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Review

The Rapidly Evolving Role of Titin in Cardiac Physiology and Cardiomyopathy

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ABSTRACT

The giant muscle filament protein titin is encoded by a single gene consisting of 364 exons. In the past, because of its enormous size and complexity, only few titin mutations were discovered causing different cardiac and skeletal muscle conditions; however, the overall role for heritable diseases, in particular dilated cardiomyopathy (DCM), has been significantly underestimated. Recently performed systematic studies using next-generation sequencing (NGS) recognized *TTN* as the major human disease gene for DCM, but at the same time those data sets revealed that unique genetic variations are also more common in the general population than previously expected. Truncating variants in *TTN* have been reported in about 25% of patients with DCM and in 2%-3% of controls; however, most of the disease-associated truncation

RÉSUMÉ

La titine, une protéine géante constituant les myofilaments, est encodée par un seul gène constitué de 364 exons. Du fait de son énorme taille et de sa complexité, on a découvert par le passé que seules quelques mutations de la titine causaient diverses affections du muscle cardiaque et des muscles squelettiques. Cependant, le rôle global des maladies héréditaires, particulièrement celui de la cardiomyopathie dilatée (CMD), a été significativement sous-estimé. Récemment, des études systémiques ayant eu recours au séquençage de nouvelle génération (SNG) reconnaissaient le gène *TTN* comme étant le plus grand gène humain de la CMD, mais en même temps ces ensembles de données révélaient que ces variations génétiques uniques sont également plus fréquentes dans la population générale

According to Greek mythology, the universe was formed and controlled by Titans, God-like giants who created order from chaos. In the same way, the most gigantic of all proteins, titin, brings order to the sarcomere. Despite more than 25 years of research since titin was discovered, its complexity and diverse roles in health and disease are continuously evolving.

The giant muscle protein titin acts as the third filament system of the sarcomere, in addition to the actin and myosin filaments, and has various roles in muscle structure, mechanical function, and signalling. Moreover, *TTN* has been recognized as the major human disease gene for dilated cardiomyopathy (DCM). From this viewpoint, the molecular mechanisms leading to human heart disease are of particular interest. This review focuses on recent implications of genetic variations in the *TTN* gene for human health and disease and summarizes the various roles of titin in cardiac physiology and pathophysiology toward novel approaches for therapeutic interventions.

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See page 1357 for disclosure information.

Structure and Function of Titin and Its Role in Human Cardiac Physiology

The giant muscle protein titin (TTN), the largest known protein in nature, represents the third filament system in cardiac and skeletal muscle and spans half a sarcomere in longitudinal direction from the Z-disk to the M-line (Fig. 1, A and B).[1,2] The up to 4-MDa protein, encoded by a single gene on chromosome 2q31 consists of 4 structurally and functionally distinct regions. The N-terminal titin is composed of alternatively spliced Z-repeats and multiple immunoglobulin (Ig) domains embedded in the sarcomeric Z-disk. Here it acts as an anchor molecule and is bound to various Z-disk proteins. The remaining TTN protein is divided into the elastic I-band region consisting of tandem Ig-like domains and the proline (P), glutamate (E), valine (V), and lysine (K)-rich (PEVK) portion, which acts as the molecular spring. The stabilizing A-band region, which binds to the thick muscle filaments, contains Ig-like and fibronectin type III domains and adjoins the M-band region embedding the C-terminus in the M-line and containing the unique serine-threonine kinase domain modulating titin expression and turnover (Fig. 1B).[3-5]

Titin has numerous roles in the heart and is also known to be highly variably regulated in cardiac development, health, and disease.[6,7] Titin acts as the primary scaffold for the correct organization and assembly of the sarcomere and is the defining structural element of the sarcomere, with a single molecule

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variants were found in constitutively expressed exons across the gene and in A-band titin, which is abundant in both major cardiac isoforms N2B and N2BA. Titin isoform composition and switch is an important factor for determination and modulation of titin-based stiffness in health and heart disease. Moreover, other factors, including post-translational modification resulting from phosphorylation and oxidative modifications of titin spring elements contribute at the cellular level to titin's stiffness. A better understanding of titin's role in cardiac (patho)physiology will achieve further insights into the molecular mechanisms leading to heart failure and arrhythmias in patients with DCM caused by titin truncation mutations and may provide potential targets for future therapeutic interventions.

