Subtle Abnormalities in Contractile Function Are an Early Manifestation of Sarcomere Mutations in Dilated Cardiomyopathy

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Background—Sarcomere mutations cause both dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM); however, the steps leading from mutation to disease are not well described. By studying mutation carriers before a clinical diagnosis develops, we characterize the early manifestations of sarcomere mutations in DCM and investigate how these manifestations differ from sarcomere mutations associated with HCM.

Methods and Results—Sixty-two genotyped individuals in families with sarcomeric DCM underwent clinical evaluation including strain echocardiography. The group included 12 subclinical DCM mutation carriers with normal cardiac dimensions and left ventricular ejection fraction (LVEF ≥55%), 21 overt DCM subjects, and 29 related mutation (-) normal controls. Results were compared with a previously characterized cohort of 60 subclinical HCM subjects (sarcomere mutation carriers without left ventricular hypertrophy). Systolic myocardial tissue velocity, longitudinal, circumferential, and radial strain, and longitudinal and radial strain rate were reduced by 10%–23% in subclinical DCM mutation carriers compared with controls ($P<0.001$ for all comparisons), after adjusting for age and family relations. No significant differences in diastolic parameters were identified comparing the subclinical and control cohorts. The opposite pattern of contractile abnormalities with reduced diastolic but preserved systolic function was seen in subclinical HCM.

Conclusions—Subtle abnormalities in systolic function are present in subclinical DCM mutation carriers, despite normal left ventricular size and ejection fraction. In contrast, impaired relaxation and preserved systolic function appear to be the predominant early manifestations of sarcomere mutations that lead to HCM. These findings support the theory that the mutation's intrinsic impact on sarcomere function influences whether a dilated or hypertrophic phenotype develops. **(*Circ Cardiovasc Genet*. 2012;5:503-510.)**

Key Words: contractility ■ dilated cardiomyopathy ■ echocardiography ■ hypertrophic cardiomyopathy ■ sarcomere

Dilated cardiomyopathy (DCM) is an important cause of heart failure and the leading indication for cardiac transplantation in the United States.[1] However, the underlying cause is often difficult to identify. Because familial disease is present in at least 30% of idiopathic DCM,[2] genetic causes are a significant contributor to disease. To date, >40 genes have been implicated, including those encoding sarcomere proteins.[3] Although sarcomere mutations were initially identified as the cause of hypertrophic cardiomyopathy (HCM),[4] recent studies have shown that they also play an important role in DCM, accounting for ≈10%–20% of familial disease.[5–7] The mechanisms by which different mutations in the same genes lead to seemingly opposite clinical phenotypes have not been elucidated. Experimental models suggest that calcium sensitivity and force generation are diminished with DCM mutations, but enhanced in the presence of HCM mutations.[8–15] These findings suggest that intrinsic differences in the biophysical effects of sarcomere mutations trigger different cellular pathways. This ultimately results in a thick heart with vigorous systolic function in 1 pathology, and a dilated, poorly functioning heart in the other.

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The clinical expression of sarcomere mutations in both HCM and DCM varies with age, although the majority of mutation carriers will likely develop clinically overt disease over their

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lifetimes. Severe manifestations of sarcomeric DCM can be prominent early in life,[16] but there can also be an earlier latent phase when mutation carriers are asymptomatic and have seemingly normal cardiac structure and function. Through systematic and comprehensive study of these subclinical mutation carriers, the early effects of sarcomere mutations can be characterized, before disease is fully manifest.

Such investigations in subclinical HCM mutation carriers have shown that reduced myocardial relaxation, impaired energetics, and increased collagen synthesis are early consequences of sarcomere mutations in HCM, detectable before development of left ventricular hypertrophy (LVH).[17–21] Analogous studies have not been performed in subclinical sarcomeric DCM. Therefore, we evaluated subclinical DCM mutation carriers with normal left ventricular (LV) size and left ventricular ejection fraction (LVEF) to identify early consequences of sarcomere mutations in DCM, and to explore how their effects differ from mutations that give rise to HCM.

