Four of the five missense mutations were novel (Arg134Gly, Arg151Cys, Arg159Gln, Arg205Trp), and one was previously reported with hypertrophic cardiomyopathy (Glu244Asp). Based on the clinical, pedigree and molecular genetic data these five mutations were considered possibly or likely disease causing. To further clarify their potential pathophysiologic impact, we undertook functional studies of these mutations in cardiac myocytes reconstituted with mutant troponin T proteins. We observed decreased Ca^{2+} sensitivity of force development, a hallmark of DCM, in support of the conclusion that these mutations are disease-causing.

Conclusions—We conclude that the combination of clinical, pedigree, molecular genetic and functional data strengthen the interpretation of *TNNT2* mutations in DCM.

Keywords

dilated cardiomyopathy; genetics

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Introduction

A key issue in cardiovascular genetic medicine is determining if a putative mutation in a gene known to be associated with dilated cardiomyopathy (DCM) is indeed causative of disease. While a molecular genetic diagnosis may not necessarily guide treatment or impact outcome of a proband with advanced disease, pre-symptomatic at-risk relatives who carry the disease-causing mutation can undergo heightened clinical surveillance, and with evidence of early disease, medical and/or anti-arrhythmic device therapy may prevent disease progression or life-threatening complications. This issue is critically important because DCM usually presents with advanced, life-threatening disease. Recent guidelines suggest that a genetic etiology should be considered even in the absence familial DCM,[1] further favoring the benefits of genetic testing in this population. That treatment may prevent or delay DCM progression in pre-symptomatic at-risk relatives provides a powerful rationale for molecular genetic testing.

Despite these clear benefits, molecular diagnosis of DCM in a proband is hampered by extensive allelic heterogeneity (see[2-4] for review). Several dozen mutations, almost all of them private missense, have been reported in the genes more commonly implicated in genetic DCM such as *LMNA*,[5-8] *MHY7*,[9-12] or *TNNT2*,[9, 11-15] encoding the lamin A/C, beta myosin heavy chain, and cardiac troponin T proteins, respectively. Further, locus heterogeneity is a prominent feature of genetic DCM, as private mutations in more than 20 other genes have also been implicated.[2-4] Such complicating factors make assessing pathogenicity difficult. While assigning pathogenicity of a mutation is usually based on its rarity in the population, the alteration of a conserved amino acid, and family studies showing segregation with disease among multiple affected relatives or negative results in the unaffected parents of a sporadic case, the approach we used in our original resequencing report,[12] this approach heavily relies on prediction models, significant clinical follow-up resources and the cooperation of family members. A large DCM mutation database provides a practical alternative, but years of data accumulation will be necessary before its full implementation. Therefore, providers currently making use of genetic testing in their practice must decide in favor of or against increased frequency of cardiovascular screening despite limited data.

With the advent of genetic cardiomyopathy guidelines,[1] and with genetic testing rapidly becoming available for greater numbers of DCM genes[16] at lower cost, these issues will only become more problematic. Because of these issues, we reasoned that relevant functional studies, when combined with pedigree and molecular genetic data, may be valuable in interpreting molecular results in DCM, particularly when affected family members are not available to assess segregation.

Troponin T cardiomyopathy is an aggressive, early onset disease accompanied by considerable morbidity and mortality. Elegant functional studies have previously demonstrated characteristic shifts in calcium sensitivity and force of contraction with DCM and hypertrophic cardiomyopathy (HCM) resulting from *TNNT2* mutations.[17-23] We therefore selected this gene for study, and now present the combined clinical, family, molecular genetics and functional data for troponin T DCM.

Materials and Methods

Patient Population

Written, informed consent was obtained from all subjects, and the OHSU Institutional Review Board approved the project. The study included 313 probands (291 Caucasians, of whom seven were of Hispanic descent; 16 African-Americans, three Asians and three Native Americans/ Alaskan Natives), and used methods of clinical categorization of FDC versus IDC as previously described.[12, 24] A composite clinical description of 304 probands and their families and our

FDC/IDC categories have previously been described24 in detail; 302 of those 304 and an additional 11 IDC and FDC probands were included in the present study.