que ce à quoi l'on s'attendait antérieurement. Des variants de troncature dans le gène *TTN* ont été rapportés chez environ 25 % des patients souffrant de CMD et chez 2 % à 3 % des témoins. Cependant, la plupart des variants de troncature associés à la maladie étaient observés dans les exons exprimés de manière constitutive dans tout le gène, ainsi que dans la titine de la bande A, qui est abondante dans les deux principaux isoformes cardiaques N2B et N2BA. La composition et la commutation des isoformes de la titine sont des facteurs importants de la détermination et de la modulation de la rigidité de la titine chez les patients en santé et ayant une cardiopathie. De plus, d'autres facteurs, dont la modification post-translationnelle résultant de la phosphorylation et des modifications oxydatives des éléments élastiques de la titine contribuent sur le plan cellulaire à la rigidité de la titine. Une meilleure compréhension du rôle de la titine dans la (patho) physiologie cardiaque nous enrichira de connaissances plus approfondies sur les mécanismes moléculaires menant à l'insuffisance et aux arythmies cardiaques chez les patients qui souffrent d'une CMD causée par les mutations par troncature de la titine et pourra fournir des cibles potentielles aux interventions thérapeutiques futures.

spanning across all regions from the Z-disk to the M-line. On a broader scale, titin is a major determinant of the myocardium's passive stiffness and elasticity. When the sarcomere is stretched during diastole, the I-band segments gradually lengthen and develop passive tension. Together with the extracellular matrix, this force defines passive myocardial stiffness. Titin-dependent passive tension can be significantly altered in different disease states and physiological processes, for instance by post-translational modifications and isoform composition.[8-13] In addition, titin is not only important for passive force but also has a modulating role in active force development because of its length-dependent activation, a determinant of the Frank-Starling relation.[14-16] Finally, titin is also involved in many more cellular processes, such as biomechanical sensing and cell signalling.[17,18]

The *TTN* gene consists of 363 coding exons, which are differentially spliced and could theoretically generate more than 1 million splice variants.[4,11,19] However, only 7 isoforms are described with reference numbers (RefSeqs), ranging from the longest canonical isoform (meta-transcript; NM_001267550) modelled from all 363 exons to the shortest Novex-3 isoform (NM_133379.3), which is truncated at the C-terminal and is expressed in all striated muscles (Fig. 1C).

Titin significantly contributes to muscle elasticity, which is mainly determined by variations in the length of the elastic I-band consisting of more or less Ig-like domains and PEVK repeats.[11] As a general rule, longer titin isoforms with longer PEVK repeats in the I-band impart more elasticity, whereas shorter isoforms provide more passive stiffness. Therefore, expression ratios of longer and shorter isoforms in different types of striated muscle tissue contribute to the elastic properties of the specific muscle. In adult cardiac muscle, 2 major isoforms are present: the long compliant N2BA (NM_001256850) and the shorter stiff N2B form (NM_003319.4) (Fig. 1C). Healthy adult human hearts express 30%-40% N2BA and 60%-70% N2B isoforms in addition to low abundant short novex isoforms. Moreover, the right ventricle expresses more N2BA than does the left ventricle, and the unique isoform in fetal heart is N2BA, which is gradually replaced by N2B in perinatal development, causing increasing titin-based stiffness.[8,10,13,20] Variable isoform expression and *TTN* splicing have become of great importance in different cardiac disease states, including inherited cardiomyopathies.[9,21,22] Moreover, the discovery of the RNA splicing factor RBM20 has been suggested to regulate titin isoform composition in diseased myocardium. Interestingly, whether genetic variations in *TTN* have a disease-causing effect or not is mainly determined by alternative *TTN* splicing in the heart.[19,22-24]

Inherited Titinopathies

With the introduction of NGS, it became clear that titin—encoded by the largest gene in the human genome—harbors an extensive amount of genetic variation. A current view for titin variants in the 6500 National Heart Lung and Blood Institute (NHLBI) Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) reports more than 3000 different variants found in the European/American population and more than 2500 variants in the African American population, demonstrating titin's enormous genetic variability. However, the ability to classify titin variants as disease-associated or benign/innocent variants remains a major challenge. In fact, numerous NGS projects have excluded titin from their analysis pipeline because of the sheer number of variations and difficulties in interpretation of variants in regard to their significance in health and disease. To date, the Exome Aggregation Consortium (ExAC) in Cambridge, Massachusetts (http://exac.broadinstitute.org), consisting of exome sequencing data from a variety of large-scale sequencing projects, is still lacking titin sequencing data. However, knowledge about genetic variants in titin is quite important because there are at least 10 different human conditions affecting the heart or skeletal muscle (or both) associated with titin mutations. The following sections systematically summarize the evolving role of titin for inherited