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Severe Disease Expression of Cardiac Troponin C and T Mutations in Patients With Idiopathic Dilated Cardiomyopathy

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OBJECTIVES We performed genetic investigations of cardiac troponin T (*TNNT2*) and troponin C (*TNNC1*) in 235 consecutive patients with idiopathic dilated cardiomyopathy (DCM) to evaluate prevalence of mutations and associated disease expression in affected families.

BACKGROUND Recently, mutations in sarcomeric genes have been reported in DCM. However, the prevalence, penetrance, and clinical significance of sarcomere gene mutations in large consecutive cohorts of DCM patients are poorly defined.

METHODS Mutation detection was performed by fluorescent SSCP/DHPLC analysis and direct sequencing. The functional effects of mutations on interactions within the troponin complex were assessed by a two-hybrid luciferase assay.

RESULTS A total of 43% (102 of 235) of the study cohort had familial DCM. One *TNNC1* and four *TNNT2* (three novel) mutations were identified in one and four families, respectively. The prevalence of *TNNC1*/*TNNT2* mutations in familial DCM was 5% with a penetrance of 100%. A total of 21 mutation carriers were identified; 6 underwent cardiac transplantation, 5 died of heart failure, and 4 died suddenly at a mean age of 29 years, while 6 remained stable on medication. Functional studies showed significant impairment of mutated troponin interaction compared with wild-type control, indicating an altered regulation of myocardial contractility.

CONCLUSIONS Cardiac troponin C was identified as a novel DCM gene. The disease expression associated with *TNNC1* and *TNNT2* mutations was severe with complete penetrance. The data suggest that mutation analysis of the troponin complex in DCM patients may prove valuable in early identification of individuals with an adverse prognosis and a high risk of premature death. This may lead to improved management and survival. (J Am Coll Cardiol 2004;44: 2033–40) © 2004 by the American College of Cardiology Foundation

Idiopathic dilated cardiomyopathy (DCM) is the most common cause of heart failure and cardiac transplantation in the young. The condition is characterized by unexplained left ventricle dilation, impaired systolic function, and nonspecific histologic abnormalities dominated by myocardial fibrosis. Patients may experience severe disease complications including arrhythmia, thromboembolic events, and sudden death. Recent studies have suggested familial disease in 30% to 50% of cases. Autosomal dominant transmission is most frequent followed by recessive, X-linked, and mitochondrial inheritance. Most affected families present with a "pure" cardiac phenotype while syndromic features are less frequent. Disease-causing mutations have been reported in genes encoding cytoskeletal and nuclear envelope proteins and, more recently, in sarcomeric contractile protein genes (1–3). At present, 10 different mutations in 12 families with autosomal dominant DCM have been identified in the sarcomeric genes for alpha-cardiac actin (*ACTC*), alpha-tropomyosin (*TPM1*), cardiac troponin T (*TNNT2*), beta-myosin heavy chain (*MYH7*), and titin (*TTN*) (4–11). In addition, we recently reported the first recessive mutation in cardiac troponin I (*TNNI3*) in a small DCM family with early and severe disease expression (10).

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The fact that DCM mutations have been identified in *TNNT2* and *TNNI3* led us to investigate whether mutations in the remaining part of the troponin complex (cardiac troponin C [*TNNC1*]) were present in DCM. This study examines the relationship between genotype and clinical phenotype in families with mutations in *TNNC1* and *TNNT2* identified by mutation analysis of 235 consecutive DCM patients and their relatives. In addition, we report the effect of *TNNC1* and *TNNT2* mutations identified on inter-troponin interactions by the use of a qualitative mammalian two-hybrid luciferase assay.

METHODS

Clinical investigations. The local research ethics committee approved the study, and informed consent was obtained from all participants.

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Abbreviations and Acronyms

DCM = dilated cardiomyopathy
DHPLC = denaturing high performance liquid chromatography
F-SSCP = fluorescent SSCP
LVEDD = left ventricular end-diastolic dimension
LVESD = left ventricular end-systolic dimension
TNNC1 = cardiac troponin C
TNNI3 = cardiac troponin I
TNNT2 = cardiac troponin T

The study cohort consisted of 235 consecutive and unrelated DCM probands who were evaluated in a dedicated cardiomyopathy clinic from 1995 to 2002. Relatives of DCM probands in whom a *TNNT2* or *TNNC1* mutation was identified were invited for clinical assessment. All participants underwent physical examination, 12-lead electrocardiogram, transthoracic two-dimensional echocardiography, and Doppler studies. Cardiac catheterization was performed in patients aged >40 years, and in those individuals with symptoms of ischemic heart disease or exercise-induced ST-segment changes consistent with myocardial ischemia. Familial disease was defined where one or more relatives had DCM identified by clinical investigation or had a family history of unexplained premature cardiac death <40 years.