Methods

Study Subjects

The study cohort consisted of genotyped DCM patients and their relatives, identified through research protocols or clinical evaluation at Brigham and Women's Hospital, Boston, MA. Genetic status was determined in all subjects by direct DNA sequencing of sarcomere genes.[22] Informed consent was obtained from all participants in accordance with the guidelines of the Institutional Review Board of Partners Healthcare. Study subjects were assessed by history, physical examination, and echocardiography. Individuals were excluded if they had sustained arrhythmias, ventricular pacing, insufficient echocardiographic image quality, or if they were found to have coexistent conditions that may lead to contractile dysfunction (eg, uncontrolled systemic hypertension (>140/90 on medications), coronary artery disease, or valvular heart disease). Subjects taking cardiac medications were included.

Individuals were assigned to 3 different status groups: overt DCM, subclinical DCM, or related normal control. The overt DCM group consisted of sarcomere mutation carriers with clinical features of DCM, defined as LVEF <55% or left ventricular enlargement according to published normal values.[23] In subjects <18 years of age with body surface area <2.0 m^2, LV enlargement was considered present if LV diastolic dimension Z-scores were >2.[24] The subclinical DCM group was comprised of mutation carriers with normal LVEF (≥55%) and LV dimensions. Control subjects were healthy, mutation-negative family members of similar age to subclinical DCM subjects.

A previously described cohort comprised of both individuals with subclinical HCM (n=60) and mutation-negative related controls (n=40)[18] was used as a comparison sample to investigate differences between sarcomere mutations associated with subclinical DCM versus HCM. Individuals with subclinical HCM were sarcomere mutation carriers without echocardiographic LVH (defined as maximal wall thickness <12 mm; Z-scores <2 in subjects <18 years of age). Controls were mutation negative, healthy relatives without LVH. They were evaluated in the same manner as the DCM cohort.

Electrocardiography

Standard 12-lead ECGs were obtained at the time of echocardiographic examination with subjects resting quietly in the supine position. All electrocardiograms were analyzed by a single investigator blinded to clinical, genetic and echocardiographic information. Published criteria for defining ECG abnormalities were used.[25] Nonspecific ST or T-wave abnormalities were considered present if there were abnormalities in the ST-segment or T-waves that did not meet criteria for T-wave inversion or ST-segment depression. Electrocardiographic LVH was considered present if any criteria were met, including Cornell, Sokolow-Lyon, or Romhilt-Estes.[26]

Echocardiographic Protocol

Echocardiographic studies were performed with a Vivid-7 ultrasound system (GE Medical Systems, Horten, Norway), including standard 2-dimensional, M-mode, spectral and color Doppler and tissue Doppler interrogation. Offline image analysis was performed using commercial software (EchoPAC 5.2.0, GE Medical Systems, Milwaukee, WI) by a single cardiologist blinded to clinical and genetic status.

Standard measurements were made according to criteria established by the American Society of Echocardiography,[23,27] including cardiac dimensions, mitral inflow parameters, calculation of LVEF (modified biplane Simpson's method), and tissue Doppler myocardial velocities in systole (S'), early (E') and late (A') diastole at the lateral, septal, anterior, and inferior aspects of the mitral annulus. The average of 3 cardiac cycles is reported. Global S', E' and A' values are the mean of regional tissue Doppler myocardial velocity values.

Longitudinal, radial, and circumferential strain analyses were performed.[28–33] During image acquisition, frame rates were maximized by narrowing the color sector to isolate individual walls, which were oriented parallel to the sample beam. Speckle tracking (2-dimensional strain, GE Medical Systems) values for peak systolic strain (ε_{sys}) and systolic strain rate (SSR) were determined in all of these views. The endocardium was manually traced and myocardial motion tracked with automated software. Image and tracking quality were verified manually and with the software's automated quality grading scale. Segments were rejected if adequate quality could not be obtained despite manual correction. Longitudinal ε_{sys} and SSR were determined in 12 segments from the basal, middle, and apical segments of the septal, lateral, anterior, and inferior walls in the apical 4- and 2-chamber views and averaged to calculate the global longitudinal ε_{sys} and SSR. Radial and circumferential ε_{sys} and SSR were determined in 6 segments from mid-ventricular parasternal short axis images using 2-dimensional strain and averaged to calculate global values.

Longitudinal ε_{sys} and SSR measurements were repeated on a subset of subjects by the primary echocardiographer (N.K.L.) and by a second experienced echocardiographer (C.Y.H.) to assess reproducibility. Interclass correlation coefficients were calculated and revealed excellent inter- and intraobserver variability for ε_{sys} and SSR measurements (average intraobserver interclass correlation coefficient =0.72; average interobserver interclass correlation coefficient =0.78).