Genetic analysis

Genomic DNA was extracted from whole blood and was sequenced in both directions to detect nucleotide variants in *TNNT2*, cardiac troponin T, as previously described.[12] All exons and intron/exon boundaries were PCR amplified by standard methods at SeattleSNPs under contract to the NHLBI resequencing service. Amino acid numbering was after Townsend et al [25] and gene structure was after Gomes et al [26] (Figure 1.A).

Samples from probands identified by the resequencing service as carriers of protein-altering variants, as well as any available samples from their relatives were resequenced in our laboratory for confirmation and segregation analysis. Nucleotide changes were only evaluated if they were absent from all 253 control samples analyzed at the resequencing center (188 Caucasian, 24 African-American, 22 Asian and 19 Hispanic). Putative disease-causing nucleotide alterations identified in African-American samples were further evaluated in an additional 169 control African-American DNA samples in our laboratory, for a total of 193 African-American controls (386 chromosomes).

Functional studies in porcine skinned cardiac fiber preparations

Human cardiac wild type troponin T (HCTnT-WT) was previously cloned in our laboratory, [27] and used as a template for overlapping PCR with primers designed to replace those amino acids present in the DCM troponin T mutant proteins. Recombinant human cardiac troponin T (HCTnT), troponin I and troponin C proteins were prepared as previously reported.[26, 28] Formation of human troponin I and troponin C complexes was performed as previously described.[28] Skinned papillary muscles were obtained from the left ventricles of fresh pig hearts obtained at a local slaughterhouse. Small bundles of fibers were isolated and treated overnight in a pCa 8.0 relaxing solution containing 1% Triton X-100 as previously described. [26, 28] After the overnight treatment, fibers were then transferred to a pCa 8.0 solution containing 50% glycerol, without Triton X-100 and stored at - 20°C. This preparation was used to dissect out porcine muscle fiber bundles that were mounted onto a force transducer (Supplemental Figure 1A). Fiber force development was evaluated with increasing Ca^{2+} concentration from pCa 8.0 - 4.0 (human wild-type and mutant troponin T proteins were incorporated into the fibers as described in the supplemental figure A2). Data were analyzed using the following equation: % Change in force $= 100 \times [Ca^{2+}]^n / ([Ca^{2+}]^n + [Ca^{2+}_{50}]^n)$ where "$[Ca^{2+}_{50}]$" is the free $[Ca^{2+}]$ which produces 50% force and "n" is the Hill coefficient, as previously described.[26, 28] The experimental results were reported as $X \pm S.E.M.$, and analyzed for significance using unpaired Student's *t* test at $p < 0.05$.

Results

Molecular genetic data

Bidirectional sequencing of *TNNT2* was carried out on DNA specimens from 313 unrelated probands with IDC or FDC, as described in our preliminary report.[12] Six protein-altering variants, none of which were present in 253 control specimens, were identified in nine of 313 probands (2.9%) (Table 1),[9, 13, 15, 18, 29] of whom eight were Caucasian (one of Hispanic descent, Pedigree B), and one was African-American (Pedigree I). The variant in Pedigree I was not identified in an additional 193 African-American control DNAs. Of the six variants, five were missense mutations, all of which altered highly conserved amino acids (Figure 1.B). The sixth was the Lys210del mutation, previously reported in multiple families associated with DCM.[9, 13, 18] Three of the six variants have previously been reported in association with

DCM[9, 13, 15, 18] or HCM[29] (Table 1). *TNNT2* mutations identified here and those previously reported in DCM and HCM show marked allelic heterogeneity (Figure 1.A).

Clinical data

Preliminary clinical assignments of were made for each mutation[12] and are provided (Table 1). Clinical characteristics (Table 2) and pedigree structures (Figure 1.C) are presented.

The variant in pedigree A (Arg134Gly) segregated with disease in other affected family members and was therefore considered likely disease causing.

The proband in Pedigree B whose parents were second cousins, was homozygous for an Arg151Cys alteration, and was the only clinically affected member of her family. DNA analysis indicated that each parent was heterozygous for the alteration. We previously reported this mutation as likely disease-causing[12] in part because of the homozygous state and in part because of the characteristic clinical behavior of *TNNT2* mutations to cause early onset, aggressive disease as observed in the proband. However, the pathogenicity of this mutation was not certain, as one sibling also carried the homozygous mutation and had no evidence of disease. Further, the proband's asymptomatic parents were carrying the mutation in the heterozygous state. All previous reports of *TNNT2* mutations, including all others herein, were heterozygous and had been sufficient to cause the DCM phenotype, and the older age of the proband's parents should have increased their probabilities of manifesting disease.