Echocardiography. Standard measurements of left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) were performed, and fractional shortening was calculated as (LVEDD − LVESD/LVEDD) × 100. Dimensions were corrected for age and body surface area (BSA) according to the formula of Henry [(LVEDD)(percent predicted) = measured LVEDD/predicted LVEDD × 100; predicted LVEDD = $[45.3 \times BSA^{0.3}] - [0.03 \times age] - 7.2$ (12).

Dilated cardiomyopathy was diagnosed in accordance with World Health Organization diagnostic criteria when echocardiography identified unexplained left ventricle dilation and impaired contractile performance: left ventricular end diastolic diameter >117% predicted for age and body surface area and a fractional shortening <25%. Relatives of DCM probands were considered to be affected if they had unexplained left ventricle enlargement >112% of predicted value or experienced unexplained heart failure or sudden cardiac death at <40 years of age (1,13). Patients with symptoms or signs of skeletal muscle disease were excluded from the study.

Mutation analysis. Genomic deoxyribonucleic acid was obtained, and protein encoding exons of *TNNT2* and *TNNC1* were amplified using standard protocols (primer sequences and conditions for polymerase chain reaction amplification available upon request). Mutation analysis of *TNNT2* was performed by fluorescent SSCP (F-SSCP) and direct sequencing of abnormal conformers. As reported previously, the sensitivity of F-SSCP to identify sequence variations in *TNNT2* was 100% (14); *TNNC1* was investigated by denaturing high performance liquid chromatography (DHPLC) analysis and abnormal elution profiles subjected to direct sequencing. Initially, 50 patients were investigated for mutations in *TNNC1* by direct sequencing without identifying any sequence variations. Subsequent DHPLC analysis of the remaining patients identified one

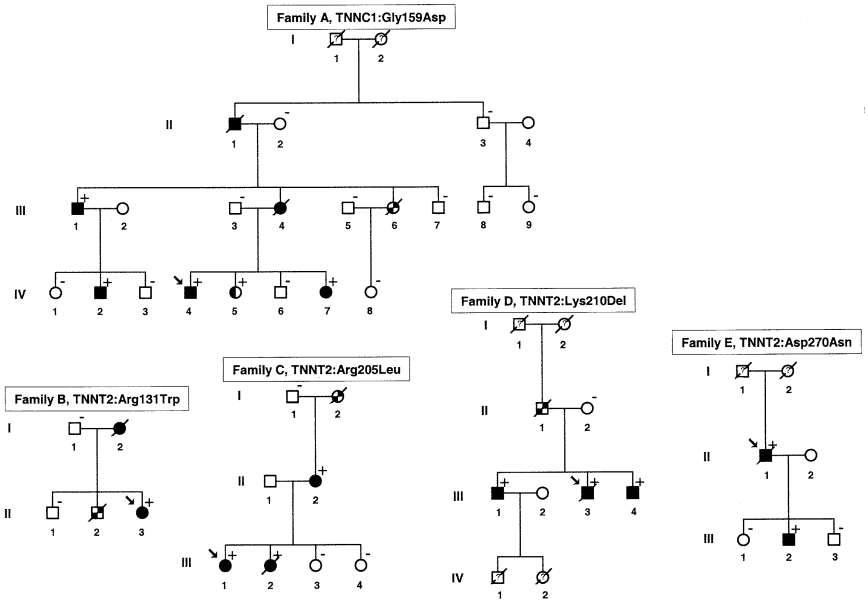

Figure 1. Pedigree drawings of dilated cardiomyopathy (DCM) families affected by cardiac troponin C and T mutations. All individuals had normal left ventricular wall thickness by echocardiography, and no patients had atrioventricluar block or impaired skeletal muscle function. **Squares** = male family members; **circles** = female family members; **symbols with slash** = deceased individuals; **open symbols** = unaffected individuals; **solid symbols** = individuals affected by DCM; **half-solid symbols** = individual with left ventricle enlargement; **checkered symbols** = individuals who died suddenly; **question marks** = unknown clinical status; **plus signs** = presence of mutation; **minus signs** = absence of mutation.

