

QUARTERLY FOCUS ISSUE: HEART FAILURE

Editorial Comment

Tropomyosin and Dilated Cardiomyopathy

Revenge of the Actinomyosin “Gatekeeper”*

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One of the most important recent advances in our understanding of the basic mechanisms of genetic cardiac disease has been the central role played by mutations in structural proteins. Nearly 2 decades ago Geisterfer-Lowrance et al. (1) published the first linkage of a mutation in the *beta-MyHC* gene to hypertrophic cardiomyopathy (HCM). Since that seminal finding, mutations in a broad array of proteins that contribute to the structural and functional integrity of components of the cardiac sarcomere, myocellular cytoskeleton, and the sarcolemma have been definitively linked to a vast range of clinical cardiomyopathies (2). Sarcomeric protein mutations represent a particularly intriguing subset of the genetic cardiomyopathies in that independent mutations within the same gene and often within the same functional domain can cause widely divergent, clinically relevant patterns of pathogenic ventricular remodeling.

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To date, mutations in virtually all of the known sarcomeric proteins have been associated with dilated cardiomyopathy (DCM) (3). Although the phenotypic variability that is characteristic of the HCM-linked sarcomeric gene mutations is also present in the DCM subgroup, important clinical similarities have been identified. In 2001 an extensive multi-kindred study first linked mutations in *MHY7* and *TNNT2* to DCM. Of note, a high frequency of early-onset ventricular dilation and congestive heart failure in children and young adults, extensive early sudden cardiac death, and no evidence of the signature histopathological findings of HCM were observed, supporting the hypothesis that the observed dilation was a primary event, as opposed to a secondary end point of hypertrophic heart disease (4). A subsequent report not only identified novel DCM-causing mutations in genes encoding the thin filament proteins cTnC and cTnT, it further confirmed the

distinct clinical phenotypes that arise in DCM linked to sarcomeric protein mutations (5).

In this issue of the *Journal*, Lakdawala et al. (6) present the first extensive linkage study to establish the pathogenicity of alpha-tropomyosin (TM) in DCM. The patient cohort is particularly robust, encompassing 2 unrelated, multigenerational families that carry an aspartate-to-asparagine substitution at residue 230 of *TMP1* (D230N). Both the large sizes of the affected families and the wealth of available clinical information (including the childhood presentation of disease for several adults) result in a particularly informative match between genotype and phenotype.

Several aspects of the clinical expression in affected individuals are notable. First, the initial presentation is “bimodal,” occurring either in early childhood (including a striking number of infants with severe left ventricular systolic failure) or in middle age, where mild, largely asymptomatic left ventricular dilation is observed. Second, the clinical response to supportive and standard medical heart failure therapy was substantial because of a significant and sustained improvement in contractile function that was observed in 3 of 5 children presenting in infancy, an interesting finding in an autosomal-dominant disorder caused by a structural mutation. Note that the improvement was not universal; 2 individuals (one from each family) diagnosed in childhood required cardiac transplantation in early adulthood and another died during the initial heart failure workup at age 13 years. Third, although most of the affected patients presenting in middle age are asymptomatic, several exhibit evidence of ongoing or triggered remodeling, suggesting a subclinical decrease in baseline function that may represent a continuing susceptibility to cardiac stress conferred by the presence of the D230N *TMP1* substitution. Thus, the current study both confirms and significantly extends our understanding of the sarcomeric DCM phenotype.

The family studies are complemented and extended by a series of in-solution in vitro assays to evaluate the potential effect of the *TMP1* D230N substitution on contractile function at the level of the cardiac sarcomere. Incorporation of a human TM carrying the D230N mutation in the actin-TM-activated ATPase assay revealed a significant

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

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decrease in both the Ca^{2+} dependence of ATPase activity and the maximal ATPase activity as compared with both wild-type and the previously described HCM-linked D175N mutation. In addition, a marked decrease in the Ca^{2+} affinity and cooperativity of Ca^{2+} binding to cTnC was observed in reconstituted thin filaments carrying the *TMP1* D230N substitution. These findings are in full agreement with previous studies in which the investigators used DCM mutations linked to thin filament proteins and support the proposed hypothesis that DCM-linked sarcomeric mutations decrease force generation and lead to a primary dilation of the left ventricle (7,8).

It has been suggested that sarcomeric cardiomyopathies represent the epitome of the interplay between structure and function. It is illustrative to note that mutations in *TMP1* that cause cardiomyopathies are relatively rare and are clustered in discrete regions of the protein. The link between structure and function in TM is precise. Alpha-tropomyosin can reside in 1 of 3 positions or “states” along the actin filament that are determined by the Ca^{2+} binding state of cTnC. At low Ca^{2+} , TM is localized to the outer domain of actin and participates in an effective block of the myosin binding site on actin, thus preventing the formation of the work-performing cross bridges that drive contraction. At high Ca^{2+} , a complex set of allosteric changes in the protein-protein interactions that govern the dynamic position of TM are activated with a resultant shift of the molecule to the inner actin domain, allowing for full myosin head access and strong cross-bridge formation.