The Arg159Gln *TNNT2* nucleotide alteration in the proband of Pedigree C was considered possibly disease-causing[12] (Table 1) as it predicted the replacement of a conserved amino acid; however, no additional pedigree information was available to assess segregation with disease.

The proband in pedigree D carried an Arg205Trp alteration. Because a previous report had identified an Arg205Leu mutation in association with DCM, we therefore assigned Arg205Trp as likely disease causing. However, neither functional nor segregation data to support this assignment were available for our proband or in the prior report.[15]

The Lys210del mutation had been shown to be disease-causing in several pedigrees with excellent segregation data,[9, 13, 15] and was observed in unrelated probands from four families (E, F, H; G has been described by others[9] and is not shown).

The *TNNT2* Glu244Asp variant in the proband of Pedigree I was previously reported in a patient with HCM,[29] however, the proband had a very dilated left ventricle and marked reduction in systolic function consistent with DCM (Table 2). This mutation was previously assigned by us as likely disease-causing[12] (Table 1) because of the prior HCM report. However, segregation data were lacking in the HCM report and in our pedigree.

The median age of onset of the 30 affected individuals with DCM from the nine pedigrees was 32.5 years, with a mean age of 31.1 years (range 0.5 to 72 years) (Table 2). Twelve of 30 (40%) had disease onset by age 20 years, 14 of 30 (47%) had disease onset by 30 years, and 21 of 30 (70%), had disease onset by 40 years.

Functional Data

The Ca^{2+} sensitivity of force development obtained in porcine cardiac skinned fibers, when comparing the pCa-force relationship of fibers exchanged with human wild-type or DCM mutant troponin T proteins reconstituted with the human cardiac troponin I and troponin C complex, demonstrated reduced sensitivity to calcium consistent with DCM in our[18, 20, 21] and other[17-19, 22, 23] prior reports. The Arg151Cys, Arg159Gln and Arg205Trp decreased Ca^{2+} sensitivity of force development of 0.09, 0.07 and 0.07 log units of $[Ca^{2+}]$, respectively

(Table 3). The Arg134Gly mutation did not display changes in Ca^{2+} sensitivity compared to the wild-type, but increased the maximal force development (Table 3). The same functional phenotype (no changes in Ca^{2+} sensitivity and increased force) was previously observed with the Glu244Asp mutation.[20] This could be associated with a distinct functional phenotype of some DCM mutants or a specific functional phenotype for mutations that start as hypertrophic and progress to dilated cardiomyopathy.[30] The pCa_{50} ([Ca^{2+}] needed to reach 50% of maximal force), Hill coefficient (a cooperativity index of thin filament activation) and maximal force values are shown in Figure 2 and Table 3. To address whether the troponin DCM mutants had altered affinity for the thin filament and subsequent poor incorporation into the fibers, we measured the unregulated tension at low Ca^{2+} concentration (pCa 8.0) after displacement and before reconstitution with the troponin I and troponin C binary complex. As more exogenous troponin T incorporates into the thin filament and more endogenous troponin is displaced, there is a greater amount of tension generated at low Ca^{2+} levels since there is less troponin available to inhibit force (see supplemental figure A for additional detail). Fibers incubated with the Arg134Gly mutant showed a reduced ability (38% Ca^{2+} unregulated force) to displace the endogenous porcine troponin complex compared to the human wild-type protein (87% Ca^{2+} unregulated force) (Table 3). The other troponin T mutants did not show changes in their incorporation into the thin filament. This finding indicates that the Arg134Gly mutant may exert its effects through changes in protein-protein interaction that alter thin filament and contractile function via mechanisms independent of calcium sensitivity to give the human DCM phenotype.

