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Familial Dilated Cardiomyopathy Caused by an Alpha-Tropomyosin Mutation

The Distinctive Natural History of Sarcomeric Dilated Cardiomyopathy

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Objectives   We sought to further define the role of sarcomere mutations in dilated cardiomyopathy (DCM) and associated clinical phenotypes.

Background   Mutations in several contractile proteins contribute to DCM, but definitive evidence for the roles of most sarcomere genes remains limited by the lack of robust genetic support.

Methods   Direct sequencing of 6 sarcomere genes was performed on 334 probands with DCM. A novel D230N missense mutation in the gene encoding alpha-tropomyosin (*TPM1*) was identified. Functional assessment was performed by the use of an in vitro reconstituted sarcomere complex to evaluate ATPase regulation and Ca^{2+} affinity as correlates of contractility.

Results   *TPM1* D230N segregated with DCM in 2 large unrelated families. This mutation altered an evolutionarily conserved residue and was absent in >1,000 control chromosomes. In vitro studies demonstrated major inhibitory effects on sarcomere function with reduced Ca^{2+} sensitivity, maximum activation, and Ca^{2+} affinity compared with wild-type *TPM1*. Clinical manifestations ranged from decompensated heart failure or sudden death in those presenting early in life to asymptomatic left ventricular dysfunction in those diagnosed during adulthood. Notably, several affected infants had remarkable improvement.

Conclusions   Genetic segregation in 2 unrelated families and functional analyses conclusively establish a pathogenic role for *TPM1* mutations in DCM. In vitro results demonstrate contrasting effects of DCM and hypertrophic cardiomyopathy mutations in *TPM1*, suggesting that specific functional consequences shape cardiac remodeling. Along with previous reports, our data support a distinctive, age-dependent phenotype with sarcomere-associated DCM where presentation early in life is associated with severe, sometimes lethal, disease. These observations have implications for the management of familial DCM.   (J Am Coll Cardiol 2010;55:320–9) © 2010 by the American College of Cardiology Foundation

Dilated cardiomyopathy (DCM) is an important cause of heart failure (HF) and a leading indication for heart transplantation in children (1) and adults (2). During the past decade, there has been increasing recognition of the important contribution of genetic etiologies in causing "idiopathic" DCM, with family studies suggesting that 30% to 50% of the disease is inherited (3,4). However, clinical manifestations may be highly variable and obscure the identification of familial disease.

In contrast to hypertrophic cardiomyopathy (HCM), where sarcomere mutations cause the majority of disease (5), the genetics of DCM are more diverse and not as well defined. Mutations in a broad spectrum of genes have been implicated, including those encoding sarcomere proteins, components of the cytoskeleton, and mitochondrial proteins (3,6). Through comprehensive sequence analyses of sarcomere genes in 334 DCM probands we identified 2 unrelated, multigenerational families with DCM that shared a variant in the gene encoding alpha-tropomyosin (*TPM1*). Alpha-tropomyosin is an alpha-helical, coiled-coil homodimeric thin filament protein that participates in the Ca^{2+} regulation of contraction and acto-myosin interaction. Although *TPM1* mutations are known causes of HCM, their pathogenicity in DCM has been inconclusive because they have previously been identified only in individual patients (7).

We report genetic segregation of a *TPM1* mutation and its in vitro functional consequences on sarcomere function. Together these data provide strong evidence that *TPM1* mutations are pathogenic in DCM and extend knowledge about the pathogenesis and clinical course associated with sarcomere gene mutations.

Methods

Genetic analysis. Genomic DNA was isolated from blood by the use of standard methods (6) in probands with DCM. Direct DNA sequence analysis of all coding regions and intron/exon boundaries was performed in 6 sarcomere genes: myosin binding protein-C (*MYBPC3*); beta-myosin heavy chain (*MYH7*); cardiac troponin-T (*TNNT2*); cardiac troponin-I (*TNNI3*); alpha-tropomyosin (*TPM1*); and alpha-actin (*ACTC1*). To calculate the statistical likelihood that a genetic variant was associated with disease, logarithm (base 10) of odds (i.e., LOD) scores were calculated by the use of Vitesse (version 2.0, Bournemouth, Dorset, United Kingdom) for Mac/PC, assuming 80% DCM penetrance at 30 years and an allele frequency = 0.005. Haplotype analysis was performed to determine family relatedness by characterizing flanking single nucleotide polymorphisms in relevant families.

Actin–tropomyosin-activated myosin ATPase assay. Bacterial expression constructs in pMW172 encoding human wild-type and D230N (mutant) Ala-Ser-alpha-tropomyosin were created by 2-step polymerase chain reaction site-directed mutagenesis. The Ala-Ser N-terminal addition to *TPM1* compensates for the absence of N-terminal acetylation in the bacterially produced peptide (8). Mutant or wild-type Ala-Ser-alpha-tropomyosin were expressed with wild-type troponin I, troponin T, and troponin C in BL21 (DES) pLysS *Escherichia coli* cells and subsequently purified according to established protocols (9). Actin and myosin subfragment-1 (S-1) were obtained from rabbit skeletal muscle by standard procedures (10). Wild-type cardiac troponin complex was reconstituted from individual subunits by the use of stepwise dialysis and gel

filtration as previously described (11). Thin filaments were reconstituted at an actin, Ala-Ser-alpha-tropomyosin, and troponin ratio of 7:1:1, respectively.

