EDITORIAL COMMENT

Genetics of Dilated Cardiomyopathy

More Genes That Kill*

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Dilated cardiomyopathy (DCM), characterized by left ventricular dilation and systolic dysfunction, is the most common form of heart muscle disease, comprising 60% of the cases of identified cardiomyopathies (1). The disorder is clinically heterogeneous, ranging from affected individuals with clinical presentations of severe symptoms, including heart failure, sudden death, or resuscitated sudden death, to asymptomatic individuals. The underlying etiologies of heart failure, sudden death, or resuscitated sudden death, to with clinical presentations of severe symptoms, including heart failure, sudden death, or resuscitated sudden death, to asymptomatic individuals. The underlying etiologies of DCM include ischemic heart disease, acquired disease caused by viral infection or cardiac toxins, metabolic disorders, and genetic causes. In the latter case, ~30% to 40% of patients with DCM have a familial form of DCM.

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Over the past decade, genetic heterogeneity in DCM has been demonstrated, and many of the causative genes have been identified. These genes appear to encode mutant proteins of the cytoskeleton, sarcolemma, and sarcomere (1), and we previously speculated that the linkage of the sarcolemma, cytoskeleton, and sarcomere would comprise the “final common pathway” of DCM (2).

Little in the way of genotype-phenotype correlations have been published for familial DCM, in part due to the “private mutation” nature of the genes identified and the relatively small number of mutations in any one gene. However, evaluation of the patients and families described as having genetic mutations to date have commonly had severe outcomes. Because DCM is the most common diagnosis requiring heart transplantation and has been reported to result in a 50% five-year mortality rate, it is not surprising that many of the patients studied have poor outcomes. Whether or not the mutated genes identified typically result in a poor prognosis or there is selection bias has not been clarified.

In the study reported by Mogensen et al. (3) in this issue of the Journal, mutations in the genes encoding the sarcomeric proteins troponin C (TnC) and troponin T (TnT) were identified in five families with inherited DCM, including one family with troponin C (TNNC1) mutations and four families with troponin T (TNNT2) mutations. In the families studied, penetrance was 100% and the outcomes were poor, with nine deaths in the 21 gene carriers (43%), including five from heart failure and four with sudden death. In these patients, the mean age at death was 29 years. Another six patients underwent transplantation (29%), with the remaining patients appearing stable while receiving long-term therapy. The authors suggest that mutations in these genes are poor prognostic indicators. Functional studies using the two-hybrid luciferase assay to determine the effects of mutations on sarcomere function suggest that alterations in troponin interactions occur, leading to altered regulation of myocardial contractility.

Several points should be raised. The youngest affected individuals reported were 16 years of age, with none apparently developing disease during early life. Is this due to selection bias, lack of early evaluation, or age-dependent onset due to the need for long-standing mechanical stress? How does the contractile apparatus abnormality lead to the clinical phenotype, and what is the mechanism of sudden death?

Several possibilities exist. These possibilities reflect not only the highly ordered and interactive network of proteins that make up the cardiac sarcomere and determine the force and shortening capability of the myocardium, but also the role of sarcomeric proteins in Ca²⁺ homeostasis. Troponin C is the major Ca²⁺ buffer in the cardiac myocyte, and it is important to understand that the affinity of cardiac troponin C (cTnC) for Ca²⁺ is not a constant. Changes in the ability of TnC to bind and/or hold on to Ca²⁺ would be expected to affect intracellular Ca²⁺, Troponin C Ca²⁺ affinity is regulated by the state of other thin-filament proteins (e.g., phosphorylation of cardiac troponin I [cTnI]), which lowers the affinity, and by force-generating cross-bridges, which increases the affinity. The latter is a significant factor in Starling’s law, in which it appears that increases in length, which are associated with an increased number of cross-bridges reacting with the thin filament, also increase the affinity of TnC for Ca²⁺ and determine the slope of the ventricular end-systolic pressure-volume relationship (4).

Regions of TnT and TnC affected by the DCM mutations reported by Mogensen et al. (3) are critical to protein-protein interactions that signal activation of the actin-cross-bridge reaction. These interactions are triggered by Ca²⁺ binding to TnC, but are fully elaborated and sustained by feedback effects of force-generating cross-bridge interactions with the thin filaments (4). The binding of cross-bridges to the thin filament promotes the binding of near-neighbor cross-bridges and the cooperative spread of activation laterally along the thin filament. Moreover, as mentioned earlier, strong force-generating interactions of
the cross-bridges with the thin filament induce an increase in the affinity of TnC for Ca\(^{2+}\) (4).