Table 1. Clinical Characteristics of DCM Families With *TNNC1* or *TNNT2* Mutations

ID	Age/Gender	NYHA	Echocardiography		Diagnosis and Outcome
			LVEDD	FS%	
Family A, *TNNC1*: *Gly159Arg*					
IV-4*	21/M	4	NA	NA	DCM, HTx age 22
IV-5‡	36/F	1	54	37	LVE, current age 37
IV-7	20/F	2	57	21	DCM, current age 22
III-4*	45/F	4	NA	NA	DCM, heart failure death age 45
III-1*	52/M	4	81	8	DCM, HTx age 52
IV-2*	22/M	4	83	11	DCM, HTx age 22
III-6	21/F	NA	NA	NA	Sudden death age 21
II-1	62/M	3	NA	NA	Heart failure death age 62
Family B, *TNNT2*: *Arg131Trp*					
II-3	23/F	3	83	10	DCM, current age 28
II-2	16/M	1	NA	NA	Sudden death age 16
I-2	34/F	3	59	15	DCM, heart failure death age 34
Family C, *TNNT2*: *Arg205Leu*					
III-1*	16/F	3	68	6	DCM, HTx age 16
III-2§	20/F	4	NA	NA	DCM, heart failure death age 20
II-2	45/F	2	50	21	DCM, current age 48
I-2	24/F	1	NA	NA	Sudden death age 24
Family D, *TNNT2*: *Lys210del*					
III-3†	25/M	3	74	14	DCM, heart failure death age 26
III-4*	21/M	4	NA	NA	DCM, HTx age 22
III-1	35/M	1	49	24	DCM, current age 36
II-1	36/M	1	NA	NA	Sudden death age 36
Family E, *TNNT2*: *Asp270Asn*					
II-1†	38/M	4	83	10	DCM, HTx age 38, died age 44
III-2	19/M	1	53	21	DCM, current age 21

*Autopsy of diseased individuals and examination of explanted hearts showed chamber dilation with cardiac weights ranging from 450–540 g; †Histology of biopsies obtained antemortem were consistent with DCM; ‡Individual IV-5 was assumed to be affected because she had LV enlargement (115% of predicted value) and an abnormal electrocardiogram with T-wave inversion in V1–V2 and left-axis deviation; §Died of heart failure despite implantation of LV assist device.

DCM = dilated cardiomyopathy; FS = fractional shortening; HTx = cardiac transplant; LVE = left ventricle enlargement; LVEDD = left ventricle end-diastolic dimension; NA = not available; NYHA = New York Heart Association.

sequence variation indicating a high sensitivity of this investigation as previously reported (14). When a mutation was identified, a second blood sample from the affected patient was reanalyzed to confirm the initial finding by direct sequencing, F-SSCP, or DHPLC analysis.

Functional studies. To delineate the effect of *TNNC1* and *TNNT2* mutations identified on inter-troponin interactions, we used a qualitative mammalian two-hybrid luciferase assay. The *TNNT2* cDNA was cloned into the pBIND plasmid (Promega, Madison, Wisconsin) and the *TNNI3* and *TNNC1* cDNA were cloned into the pACT plasmid (Promega). The cloning result was confirmed by direct sequencing and compared with accession numbers X90780 (*TNNI3*), AY044273 (*TNNT2*), and M37984 (*TNNC1*) (15). Mutations identified in *TNNT2* (R131W, R205L, δK210, D270N, and a recognized polymorphism, K253R) and *TNNC1* (G159D) were introduced into pBIND-*TNNT2* and pACT-*TNNC1*, respectively, by oligonucleotide-mediated site-directed mutagenesis. HEK293 cells were cotransfected with equimolar amounts of pBIND-*TNNT2* (wild-type [wt] or mutant), pACT-*TNNI3*, or pACT-*TNNC1* (wild-type or mutant) and the pG5Luc reporter plasmid. After 48 h, cell lysates were assayed for *Photinus* and *Renilla* luciferase using the Promega dual-luciferase assay system. Data was normalized against *Renilla* luciferase activity to account for differences in transfection efficiency. The values for the interaction between wild-type protein troponin T (cTnT), I (cTnI), and C (cTnC) were arbitrarily set to 100%. The values for interactions between mutant cTnT and cTnC or cTnI were expressed relative to this. All experiments were repeated 10 times. The values for luciferase activity resulting from interaction of mutated cTnT–wild-type cTnI and mutated cTnT–wild-type cTnC were compared with luciferase activities obtained from interaction of wild-type cTnT–wild-type cTnI and wild-type cTnT–wild-type cTnC by use of a

Table 2. Conservation of Cardiac Troponin T (cTnT) Amino Acid Residues Affected by Mutations

Protein Encoding Exon	Exon 10					Exon 13										Exon 15				
Amino Acid Position	131					205					210					270				
Human cTnT	E	N	**R**	K	K	T	E	**R**	E	K	K	K	**K**	I	L	I	N	**D**	N	Q
Family B	.	.	**W**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Family C	.	.	.	.	.	.	.	**L**	.	.	.	.	.	.	.	.	.	.	.	.
Family D	.	.	.	.	.	.	.	.	.	.	.	.	**del**	.	.	.	.	.	.	.
Family E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**N**	.	.
Bovine cTnT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rat cTnT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mouse cTnT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rabbit cTnT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chicken cTnT	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	V	S	.	H	.
Zebrafish cTnT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	F	V	S	.	H	.
Ovine cTnT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.
Ascidian cTnT	.	S	.	.	Q	.	.	.	.	.	.	.	.	L	.	V	N	E	Y	M