Discussion

A great deal of progress has recently been made in understanding the genetic basis of DCM with mutations identified in more than 20 genes (reviewed in 2-4). This progress has led to the recent release of clinical guidelines for the genetic cardiomyopathies,1 and to a rapid expansion of clinical molecular genetic testing. However, molecular diagnosis of DCM is challenging. For example, genetic DCM, usually presenting as adult-onset disease, varies greatly in its age of onset. Thus, even if first degree relatives are available for molecular genetic testing, family members carrying the putative disease-causing mutation may have little or no evidence of disease, confounding efforts to assess segregation of the mutation with disease and therefore confirmation of the variant as disease-causing. Yet paradoxically, it is these family members who may most benefit from clinical surveillance and early intervention to prevent disease progression. Further, although not yet reported for *TNNT2*, sequencing the many genes implicated in DCM will likely reveal increasing numbers of variants of unknown significance. Ongoing research integrating molecular, clinical and functional data, as shown here, may facilitate clinical management by adding to the interpretation of gene variations thought to represent disease-causing DCM mutations.

In this study the molecular, clinical and pedigree data for troponin T mutations in these families were supplemented with functional studies with mutant troponin T proteins reconstituted into porcine cardiac myocytes. This approach yielded highly informative calcium sensitivity and maximal force response data that was helpful to augment molecular genetic data for the identified novel mutations.

Aside from conduction and rhythm perturbations observed with *LMNA* and *SCN5A* mutations, 2-4 few genotype/phenotype correlations exist for genetic DCM. However, one useful genotype/phenotype correlation may be the fully penetrant, early onset, aggressive disease observed in this and prior reports9, [15] of troponin T DCM. In the 30 affected subjects herein, the median age of onset was 32.5 years, with an average age of onset of 31.1 years; almost one-half (47%) of those affected had disease onset by 30 years of age. This compares with an

earlier analysis from our research database of 304 probands and 166 family members affected with FDC/IDC, where the average age at diagnosis was 43 years.[24]

The mechanism of the early onset and aggressive nature of troponin T cardiomyopathy is likely related to the severity of disruption of the force-generating components of the thick and thin filaments. Functional reports of DCM mutations have shown decreased Ca^{2+} sensitivity of the myofilament in the tropomyosin/troponin regulatory complex.[17-19, 22, 23] Cardiac troponin T is the subunit that connects the troponin complex to the thin filament. The additional subunits are troponin C that binds Ca^{2+}, and troponin I that binds to actin and is involved in inhibition of muscle contraction.[31-33] This decrease in Ca^{2+} sensitivity of force development is sufficient to alter the contractility dynamics of the heart leading to systolic dysfunction, as previously observed for the Lys210 deletion and the R141W mutants.[18]

While skinned porcine myocytes present an ideal model to evaluate functional changes in troponin cardiomyopathy, other systems able to evaluate the functional impact of mutations of other DCM genes (e.g., those encoding contractile, channel, calcium handling and other proteins) could also augment clinical and molecular genetic data to help confirm that a specific sequence variant causes DCM.

