It’s Never Too Early to Look
Subclinical Disease in Sarcomeric Dilated Cardiomyopathy

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The observation that ≈30% of nonischemic dilated cardiomyopathy (DCM) is genetic in origin represents one of the most important advances in modern cardiology and has transformed our view of this common disorder.1 Equally surprising is the more recent finding that sarcomeric genes previously linked to the development of hypertrophic cardiomyopathy (HCM) were also causative in the pathogenesis of DCM.2 Mutations in most of the protein components of the cardiac sarcomere have now been linked to DCM, many of them arising in close proximity to known HCM mutations, and provide a unique challenge: how can such divergent patterns of ventricular remodeling arise from such closely related structural changes? Although it may appear to be straightforward that discrete alterations in sarcomeric function caused by gene mutations could eventually lead to either left ventricular (LV) dilation or hypertrophy, our lack of understanding regarding the earliest clinical stages of DCM before the effects of secondary changes are manifest has precluded mechanistic insight. In this issue of Circulation Genetics, Lakdawala et al used a unique and well-characterized sarcomeric DCM cohort to directly determine whether abnormal systolic function can be detected in genotype-positive patients with normal LV ejection fraction and size. Their robust approach, including determination of echo strain and systolic myocardial tissue velocity and comparisons among subclinical DCM, overt DCM, and control (genotype-negative) family members across 4 independent mutation groups, revealed a significant decrease in systolic function in the subclinical group. These results complement their previous findings that gene mutations linked to HCM cause diastolic dysfunction in subclinical cohorts and fully establish that the primary mechanisms driving diverse ventricular remodeling in sarcomeric cardiomyopathies are tightly coupled to the underlying changes in sarcomere dynamics.3

Since the publication of the first linkage studies in 1990, >1000 mutations in genes encoding the protein components of the cardiac sarcomere have been associated with the development of cardiomyopathic remodeling.3 Although the disorder is unique in that the overall structure and function of the affected proteins are well established, this extensive biophysical understanding has not yet translated to patient care, and the lack of genotype–phenotype correlation has hindered the use of genotype to predict prognosis and direct clinical management. Indeed, due in part to the characteristic phenotypic heterogeneity in patients with sarcomeric cardiomyopathies, it has been suggested that the goal of genotype-driven patient management may not be attained.4 Two major observations have refocused efforts to establish robust genotype–phenotype relationships in HCM and DCM. First, the biophysical basis of the clinical disorder was established by the overall concordance among the in vitro studies in identifying precise alterations in sarcomeric function coupled to the persistent finding in animal models that contractile dysfunction preceded histopathology. Second, longitudinal studies of genotyped cohorts have begun to establish the progressive nature of the ventricular remodeling caused by sarcomeric mutations.5,6 These findings mirrored those in animal models, where initial abnormalities in sarcomeric function led to activation of multiple downstream myocellular signaling pathways that drove cardiac remodeling. The latter observation is similar to the difficulties in determining the cause in patients with congestive heart failure; in that end-stage, remodeled tissue is temporally removed from the initial pathogenic process. Thus, to mechanistically couple a primary biophysical abnormality to a specific pattern of ventricular remodeling, it is imperative to identify and study the earliest stages of cardiomyopathy, exactly the approach taken in the current study.

To directly address this central question, the authors have assembled a unique clinical cohort drawn from 5 independent families carrying mutations in 3 sarcomeric proteins previously linked to DCM (MYH7, TMP1, and TNNT2). Although the statistical power was insufficient to elucidate mutation-specific information and the reliance on families for recruitment may limit the ability to address some questions raised by the data (eg, the intriguing sex differences), the multigenerational cohort yielded 3 well-defined groups for study. The main subgroup (subclinical DCM) was composed of 12 genotype-positive patients with baseline clinical and standard echo parameters largely indistinguishable from genotype-negative controls, including normal LV size and ejection fraction. Of note, many of these parameters exhibited clear differences compared with the genotype-positive subgroup with overt DCM. Application of more sensitive global systolic echo methodologies, including tissue Doppler and strain imaging, revealed striking differences in nearly all indices within the subclinical group compared with controls. As noted by the authors, the decreases in global peak systolic myocardial tissue velocity, longitudinal peak systolic strain, and strain rates all remained significant after controlling for LV geometry and ejection fraction. Although

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483
findings in the current study. Analysis of molecular motor detected before overt histopathology, mirroring the clinical DCM phenotype, but also deficits in contractile function were both cases, homozygous animals not only recapitulate the Troponin T mutations have been modeled in mice, and, in this paradigm. Both the S532P myosin and del210K cardiac the mutations studied in the current report generally fit within of exogenously expressing myosin is a significant limitation), specially for the myosin motor mutations, where the challenge of impairment in high-resolution systolic and diastolic indices, respectively. This robust coupling between cardiac dysfunction in early disease states and the eventual clinical phenotype is not only clinically relevant, it again reinforces the importance of elucidating the primary biophysical mechanisms whereby individual mutations cause these precise pathophysiological responses in cardiac muscle.