Period indicates amino acid identity. Accession numbers for rat, mouse, chicken, rabbit, bovine, ovine, zebrafish, and ascidin troponin T are: NM_012676, NM_011619, M10013, A25345, AF175558, P50751, NP_690853, D50867, respectively (15).

t test. The values for luciferase activity were distributed in accordance with a normal distribution, and an F-test showed homogeneous distribution of variances (data not shown). Likewise, values for luciferase activity resulting from interaction of mutated cTnC–wild-type cTnI and mutated cTnC–wild-type cTnT were compared with luciferase activities obtained from interaction of wild-type cTnC–wild-type cTnI and wild-type cTnC–wild-type cTnT by use of same *t* test and conditions. Control experiments consisting of cotransfections of pBIND, pG5Luc, and pACT-*TNNC1* or pACT-*TNNI3* (wild type and mutants) as well as pBIND-*TNNT2* (wild-type and mutants), pG5Luc, and pACT were performed without observing any interaction (data not shown).

RESULTS

Characteristics of the study cohort. A total of 43% (102 of 235) of the study cohort had one or more relatives with DCM identified by clinical investigation or a family history of premature cardiac death at <40 years of age. A total of 85% of these families (87 of 102) exhibited an autosomal dominant mode of inheritance, whereas the remaining 15% (15 of 102) transmitted the condition consistent with autosomal recessive inheritance, although autosomal dominant inheritance with incomplete penetrance could not be excluded. Inheritance patterns suggestive of maternal or X-linked inheritance were not observed.

Clinical characteristics of genotype-positive families. Mutation analysis of *TNNC1* in family A identified a missense mutation that was predicted to result in a G159D amino acid substitution (Fig. 1, Table 1). The mutation was present in all affected individuals and absent in 200 chromosomes from ethnically matched control individuals. The proband, IV-4, had a sudden onset of heart failure symptoms at the age of 21 years. His cardiac function deteriorated rapidly, and he underwent cardiac transplantation two months after initial presentation. Two additional individuals, III-1 and IV-2, received cardiac transplants at the age of 52 and 22 years; III-1 had been evaluated at the age of 50 years at the time of his son's transplant of which he had normal systolic function. However, two years later he developed dyspnea and edema of the lower extremities due to heart failure and required transplantation two months after onset of symptoms. The mother of the proband, III-4, who was an obligate mutation carrier, died at the age of 45 years awaiting cardiac transplant, while II-1 died of heart failure (age 62 years), after eight months of medical treatment. Postmortem examination showed dilation of all cardiac chambers, and a heart weight of 540 g; III-6 experienced unexplained sudden death at the age of 21 years; IV-7 was diagnosed with DCM at the age of 17 years and has been stabilized on angiotensin-converting enzyme inhibitors for the past year, whereas IV-5, age 36 years, has left ventricle enlargement on echocardiography and electrocardiographic abnormalities including T-wave inversion in V1 to V2 and left-axis deviation.

Mutation analysis of *TNNT2* identified four different mutations in four families of which three were novel (family B, R131W; family C, R205L; family E, D270N) and one has previously been reported (family D, δK210) (Fig. 1, Table 1) (7,9). The mutations identified segregated with the disease in each family and were absent in 200 chromosomes from ethnically matched control individuals and 1,520 chromosomes from patients with hypertrophic cardiomyopathy. The disease expression in the four families with *TNNT2* mutations was similar to family A in severity. Three individuals (family C, III-1; family D, III-4; family E, II-1) received cardiac transplants at the age of 16, 22, and 38 years, respectively. Three individuals died of heart failure (family B, I-2; family C, III-2; family D, III-3), three died suddenly (family B, II-2; family C, I-2; family D, II-1), whereas four have remained stable on conventional heart failure therapy.

In this study, 21 individuals were identified with mutations in *TNNC1* (n = 8) and *TNNT2* (n = 13) (Fig. 1, Table 1). Five died of heart failure, and six received a cardiac

Table 3. Conservation of Cardiac Troponin C (cTnC) Amino Acids Affected by Mutation

Protein Encoding Exon					
Amino acid position			**159**		
Human cTnC	M	K	**G**	V	E
Family A	.	.	**D**	.	.
Bovine cTnC	.	.	.	.	.
Mouse cTnC	.	.	.	.	.
Chicken cTnC	.	.	.	.	.
Quail cTnC	.	.	.	.	.
Xenopus cTnC	.	.	.	.	.
Zebrafish cTnC	.	.	.	.	.
Pufferfish cTnC	.	.	.	.	.
Lamprey cTnC	.	.	.	.	D