Although most of the mutations described in the report by Mogensen et al. (3) have not been thoroughly tested with regard to effects on myofilament function, the deletion mutation cTnT K210 has been reported to induce a depression in the myofilament response to Ca\(^{2+}\) (5). It is likely that the same effect occurs with the R205L mutation. The region surrounding K210 and R205 has been demonstrated to be critical in the control of the myofilament response to Ca\(^{2+}\). Protein kinase C (PKC)-dependent phosphorylation of a T203, T203E, or T203A mutation induces a significant depression in the maximum tension and a decrease in myofilament sensitivity to Ca\(^{2+}\) (6). Moreover, these changes also induce a decrease in filament sliding velocity, suggesting that afterload velocity of contraction would be reduced, leading to a reduction in power. Protein kinase C is activated in heart failure (4), and it will be of interest to determine whether the mutations linked to DCM are exacerbated with PKC-dependent phosphorylation of nearby residues. The region surrounding K210 is localized in the N-cap of a TnT-alpha helix, and molecular modeling has indicated that modification in this N-cap would induce large structural modifications likely to be of significance in transmission of the cTnC Ca\(^{2+}\)-binding signal to tropomyosin (Tm) (6). The result is a systolic abnormality that would contribute significantly to maintenance of cardiac output at elevated end-diastolic volumes, abnormal stretching of the myocardium, and altered gene expression (4). These alterations, which are secondary to the primary defect in the sarcomere, are likely to alter Ca\(^{2+}\) fluxes and be critical in the course of the syndrome, leading to sudden death. Triggered arrhythmias are generated by altered Ca\(^{2+}\) fluxes and delayed afterdepolarizations (DADs). Important factors include alterations in Na/Ca exchange activity and depression in I_{Ks}, the outward current occurring mainly during the resting membrane potential (7). It is apparent, however, that these same alterations may be directly related to mutations in the sarcomere and their effects on cellular Ca\(^{2+}\) fluxes and ionic currents.

Variations in the effects of force-generating cross-bridges on TnC Ca\(^{2+}\) affinity may play a significant role in triggered arrhythmias in hearts with DCM-linked mutations in thin-filament proteins. The mechanism for induction of these arrhythmias involves heterogeneous excitation-contraction (EC) coupling in serially coupled elements of the myocardium (8). Stretches or releases of relatively inactive regions of the myocardium by adjacent, fully active regions lead to a release of Ca\(^{2+}\) from the myofilaments. A release of Ca\(^{2+}\) from the myofilaments is known to occur with changes in length that release bound cross-bridges, thus reducing the affinity of TnC for Ca\(^{2+}\) (8). Triggered, propagated contractions and Ca\(^{2+}\) waves that occur as a result of the release of myofilament Ca\(^{2+}\) are likely to be accompanied by DADs, possibly by the effects on electrogenic Na/Ca exchange or by nonselective sarcolemmal channels. These effects are reminiscent of the triggered arrhythmias occurring in nonischemic heart failure (7). An example of how such a mechanism might contribute to DADs is the abnormality associated with ischemic zones adjacent to well-perfused regions of myocardium. In the case of hearts expressing mutant TnT or TnC, if a mosaic exists, one might imagine similar regions of heterogeneous EC coupling with variations in stresses and strains between regions of the heart expressing mutant and wild-type proteins, which produces a similar effect. Interestingly, the mechanical effects induced by ischemia and by expression of the cTnT-delta K210 mutant are quite similar in involving a depression in the myofilament response to Ca\(^{2+}\).

Linkage of the G159D mutation in the C-terminal lobe of cTnC to DCM is especially interesting in view of its novelty and its location in a region that reacts tightly with an N-terminal region of cTnI at amino acids 33 through 80. The C-terminal lobe of TnC is generally considered to be structural in that, unlike the case with the N-terminal lobe, Ca\(^{2+}\) exchange with the C-terminal lobe is much too slow to occur within a beat of the heart. However, modulation of the interactions of this region of TnC with TnI has been reported to have a significant impact on the force and shortening of cardiac myofilaments. For example, phosphorylation of TnI-S43 and -S45 or mutations S43E, S43E, or S43A, S45A all result in a depression maximum tension (4). Thus, the charge change in the TnC mutation G159D may also modify an interaction of cTnI with cTnC, leading to a reduction in tension-generating capability, shortening, and power generation by the heart. The mechanism for these changes could be related to a defect in transmission of cTnC Ca\(^{2+}\) binding to Tm, but also altered feedback effects of force-generating cross-bridges on the thin filament. Troponin C G159 appears to comprise a C-cap of the terminal alpha helix of TnC and thus may be of considerable structural significance. It will be of great interest to determine the effects of the G159D mutation of cTnC on the function of the sarcomeric lattice of proteins.

Caution, however, should be maintained regarding genotype-phenotype correlation interpretations. Many previous studies on cardiac genetics have suggested “benign” or “malignant” outcomes related to gene mutations (9,10), and many of these have later been shown to be inconsistent and nonspecific (11). Whether these mutations determine the outcome or whether the small number of patients and potential selection bias is responsible for the appearance of a cause and effect relationship remains to be proven.

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