In conclusion, the functional data obtained for the six *TNNT2* mutations reported in this study suggests that these variants are highly likely to be causative of DCM considering the cumulative data from molecular genetic, clinical, pedigree and functional studies. Adding functional data to existing clinical and genetic data will be key to understanding the molecular etiology of genetic DCM while moving the cardiovascular genetic medicine field ahead.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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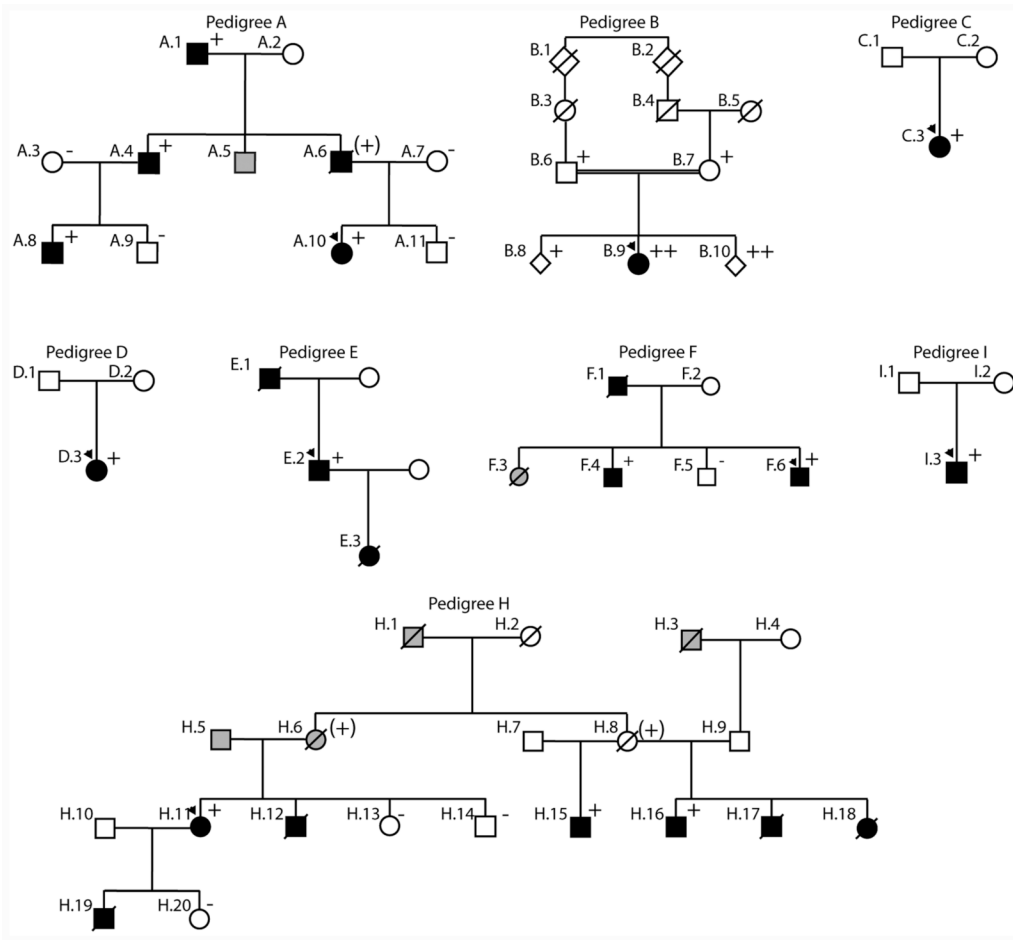
Figure 1. The *TNNT2*, cardiac troponin T gene structure, cardiac troponin T amino acid conservation, and pedigrees of cardiac troponin T associated cardiomyopathy

A. The *TNNT2*, cardiac troponin T gene structure. The *TNNT2* gene is shown, and the adult isoform of cardiac troponin T, encoded by exons 2-17, is a total of 288 amino acids in length.

The locations of the 6 mutations presented are labeled with their respective letters (A through I); other published DCM mutations are labeled J through N. Mutations reported to cause hypertrophic cardiomyopathy are labeled 1 through 32. Glu244Asp, previously reported in association HCM and reported here in association with DCM is identified as both (I, 24). Exon numbering[25] and amino acid numbering[26] is per prior reports. Binding regions for troponin C, troponin I, tropomyosin and cardiac actin are shown. HCM mutations are shown in Supplemental Table A.

B. Amino acid conservation. Altered amino acids are shown for each missense mutation on the first line. The wild-type human sequence is shown, followed by rat, mouse, chicken, zebrafish and drosophila. All variants were conserved to zebrafish or drosophila except Arg159Gln (rat, mouse).

