

Molecular Diagnosis in 100% of Dystrophinopathies

Are We There Yet?

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Duchenne muscular dystrophy (DMD) is experiencing its renaissance. After being the first muscular dystrophy in which the disease gene has been identified, it is now the first muscular dystrophy to be treated, in some cases, with personalized, dystrophin-restoring therapy.¹ In spite of these tremendous advancements in the treatment of DMD, in this issue of *Neurology® Genetics*, Waddel et al.² represent the challenges in achieving a genetic diagnosis in a cohort of male patients with elevated serum creatine kinase, dystrophin protein studies suggesting an underlying dystrophinopathy, yet normal multiplex ligation-dependent probe amplification and exomic sequencing.

The authors combined omic and bioinformatic analyses to identify causative mutations in their small cohort of undiagnosed patients. This is an important, well-designed, and timely study, which through intelligent use of updated omic resources and experimental data addresses several important issues in the field of dystrophinopathies.

First, the importance to achieve a molecular diagnosis in any suspected dystrophinopathy to allow patients to access potential therapies and families to receive appropriate genetic counselling. Although most of the currently available therapies to restore dystrophin expression (bioactive molecules that modulate the translation machinery reading through premature stop codons during messenger RNA [mRNA] translation, or treating RNA expression with anti-sense oligonucleotides) are not amenable to this cohort of patients harboring large structural rearrangements of the *DMD* gene, the foreseen availability of gene therapy potentially targeted to any DMD patients, regardless of the *DMD* mutation is emerging and likely it will deeply impact prognosis.^{1,3} Under these premises, the achievement of the molecular diagnosis in DMD is of a foremost relevance. The experimental approach used by Waddel et al.² demonstrates also the relevance of muscle biopsy in selected cases of unsolved muscular dystrophy. *DMD* mRNA isolated from skeletal muscle tissue will allow the localization of sequence anomalies in the transcript pointing to deep intronic causative mutations. Indeed, only the complementary use of genome sequencing, transcriptomics, and dystrophin protein studies have allowed a successful mutation identification in this cohort of patients.

Second, the authors attempted to define the amount of full-length dystrophin capable to rescue clinical phenotype using the remnant levels of normally spliced dystrophin *DMD* mRNA in patients with complex splicing mutations. The *vexata quaestio* “how much dystrophin is enough?” is now receiving a new answer.

It is well known that dystrophin is the strongest genetic modifier of the phenotype in the clinical spectrum of dystrophinopathies. If everybody agrees that “the more the better,” more controversies emerge to define the minimum amount necessary to be clinically relevant. Preclinical and single patient reports suggest that 15%–20% homogeneous dystrophin expression is sufficient to completely protect against eccentric contraction-induced injury, but still the debate is active.^{4,5} Waddel et al. using skeletal muscle biopsies reveals that normal dystrophin level $\sim 15 \pm 2\%$ correlates to the

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milder spectrum of dystrophinopathies (i.e., myalgia without overt weakness), $\sim 10 \pm 2\%$ levels of dystrophin predicts a Becker muscular dystrophy phenotype with mild weakness and cardiac involvement, and 0%–5% levels of dystrophin results in severe Becker muscular dystrophy or DMD.² These predictions are in line with early studies in which level of dystrophin 29%–57% of normal was shown to be sufficient to avoid muscle weakness in X-linked dilated cardiomyopathy families.⁶ The novelty in the approach by Waddel et al is the estimate of normal dystrophin vs the amount of internally deleted dystrophin able to modulate the phenotype.¹ In the latter case, not only the amount but also the deleted region of the dystrophin gene is relevant to predict phenotype. Indeed, different regions in dystrophin have variable significance for its function, and thus, the phenotype prediction remain more complex and likely less precise. Upcoming clinical studies in the field of gene therapy will show how this “qualitative” factor will interact with sheer dystrophin quantity to determine the efficacy of various “microdystrophins” engineered into AAV vectors.

This study has impact for its translational implications in the emerging field of personalized medicine in dystrophinopathies

and will help assessing the efficacy of dystrophin-restoring therapies.

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Disclosure

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