Assays were performed as previously described with the use of $0.5-\mu mol/l$ myosin S-1 and thin filaments reconstituted with the use of $3.5-\mu mol/l$ actin, $0.5-\mu mol/l$ tropomyosin, and $0.5-\mu mol/l$ troponin in $50-mmol/l$ KCl, $5-mmol/l$ piperazine-N,N'-bis(2-ethanesulfonic acid), $3.87-mmol/l$ $MgCl_2$, and 0.25 $mmol/l$ dithiothreitol, pH 7.0, at 37°C. The free Ca^{2+} concentration was set by the use of $1-mmol/l$ ethylene glycol tetraacetic acid and the appropriate concentration of $CaCl_2$ as previously described (11). Phosphate release was determined colorimetrically by standard protocols.

Measurement of Ca^{2+} affinity by the use of 2-[4′-(iodo-acetamido)aniline]-naphthalene-6-sulfonate (IAANS) troponin. Thin-filament Ca^{2+} affinity was measured by the use of IAANS label bound to Cys35 of recombinant human troponin C (12); a reporter of Ca^{2+} binding to the low-affinity site of troponin C (site II) (12). Thin filaments were reconstituted with $21-\mu mol/l$ actin, $3-\mu mol/l$ Ala-Ser-alpha-tropomyosin, and $3-\mu mol/l$ IAANS troponin. The final buffer concentration of ethylene glycol tetraacetic acid was $1 \ mmol/l$, and the free Ca^{2+} concentration was set by use of the appropriate concentration of $CaCl_2$ as previously described (12).

Steady-state fluorescence measurements (excitation 325 nm, emission 455 nm) were made with the use of a RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan) at 22°C. The change in fluorescence (ΔF) was monitored as the Ca^{2+} was titrated with final ΔF values adjusted for the difference in assay mix volume after each incremental addition of $10 \ mmol/l$ $CaCl_2$. The adjusted and normalized ΔF was plotted as a function of Ca^{2+} concentration and the resultant curves fitted to the Hill equation, a measure of the cooperativity of binding between Ca^{2+} and the thin filament.

Clinical evaluation. Families with apparent familial DCM were recruited for genetic research. Family members were evaluated through history, physical examination, electrocardiography, and echocardiography. Echocardiographic dimensions were represented as *z*-scores for subjects younger than 16 years of age and left ventricular end-diastolic internal diameter (LVIDD *z*-score >2.0 was considered enlarged. The left ventricular ejection fraction (LVEF) was calculated by use of the modified Simpson's method. Subjects were considered affected with DCM if they had any of the following: clinical HF with left ventricular (LV) enlargement or systolic dysfunction; asymptomatic LV enlargement, that is, LV dimensions greater than the reference normal dimensions in adults (13) or *z*-score >2 in children;

Abbreviations and Acronyms

DCM = dilated cardiomyopathy

HCM = hypertrophic cardiomyopathy

HF = heart failure

LV = left ventricular/ventricle

LVIDD = left ventricular end-diastolic internal diameter

LVEF = left ventricular ejection fraction

or asymptomatic LV systolic dysfunction (LVEF <55%). Clinical information on deceased subjects was obtained from their relatives and medical records whenever possible. All subjects provided informed consent in accordance with the guidelines of the University of Chicago and Brigham and Women's Hospital Human Subjects Committees.

Statistical analysis. SAS version 9.1 (SAS Institute, Cary, North Carolina) was used to generate Kaplan-Meier curves to describe disease penetrance and event-free survival. Statistically significant differences in Ca^{2+} affinity and myosin ATPase activity were determined by the use of an unpaired Student *t* test (InStat, GraphPad Software Inc., La Jolla, California), with significance values defined as $p < 0.05$.

Results

Genetic analysis. A total of 334 subjects with DCM underwent sarcomere gene sequence analysis. A guanine to adenine (G>A) substitution at residue 688 in the gene encoding alpha-tropomyosin (*TPM1*) was identified in 2 probands with familial disease (Online Fig. 1). This variant is predicted to substitute a highly conserved, negatively charged aspartate residue (D, position 230) (Online Table 1) with a neutral asparagine (N) on the surface of tropomyosin (Fig. 1) (14).

TPM1 D230N segregated with DCM in 2 large Caucasian families (Figs. 2A and 2B). Haplotype analysis was performed by characterizing flanking single nucleotide polymorphisms, which are found to occur at frequencies of 24% and 52%, respectively, in the general population. Disease haplotypes were different in the 2 families (Fig. 2C), indicating that the families were unrelated and that *TPM1* D230N arose independently in each family.

The *TPM1* D230N variant was present in all affected members in families A and B and absent from 21 of 25 unaffected adult family members as well as >1,000 unrelated Caucasian control chromosomes. The combined calculated LOD score was 5.22. Therefore, we concluded that *TPM1* D230N caused DCM in these 2 unrelated families.