C. Pedigrees of cardiac troponin T associated cardiomyopathy. Pedigrees have been labeled by letter, which correspond to their respective mutation as shown in panel A and given in Tables 1 and 2. Squares represent males, circles females. An arrowhead denotes the proband. A diagonal line marks deceased individuals. Solid symbols indicate idiopathic dilated cardiomyopathy with or without heart failure; shaded symbols represent any cardiovascular abnormality. Open symbols represent unaffected individuals. The presence or absence of the pedigree's *TNNT2* mutation is indicated by a + or - symbol, respectively. Obligate carriers are noted in parenthesis, (+). Pedigree G, previously published by others,[5] is not shown.
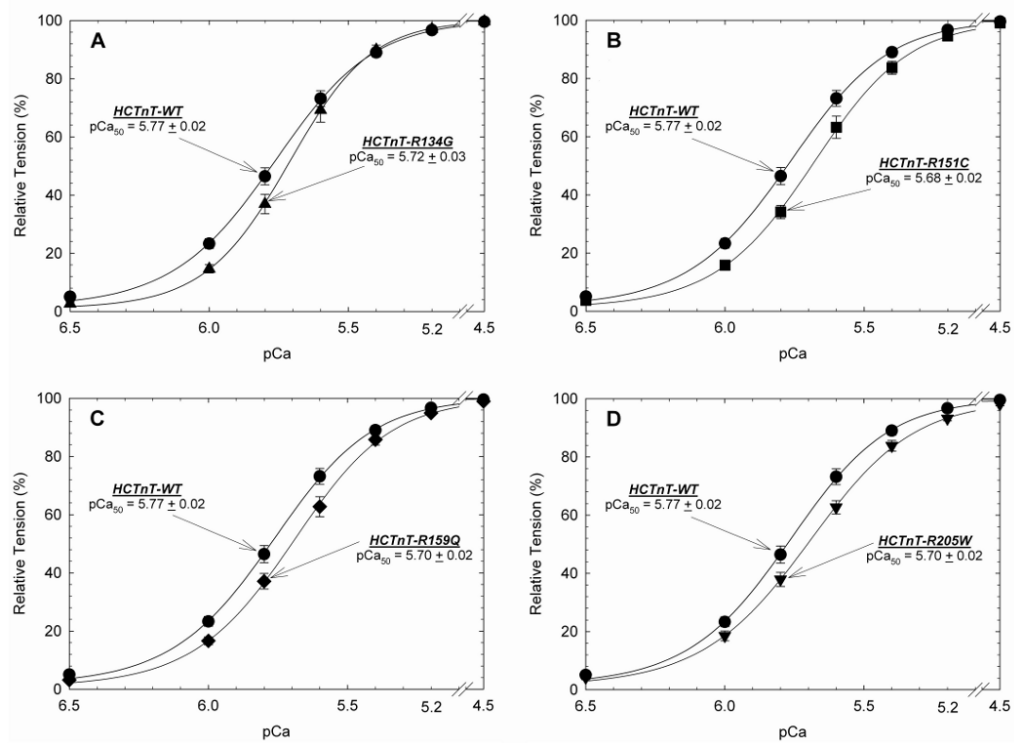
Figure 2. Normalized pCa-Force relationship in skinned cardiac muscle fibers
The wildtype (WT) and DCM-HCTnTs replaced native CTnT in the procedure shown in Figure 2.A) HCTnT-WT containing fibers were compared to DCM-mutant HCTnT-R134G B) HCTnT-WT containing fibers compared to DCM-mutant HCTnT-R151C C) HCTnT-WT containing fibers compared to DCM-mutant HCTnT-R159Q D) HCTnT-WT containing fibers compared to DCM-mutant HCTnT-R205W. The Ca^{2+} dependence of force development remained unchanged in native porcine fibers and after HCTnT-WT substitution (See Table 3). Data in each panel is an average of 7-9 experiments and the mean is shown as mean ± S.E.

Table 1

Nonsynonymous mutations in *TNNT2*, cardiac troponin T

| Pedigree | Molecular Genetic Data | | Clinical and Pedigree Data | | | | | Functional Data |
	Nucleotide change*	Amino Acid Change[†]	Race	Diagnosis, FDC or IDC	Segregation[‡]	Disease-Associated Status in Prior Report[12]	Conservation[§]	Previously reported cardiovascular disease	Reference	Calcium sensitivity
A	13712C>G	Arg134Gly	Non-Hispanic White	FDC	Yes	Likely	Yes			decreased
B	13763C>T[//]	Arg151Cys	Hispanic White	IDC		Likely	Yes			decreased
C	14679G>A	Arg159Gln	Non-Hispanic White	IDC		Possibly	Yes			decreased
D	16080C>T	Arg205Trp	Non-Hispanic White	IDC		Likely	Yes	DCM[¶]	15	decreased
E	16096_16098delAGA	Lys210del	Non-Hispanic White	FDC		Likely	N/A	DCM	9, 13, 15, 18	decreased
F	16096_16098delAGA	Lys210del	Non-Hispanic White	FDC	Yes	Likely	N/A	DCM	9, 13, 15, 18	decreased
G	16096_16098delAGA	Lys210del	Non-Hispanic White	FDC	Yes	Likely	N/A	DCM	9, 13, 15, 18	decreased
H	16096_16098delAGA	Lys210del	Non-Hispanic White	FDC	Yes	Likely	N/A	DCM	9, 13, 15, 18	decreased
I	16742G>T	Glu244Asp	Non-Hispanic African American	IDC		Possibly	Yes	HCM	29	decreased

*
Nucleotide numbering is per the SeattleSNPs resequencing service.

