Understanding Titin Variants in the Age of Next-Generation Sequencing—A Titanic Challenge

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The titin gene (*TTN*), with its 364 exons, encodes the largest human protein. It gives rise to a dizzying array of alternatively spliced isoforms differentially expressed in various skeletal muscles, heart, and in development. Titin is not only the main spring element of the sarcomere, extending all the way from the Z-disc to the M-band, but it is also a stretch sensor and is involved in atrophy and other signaling pathways while interacting with a large and growing number of proteins, exerting many control and regulatory functions in muscles.[1] No transcriptional unit exemplifies the unique diagnostic challenges posed by such a large and complex gene better than *TTN*. Until recently, the biggest challenge was to simply fully sequence *TTN*. Now, next-generation sequencing panels as well as whole-exome platforms have made *TTN* accessible to full-length testing on a routine basis. This development has rapidly increased diagnostic yields while amplifying the challenges posed by an increasingly large number of sequence variants of uncertain significance resulting from this high-throughput sequencing. These findings highlight the urgent need to confidently clarify the relevance of these variants. Savarese et al[2] do an admirable job of illustrating and addressing these challenges.

Related article page 557

The discovery of the first skeletal muscle titinopathy caused by a mutation in the last exon in the M-band of *TTN* by Hackman et al[3] in Finnish patients with dominant tibial muscular dystrophy (and its recessive occurrence as LGMD2J) was a titanic undertaking of manual sequencing.[4] Laborious targeted exploration of the *TTN* sequence then resulted in the discovery of additional skeletal muscle *TTN* disorders, including hereditary myopathy with early respiratory failure,[5] recessive tibial muscular dystrophy, and the recessive Salih myopathy with early cardiomyopathy.[6] With next-generation sequencing facilitating easy surveillance of the entire *TTN* gene, mutations are now discovered at a much more accelerated pace. However, at the same time, variants of unknown significance in *TTN* are also emerging at an even faster pace while the understanding of the normal genomic landscape of *TTN* and its variations remain far from complete. This knowledge gap seriously hampers the proper assignment of pathogenicity of variants detected in a patient undergoing a diagnostic evaluation.

While some types of variants, such as those that are predicted to result in truncation, seem convincing as disease-causing alleles at face value, others, including missense variants, are much less clear and pose the greatest challenge.[7,8] While individually often rare, missense variants as a group are very common in the *TTN* gene. Unfortunately, in silico prediction of the effect of such variants currently is not very reliable for *TTN*. Furthermore, there is good evidence that there are mutations in *TTN* that are not detectable on currently used next-generation sequencing–based platforms (such as some deletions and duplications, inversions, or deep intronic mutations). These uncertainties can have significant consequences in that a titinopathy diagnosis may be either erroneously declared or prematurely discarded. Both scenarios can have consequences for genetic counseling, clinical management, and further diagnostic workup.

It is therefore essential to be able to assign positive or negative plausibility to a titinopathy diagnosis, in particular if the *TTN* genotype found in the patient is incomplete or unresolved. Such scenarios arise when only 1 recessive variant has been detected when 2 would be expected; when there is 1 clear recessive variant, such as a truncation, but in compound heterozygosity with a rare missense variant of uncertain significance; and, perhaps most challenging, when only suspicious-looking missense mutations are found. The diagnostic elements required for this plausibility process rely on the clinician as much as the geneticist, especially because approaching the diagnosis in this situation is an iterative process, returning to all available clinical, histological, genomic, and segregation data.[9]

Savarese et al[2] demonstrate this process. They resequenced 504 undiagnosed patients on a next-generation multigene panel, identifying 9 newly confirmed and 4 potential patients with titinopathy carrying previously reported mutations (ie, hereditary myopathy with early respiratory failure–associated and tibial muscular dystrophy–associated mutations) as well as novel mutations. For recessively acting mutations, they identified clear biallelic truncations, and they also addressed the less obvious diagnostic scenarios outlined above. In particular, Savarese et al[2] included patients with a missense variant in compound heterozygosity with a truncation, patients with an apparently missing second mutation, and patients with rare and potentially pathogenic missense variants on both alleles. The article puts forward a basic algorithm,[2] outlining the elements involved to clarify such situations, which importantly also emphasizes the power of variant segregation studies in the family. In their approach, they also include Western blotting for *TTN*. This is an important addition to the diagnostic toolbox to show potential truncation at the protein level, which is currently only available on a research basis. An important point mentioned in this algorithm is that deep phenotyping needs to be an essential part in the diagnostic approach to support or disprove a diagnosis of titinopathy.