In vitro alpha-tropomyosin functional studies. To investigate the functional impact of *TPM1* D230N, Ca^{2+} regulation of actin-tropomyosin–activated myosin S-1 ATPase was evaluated as an in vitro correlate of contraction. Thin filaments reconstituted with wild-type Ala-Ser-alpha-tropomyosin had a maximally activated rate of ATP turnover of 4.08 ± 0.01 s^{-1}. The concentration of Ca^{2+} at half-maximal ATP-turnover (pCa_{50}) was 6.47 ± 0.02 (n = 5) (Fig. 3A). In contrast, thin filaments reconstituted with D230N Ala-Ser-alpha-tropomyosin produced a lower maximum ATPase turnover rate (2.95 ± 0.11 s^{-1}, n = 3, p < 0.001) and reduced Ca^{2+} sensitivity ($pCa_{50} = 6.20 \pm 0.04$, n = 3, p < 0.05) (Fig. 3A). Filaments reconstituted with a 1:1 mixture of wild-type/D230N Ala-Ser-alpha-tropomyosin (the expected ratio in situ) also showed a depressed maximally activated rate (3.26 ± 0.21 s^{-1},
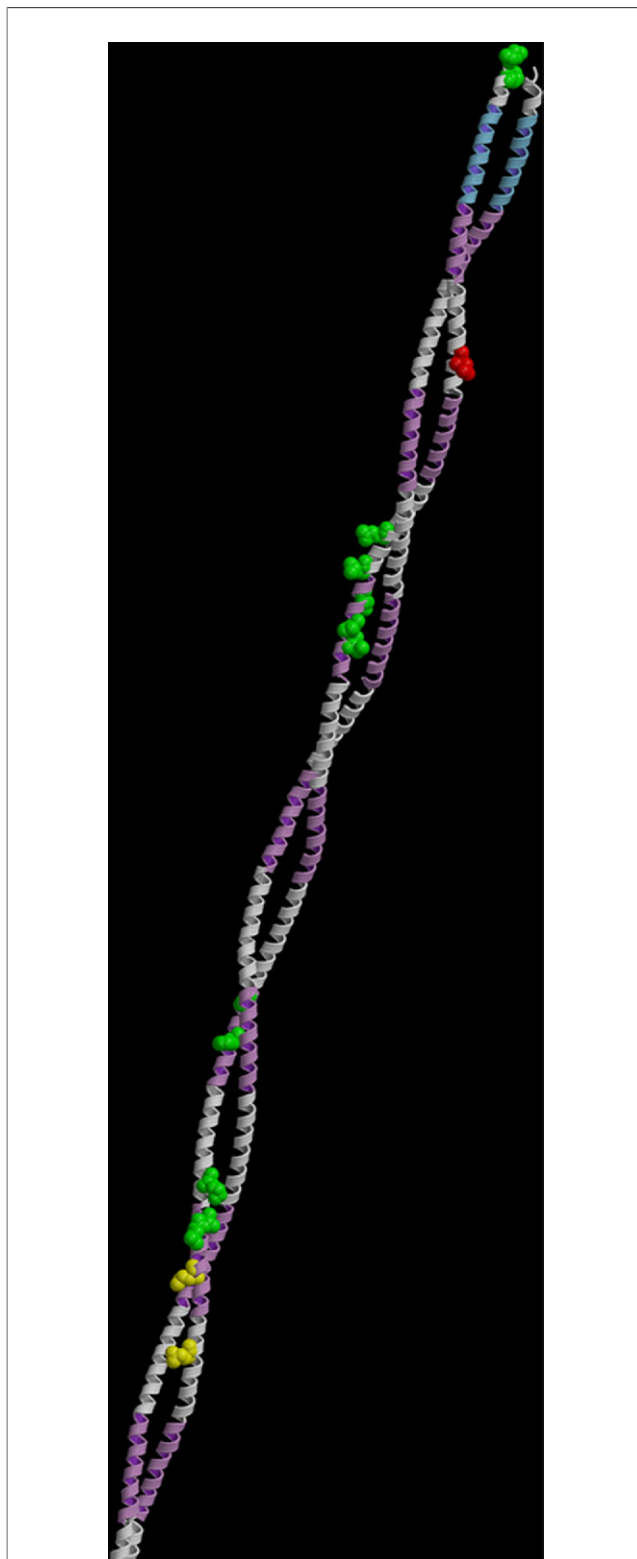