Statistical Analysis

Patient characteristics are presented for each of the 3 groups (overt DCM, subclinical DCM, controls) using mean values and simple SD, unless inappropriate due to the need for adjustment for relevant covariates. Specifically, echocardiographic strain and tissue Doppler were adjusted for age as well as family relations. Adjusted means and SEs are reported for these parameters. To test for significant differences among the 3 groups, pairwise comparisons were performed with clustered regression to adjust for the influence of relationships between family members. ANOVA was performed using the GenMod procedure in SAS to account for these relationships, assuming an exchangeable correlation structure. A P value <0.017 was considered statistically significant to apply post hoc Bonferroni correction for multiple comparisons across the 3 status groups. Logistic regression was used to evaluate the ability of echocardiographic tissue Doppler and strain parameters to distinguish subclinical DCM subjects from controls**.** Similar analyses were performed to compare subclinical DCM and HCM sarcomere mutation carriers. For these comparisons, a P value <0.05 was considered statistically significant, adjusting for age, sex, and family relations. Statistical analysis was performed with SAS version 9.1 (SAS Institute Inc, Cary, NC).

Results

Clinical Characteristics and Basic Echocardiographic Parameters

A total of 62 individuals from 5 DCM families were studied, including overt DCM (n=21), subclinical DCM (n=12), and mutation (-) healthy relatives serving as normal control

subjects (n=29). Subjects had mutations in β-myosin heavy chain (*MYH7*; S532P n=15 and A893V n=5), α-tropomyosin (*TPM1*; D230N n=9), and cardiac troponin T (*TNNT2*, K210del n=4). The clinical characteristics and basic echocardiographic parameters are summarized in Table 1.

All subclinical and control subjects were asymptomatic and had normal basic echocardiographic studies. Two subclinical subjects were receiving an angiotensin receptor blocker or β-blocker; 1 to treat mild hypertension, the other was started on off-label therapy when confirmed to carry a sarcomere mutation. Exclusion of these subjects did not change the overall study results (data not shown). The majority of subjects had normal ECG tracings, although nonspecific ST-segment and T-wave abnormalities were more prevalent in subclinical DCM compared with normal controls, (25% versus 5%, respectively; $P<0.001$) (online-only Data Supplemental Table I).

Subjects with overt DCM had modest symptoms (95% New York Heart Association class I-II) and 52% were receiving medical therapy with either angiotensin-converting enzyme inhibitors or β-blockers. By definition, this cohort had significantly larger LV dimensions and lower LVEF compared with both the control and subclinical DCM groups (Table 1). Two thirds of overt DCM subjects had at least 1 ECG abnormality, most frequently nonspecific ST-segment and T-wave (online-only Data Supplemental Table I).

Assessment of Systolic Function

Adequate tissue Doppler waveforms were obtained in all subjects. Circumferential ε_{sys} and SSR could be measured in 89% and 69% of subjects respectively, radial ε_{sys} and SSR in 77% and 69% radial, and longitudinal ε_{sys} and SSR in 100% and 97%. The frequency of interpretable walls was similar in the 3 status groups.

Evaluation of systolic function is summarized in Table 2 (see online-only Data Supplemental Table II for data on individual subjects). Although the LVEF of all subclinical subjects was normal (LVEF 59±3%) and not significantly different from controls (62±5%; $P=0.07$), subclinical mutation carriers had a significant reduction in all of the more sensitive metrics of global systolic function, with the sole exception of global circumferential SSR. As illustrated in Figure 1, global peak systolic myocardial tissue velocity (global S') was 16% lower in subclinical DCM compared with controls (7.6±0.3 cm/s versus 9.0±0.2 cm/s; $P<0.001$). Analyzing the individual components of global S' (ie, values of the septal, lateral, inferior and anterior walls) did not reveal significant regional differences in systolic function in any group. Echocardiographic strain analysis similarly showed reduced systolic function in subclinical DCM. Global circumferential, radial and longitudinal ε_{sys} were 10%, 23%