It is important to note that these movements represent a continuum across the actin surface and thus can be highly modulated (9,10). A further level of complexity is imposed by the head-to-tail overlap of adjacent TM molecules. The end result is a continuous, flexible coiled-coil that extends the entire length of the thin filament, linking adjacent functional units and influencing cooperativity via a yet unknown mechanism (11). In this context, the complex effects of the *TMP1* D230N mutation are likely to reflect a functional disruption at multiple levels.

Although residue 230 of TM is not within the physical head-to-tail overlap region, it is in proximity and is likely to be involved in important cTnT-TM interactions that are, in part, governed by electrostatic interactions that may be disrupted by the loss of the negatively charged aspartate residue. The subsequent alteration in the central relationship between the Ca^{2+} “transducer,” troponin, and the actinomyosin “gatekeeper,” TM, may significantly alter the distribution of TM across the actin surface and impair normal cross-bridge activation, an effect that may be transmitted across adjacent functional units linked by the mutant TM.

It would be interesting to determine whether the HCM-linked D175N mutation altered the Ca^{2+} -activated physical distribution of TM in the “opposite” direction as compared to the DCM-causing D230N mutation. The end result would likely be the complex and large effects on myofila-

ment activation as were measured in the current study. Recent advances in high-resolution imaging and single myofibril force measurements will likely inform this basic question and yield important information regarding the biophysical role of TM in human DCM.

The basic question remains as to how a single amino acid substitution in a component of the thin filament can result in such a complex cardiovascular phenotype. Although the answer to this seemingly eternal question remains unclear, the striking clinical similarities between the 2 unrelated families in the current study afford a rare opportunity to begin to link genotype to phenotype in DCM. The well-defined clinical “states” provide a framework for further studies. One important consideration, however, is that ventricular remodeling represents the end point of many different, often initially compensatory myocellular responses that are secondary to the initial mutation at the level of the sarcomere. For example, animal models of HCM-linked sarcomeric mutations have exhibited downstream effects on Ca^{2+} handling, beta-adrenergic signaling, and myocardial energetics (12,13). Thus, as noted by the investigators, the striking improvement of 3 of 5 affected infants may be the result of a 2-hit mechanism whereby the D230N *TMP1* mutation confers susceptibility to myocardial injury.

Along similar lines, the downstream effects of the primary loss of sarcomeric contractile function may lead to a new molecular “set-point,” with a corresponding decrease in available cardiac reserve, thus rendering the myocardium particularly sensitive to stresses such as systemic illness or the plasma volume increases that occur in pregnancy. Aggressive medical therapy may provide adequate support to allow the return to this new equilibrium state. Finally, the clinical phenotypic changes may also reflect changes in sarcomeric protein isoforms that may attenuate the primary effects or alterations in mutant protein dose over time. Like all translational studies there are many remaining questions, but many of them can be addressed, and the current study provides a wealth of information that will have an impact on both clinical management of early-onset DCM and our understanding of the delicate interplay between sarcomeric structure and ventricular function.

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REFERENCES

1. Geisterfer-Lowrance AA, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy. A α cardiac myosin heavy chain gene missense mutation. *Cell* 1990;62:999–1006.
2. Ahmad F, Seidman JG, Seidman CE. The genetic basis for cardiac remodeling. *Annu Rev Genomics Hum Genet* 2005;6:185–216.

3. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MR, Towbin JA. Genetic evaluation of cardiomyopathy—a Heart Failure Society of America practice guideline. *J Card Fail* 2009;15:83–97.
 4. 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.
 5. Mogensen J, Murphy RT, Shaw T, et al. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 2004;44:2033–40.
 6. Lakdawala NK, Dellefave L, Redwood CS, et al. Familial dilated cardiomyopathy caused by an alpha-tropomyosin mutation: the distinctive natural history of sarcomeric dilated cardiomyopathy. *J Am Coll Cardiol* 2010;55:320–9.
 7. Chang AN, Harada K, Ackerman MJ, Potter JD. Functional consequences of hypertrophic and dilated cardiomyopathy-causing mutations in alpha-tropomyosin. *J Biol Chem* 2005;280:34343–9.
 8. Carballo S, Robinson P, Otway R, et al. Identification and functional characterization of cardiac troponin I as a novel disease gene in autosomal dominant dilated cardiomyopathy. *Circ Res* 2009;105:375–82.
 9. Lehman W, Rosol M, Tobacman LS, Craig R. Troponin organization on relaxed and activated thin filaments revealed by electron microscopy and three-dimensional reconstruction. *J Mol Biol* 2001;307:739–44.
 10. Galińska-Rakoczy A, Engel P, Xu C, et al. Structural basis for the regulation of muscle contraction by troponin and tropomyosin. *J Mol Biol* 2008;379:929–35.
 11. Boussouf SE, Geeves MA. Tropomyosin and troponin cooperativity on the thin filament. *Adv Exp Med Biol* 2007;592:99–109.
 12. Javadpour MM, Tardiff JC, Pinz I, Ingwall JS. Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. *J Clin Invest* 2003;112:768–75.
 13. Baudenbacher F, Schober T, Pinto JR, et al. Myofilament Ca²⁺ sensitization causes susceptibility to cardiac arrhythmia in mice. *J Clin Invest* 2008;118:3893–903.
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- Key Words:** dilated cardiomyopathy ■ hypertrophic cardiomyopathy ■ heart failure ■ sarcomere ■ thin filament.