Titin and centronuclear myopathy

The tip of the iceberg for *TTN*-ic mutations?

James J. Dowling, MD, PhD

Correspondence to Dr. Dowling: jamedowl@umich.edu

Neurology® 2013;81:1189–1190

Congenital myopathies are a clinically and genetically heterogeneous group of disorders that typically present in infancy with low muscle tone and diffuse muscle weakness.[1] They are defined and subdivided by characteristic features observed on muscle biopsy. Once thought to be extremely rare, it is now clear that congenital myopathies are a commonly encountered clinical entity. One recent study approximates the US pediatric prevalence to be 1:20,000.[2] Despite increased recognition and improved diagnostics, it is estimated that nearly 50% of affected individuals do not have a genetic diagnosis. The lack of genetic resolution is a major barrier for patients/families, clinicians, and researchers, hindering prognostics, recurrence counseling, expectant care, and treatment development. This diagnostic chasm, however, is now being bridged, owing in large part to the utilization of new DNA sequencing technologies. The application of next-generation sequencing has revolutionized gene discovery,[3] and its use for congenital myopathies has identified novel disease genes[4] and has uncovered mutations in known disease genes in new and unexpected clinical contexts.[5]

In this issue of *Neurology*®, Ceyhan-Birsoy et al.[6] report the results of whole-exome sequencing applied to genetically unsolved cases of centronuclear myopathy (CNM), a condition defined by the appearance of internally located nuclei in at least 25% of muscle fibers from a muscle biopsy.[7] This study represents a beautiful merging of next-generation sequencing with an extensively developed and characterized clinical cohort that shares an overarching histopathologic diagnosis. This cohort, assembled by Dr. Alan Beggs through collaborative contributions from multiple clinicians throughout the world, represents an ideal starting point for rare disease gene discovery. The major finding from this study is the identification of recessive mutations in titin (*TTN*) in 5 of 29 individuals with CNM. The important messages from this study are that 1) *TTN* mutations are a common cause of muscle disease, 2) identifying pathogenic *TTN* mutations is tricky and likely requires confirmatory experimentation, and 3) whole-exome sequencing is still only scratching the surface for gene discovery.

Titin, as its name would suggest, is a massive gene. It is composed of 363 exons and is subject to extensive differential splicing, making it one of the largest and most complex genes in the genome. The full-length titin protein is 3.8 million Da, and serves multiple functions, including key roles in establishing muscle elasticity and determining passive muscle force.[8] Given its large size and critical functions, it is not surprising that mutations of *TTN* cause muscle disease. In fact, *TTN* mutations have been identified in several rare myopathies: adult-onset tibial muscular dystrophy/limb-girdle muscular dystrophy 2J,[9] hereditary myopathy with early respiratory failure,[10,11] and early-onset myopathy with fatal cardiomyopathy.[12] Titin mutations are also a common cause of dilated cardiomyopathy.[13] Now, with the addition of mutations in patients with CNM, it seems clear that *TTN* mutations are associated with a very broad range of muscle conditions.

Adding the present study to the existing cases of *TTN* mutations yields a diverse clinical picture. There are both pediatric and adult-onset cases, and cases with predominantly proximal muscle weakness vs those with a distinctively distal pattern. Mutations can be de novo/dominant or recessive, and include missense, nonsense, and splice site changes. No consistent histopathologic pattern unites the cases, though multiple internal nuclei are mentioned for several, and the observation of some type of disorganized myofibrillar material appears common. Therefore, *TTN* mutations need to be considered as potential culprits in myriad clinical and histopathologic settings. Furthermore, a high index of suspicion is needed for *TTN* mutations because of the known association with cardiomyopathy and progressive respiratory failure, potentially fatal complications of disease.

The study by Ceyhan-Birsoy et al. introduces several other important issues. One is the importance of validation studies for sequence variants identified by next-generation sequencing. All 5 identified patients had some type of confirmatory study performed to establish the pathogenicity of the *TTN* sequence variants. Analysis included studies to evaluate RNA

See page 1205

From the Division of Pediatric Neurology, University of Michigan Medical Center, Ann Arbor.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the author, if any, are provided at the end of the editorial.

splicing abnormalities (including mini-splicing assays) and immunofluorescence with multiple antibodies to determine protein expression. The issue of validation experimentation is a critical one to consider as next-generation sequencing reaches the clinical arena. For example, how does one interpret a novel variant in *TTN* discovered through clinical exome sequencing or through a next-generation sequencing panel? Should it be considered causative or, instead, a variant of uncertain significance? This question is particularly troublesome for genes like *TTN* and *RYR1*, which are likely to be both common causes of muscle disease and frequent sources of non-disease-causing variants. Future development of clinically applicable and available validation techniques is thus necessary as genetic diagnostic evaluations increasingly utilize next-generation sequencing.

A final note concerns the current success rate of whole exome–based gene discovery projects. The current study found *TTN* mutations in 5/29 individuals, leaving the majority of the cohort without a known cause. While it is certain that mutations in additional genes will ultimately be identified in this cohort, the results of the present analysis emphasize that 1) 1 or 2 genes are unlikely to account for the majority of cases in any cohort of undiagnosed patients, regardless of the level of uniformity of the cases, and 2) next-generation sequencing is not yet the ultimate solution for gene discovery. These facts reflect the immense complexity of our genetic material as well as our still relatively primitive ability to analyze the gene variant data derived from next-generation sequencing in a meaningful way.

STUDY FUNDING

No targeted funding reported.

DISCLOSURE

The author reports no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

REFERENCES

1. Romero NB, Clarke NF. Congenital myopathies. Handb Clin Neurol 2013;113:1321–1336.
2. Amburgey K, McNamara N, Bennett LR, McCormick ME, Acsadi G, Dowling JJ. Prevalence of congenital myopathies in a representative pediatric united states population. Ann Neurol 2011;70:662–665.
3. Singleton AB. Exome sequencing: a transformative technology. Lancet Neurol 2011;10:942–946.
4. Majczenko K, Davidson AE, Camelo-Piragua S, et al. Dominant mutation of CCDC78 in a unique congenital myopathy with prominent internal nuclei and atypical cores. Am J Hum Genet 2012;91:365–371.
5. Davidson AE, Siddiqui FM, Lopez MA, et al. Novel deletion of lysine 7 expands the clinical, histopathological and genetic spectrum of TPM2-related myopathies. Brain 2013;136:508–521.
6. Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, et al. Recessive truncating titin gene, *TTN*, mutations presenting as centronuclear myopathy. Neurology 2013;81:1205–1214.
7. Jungbluth H, Wallgren-Pettersson C, Laporte J. Centronuclear (myotubular) myopathy. Orphanet J Rare Dis 2008;3:26.
8. Fukuda N, Granzier HL, Ishiwata S, Kurihara S. Physiological functions of the giant elastic protein titin in mammalian striated muscle. J Physiol Sci 2008;58:151–159.
9. 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;64:636–642.
10. Ohlsson M, Hedberg C, Bradvik B, et al. Hereditary myopathy with early respiratory failure associated with a mutation in A-band titin. Brain 2012;135:1682–1694.
11. Pfeffer G, Elliott HR, Griffin H, et al. Titin mutation segregates with hereditary myopathy with early respiratory failure. Brain 2012;135:1695–1713.
12. 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;61:340–351.
13. Herman DS, Lam L, Taylor MR, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med 2012;366:619–628.