Table 1. Clinical and 2-Dimensional Echocardiographic Characteristics

	Subclinical DCM n=12	*P** Subclinical vs Control	Overt DCM n=21	*P** Subclinical vs Overt	Related Normal Control n=29	*P*-value* Overt vs Control
Age, y (range)	24.6±18.5 (3–50)	0.59	35.0±20.1 (8–71)	0.06	22.1±16.3 (3–66)	0.01
Female, % (female/male)	92% (11/1)	0.09	67% (14/7)	0.21	62% (18/11)	0.74
Gene, n						
MYH7	9		11			
TPM1	3		6			
TNNT2	—		4			
Medical therapy, n						
ACEi/ARB	2		9		1	
β-blocker	1		9		1	
BSA, m^2	1.4±0.5	0.001	1.8±0.3	0.01	1.6±0.5	0.74
Heart rate, bpm	74±15	0.13	65±11	0.04	69±9	0.01
SBP, mm Hg	109±20	0.59	118±13	0.37	117±13	0.03
DBP, mm Hg	68±15	0.93	67±13	0.55	70±7	0.32
NYHA class, n						
I			16			
II			4			
III			1			
IVS, mm	7.0±1.4	0.004	7.6±1.7	0.14	7.8±1.6	0.71
LVEDD, cm	4.3±0.7	0.65	5.4±0.7	<0.001	4.3±0.6	<0.001
LVESD, cm	3.1±0.6	0.09	4.4±0.7	<0.001	2.8±0.5	<0.001
LVEF, % [range]	59±3 [55–65]	0.07	44±10 [19–57]	<0.001	62±5 [55–71]	<0.001
LA diameter, cm	3.0±0.7	0.27	3.7±0.6	<0.001	3.3±0.6	0.07

Values expressed as unadjusted mean±SD.

MYH7 indicates β-myosin heavy chain; *TPM1*, α-tropomyosin; *TNNT2*, cardiac troponin T; ACEi, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; NYHA, New York Heart Association; IVS, interventricular septal thickness; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; LVEF, left ventricular ejection fraction; and LA, left atrium.

*$P<0.017$ is considered statistically significant and reflects adjustment for age and family relations.

Table 2. Assessment of Systolic Function by Tissue Doppler and Strain Imaging

	Subclinical DCM n=12	*P** Subclinical vs Control	Overt DCM n=21	*P** Subclinical vs Overt	Normal Related Control n=29	*P** Overt vs Control
Global S', cm/s	7.6±0.3	<0.001	7.3±0.3	0.50	9.0±0.2	<0.001
Septal S'	6.8±0.3	0.005	6.3±0.3	0.30	8.1±0.4	<0.001
Lateral S'	7.5±0.2	<0.001	8.4±0.4	0.014	9.8±0.2	<0.001
Inferior S'	7.4±0.1	<0.001	6.8±0.3	0.06	9.1±0.3	<0.001
Anterior S'	8.1±0.6	0.17	7.5±0.4	0.35	9.0±0.3	<0.001
Global ε_{sys}, %						
Circumferential	−15.4±0.2	<0.001	−12.1±1.0	<0.001	−17.2±0.5	<0.001
Radial	39.8±2.6	<0.001	27.6±0.9	<0.001	51.8±0.3	<0.001
Longitudinal	−17.2±0.6	<0.001	−15.5±0.4	0.018	−20.3±0.6	<0.001
Global SSR, 1/sec						
Circumferential	−1.27±0.17	0.34	−0.97±0.06	0.06	−1.39±0.07	<0.001
Radial	1.41±0.06	<0.001	1.35±0.03	0.03	1.75±0.001	<0.001
Longitudinal	−1.09±0.03	<0.001	−0.86±0.01	<0.001	−1.31±0.02	<0.001

Values expressed as mean±SE, adjusted for age and family relations.
S' indicates peak systolic myocardial tissue velocity; $\varepsilon_{sys,}$ peak systolic strain; SSR, peak systolic strain rate.
*P<0.017 is considered statistically significant.

and 15% lower in subclinical DCM compared with controls, respectively (P≤0.001 for all comparisons). Radial and longitudinal SSR were significantly reduced by 22% and 15% in subclinical DCM compared with controls. The difference in global S', longitudinal ε_{sys}, and longitudinal SSR remained significant after controlling for LVEF, internal dimension or volume, the presence of nonspecific ST-segment and T-wave changes on ECG, or excluding the 2 subclinical subjects who were taking medications (P<0.01 for all comparisons).