Figure 1   **Molecular Model of Alpha-Tropomyosin**

Alpha-tropomyosin is an alpha-helical coiled-coil that interacts with actin (**violet** functional domains) and troponin T (**blue**). The locations of mutations associated with dilated cardiomyopathy (**yellow**) and hypertrophic cardiomyopathy (**green**) are indicated. The D230N mutation is shown in **red**.
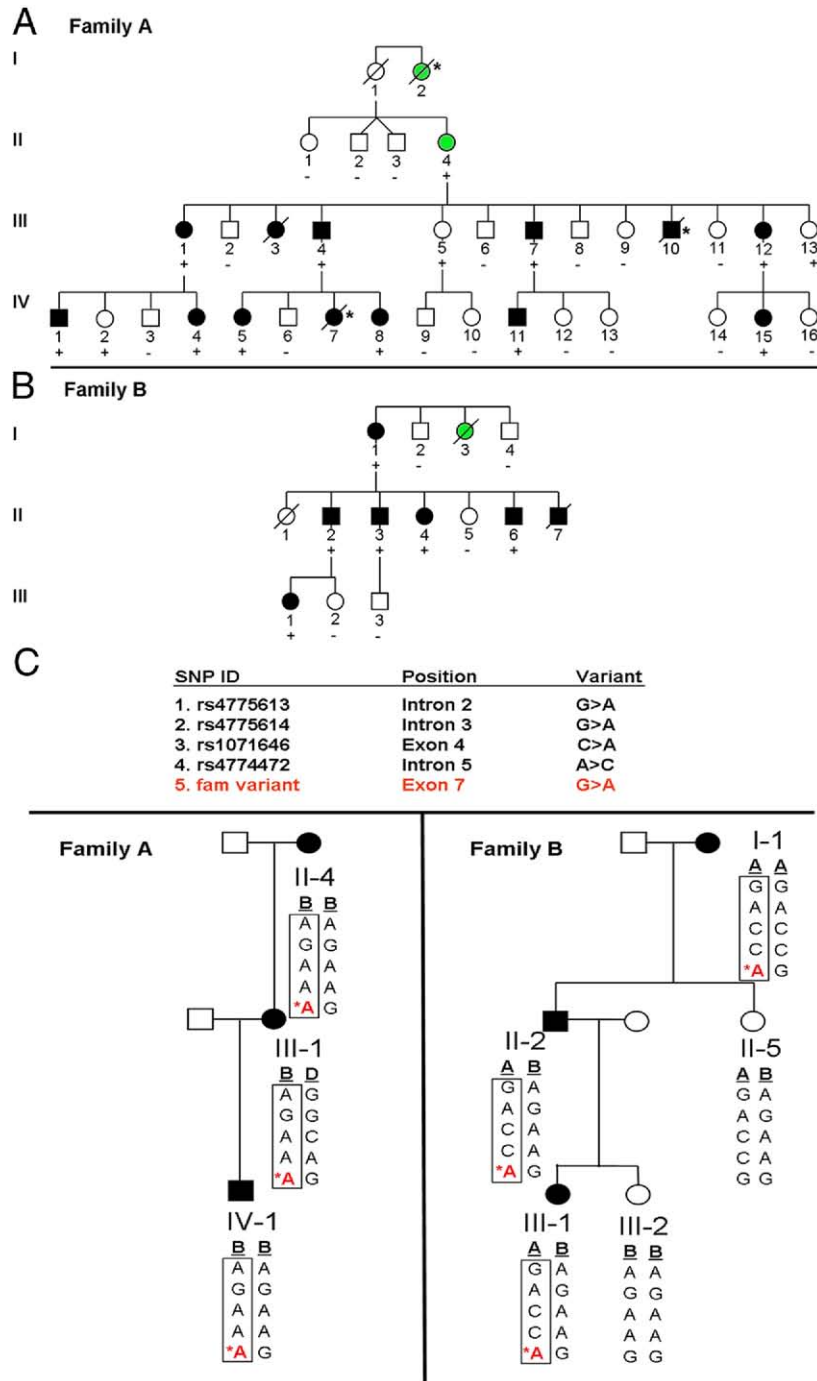
Figure 2   Pedigrees and Haplotype Analysis of Families With Autosomal-Dominant DCM Due to D230N Mutation in Alpha-Tropomyosin

Pedigrees are shown for 2 unrelated families (family A **[A]** and family B **[B]**) with dilated cardiomyopathy (DCM). **Squares** indicate male family members, **circles** indicate female family members, **solid symbols** indicate DCM, **open symbols** unaffected members, **green symbols** indicate uncertain clinical status, and **slashes** indicate deceased members. Genotype results are indicated by (+) = D230N present and (−) = mutation absent. *Sudden cardiac death. **(C)** Haplotype analysis indicates that the families are unrelated and that the D230N (688G>A) *TPM1* mutation arose independently in these families. Several family members from each kindred were genotyped at 4 loci in the *TPM1* gene (rs4775613, rs4775614, rs1071646, rs4774472), comprising 4 common haplotypes in Caucasians (A, B, C, D) as described by Hap-Map. The D230N mutation (fam variant) is shown in **red** with an **asterisk**.
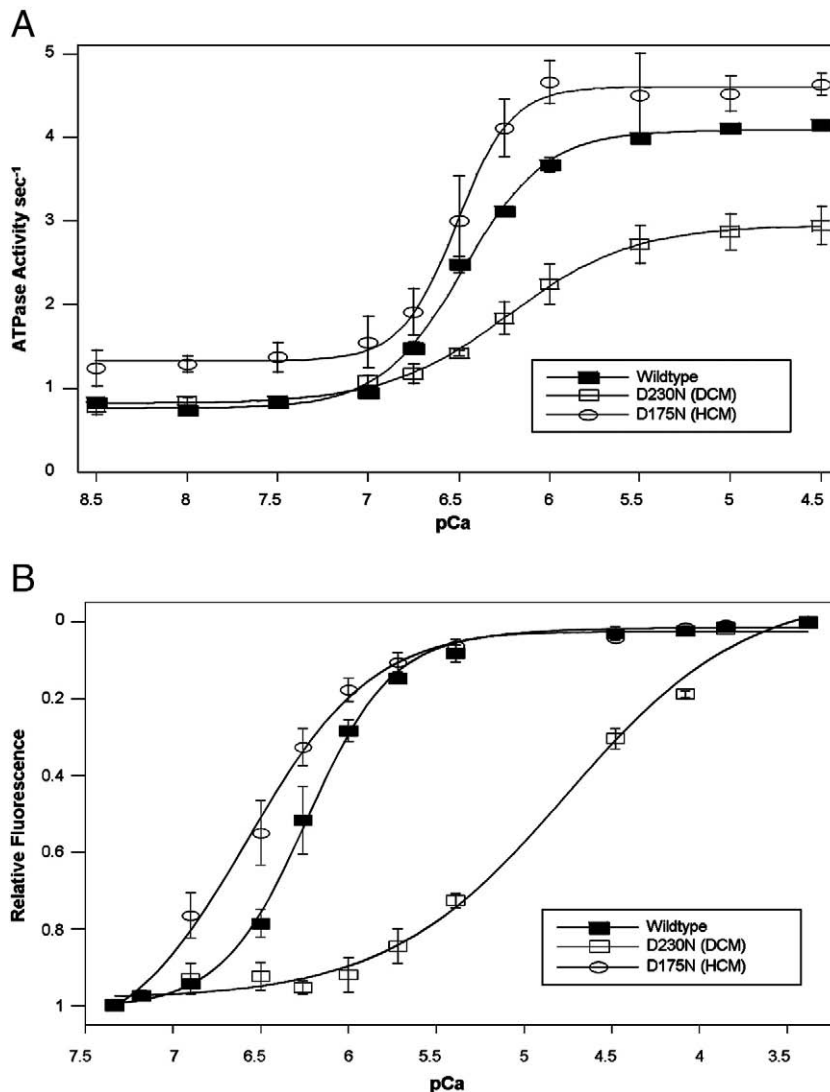
Figure 3   **Functional Properties of the D230N Mutation in Alpha-Tropomyosin**