Accession numbers for mouse, bovine, chicken, quail, xenopus, zebrafish, pufferfish, and lamprey troponin C are: M29793, P02590, D13037, M29722, AB003080, AF434188, AF434190, AB00856, respectively (15).

transplant (average age: 33 years, range: 20 to 62 years) <6 months after initial diagnosis (range: 2 to 8 months). Four experienced sudden unexplained death (average age: 24 years, range: 16 to 36 years). In total, 71% (15 of 21) experienced premature cardiac death or underwent cardiac transplantation. Six mutation carriers are currently alive (five with DCM and one with left ventricular enlargement) and have remained stable (New York Heart Association functional class I) on heart failure therapy during an average observation period of 30 months (range: 20 to 150 months) (Table 1). The average number of premature cardiac deaths per family with troponin disease (2.6 deaths/family) was significantly higher compared with the death ratio observed in the remaining families of the cohort with hereditary DCM (0.7 deaths/family) ($p < 0.001$, Fisher exact test). The penetrance of mutations in both *TNNC1* and *TNNT2* was 100%.

Histology. Myocardial tissue from eight patients was available for histologic evaluation (two biopsies obtained antemortem [D: III-3; E: II-1], two hearts from autopsies [A: III-4; C: II-2], and four explanted hearts [A: III-1, IV-2, IV-4; C: III-1]). All specimens showed varying degrees of nonspecific abnormalities including myocyte hypertrophy, increased interstitial fibrosis, and endocardial thickening with smooth muscle cells characteristic of DCM (16). There was no significant myocyte disarray characteristic of hypertrophic cardiomyopathy or features suggesting storage disease in any of the specimens evaluated (17,18).

Functional studies of troponin C and T mutations. The mutations identified were all localized in conserved and functionally important domains of the proteins involved in the interaction with other troponin subunits and/or tropomyosin (Tables 2 and 3, Fig. 2) (19–23). The effect of the mutations on cTnI, cTnT, and cTnC protein interactions was qualitatively assessed by a mammalian two-hybrid assay (Fig. 3). There was a significant impairment of mutated $cTnT_{R131W,\ R205L,\ \delta K210,\ D270N}$–$cTnI_{wt}$ and $cTnT_{R131W,\ R205L,\ \delta K210,\ D270N}$–$cTnC_{wt}$ protein interactions compared with wild-type controls (*t* test: $p < 0.001$). Likewise, a significant impairment of mutated $cTnC_{G159D}$–$cTnT_{wt}$ protein interaction was observed, whereas the interaction between $cTnC_{G159D}$–$cTnI_{wt}$ was significantly enhanced (p values for both experiments <0.001). No significant change was observed after similar inter-troponin protein interaction studies of a recognized cTnT amino acid polymorphism (K253R), which appeared with a frequency of 2.3% in controls (Fig. 3).

All mutations identified were believed to be disease-causing because: 1) they co-segregated with disease in each of the five families; 2) no sequence variations were identified in 200 ethnically matched control chromosomes investigated in *TNNT2* and *TNNC1* or 1,520 chromosomes from hypertrophic cardiomyopathy patients investigated in *TNNT2*; 3) the mutations were localized in conserved and functionally important regions of the genes; and 4) functional studies of the mutated proteins showed altered troponin protein-protein interactions.

DISCUSSION

Clinical and genetic investigations. Cardiac troponin C was identified as a novel disease gene in DCM. In addition, four disease-causing mutations were found in *TNNT2*, whereas our previous investigation of the same cohort of patients for autosomal dominant mutations in *TNNI3* was negative (10). The overall frequency of mutations in the troponin complex in familial DCM was 6% (6 of 102) including a previously reported recessive *TNNI3* mutation (10). The disease expression appeared malignant because 71% (15 of 21) of mutation carriers experienced premature cardiac death or received a cardiac transplant mostly by the fourth decade with an average duration of six months from

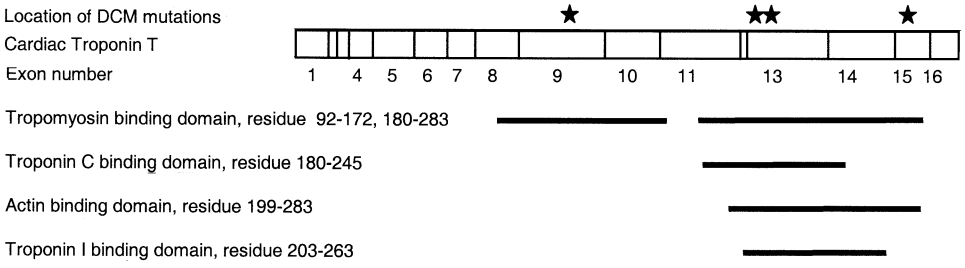

Figure 2. Schematic representation of the human cardiac troponin T gene showing interaction sites with other thin filament sarcomeric contractile proteins (18–22). The **stars** indicate mutations identified in patients with dilated cardiomyopathy (DCM), R131W in family B, R205L in family C, δK210 in family D, and D270N in family E.