Systolic dysfunction was more pronounced in overt DCM. All metrics of global S', ε_{sys} and SSR were 19% to 47% lower compared with normal controls (P<0.001 for all comparisons, Table 2), including a significant reduction in circumferential SSR.

In family members with normal 2-dimensional echocardiograms, we tested the ability of systolic parameters (S', ε_{sys} and SSR) to discriminate subclinical mutation carriers at risk for developing DCM from healthy relatives without mutations.

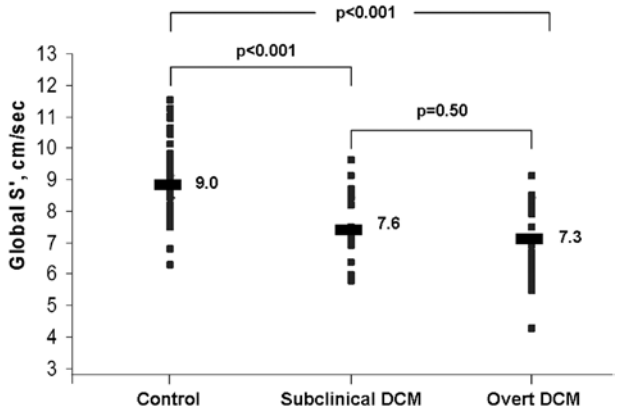

Figure 1. Systolic function is reduced in subclinical dilated cardiomyopathy (DCM). Global peak systolic myocardial tissue velocity (S') was reduced in subclinical DCM compared with controls. Black dots represent individual unadjusted global S' values. The horizontal black bar indicates mean global S', adjusted for age and family relations. *P* values also reflect this adjustment.

Global S' had reasonable diagnostic accuracy for identifying mutation carriers (area under the receiver operating characteristic curve = 0.82). After adjustment for age, sex, and family relations, the odds of carrying a sarcomere mutation increased substantially for every 1 cm/sec decrement in global S' (odds ratio, 3.2; 95% confidence interval, 2.5 to 4.1; P<0.001). Similar predictive value was observed for septal S', global longitudinal ε_{sys}, and global longitudinal SSR (data not shown).

Assessment of Diastolic Function

Evaluation of diastolic function is summarized in Table 3. In contrast to systolic function, diastolic function was relatively preserved in subjects with subclinical DCM. No significant differences in mitral inflow parameters or early diastolic myocardial velocities were identified. In overt DCM, global early diastolic tissue velocity (E') was significantly reduced compared with controls.

Early Manifestations of Sarcomere Mutations in DCM Compared With HCM

To explore if early phenotypes differ between sarcomere mutations that result in HCM versus DCM, the subclinical DCM cohort was compared with a previously characterized cohort of subclinical HCM sarcomere mutation carriers without echocardiographic LVH.[18] Although LVEF was normal in both cohorts, it was lower in subjects with subclinical DCM. Moreover, tissue Doppler myocardial velocities and strain measures of systolic function were all significantly reduced in subclinical DCM mutation carriers compared with subclinical HCM (Table 4). Compared with separate normal control cohorts comprised predominantly of healthy, mutation-negative relatives of study subjects, subclinical DCM mutation carriers had reduced systolic and preserved diastolic function, whereas subclinical HCM mutation carriers showed the opposite pattern of contractile dysfunction with preserved systolic and reduced diastolic function (Figure 2).

Table 3. Assessment of Diastolic Function

	Subclinical DCM n=12	*P** Subclinical vs Control	Overt DCM n=21	*P** Subclinical vs Overt	Normal Related Control n=29	*P** Overt vs Control
Mitral peak E wave velocity, m/s	0.88±0.02	0.93	0.82±0.06	0.17	0.88±0.02	0.21
Mitral peak A wave velocity, m/s	0.55±0.07	0.62	0.47±0.04	0.11	0.52±0.03	<0.001
E/A ratio	1.8±0.2	0.69	1.9±0.1	0.58	1.7±0.1	0.03
E deceleration time, ms	165±6	0.11	162±7	0.72	180±4	0.05
Global E', cm/s	13.0±0.7	0.21	12.1±0.5	<0.001	14.0±0.2	<0.001
E/E' ratio	6.8±0.6	0.44	7.6±0.9	0.04	6.5±0.3	0.09

Values expressed as mean±SE, adjusted for age and family relations.
*P<0.017 is considered statistically significant.