In vitro functional analyses of the D230N mutant alpha-tropomyosin show that at any activating-level of Ca^{2+}, the D230N mutation resulted in diminished sarcomere function and Ca^{2+} affinity. **(A)** Ca^{2+} sensitivity of thin-filament regulation of actin-tropomyosin–activated myosin ATPase activity. Compared with the wild type, maximal ATPase turnover and pCa_{50} were significantly reduced ($p < 0.05$) in the DCM mutant alpha-tropomyosin (D230N). In contrast, both parameters were significantly increased ($p < 0.05$) in the HCM mutant protein (D175N). **(B)** Ca^{2+} binding to the thin filament causes a decrease in spectrofluorescent intensity. The Ca^{2+} affinity of reconstituted thin filaments containing D230N alpha-tropomyosin DCM mutant was significantly reduced ($p < 0.001$) compared with wild-type alpha-tropomyosin. In contrast the D175N HCM mutant was associated with significantly increased Ca^{2+} affinity ($p < 0.05$). HCM = hypertrophic cardiomyopathy; other abbreviations as in Figure 2.

$p < 0.001$) and Ca^{2+} sensitivity ($pCa_{50} = 6.20 \pm 0.07$, n = 3, $p < 0.05$). The maximally inhibited rate at pCa 8.5 (0.75 ± 0.02 s^{-1}) was not significantly affected by the presence of the mutation.

The functional impact of this *TPM1* D230N mutation is strikingly different than a *TPM1* mutation that causes HCM (D175N) (15). Thin filaments reconstituted with the HCM-mutant protein resulted in regulation with greater Ca^{2+}-sensitivity ($pCa_{50} = 6.58 \pm 0.04$, n = 3, $p < 0.05$) and increased maximum ATP turnover (4.55 ± 0.09 s^{-1}, n = 3, $p < 0.001$) relative to wild-type tropomyosin (Fig. 3A).

To determine whether the observed decrease in Ca^{2+} sensitivity of contractility was due to an actual change in Ca^{2+} affinity rather than an apparent change caused by altered troponin-tropomyosin switching, thin filaments were reconstituted with the use of actin, IAANS-labeled troponin C, and wild-type, D230N, or a 1:1 wild-type/DCM-mutant mixture tropomyosin. Wild-type thin filaments bound Ca^{2+} with a pCa_{50} of 6.24 ± 0.02 with a Hill coefficient (n_H) of 1.74 ± 0.12 (Fig. 3B). In contrast, there was a dramatic decrease in the affinity of filaments containing D230N ($pCa_{50} = 4.76 \pm 0.09$; $p < 0.001$), and the Hill

coefficient was also significantly reduced ($n_H = 0.85 \pm 0.10$; $p < 0.001$) (Fig. 3B), indicating decreased cooperativity of Ca^{2+} binding. Experiments in which we used the 1:1 wild-type/DCM-mutant mixture also showed reduced Ca^{2+} affinity ($pCa_{50} = 5.54 \pm 0.08$) and lower cooperativity ($n_H = 1.04 \pm 0.23$ $p < 0.001$) (data not shown in figure). In contrast, thin filaments reconstituted with the HCM mutant protein were found to have significantly increased Ca^{2+} affinity compared with wild-type tropomyosin ($pCa_{50} = 6.57 \pm 0.09$; $p < 0.05$), consistent with the increased Ca^{2+} sensitivity observed in the ATPase assay (Fig. 3B).

Clinical features of TPM1 D230N. The clinical manifestations and course of DCM in families A and B are summarized in Table 1. No family members with *TPM1* D230N had evidence of cardiac conduction disease, and with one exception, none had evidence of skeletal myopathy. Abnormal cardiac dimensions and contractile parameters were identified in 16 of 20 mutation carriers, indicating a penetrance of 80% for DCM in the 2 families. As seen in Figure 4, the development of DCM occurred over a wide spectrum of ages. However, there were striking differences in clinical course on the basis of age of presentation, with adverse outcomes occurring early in life (Fig. 4).

Family A. Family A was notable for severe HF and sudden death in young children. Subjects I-2, III-10, and IV-7 died suddenly at ages 3 years, 13 months, and 5 months, respectively, without previous evidence of heart disease. Two siblings, IV-5 and IV-8, presented with DCM and advanced HF at 5 months of age (LVEF 27% and 29%) and were given the presumptive diagnosis of myocarditis. In IV-5, adenovirus DNA was detected by polymerase chain reaction on endomyocardial biopsy. Both had substantial recovery of LV systolic function and resolution of symptoms after receiving standard medical care at the time of presentation (digoxin and furosemide). Notably IV-8, now age 21 years, participates in marathons.

Subjects III-3 and IV-1 developed end-stage HF refractory to medical therapy as teenagers. III-3 presented at age 13 with refractory HF, underwent mitral and tricuspid valve replacement for functional regurgitation, and died weeks later of multisystem organ failure before genetic testing. IV-1 was asymptomatic when diagnosed with DCM at 7 years during family screening but developed severe HF at age 17 years and underwent transplant at 18 years of age. Histologic examination of his explanted heart revealed nonspecific changes consistent with DCM, without myocyte disarray, marked fibrosis, or inflammation to suggest either end-stage HCM or myocarditis.

