Mutations in the Sensitive Giant Titin Result in a Broken Heart

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Truncations of Titin Causing Dilated Cardiomyopathy
Herman et al

The specialized cytoskeleton of striated muscle cells consists of highly ordered structures, the single contractile units termed sarcomeres. Although once believed to be simply a scaffold for the assembly of structural proteins to generate force and motion, it is now widely recognized that the sarcomeric cytoskeleton is dynamic and is intimately involved in numerous cellular signaling processes that respond to a wide range of extracellular cues.

Sarcomeres in vertebrates are built of an intricate array of myosin-containing thick filaments, actin-containing thin filaments, and titin filaments (Figure, A), along with a plethora of other structural and regulatory proteins. Titin (also known as connectin) is the largest protein discovered in humans to date.1,2 The human titin gene is enormous; at 363 exons, it encompasses the largest number of exons in any single gene and is predicted to encode up to 38 138 residues, or a 4.2-MDa protein.3 Remarkably, a single molecule of titin spans half the length of a sarcomere with its NH2 terminus embedded in the Z-disc and its COOH terminus anchored at the center of the sarcomere, the M-line (Figure, A). This corresponds to a molecular length of approximately 0.9 to 1.5 μm, depending on sarcomere stretch! Because titin filaments from opposite half-sarcomeres fully overlap in the Z-discs and at the M-line, titin filaments form a continuous system within myofibrils.

Although many unique functions for titin have been described, it is most known for its function as a molecular spring; it limits the range of motion of the sarcomere in tension, thus contributing to the passive stiffness of muscle.4–6 The elasticity of titin is modulated through its structure and its isoform diversity generated by alternative splicing from the single transcript of a titin gene. Its structure is highly modular, composed mostly of immunoglobulin domains, fibronectin-like domains, and elastic linker regions, which are responsible for resting (passive) elasticity of muscle.7 These domains unfold when the protein is stretched and they refold when the tension is removed. This force centers the thick filaments in the middle of the sarcomere, sustains sarcomere length homogeneity, and is thus the ultimate regulator of passive tension that determines diastolic filling in the heart.

Titin not only is the main source of passive elasticity in the sarcomere but also it modulates active contractile force.8 It is a hub for signal transduction,9 and it is a proposed (but not yet proven) molecular ruler for sarcomere assembly.10 Furthermore, much attention has been focused on the role of titin as an exquisitely sensitive sensor of mechanical load that triggers adaptations in response to injury and disease.11,12 In particular, the single catalytic kinase domain near the C terminal end in the M-band of titin appears to be pivotal in sensing mechanical load. Single-molecule data suggest that the opening of the titin kinase active site could be achieved by mechanical unfolding of the C-terminal autoinhibitory tail, providing an incredibly sensitive and efficient link between tension and signal transduction pathways required for cellular remodeling.13

Because of its length, complexity, and wide-ranging functions, titin is a chief candidate for disease-causing mutations. Not surprisingly, because of the enormous size of the coding sequence of titin, it has been technically challenging to investigate its role in disease. However, even with these hurdles, point mutations in this giant molecule have been linked to various human myopathies, including hereditary skeletal myopathy with early respiratory failure and tibial muscular dystrophy.12,14,15

In a milestone article published in the February 16, 2012 edition of The New England Journal of Medicine, Seidman et al conquered previous challenges to identify titin-based myopathies (titinopathies). They took advantage of access to genomic DNA isolated from hundreds of subjects in the United States, Italy, and England, coupled with filter-based hybridization capture and next-generation sequencing of 145kb of the TTN gene, including all the annotated exons and splice sites.16 Notably, their work concluded that TTN truncating mutations are the most common known genetic cause of idiopathic dilated cardiomyopathy (DCM). This is a heart muscle disease that underlies one-third of cases of congestive heart failure in which patients are at risk for sudden cardiac death. Specifically, DCM is a disorder in which the systolic pump function of the heart is impaired, leading to progressive weakening and thinning of the ventricular walls and enlargement of the left ventricular cavity, resulting in inefficient delivery of oxygen to the body. It has been reported that

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>30% (maybe up to 50%) of all DCM cases are familial, exhibiting dramatic genetic, phenotypic, and symptomatic heterogeneity.\(^7\)

The Seidman laboratories analyzed DNA from 312 patients with idiopathic DCM. Using next-generation sequencing, they determined that 27% of DCM subjects (54/203), 1% of hypertrophic cardiomyopathy subjects (7/249), and 3% of age-matched subjects with no evidence of heart disease (3/231) contained a single mutation in the TTN gene. Combining data using both next-generation sequencing and the less sensitive traditional dideoxy sequencing (Sanger method), they identified 72 unique mutations in DCM subjects that altered full-length titin. These mutations included 25 nonsense, 23 frameshift, 23 splicing, and 1 large tandem insertion. The penetrance of the mutation was high (>95%) in subjects aged older than 40 years. Because DCM is a condition with significant underlying genetic etiology, it was not too surprising that TTN mutations and DCM were discovered to be co-inherited in the subjects whose families participated in their investigation (combined lod score, 11.1). Although a few families with TTN mutations had been positively linked to DCM,\(^{18,20}\) the findings from the Seidman study are particularly remarkable because their data suggest that >25% of all DCM cases result from mutations in titin. This is a higher percentage than predicted for all other (>40) genes reported to be linked to DCM put together! The other mutations already identified encode primarily cytoskeletal components involved in a wide variety of cellular compartments and pathways that contribute to the mechanotransduction apparatus that allow cardiomyocytes to sense and respond to changes in contractility. These include components of the contractile thick and thin filaments, Z-disc (ie, boundary of each sarcomere), costamere (ie, structural network linking the sarcomeric components to the sarcolemma), extracellular matrix, intermediate filaments, nuclear envelope, and transmembrane proteins, as well as components of gene transcription and splicing machinery and molecules involved in calcium regulation and handling.\(^{21}\) The broad range of previously described causative gene mutations linked to DCM has confounded genetic screening approaches. The identification of such a significant genetic contributor to the clinical burden of DCM represents a welcome addition of order to a chaotic set of syndromes.