Discussion

Sarcomere mutations are an important cause of both hypertrophic and DCM, accounting for ≈60% of HCM and ≈10%–20% of DCM.[4–7] However, the pathways leading from mutation to clinically overt disease are not well understood. One strategy to gain insight into pathogenesis is studying sarcomere mutation carriers before they develop diagnostic clinical findings. This approach is particularly challenging in DCM because sarcomere mutations are relatively rare, and the duration of the subclinical phase is inconsistent and unpredictable. Presentation with severe disease is well-described in infancy and early childhood, but in the same families, middle aged adults have presented with mild manifestations.[16,22,34] The reasons underlying variability are unclear, but may reflect temporal differences in the expression and effects of sarcomere mutations,[12] or an increased susceptibility to an acquired second hit conferred by the mutation. As a result of these challenges, individuals with subclinical DCM are hard to identify and the early manifestations of sarcomere mutations have not been well-characterized in this population.

Table 4. Comparison of Subclinical HCM and DCM

	Subclinical DCM n=12	Subclinical HCM n=60	*P**
Age, y (range)	24.6±18.5 (7–54)	26.2±12.1 (3–50)	0.75
Female, % (female/male)	92% (11/1)	65% (39/21)	0.003
Disease gene, subjects			
MYH7	9	27	
TPM1	3	—	
MYBPC3	—	23	
TNNT2	—	6	
TNNI3	—	4	
IVS, mm	7.0±1.4	8.8±1.3	<0.001
LV ejection fraction, % (range)	59±3 (55–65)	69±7 (52–86)	<0.001
Global S' cm/s†	8.0±0.2	9.3±0.2	<0.001
Global longitudinal ε_{sys}, %†	−17.9±0.6	−22.0±0.4	<0.001
Global longitudinal SSR, 1/sec†	−1.06±0.03	−1.39±0.03	<0.001

DCM indicates dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; *MYH7*, β-myosin heavy chain; *TPM1*, α-tropomyosin; *MYBPC3*, cardiac myosin-binding protein C; *TNNT2*, cardiac troponin T; *TNNI3*, cardiac troponin I; IVS, interventricular septal thickness; LV, left ventricular; S' = peak systolic myocardial tissue velocity; $\varepsilon_{sys,}$ systolic strain; and SSR, systolic strain rate.
*P adjusted for age and family relations.
†Mean point estimates±SE for tissue Doppler and strain parameters are adjusted for age, sex and family relationships. These point estimates differ from those occurring in the 3 DCM subgroups represented in Tables 2 and 3, due to adjustments for subclinical HCM with different relationships.