Given the relatively small size of the subclinical DCM group, the authors were reasonably circumspect in the extrapolation of their results to the broader questions of genotype–phenotype correlation and eventual patient management in genetic cardiomyopathies. Nonetheless, the current study raises important questions that will spur both basic and clinical investigations. As noted in an extensive recent review by Moore et al., HCM-linked myosin mutations tend to enhance contractility, whereas the DCM-linked subset decrease contractile function. Although biophysical data are limited (especially for the myosin motor mutations, where the challenge of exogenously expressing myosin is a significant limitation), the mutations studied in the current report generally fit within this paradigm. Both the S532P myosin and del210K cardiac Troponin T mutations have been modeled in mice, and, in both cases, homozygous animals not only recapitulated the DCM phenotype, but also deficits in contractile function were detected before overt histopathology, mirroring the clinical findings in the current study. Analysis of molecular motor function for the S532P myosin isolated from homozygous animals revealed significant decreases in actin sliding velocity and ATPase activity, in direct contrast to previous results from the HCM-linked R403Q myosin mice. The combination of the accumulating evidence from animal models and in vitro studies provides strong support for the hypothesis that HCM and DCM mutations cause differential disease states via precise (and opposite) modulation of sarcomeric efficiency. The link between these changes, however, and the diverse ventricular remodeling patterns remains unclear.

From the standpoint of sarcomeric dynamics, structure, and function, the varied functional roles of the 4 independent protein mutations are intriguing. Why would 4 such disparate mutations cause such a similar clinical phenotype? All 4 mutations are located within highly conserved regions of the affected proteins (Figure). The MYH7 mutations S532P and A893V occur in the globular N-terminal domain of the myosin motor. Specifically, the S532P mutation is within the actin–myosin interface, whereas the A893V mutation is immediately distal to the regulatory light chain–binding domain in close proximity to the transition between the neck and hinge. Although no biophysical studies have been performed on the A893V mutation, this unique linker region of the motor is thought to, in part, modulate intermolecular interactions between the 2 myosin molecules. Either of these domains could thus alter motor efficiency. Comparing the effects of either mutation on predicted secondary structure using the PSIPRED algorithm reveals potentially significant effects on local structure (Figure). At residue 532 in the actin-binding domain, the substitution of the nonpolar Phe residue for Ser is predicted to decrease the overall helical character of the region and lengthen the linker domain. The relatively conservative Ala to Val substitution at residue 893 also significantly decreases the helical nature of the region, in this case, appearing to favor a structure more consistent with a β-sheet. Either of these structural changes would be predicted to alter both local and distant inter- and intramolecular protein interactions and contribute to a decrease in motor efficiency. Computational modeling of protein dynamics would provide additional mechanistic detail. Although it is relatively straightforward to envision the role of structural changes in myosin as a cause of altered motor function, the molecular effects of the thin filament mutations TMP1 D230N and TNNT2 del210K are less clear. The regulatory thin filament directly determines the access of actin and myosin, in part by allosterically modulating the position of tropomyosin in response to calcium binding to cardiac troponin C. Until recently, the complexity of the protein–protein interactions within the complex and the lack of high-resolution structure for the N-terminal domain of cardiac troponin T have complicated disease insight for thin filament mutations. Transgenic mouse models of HCM-linked cardiac troponin T mutations have demonstrated both significantly altered energetics and inefficient ATP use that can be partially rescued by genetically switching the myosin isoform in vivo. These results support a direct role for the troponin complex in modulating cross-bridge dynamics that may, in part, explain how thin filament mutations can cause similar clinical phenotypes compared with thick filament mutations. Interestingly, the local structural and functional effects are likely to be different than the myosin mutations, although both the TMP1 and TNNT2 mutations in the current study also occur in highly helical domains. This is illustrated in the secondary structure predictions (Figure), where no change in helical character is observed for either mutation. Depending on the position of the mutated residue within the helical array (eg, D230N faces away from the inner coiled-coil structure), the effects of the mutations are likely to influence protein–protein interactions within the complex via changes in electrostatics and flexibility. Again, molecular dynamics will be a useful approach in determining the molecular mechanisms.
support to the emerging consensus that deficits in contractile function drive the development of DCM. Although at present the high-resolution echo techniques used in the current study are not sufficiently predictive to be used to identify relatives at risk, it is likely to change as larger patient cohorts are obtained. This latter point is key, because this study illustrates the way forward for future work, with a focus on genotyped, multigenerational cohorts and careful longitudinal characterization of clinical phenotypes from the earliest stages of disease. The techniques described here will clearly be useful in following the progression of the cardiac dysfunction, a crucial next step in developing a more robust understanding of the natural history of HCM and DCM. The demonstration that diverse protein mutations can cause similar patterns of early ventricular remodeling will help focus efforts to develop more functionally driven molecular studies where the structural and dynamic effects on single proteins can be better integrated into multiprotein in silico and in vitro approaches and finally provide insight as to how mutations in proteins of the cardiac sarcomere cause distinct patterns of ventricular remodeling. The eventual results will be fully translational in that they will identify unique points of therapeutic intervention and move us closer to the goal of using genotype to inform clinical management in this not uncommon disorder.

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Disclosures
None.

Figure. Homology alignments and secondary structure plots for mutation sites and immediate flanking regions. WT indicates wild-type sequence.
References


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