Deep phenotyping as a diagnostic tool draws on the widening spectrum of manifestations associated with *TTN* mutations. This spectrum has expanded considerably since the routine use of next-generation sequencing and will continue to expand in particular for the recessive or, more accurately, biallelic cases (ie, some recessive skeletal muscle disease alleles may also be acting as dominant for conferring a risk for dilated cardiomyopathy[10]). The initial reports of recessive *TTN* disorders have mostly included only those patients with convincing biallelic mutations, which are predicted to be truncating mutations, and if a missense mutation was included, it had been worked up carefully for its functional effect.[6,11,12]

As more and more cases of titinopathy are recognized and reported, the gestalt of *TTN* mutation–compatible phenotypes across age groups is starting to emerge. In addition to the clinical phenotype on examination, the deeper phenotype also includes *TTN*-compatible muscle imaging patterns, such as involvement of the tibialis anterior and of the semitendinosus muscle in the thigh,[13] and the muscle histological appearances that are compatible with *TTN* mutations (the histotype). The increasing knowledge about the functional consequences of the recognized mutations (the physiotype), including Western blot analysis (as convincingly laid out in Savarese et al[2]), together with modeling mutations in animal and in silico models will add a powerful layer to the diagnostic approach. While none or few phenotypic, histological, or functional features in isolation may be entirely diagnostic or specific for a *TTN*-related disorder, their interplay with the genotype creates a good number of combinations that would be *TTN* compatible as well as those that would be *TTN* incompatible, thus helping to assign plausibility to a still tentative interpretation of the genotype. A typical example would be a patient with a convincing truncating *TTN* mutation and a missense *TTN* variant of unknown significance in compound heterozygosity, ie, inherited from either parent. If the patient has a phenotype and muscle imaging pattern that is recognizable as falling within the spectrum of emerging recessive titinopathy and additionally has a consistent *TTN* histotype (for instance, frequent nuclear centralization and some corelike areas), it would be justified to now assign the missense mutation putative causality. However, if only 1 convincing *TTN* mutation has been found in a patient with the same compatible phenotype and histotype , one would then strongly suspect that there might be a second, as-of-yet hidden mutation in *TTN* on the other allele that needs to be addressed with different technology (such as whole-genome sequencing and RNA sequencing).[14]

Ideally, this accumulating collective genotypic, functional, and phenotypic knowledge needs to be captured in a central and accessible database platform with the goal of an ever-improving prediction algorithm for determining the effect (physiotype) of any given *TTN* variant and its associated phenotypes.[15] At some point, it may then be possible to confidently diagnose patients with 2 *TTN* missense alleles, as patients with this genetic scenario must undoubtedly exist. Clearly, the time to take initiative and accept *TTN*'s titanic challenge is now.

ARTICLE INFORMATION

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REFERENCES

1. Gautel M. Cytoskeletal protein kinases: titin and its relations in mechanosensing. *Pflugers Arch*. 2011;462(1):119-134.

2. Savarese M, Maggi L, Vihola A, et al. Interpreting genetic variants in titin in patients with muscle disorders [published online February 12, 2018]. *JAMA Neurol*. doi:10.1001/jamaneurol.2017.4899

3. Hackman P, Vihola A, Haravuori H, et al. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. *Am J Hum Genet*. 2002;71(3):492-500.

4. Udd B, Vihola A, Sarparanta J, Richard I, Hackman P. Titinopathies and extension of the M-line mutation phenotype beyond distal myopathy and LGMD2J. *Neurology*. 2005;64(4):636-642.

5. Ohlsson M, Hedberg C, Brådvik B, et al. Hereditary myopathy with early respiratory failure associated with a mutation in A-band titin. *Brain*. 2012;135(pt 6):1682-1694.

6. Carmignac V, Salih MA, Quijano-Roy S, et al. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. *Ann Neurol*. 2007;61(4):340-351.

7. Chauveau C, Rowell J, Ferreiro A. A rising titan: TTN review and mutation update. *Hum Mutat*. 2014;35(9):1046-1059.

8. Savarese M, Sarparanta J, Vihola A, Udd B, Hackman P. Increasing role of titin mutations in neuromuscular disorders. *J Neuromuscul Dis*. 2016;3(3):293-308.

9. Foley AR, Donkervoort S, Bönnemann CG. Next-generation sequencing still needs our generation's clinicians. *Neurol Genet*. 2015;1(2):e13.

10. Roberts AM, Ware JS, Herman DS, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci Transl Med*. 2015;7(270):270ra6.

11. Chauveau C, Bonnemann CG, Julien C, et al. Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Hum Mol Genet*. 2014;23(4):980-991.

12. Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, et al. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology*. 2013;81(14):1205-1214.

13. Mahjneh I, Lamminen AE, Udd B, et al. Muscle magnetic resonance imaging shows distinct diagnostic patterns in Welander and tibial muscular dystrophy. *Acta Neurol Scand*. 2004;110(2):87-93.

14. Cummings BB, Marshall JL, Tukiainen T, et al; Genotype-Tissue Expression Consortium. Improving genetic diagnosis in mendelian disease with transcriptome sequencing. *Sci Transl Med*. 2017;9(386):eaal5209.

15. Hackman P, Udd B, Bönnemann CG, Ferreiro A; Titinopathy Database Consortium. 219th ENMC International Workshop Titinopathies International database of titin mutations and phenotypes, Heemskerk, the Netherlands, 29 April-1 May 2016. *Neuromuscul Disord*. 2017;27(4):396-407.

JAMA Neurology—The Year in Review, 2017

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On behalf of our team here at *JAMA Neurology*, I want to thank everyone who has contributed to the journal in 2017. A scientific publication is only as strong as its editors, board, reviewers,[1] authors, and readers, and we are incredibly fortunate to have so many great friends and colleagues who have taken the time to help make *JAMA Neurology* successful over the past 12 months.

It has been a busy year for us, with new peaks in numbers of submissions of major manuscripts (1945) and research manu-