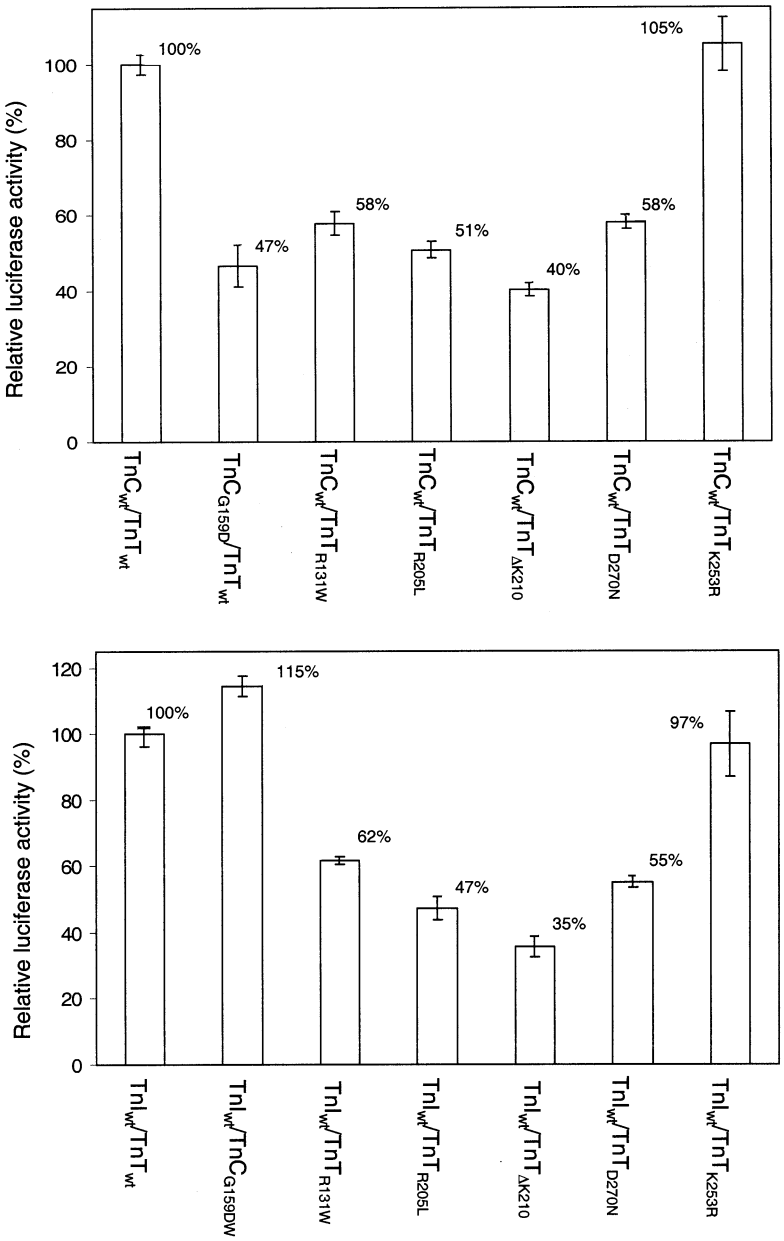

Figure 3. Effect of troponin C ($cTnC_{G159D}$) and troponin T ($cTnT_{R131W}$, $cTnT_{R205L}$, $cTnT_{\delta K210}$, $cTnT_{D270N}$) mutations on inter-troponin interactions assessed by a mammalian two-hybrid assay. All mutations impaired cTnT-cTnC and cTnT-cTnI interaction significantly ($p < 0.001$) compared with wild-type protein interaction ($cTnT_{wt}$, $cTnC_{wt}$, $cTnI_{wt}$). A recognized troponin T polymorphism ($cTnT_{K253R}$) did not change inter-troponin interaction significantly compared with wild-type troponin interaction. All experiments were repeated 10 times. **Vertical bars** = standard deviation.

diagnosis to event. Recent investigations of *TNNT2* in DCM families have identified a δK210 deletion in three unrelated families and an R143W amino acid substitution in one family (6,7,9). The clinical disease expression in these families was similarly severe, and a significant number of affected individuals experienced premature cardiac death/transplantation in the second or third decade of life. However, the disease expression reported was more variable, and healthy mutation carriers without signs and symptoms of disease were present even at older ages. The design of previous studies did not allow an estimate of the prevalence of *TNNT2* mutations in consecutive DCM patients.