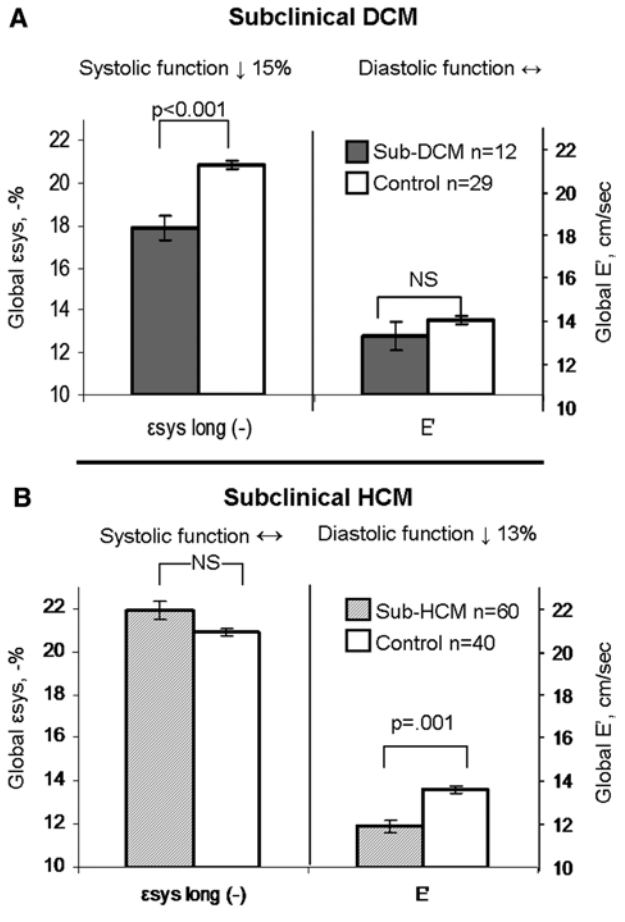

Figure 2. Sarcomere mutations associated with hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) have divergent effects on contractile function before clinical disease. **A**, Systolic function is reduced and diastolic function is preserved in subclinical DCM (subDCM) compared with controls. **B**, In contrast, systolic function is preserved and diastolic function is reduced in subclinical HCM (subHCM) compared with controls. ε_{sys} long indicates global, peak longitudinal systolic strain, %; E', global, peak early diastolic myocardial tissue velocity, cm/s; and NS, not signficant. Values are adjusted for age and family relations, error bars represent SE.

Using more sensitive tissue Doppler and strain echocardiography, we demonstrate that subclinical DCM sarcomere mutation carriers have impaired systolic function without other clinical evidence of disease. Systolic myocardial velocity, strain, and strain rate were all reduced despite normal cardiac dimensions, LVEF and diastolic function. Analogous findings have been shown in early Duchenne muscular dystrophy, highlighting the limitations of using less sensitive metrics like LVEF to characterize subtle changes in pathophysiology.[35–37]

Systolic dysfunction in subclinical DCM was mild, with substantial overlap between measurements in mutation carriers and normal controls. Although S', ε_{sys} and SSR were significantly reduced in subclinical DCM mutation carriers compared with controls, these metrics do not have sufficient predictive accuracy to support clinical decision making, or to reliably discriminate at-risk mutation carriers from healthy relatives. Our data were not intended to support using tissue Doppler myocardial velocities or echo strain imaging as diagnostic tools or as an alternative to genetic testing for identifying at-risk relatives, but rather as a means to investigate pathophysiology. The findings are intriguing because they provide the new insight that subtle, subclinical systolic dysfunction appears to be an early manifestation of DCM sarcomere mutations.

Notably, >90% of subjects in our subclinical cohort were female. Animal models of cardiomyopathy have suggested that phenotypic expression of sarcomere mutations may be less pronounced in females than males,[38–40] and similar findings have been observed in humans with sarcomeric HCM.[41] The influence of sex on the development of DCM in humans is not well characterized. In a study of DCM caused by troponin T mutations, fewer females than males (7 females versus 15 males) had overt disease.[22,34] A recent study of DCM caused by truncating titin mutations suggested that adverse events occurred a decade later in females compared with males.[42] These observations hint at a potential protective effect of female sex that may delay or diminish phenotypic expression to account for the over-representation of females in our subclinical cohort. Owing to the small sample size and the need to capture subjects through family membership in this study, it is difficult to determine if the apparent female predominance reflects a true biological difference or an artifact related to ascertainment. When controlled for sex, metrics of systolic function remained significantly reduced in subclinical DCM compared with controls. Continued study of genotyped cohorts is needed for more precise characterization of potential modifying factors.

Sarcomere Mutations in DCM and HCM: Divergent effects from a common Genetic Cause

DCM and HCM are both caused by sarcomere mutations, however, the histopathology, ventricular remodeling, and natural history of these diseases contrast starkly. These profound differences suggest that DCM and HCM result from fundamentally different upstream disease processes, despite an apparently common genetic basis. The factors that determine whether a given sarcomere mutation results in DCM or HCM are not well understood. One approach to elucidate these mechanisms is to characterize and compare the intrinsic functional properties of sarcomere mutations that cause human disease at multiple levels—in vitro, in genetically modified animal models, and in human mutation carriers before the development of clinical disease.

A wealth of basic investigation indicates that DCM sarcomere mutations alter myofilament and myocellular calcium handling and responsiveness, resulting in depressed force generation.[11] Experiments on DCM-mutant thin filaments have consistently demonstrated decreased in vitro motility, Ca^{2+} sensitivity of force generation, Ca^{2+} affinity, and maximal ATPase activity.[9,13,43] Studies on young mice genetically engineered to carry human DCM myosin heavy chain mutations demonstrate diminished contractility before the animals develop either LV dilation or obvious reduction in fractional shortening.[44] Collectively, these findings predict that DCM sarcomere mutations impair the heart's ability to generate force. This impairment may be the initial stimulus for compensatory development of left ventricular enlargement and systolic dysfunction characteristic of DCM.