By contrast, 4 mutation carriers who presented in adulthood had mild clinical courses. Subjects III-4, III-7, and III-12 developed mild exercise intolerance in their fifth decade. Clinical studies revealed mild-to-moderate LV systolic dysfunction and enlargement and symptoms resolved with medical management. II-6 was healthy before an inferior myocardial infarction at 71 years. She was

subsequently found to have mild LV systolic dysfunction (LVEF 49%) and remains asymptomatic.

Four mutation carriers (subjects III-1, IV-4, IV-11, IV-15; ages 54, 16, 22, and 8 years, respectively), had asymptomatic LV systolic dysfunction and/or enlargement. Three adult mutation carriers (III-5, III-13, IV-2), were free of symptoms and had normal LV size and systolic function.

Family B. Young members of this family also presented with severe HF. Subject III-1 developed failure to thrive at 10 weeks of age and echocardiography revealed severe LV dilation (LVIDD *z*-score 15.6) and systolic dysfunction (LVEF 15%). Transplantation was considered, but the subject had substantial recovery with basic medical therapy. By age 8 years, she was asymptomatic with only mild LV systolic dysfunction (LVIDD *z*-score 1.3, LVEF 46%). At age 20 years, II-6 underwent cardiac transplantation shortly after presenting with refractory HF symptoms and severe LV systolic dysfunction (LVEF <20%).

Subject II-7 died of HF at age 12 years, before genetic testing. In addition to DCM, he had congenital cataracts and skeletal muscle weakness. Post-mortem examination demonstrated cardiomegaly with marked endocardial and interstitial fibrosis. Skeletal muscle analyses demonstrated chronic myopathic changes but normal dystrophin staining and no inflammation, evidence of storage disease, tissue-specific atrophy, or fiber type grouping.

As in family A, the clinical course of mutation carriers in family B identified in adulthood was far less severe. I-1 had unexplained syncope at age 56 years. Cardiac studies revealed ventricular tachycardia and DCM (LVEF 25%), which improved with medical management. Three other adults (II-2, II-3, and II-4, ages 38, 36, and 33 years, respectively) had asymptomatic LV systolic dysfunction. After an uncomplicated pregnancy, II-4 had a mild further decrease in LVEF from 48% to 39%, improving to 55% with institution of an angiotensin-converting enzyme inhibitor.

Discussion

We identified a D230N missense mutation in *TPM1* as the cause of DCM in 2 unrelated multigenerational families. Unlike previously reported *TPM1* variants (E45K, E40K) associated with DCM in isolated subjects (7), we demonstrate that dominant transmission of *TPM1* D230N segregates with disease and deleteriously impacts in vitro assays of contractility, providing definitive evidence that *TPM1* mutations cause DCM. Furthermore, these functional studies demonstrated that mutations in the same genes have different effects that may influence whether a phenotype of DCM or HCM develops. The clinical profile of these families suggests a pattern for sarcomeric DCM in which clinical outcomes differ dramatically on the basis of age of presentation.

		Age at Diagnosis*	Current Age or Age at Death†	D230N	Sex	Clinical Features	LVEF (%)	LVIDD (cm)/(z-score)	Comments
Family	Pedigree								
NYHA functional class >II									
A	I-2	—	3 yrs†	NA	F	SCD	—	—	No symptoms before SCD. No autopsy performed.
A	III-3	13 yrs	13 yrs†	NA	F	NYHA IV HF death	—	—	Died of multisystem organ failure during HF hospitalization.
A	III-10	13 months	13 months†	NA	M	SCD	—	—	No symptoms before SCD. Autopsy notable for LV dilation.
A	IV-1	7 yrs	26 yrs	+	M	NYHA IV Txp	9	8.6	Diagnosed during family screening and became progressively symptomatic culminating in cardiac transplantation at 18 yrs.
A	IV-5	5 months	28 yrs	+	F	NYHA IV with improvement	27	4.2 (13.2)	Severe HF as an infant with improvement after medical therapy (LVEF now 50%).
A	IV-7	6 months	6 months†	NA	F	SCD	—	—	SCD at age 6 months without previous symptoms. Autopsy reported LV dilation.
A	IV-8	5 months	21 yrs	+	F	NYHA IV with improvement	29	4.5 (15.2)	Severe HF as an infant with improvement after medical therapy (LVEF now 58%).
B	I-1	56 yrs	64 yrs	+	F	VT	25	5.9	Presented with symptomatic VT and severe DCM, which improved with medical therapy (LVEF now 40%).
B	II-6	20 yrs	24 yrs	+	M	NYHA IV Txp	<20	—	Cardiac transplantation at age 20 yrs.
B	II-7	9 yrs	12 yrs†	NA	M	NYHA IV HF death	—	—	Presented with DCM at 9 yrs and died of HF at 12 yrs. Also had congenital cataracts and skeletal weakness.
B	III-1	10 weeks	8 yrs	+	F	NYHA IV	15	4.8 (15.6)	Severe HF as an infant with improvement after medical therapy (LVEF now 46%).
NYHA functional class ≤II									
A	II-4	—	71 yrs	+	F	NYHA I	49	4.6	Asymptomatic. Mildly reduced LVEF identified after myocardial infarction at 71 yrs.
A	III-1	54 yrs	57 yrs	+	F	NYHA I	40	5.5	Asymptomatic but LVEF decreased during follow-up from 52% to 40%.
A	III-4	47 yrs	53 yrs	+	M	NYHA II	50	6.4	Minimal exercise intolerance developed at age 48 yrs.
A	III-5	—	53 yrs	+	F	NYHA I	56	4.8	Asymptomatic without evidence of LV enlargement or dysfunction.
A	III-7	45 yrs	50 yrs	+	M	NYHA II	50	5.7	Minimal exercise intolerance developed at age 47 yrs.
A	III-12	40 yrs	44 yrs	+	F	NYHA II	50	5.6	Palpitations and mild LV enlargement/systolic dysfunction without exercise limitation.
A	III-13	—	42 yrs	+	F	NYHA I	55	4.7	Asymptomatic without evidence of LV enlargement or dysfunction.
A	IV-2	—	23 yrs	+	F	NYHA I	59	4.8	Asymptomatic without evidence of LV enlargement or dysfunction.
A	IV-4	16 yrs	19 yrs	+	F	NYHA I	57	5.6	Asymptomatic with mild LV enlargement.
A	IV-11	22 yrs	24 yrs	+	M	NYHA I	40	5.5	Asymptomatic but LVEF decreased during follow-up from 50% to 40%.
A	IV-15	8 yrs	11 yrs	+	F	NYHA I	51	4.2	Asymptomatic with mildly reduced LVEF.
B	II-2	30 yrs	38 yrs	+	M	NYHA I	45	5.1	Asymptomatic with mildly reduced LVEF.
B	II-3	32 yrs	36 yrs	+	M	NYHA I	35	6.4	Asymptomatic but moderately reduced LVEF that decreased during follow-up from 42% to 35% with concomitant 5-mm increase in LVIDD.
B	II-4	31 yrs	34 yrs	+	F	NYHA I	39	5.6	Asymptomatic but LVEF decreased after pregnancy from 48% to 39%, normalizing with medical therapy (55%).