Utility of genetic investigations. Overall, the clinical expression of mutations in the troponin complex was associated with an adverse prognosis in DCM. However, this and other studies have reported the clinical findings in patients diagnosed over decades in which advances in pharmacologic treatment have substantially improved the prognosis of patients with heart failure. Although a limited number of patients with DCM in this study are currently alive with a relatively short observation period, it is of note that their condition appears stable on modern pharmacologic therapy with angiotensin-converting enzyme inhibitors and beta-blockers. The results of previous drug trials of patients with

heart failure have indicated a beneficial effect of prophylactic pharmacologic therapy in asymptomatic individuals with impaired systolic function (24,25). Therefore, it is reasonable to assume that early diagnosis and treatment of asymptomatic patients with hereditary DCM may also improve their prognosis (26). The many premature cardiac deaths observed suggest that regular clinical investigation of relatives at risk of disease development is warranted to ensure early diagnosis. Genetic diagnosis would be helpful in identifying unaffected mutation carriers who require follow-up and would enable termination of clinical screening of relatives with a normal genotype. In addition, knowledge of the specific disease gene and mutation present in a family may allow risk-stratification, although limited data are available in relation to sarcomeric gene mutations. So far, few families have been identified, and only the genes for *ACTC*, *TPM1*, and *TNNI3* have been fully investigated in large cohorts of DCM families with a frequency of mutations that account for <3% of all cases (4,5,10). Most other patient populations studied were small in number, and our findings reemphasize the importance of designing genetic studies in larger DCM populations to describe the prevalence of these mutations. The fact that we investigated patients with a "pure" cardiac phenotype explains that we did not observe inheritance patterns suggestive of maternal or X-linked inheritance because these conditions are often associated with symptoms of systemic disease. In maternal inheritance caused by mithocondrial gene mutations, patients typically have multiorgan disease including dilated or hypertrophic cardiomyopathy, neurological symptoms, and skeletal muscle impairment. In X-linked inheritance caused by dystrophin mutations, the disease expression is most commonly associated with both DCM and skeletal muscle impairment, although a few patients with a "pure" cardiac phenotype have been reported.

Functional studies. Myocardial contractility is largely regulated by the troponin complex that, in turn, is influenced by the concentration of intracellular Ca^{2+}. The complex is distributed along the thin filament of the sarcomere and interacts with actin and tropomyosin. During systole, Ca^{2+} binds to cTnC and introduces conformational changes of the troponin complex that attenuate the inhibitory effect of cTnI. This enables the myosin head of the thick filament to interact with actin and generate force. The Ca^{2+} concentration also influences cTnC-cTnT interaction which is important for control of sliding velocity between thick and thin filament (19–23). Interestingly, recent studies have suggested that cTnT is essential, not only for the structural integrity of the troponin complex, but also for sarcomere assembly and cardiac contractility (27).

The amino acid substitution identified in *TNNC1* (G159D) is localized in a domain of the protein constitutively occupied by Ca^{2+}. This may change the affinity for Ca^{2+} and, thereby, alter the ability of the troponin complex to regulate myocardial contractility. Also, mutations identified in *TNNT2* (R131W, R205L, δK210, D270N) occurred in well-conserved regions essential for interactions within the troponin complex or its interactions with tropomyosin or actin (19–23,28,29). The qualitative effect of the mutations on inter-troponin interactions was investigated using a mammalian two-hybrid assay, which showed significant changes for all mutant proteins compared with wild-type. A common *TNNT2* polymorphism (K253R) served as a negative control in the test system and did not change inter-troponin interactions. The results indicate that this assay may be helpful in providing supporting evidence to determine if an amino acid change identified in an affected individual is a disease-causing mutation or more likely to be a polymorphism.

Previous functional studies of one of the DCM mutations identified (*TNNT2*, δK210) showed that mutated protein reduced the Ca^{2+} sensitivity of actomyosin ATPase activity, which resulted in decreased maximum speed of muscle contraction (30,31). Thus, DCM mutations in the troponin complex may induce a profound reduction in force generation leading to impaired systolic function and cardiac dilation. In addition, it is possible that the myocardium of mutation carriers may be more susceptible to environmental influences such as viruses and toxic agents. It seems likely that the heterogeneous clinical appearance in DCM families with mutations in the troponin complex is a result of interplay between the specific mutation identified, modifier genes, and non-genetic factors in the environment.

In summary, mutations in the three proteins of the troponin complex were not uncommon in DCM. The disease expression was characterized by high penetrance and severe prognosis. The data suggest that genetic investigations of the troponin complex may help to identify a subset of DCM families at high risk of rapid disease development.