In contrast, sarcomere mutations associated with HCM have seemingly opposite effects on myofilament function. Biophysical studies on thick and thin filaments with HCM mutations have shown increased in vitro motility, Ca^{2+} sensitivity to force generation, Ca^{2+} affinity, and maximal ATPase activity.[9] Compared with DCM mutations, HCM sarcomere mutations appear to enhance motor function and increase maximal force generation.[8,14,15,45] However, diastolic abnormalities are prominent in HCM sarcomere mutations. Animal and human studies of subclinical HCM have demonstrated diminished diastolic function even when systolic function is preserved[20,21,46,47] and before the development of LVH.[12,18,47] The opposing functional effects associated with HCM and DCM sarcomere mutations are postulated to, in part, account for how mutations in the same genes give rise to such contrasting clinical phenotypes. Presumably distinct cellular pathways are triggered early in disease pathogenesis, based on how a mutation fundamentally alters sarcomere function. These pathways ultimately diverge to the development of HCM or DCM.

Our findings support this hypothesis, showing that the early effects of sarcomere mutations are different in subclinical DCM and HCM. As seen in model systems, human subclinical DCM mutation carriers have impaired systolic function but relatively preserved diastolic function compared with normal controls. In contrast, subclinical HCM mutation carriers have impaired diastolic function but relatively preserved systolic function. We acknowledge that our subclinical DCM cohort was small and may be underpowered to detect slight differences in diastolic function. Subtle diastolic abnormalities might be detected with larger numbers, or if more sensitive measures were available. Further evaluation is essential to validate and expand these findings. Additionally, it will be important to explore whether subclinical systolic dysfunction is unique to myofilament mutations, or present at the subclinical stage in all forms of genetic DCM, such as caused by mutations in titin, lamin A/C or cytoskeletal elements. Nonetheless, these findings suggest that different patterns of contractile dysfunction are present in early sarcomeric DCM and HCM. Systolic dysfunction appears to be the predominant early contractile abnormality associated with sarcomere

mutations that cause DCM, whereas diastolic dysfunction is predominant in early HCM.

Clinically overt disease is typically not present at birth in genetic cardiomyopathies. The changes set forth by the underlying mutation are initially slight and well-tolerated. As such, targeting subclinical mutation carriers may provide a key opportunity for disease modification and prevention. Although disease development is not universal among mutation carriers, the majority will develop cardiomyopathy over time. Systematic, comprehensive, and longitudinal evaluation of genotyped cohorts is critical to better characterize early phenotypes across the spectrum of genetic cardiomyopathies. In concert with ongoing basic science investigation, such knowledge will better elucidate disease pathogenesis, provide surrogate end points of disease progression and treatment response, and potentially identify therapeutic targets to limit, and ultimately prevent, the development of DCM and HCM in at-risk patients.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Sarcomere gene mutations play an important role in genetic cardiomyopathies. They are present in a prominent subset of patients with dilated cardiomyopathy (DCM) and in the majority of patients with familial hypertrophic cardiomyopathy (HCM). However, the precise mechanistic steps that lead from mutation to clinically obvious disease are not well understood. Sarcomere mutation carriers typically do not manifest disease at birth. A subclinical phase may last for decades, even indefinitely, before overt cardiomyopathy develops. This implies that sarcomere mutations can be well-tolerated and that disease-modifying treatments could potentially be developed to prolong the compensated state or prevent disease development entirely. The finding that both HCM and DCM can be caused by different mutations in the same sarcomeric proteins highlights the complex interplay between genotype and phenotype. Experimental models have demonstrated that the intrinsic biochemical and mechanical properties of mutations that give rise to DCM are different from mutations that give rise to HCM. In this study we begin to characterize the early consequences of DCM sarcomere mutations and contrast them to mutations associated with HCM in a human population. Tissue Doppler and strain echocardiography suggest that subtle systolic dysfunction is present in subclinical DCM mutation carriers, despite normal left ventricular dimensions and ejection fraction. In contrast, diastolic function appeared to be preserved. These findings are opposite those seen in subclinical HCM sarcomere mutation carriers where preserved systolic function and impaired diastolic function have been described. These differences may play a critical role in shaping downstream cardiac remodeling.