[†]
Amino acid numbering is per previous publications.

[‡]
Segregation means multiple affected carrying mutation and/or multiple nonaffected not carrying mutation; entry left blank because of insufficient clinical data and/or DNA specimens to assess segregation.

[§]
see Figure 1.B.

[//]
Homozygous mutation.

[¶]
The Arg205Trp reported here is a novel mutation; the earlier study reported Arg205Leu.

Table 2

Clinical Characteristics

Subject	Age of diagnosis, years	DCM	ECG / Arrhythmia	LVEDD, mm (Z-score)	LV septum, posterior wall thickness, mm	Ejection Fraction	Mutation present (yes, no, unknown)	Comment
Pedigree A: ARG-134-GLY								
A.1	63	yes	NA	57 mm (1.90)	NA	25%	yes	
A.4	34	yes	AF, VT, bigem, PM, ICD	58 mm (1.69)	10/10	30%	yes	
A.5	34	NA	NA	NA	NA	NA	unknown	Heart transplant at 34 yrs; no medical records
A.6	34	yes	NSSTT	63mm (3.14)	8/9	35%	yes (obligate carrier)	Died of SCD at age 36
A.8	12	yes	1AVB	51mm (1.78)	7/7	23%	yes	Asymptomatic
A.10	6	yes	NA	58 mm (6.05)	5/6	19%	yes	Heart transplant at 8 yrs
Pedigree B: ARG-151-CYS								
B.6	56	no	LAD, LVH	NA	7/7	60%	yes	
B.7	52	no	NA	51 mm (2.11)	9/9	57%	yes	Asymptomatic at 53 yrs
B.8	16/20	no	NL	50 mm (0.23)	7/7	70%	yes	Asymptomatic
B.9	19	yes	NSSTT, LVD, low voltage	65 mm (6.00)	NA	24%	yes, homozygous	Heart transplant at 19 years
B.10	16/20	no	NA	47 mm (0.77)	6/5	66%	yes, homozygous	Asymptomatic
Pedigree C: ARG-159-GLN								
C.3	20	yes	NA	65 mm (5.60)	9/8	25%	yes	HF onset associated with pregnancy
Pedigree D: ARG-205-TRP								
D.3	0.5	yes	1AVB, LVH	78 mm (7.01)	4/4	18%	yes	Congenital DCM at 4 mo; heart transplant at 12 yrs
Pedigree E: Lys 210 deletion								
E.1	?	yes	NA	NA	NA	NA	unknown	Died awaiting heart transplant at 52 yrs
E.2	31	yes	LAFB, LVH, NSSTT	83 mm (7.10)	11/10	14%	yes	Heart transplant at 31 yrs
E.3	5	NA	NA	NA	NA	NA	unknown	No medical records; died 3 mo after diagnosis
Pedigree F: Lys 210 deletion								
F.1	50	yes	NA	NA	NA	NA	unknown	DCM at autopsy