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REFERENCES

1. Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. Circulation 1996;93:841–2.
2. Seidman JG, Seidman C. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. Cell 2001;104:557–67.
3. Towbin JA, Bowles NE. The failing heart. Nature 2002;415:227–33.
4. Olson TM, Kishimoto NY, Whitby FG, Michels VV. Mutations that alter the surface charge of alpha-tropomyosin are associated with dilated cardiomyopathy. J Mol Cell Cardiol 2001;33:723–32.
5. Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. Science 1998;280:750–2.

6. Li D, Czernuszewicz GZ, Gonzalez O, et al. Novel cardiac troponin T mutation as a cause of familial dilated cardiomyopathy. Circulation 2001;104:2188–93.
7. Kamisago M, Sharma SD, DePalma SR, et al. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. N Engl J Med 2000;343:1688–96.
8. Gerull B, Gramlich M, Atherton J, et al. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. Nat Genet 2002;430:201–4.
9. Hanson EL, Jakobs PM, Keegan H, et al. Cardiac troponin T lysine 210 deletion in a family with dilated cardiomyopathy. J Card Fail 2002;8:28–32.
10. Murphy R, Mogensen J, Shaw A, et al. Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy. Lancet 2004;363:371–2.
11. Itoh-Satoh M, Hayashi T, Nishi H, et al. Titin mutations as the molecular basis for dilated cardiomyopathy. Biochem Biophys Res Commun 2002;291:385–93.
12. Henry WL, Gardin JM, Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. Circulation 1980;62:1054–61.
13. Mestroni L, Maisch B, McKenna WJ, et al. Guidelines for the study of familial dilated cardiomyopathies: Collaborative Research Group of the European Human and Capital Mobility Project on Familial Dilated Cardiomyopathy. Eur Heart J 1999;20:93–102.
14. Mogensen J, Bahl A, Kubo T, Elanko N, Taylor R, McKenna WJ. Comparison of fluorescent SSCP and denaturing HPLC analysis with direct sequencing for mutation screening in hypertrophic cardiomyopathy. J Med Genet 2003;40:E59.
15. National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. Available at: www.ncbi.nlm.nih.gov. Accessed January 27, 2004.
16. Mestroni L, Rocco C, Gregori D, et al. Familial dilated cardiomyopathy: evidence for genetic and phenotypic heterogeneity: Heart Muscle Disease study group. J Am Coll Cardiol 1997;34:181–90.
17. Baandrup U, Olsen EG. Critical analysis of endomyocardial biopsies from patients suspected of having cardiomyopathy. I: Morphological and morphometric aspects. Br Heart J 1981;45:475–86.
18. Varnava AM, Elliott PM, Sharma S, McKenna WJ, Davies MJ. Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. Heart 2000;84:476–82.
19. Perry SV. Troponin T: genetics, properties and function. J Muscle Res Cell Motil 1998;19:575–602.
20. Perry SV. Troponin I: inhibitor or facilitator. Mol Cell Biochem 1999;190:9–32.
21. Perry SV. Vertebrate tropomyosin: distribution, properties and function. J Muscle Res Cell Motil 2001;22:5–49.
22. Hinkle A, Goranson A, Butters CA, Tobacman LS. Roles for the troponin tail domain in thin filament assembly and regulation: a deletional study of cardiac troponin T. J Biol Chem 1999;274:7157–64.
23. Hinkle A, Tobacman LS. Folding and function of the troponin tail domain: effects of cardiomyopathic troponin T mutations. J Biol Chem 2003;278:506–13.
24. Poole-Wilson PA, Swedberg K, Cleland JG, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. Lancet 2003;362:7–13.
25. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. N Engl J Med 1992;327:685–91.
26. Waagstein F, Bristow MR, Swedberg K, et al. Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy: Metoprolol in Dilated Cardiomyopathy (MDC) trial study group. Lancet 1993;342:1441–6.
27. Sehnert AJ, Huq A, Weinstein BM, Walker C, Fishman M, Stainier DY. Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nat Genet 2002;31:106–10.
28. Rarick HM, Tang HP, Guo XD, Martin AF, Solaro RJ. Interactions at the NH2-terminal interface of cardiac troponin I modulate myofilament activation. J Mol Cell Cardiol 1999;31:363–75.
29. Solaro RJ, Rarick HM. Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments. Circ Res 1998;83:471–80.
30. Morimoto S, Lu QW, Harada K, et al. Ca(2+)-desensitizing effect of a deletion mutation Delta K210 in cardiac troponin T that causes familial dilated cardiomyopathy. Proc Natl Acad Sci USA 2002;99:913–8.
31. Robinson P, Mirza M, Knott A, et al. Alterations in thin filament regulation induced by a human cardiac troponin T mutant that causes dilated cardiomyopathy are distinct from those induced by troponin T mutants that cause hypertrophic cardiomyopathy. J Biol Chem 2002;277:40710–6.