*No age provided for subjects without evidence of DCM. †Age at death.

DCM = dilated cardiomyopathy; HF = heart failure; LV = left ventricular/ventricle; LVEF = left ventricular ejection fraction; LVIDD = left ventricular end-diastolic diameter z-score calculated for children age <16 yrs; NA = not available; NYHA = New York Heart Association functional class; SCD = sudden cardiac death; Txp = cardiac transplantation; VT = ventricular tachycardia.

Divergent functional consequences of sarcomere mutations may shape different patterns of cardiac remodeling. Sarcomere mutations were initially characterized as the cause of HCM but, as demonstrated in this study, can also cause DCM (6,16). The molecular mechanisms that determine whether a dilated or hypertrophic phenotype
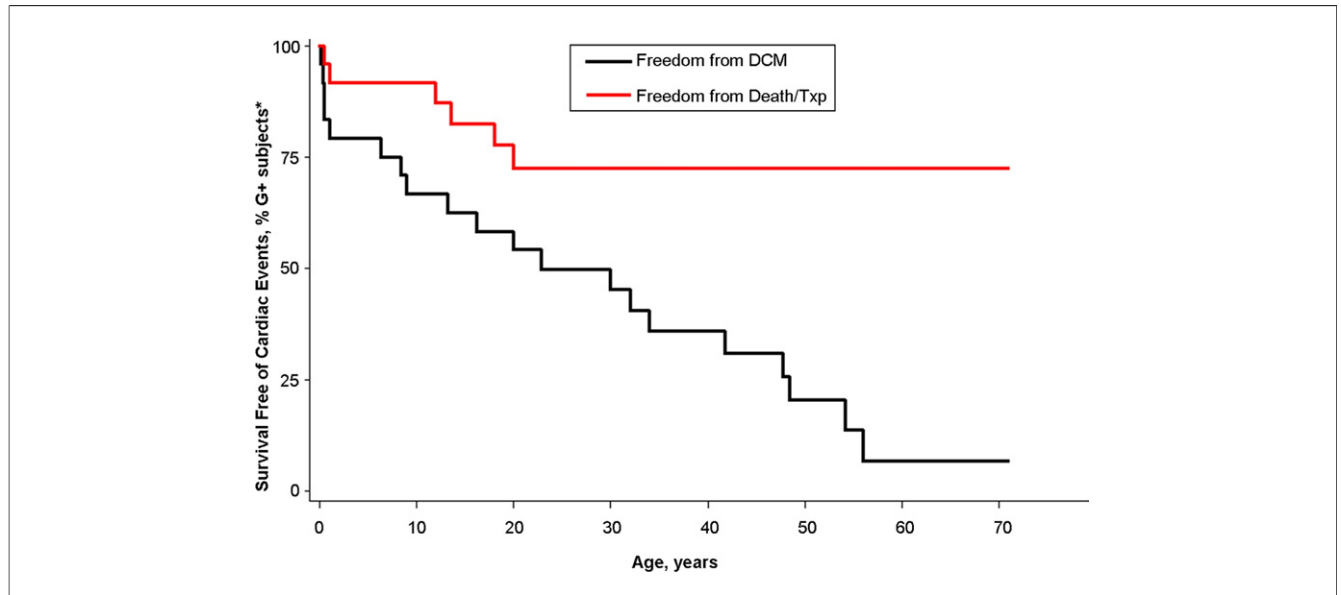
Figure 4   **Clinical Outcomes in Carriers of the D230N Alpha-Tropomyosin Mutation**

The presence of *TPM1* D230N is associated with a high risk for developing DCM but over a wide spectrum of ages, ranging from infancy to the sixth decade **(black curve)**. DCM includes both symptomatic and asymptomatic left ventricular systolic dilation and/or dysfunction. Severe clinical outcomes, cardiac death, and transplantation (Txp) were confined to young family members and distinctly absent from those presenting in adulthood **(red curve)**. *Subjects who experienced cardiac death before genotyping were assumed to be mutation carriers (G^+). Abbreviations as in Figure 2.