Subject	Age of diagnosis, years	DCM	ECG / Arrhythmia	LVEDD, mm (Z-score)	LV septum, posterior wall thickness, mm	Ejection Fraction	Mutation present (yes, no, unknown)	Comment
F.3	34	NA	NA	NA	NA	NA	unknown	Died after heart transplant at age 34; no medical records
F.4	37	yes	RAD	66 mm (3.95)	9/11	30%	yes	Asymptomatic, age 50
F.6	26	yes	1AVB, LBBB	81 mm (6.47)	11/11	15%	yes	Heart failure
Pedigree H: Lys 210 deletion								
H.1	35							
H.6	74	yes	LAD, LAFB, NSSTT	NA	NA	28%	yes (obligate carrier)	Diagnosed at 74 years; no coronary angiogram
H.8	N/A	NA	NA	NA	NA	NA	yes (obligate carrier)	Died at 34 years of cancer
H.11	59	yes	1AVB, NSSTT	58 mm (4.05)	14/11	20%	yes	Heart Failure
H.12	16	yes	1AVB, RAE, LAE, LVH	NA	NA	NA	unknown	Died at 16 years of advanced HF; DCM by autopsy
H.15	52	yes	LBBB, NSSTT	75 mm (5.00)	9/9	16%	yes	Heart failure
H.16	47	yes	NL	59 mm (2.54)	7/7	50%	yes	Asymptomatic
H.17	1	yes	NA	NA	NA	NA	unknown	Died at 1 yr, diagnosis by autopsy
H.18	12	yes	1AVB, LAE, LVH, NSSTT	NA	NA	NA	unknown	Died of DCM at 12 yrs
H.19	21	yes	LAE, RAD	NA	NA	20%	unknown	Died of DCM at 21 yrs
Pedigree I: GLU-244-ASP								
I.3	13	yes	LVH, RVH, NSSTT	70 mm (5.66)	NA	7%	yes	Severely reduced systolic function; heart failure, VAD, heart transplant 13 at yrs

1AVB, first degree atrioventricular block; AF, atrial fibrillation; bigem, bigemeny; DCM, dilated cardiomyopathy; ICD, implantable cardiac defibrillator; LAD, left axis deviation; LAE, left atrial enlargement; LAFB, left anterior fascialar block; LBBB, left bundle branch block; LVEDD, left ventricular end-diastolic dimension; LVH, left ventricular hypertrophy; NSSTT, non-specific ST-T changes; PM, pacemaker; RAD, right axis deviation; RAE, right atrial enlargement; RVH, right ventricular hypertrophy; VAD, ventricular assist device; VT, ventricular tachycardia.

Table 3

Summary of calcium-force relationship curves in porcine ventricular myocyte fibers displaced with human cardiac troponin T wild type or troponin T mutations and reconstituted with human cardiac troponin I/troponin C complex

The pCa_{50}, n_H, % maximal force and % Ca^{2+} unregulated force values are the average of a number of independent fiber experiments, and the errors are reported as S.E. values. The Ca^{2+} unregulated force was calculated after the human cardiac troponin T (HCTnT) treatment by the following equation: (F_{pCa8}/F_{pCa4}) X 100, where the F_{pCa8} (x in Figure 5) [and F_{pCa4} (y in Figure 6)] are the force at pCa 8.0 and pCa 4.0 solutions, respectively. The Ca^{2+} unregulated force reflects the efficiency of the human cardiac troponin T to displace the whole native troponin complex and its ability to incorporate into the fiber.

Human cardiac troponin T proteins	pCa_{50}	Hill coefficient, n_H	ΔpCa_{50}[a]	% Maximal Force Recovery	% Ca^{2+} Unregulated Force	Number of Experiments
Native (porcine)	5.83 ± 0.01	3.44 ± 0.09	+ 0.06	100.0	-	40
Wild-type	5.77 ± 0.02	2.50 ± 0.07	-	67.6 ± 3.7	87.2 ± 3.2	9
Arg134Gly	5.72 ± 0.03	3.09 ± 0.07	- 0.05	79.7 ± 2.5*	38.1 ± 3.0*	9
Arg 151Cys	5.68 ± 0.02*	2.64 ± 0.16	- 0.09	65.7 ± 3.4	79.8 ± 6.4	7
Arg159Gln	5.70 ± 0.02*	2.53 ± 0.06	- 0.07	65.0 ± 3.4	91.1 ± 4.3	7
Arg205Trp	5.70 ± 0.02*	2.33 ± 0.07	- 0.07	63.2 ± 3.2	92.3 ± 3.2	8
Lys210del[b]	-	-	-0.12[b]	46.8[b]	89.6[b]	-
Glu244Asp[c]	-	-	0[c]	83.4[c]	100.0[c]	-

P values compare the DCM mutant human cardiac troponin T versus the wild-type;

*
$p < 0.05$

[a]
ΔpCa_{50}: HCTnT-DCM Mutant pCa_{50} − HCTnT-WT pCa_{50}.

[b]
values obtained from prior reports[18, 21]

[c]
values obtained from a prior report[20]