There are several additional noteworthy observations reported in the Seidman study. For example, they observed that the rates of clinical outcomes together with cardiac transplantation, implantation of a left ventricular assist device, morbidity, and mortality in the DCM subjects and family members with TTN mutations did not appear to be different based on the type of mutation but were influenced by the sex of the subject. Men with a TTN mutation had adverse events at significantly earlier ages than did women. Furthermore, with the exception of one subject, the subjects with TTN truncating mutations who had DCM did not present with skeletal muscle abnormalities. This is particularly surprising because titin is also the third most abundant protein of skeletal muscle and the titin isoforms expressed in this tissue contain the kinase region. In addition, the TTN mutations identified that result in idiopathic DCM were not scattered uniformly along the length of the enormous gene. The mutations were absent from the Z-disc and M-band regions but were prominently concentrated in a region that associates with the myosin thick filament (A-band): specifically, unique mutations were found in the thin filament–associated I-band region (2), elastic PEVK region (7), A/I junction (4), and in the A-band (40) (Figure B). This is the largest part of the titin molecule and is constitutively expressed in heart and skeletal muscles. Importantly, the truncated titin molecules are missing the only kinase domain of titin as well as interacting sites for numerous important regulatory and structural components, including the following: the integral thick filament–associated protein that cross-links the thick filament and the COOH terminus of titin in an elastic manner, myomesin; the giant muscle-specific protein, obscurin; myosin-binding protein, MyBP-C; the protease calpain-3 (p94); a four-and-one-half LIM–only protein, FHL2/DRAL; and the ubiquitin ligase, muscle-specific ring finger protein-1. The titin kinase region has been proposed to be the mechanosensor that regulates muscle protein expression in a strain-dependent fashion via its interaction with muscle-specific ring finger protein-2, nbr1, and p62, which is potentially a “signosome” that communicates with the nucleus to modulate protein expres-
sion.\textsuperscript{12} How these functional sites and interactions contribute to the progression of DCM is not known.

What are possible explanations for how expression of a truncated form of titin results in DCM? Based on the extensive unique and apparently essential roles attributed to titin in the sarcomere, it is perplexing how a truncated form of titin results in DCM? Based on the full-length titin, leading to proper cardiac function without obvious structural and mechanistic alterations of cardiomyocytes under baseline conditions; this was observed in a DCM-causing TTN insertion mutation knock-in mouse.\textsuperscript{22} In response to stress, the hearts of people with titin truncation mutations could then dec ompensate, resulting in signatures of DCM. Because the Gotthardt group has generated both constitutive and conditional knockout mice missing the M-line titin kinase domain (as well as other M-line interacting sites),\textsuperscript{23} it appears that a way to understand pathogenesis would be to analyze these aged heterozygote titin–deficient mice, which may model humans with DCM-causing truncated titin.

Some proportion of titin mRNAs with nonsense mutations likely would be subject to nonsense-mediated RNA decay and protein surveillance pathways would be expected to degrade some truncated titin peptides. However, a significant amount of the truncated mutant titin would be expected to be assembled into the sarcomere because truncated titin molecules would assemble with their intact N-termini in the Z-disc. Their highly extensible region would be intact in the I-band, but the truncated molecules would be missing a significant portion of their C-terminal A-band regions, particularly missing their autocatalytic titin kinase regions and interacting sites with key structural and signaling M-line components. Interestingly, in support of this, there are several examples in primary cardiomyocyte cultures and in vivo that demonstrate the ability of titin molecules to partially incorporate in the Z-line but do not extend to the M-line.\textsuperscript{23,24} A reduction in full-length titin levels might reduce sarcomere formation and cause cardiac abnormalities. More likely, however, assembly of the truncated protein might cause DCM by a dominant-negative mechanism. Incorporation of truncated titin is likely to result in altered force transmission and to affect stretch-sensing mechanisms involving titin (eg, impairing the ability of the titin to signal that the sarcomere has lengthened abnormally). Disruption of the well-balanced stretch-sensing machinery would be predicted to result in mechanical dysregulation and subsequent cardiac remodeling to attempt to compensate for the strain. Persistent responses at the cellular level lead to global heart alterations that are highly correlated with sudden cardiac death and heart failure.\textsuperscript{21}

As exemplified by Herman et al,\textsuperscript{16} advances in molecular biological techniques are rapidly allowing for improved knowledge of mechanisms responsible for cardiac dysfunction in cardiomyopathies. DCM occurs in 1 in 2500 individuals in the United States. The diagnosis of DCM remains wide-ranging because many insults can cause DCM and, as a result, the approach to its underlying etiology is often difficult. However, taken together, the results presented by Herman et al clearly support the hypothesis that mutations in the titanic TTN gene have a pivotal role in DCM, likely accounting for more than one-quarter of all cases of idiopathic DCM (and approximately 18\% of sporadic cases). Although this study provides a giant step in discovering genetic causes of DCM, much more extensive research, most likely by incorporating next-generation sequencing, is needed to discover the remaining genetic causes of DCM. In addition, based on this study it appears warranted to incorporate next-generation sequencing analyses of TTN into clinical genetic screens.

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**References**

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