develops have not been clearly elucidated. Mutation location does not appear to be critical. As shown in Figure 1, mutations that cause HCM and DCM are closely interspersed and in the same functional domains in tropomyosin. However, the in vitro functional consequences of these mutations differ. Consistent with previous reports evaluating DCM mutations in thin filament proteins (12), our results demonstrate that D230N alters Ca^{2+} regulation in tropomyosin by reducing Ca^{2+} sensitivity, maximum activation, and Ca^{2+} affinity. Experimental models in which the investigators used mechanically loaded cardiac muscle fibers (17) have also shown a Ca^{2+}-desensitizing effect of thin filament mutations. Collectively, these DCM mutations are predicted to produce a muscle intrinsically capable of producing less force at any activating Ca^{2+} concentration. Left ventricular dilation may represent a compensatory mechanism to maintain stroke volume in the setting of reduced contractility as the result of decreased force production and/or Ca^{2+} affinity associated with the *TPM1* mutation. Activation of the neurohormonal axis may also occur, culminating in progressive cardiac failure.

Notably, these functional changes are opposite to those observed for sarcomere mutations that ultimately give rise to HCM in which Ca^{2+} sensitivity is enhanced and predicted to increase contractility (12). Figure 3 illustrates this contrast. Maximum ATPase activity, Ca^{2+} sensitivity, and Ca^{2+} affinity are decreased with the D230N DCM *TPM1* mutation but increased with the D175N HCM *TPM1* mutation. Moreover, previous

biophysical studies in which the investigators examined the thick filament demonstrated a similar pattern. Force generation and ATPase activity are decreased in myosin heavy-chain mutations associated with DCM but increased in HCM-associated mutations (18). Collectively these results suggest that fundamental differences in the functional consequences of sarcomere mutations may underlie the very disparate patterns of remodeling observed in DCM and HCM, despite an apparently common genetic etiology. We postulate that sarcomere mutations that compromise force generation may lead to a dilated phenotype, whereas a hypertrophic phenotype may arise from mutations that increase force generation.

A distinctive age-dependent phenotypic profile of sarcomere mutation DCM. Consistent with previous descriptions of DCM caused by sarcomere mutations, the clinical profiles of our families differ from both HCM caused by sarcomere mutations and DCM of other genetic etiologies, neither of which are characterized by severe disease in early childhood (19,20). As illustrated in Figure 4, marked age-dependent differences in outcomes were observed in our 2 families with *TPM1* mutations. Presentation early in life, from infancy to adolescence, was not uncommon and was associated with severe, sometimes lethal outcomes, including sudden cardiac death and refractory HF leading to death or transplantation. In contrast, a mild course was observed in relatives diagnosed as adults.

The pattern of severe childhood but mild adult-onset disease seen with the *TPM1* D230N mutation is similar to that reported with 2 other sarcomere genes, *MYH7* and

TNNT2 (6,16,21). Affected members of these families also demonstrated marked LV dysfunction and HF very early in life, with either striking recovery or progression to death or transplantation, whereas those identified in adulthood generally had mild disease. This clinical profile differs meaningfully from that observed in other genetic causes of DCM, such as that caused by lamin A/C or phospholamban mutations where manifestations typically do not develop until adulthood and are progressive (19,22). The mechanisms underlying the different clinical course in children and adults have not been defined; however, recognizing this pattern as a feature of sarcomere mutation DCM is important for appropriate family evaluation and intervention.

Moreover, although early presentation with DCM was typically severe, there was potential for remarkable improvement because 3 of 5 affected infants with the *TPM1* mutation had striking recovery of LV function. We speculate that the underlying *TPM1* mutation may confer susceptibility to myocardial injury due to viral infections (e.g., myocarditis) or systemic illness that could account for the initially dramatic clinical presentations. Factors leading to the marked improvement in a subset of these children are less clear and unlikely to be related solely to receiving basic medical therapy. However, further elucidation of the mechanisms that allowed recovery in this primary genetic cardiomyopathy may provide important insights regarding the pathogenesis and management of more common secondary forms of DCM and HF.

Conclusions and Clinical Implications

We present robust evidence that mutations in *TPM1* cause DCM and further characterization of disease pathogenesis and clinical course. In vitro functional studies provide insight into the phenotypic development of cardiomyopathy. Although sarcomere mutations are a common cause of both HCM and DCM, the functional consequences appear markedly different in these 2 diseases, with opposite effects on Ca^{2+} affinity, sensitivity, and contractility. These fundamental differences may play an important role in shaping the type of cardiac remodeling that arises.

The pattern emerging in these and other DCM families suggests that sarcomere mutations may result in a distinctive, age-dependent clinical profile. Early presentation is associated with severe, sometimes-lethal disease, although with potential for substantial recovery. In contrast, presentation in adulthood is generally benign. These observations have several important clinical implications. In pediatric-onset HF, inherited causes of cardiomyopathy often are overlooked in favor of a presumptive diagnosis of myocarditis (23), particularly if there is dramatic clinical improvement. However, in these 2 families, childhood disease was caused by the *TPM1* mutation. This finding highlights the importance of considering genetic etiologies in new-onset DCM in children and the need to consider at-risk family members. Furthermore, these findings have relevance for screening families with DCM. Current guidelines for family screening in HCM recommend that formal evaluation typically begins in adolescence (24). However, the severe disease manifestations observed in young children with DCM suggest that the identification of a sarcomere mutation should prompt aggressive screening for DCM in all first degree relatives, beginning early in life.

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▶ **APPENDIX**