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NEWS AND COMMENTARY

Duchenne Muscular Dystrophy: Stalled at the junction?

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A surprising recent study in the nematode worm, *Caenorhabditis elegans*, indicates that genetic defects in acetylcholine (Ach) transmission at the neuromuscular junction (NMJ) might partially underlie Duchenne Muscular Dystrophy (DMD).[1]

In their recent *Nature* paper, Hongkyun Kim and colleagues describe *snf*-6: a gene that encodes a new type of Ach transporter in the worm. Surprisingly, inactivation of this gene results in a phenotype almost identical to that of worms with inactivating mutations in the homolog of dystrophin, the gene that is mutated in DMD, the most common form of lethal degenerative myopathy in humans.

DMD is a progressive degenerative disease that affects cardiac, skeletal and smooth muscle, and neurons. It is caused by mutations in the dystrophin gene, which encodes a large structural protein. Dystrophin in turn organizes the dystroglycan complex (DGC), a cytoskeletal transmembrane protein complex. Mutations in genes that encode the proteins that form the DGC result in pathologies similar to DMD both in humans and animal models. Despite several decades of effort by many groups, the mechanisms that underlie the muscle damage observed in DMD have not been clearly identified.

In 1998, our group showed that the dystrophin gene is conserved in primitive animals, including the worm.[2] To our surprise, null mutations of the so-called *dys-1* gene left the *C. elegans* muscles almost unaffected, but resulted in hyperactivity and a tendency to hypercontract. Moreover, the *dys-1* mutants were hypersensitive to Ach and to the drug aldicarb, which is used in *C. elegans* as an indirect measurement of cholinergic activity. The most straightforward interpretation of this phenotype was – and remains – that the absence of dystrophin upregulates cholinergic transmission in *C. elegans*.[2] It was later shown that acetylcholinesterase (AchE) activity was slightly reduced in *dys-1* mutants.[3] However, since *dys-1* and AchE mutants have different phenotypes in *C. elegans*, it was clear that additional directions had to be explored to explain the *dys-1* phenotype.

These new findings thus come as a long-awaited part in a jigsaw puzzle. Not only are the *snf-6* mutants indistinguishable from the *dys-1* mutants, but the two mutants also behave quite similarly in combination with other mutations. In particular, in worms, as in mice, mutations of the myogenic factor *MyoD* sensitize the muscles and dramatically enhance the phenotype caused by the absence of dystrophin, resulting in a progressive myopathy.[4] The authors report that *snf-6, MyoD* double mutants also display some muscle degeneration. Moreover, they also show that the snf-6 transporter binds to the syntrophin protein (stn-1), an anchoring protein that is part of the dystrophin complex in vertebrate and *C. elegans* muscle.[5] In the absence of either dys-1 or stn-1, snf-6 expression seems reduced.

Therefore, the picture emerging from this paper is that, in *C. elegans*, the muscle-bound snf-6 transporter normally participates in eliminating Ach from the cholinergic synapse. When dystrophin or syntrophin is absent in the worm, the transporter is not properly localized, which results in an increased acetylcholine concentration at the NMJ. In the long run, this defect is deleterious since it can lead to muscle wasting in *C. elegans*. Since the *snf-6* mutant phenotype resembles that of worm dystrophin mutants, it is tempting to speculate that muscle degeneration that occurs in the absence of dystrophin is due to a reduction of snf-6 activity. The definitive test of this intriguing hypothesis would be to attempt to rescue *dys-1* mutants by overexpressing *snf-6*.

So how do these findings affect our understanding of Ach handling in general, especially as it might relate to the pathophysiology of muscular dystrophies in mice and humans? There are certainly some indications that cholinergic transmission at the NMJ might also be important in mammalian dystrophies. For example, sensitivity to aldicarb (which slows removal of Ach from the NMJ) and high Ach at NMJs are reported in mammalian dystrophy.[6] Moreover, we know that increases in AchE are likely secondary to dystrophy in *mdx* mice,[7] the murine model of DMD, and that dystrophin plays a key role in organization of the Ach receptor (AchR), which transduces the transmitter signal to the underlying muscle fiber[8] and NMJ remodeling during muscle regeneration.[9] Dystrophin deficiency in DMD and *mdx* mice was recently suggested to result in autonomic dysfunction such as reduced exercise- or shear-induced arterial dilation,[10,11] and destabilizes

nNOS protein that is important in signaling at the presynaptic region and from the muscle cytoskeleton.

However, there are well-recognized caveats to modeling human or mouse dystrophy in *C. elegans*. Dysfunction in worms is a global locomotion defect, and similar dysfunctions result from mutations of genes other than those related to *dys-1*, *snf-6* or the DGC. Worm locomotion is a relatively coarse screen for phenotypes, compared to clinical and physiology studies in humans and mice, and muscle damage may be uncoupled from the functional phenotypes, as for *dys-1* and *dyb-1* mutations that cause little or no muscle degeneration in *C. elegans*. By comparison, DMD is lethal and *mdx* mice have progressive disease and shortened lifespan, although there are differences reported in the characterization of disease in DMD patients and *mdx* mice. Homologies of the dystrophin–dystroglycan complex proteins also vary widely between *C. elegans* and mammals. In particular, as well as being the homolog of the human dystrophin gene, *dys-1* is the only known worm homolog of the human gene that encodes utrophin (*utr*). So the similarity of *snf-6* and *dys-1* mutants might also reflect the impairment of utrophin-like function, rather than dystrophin-like function, at the NMJ. Nonetheless, the phenotypic similarities suggest functional protein interactions, and the authors provide a strong basis for a potential unifying hypothesis on such neuromuscular disease etiologies.

The breadth of these new worm experiments demonstrates the advantage of this model for studying genetic neuromuscular disorders. Intriguingly, these parallel molecular 'dissections' of the dystrophin protein complex and mutations in a novel Ach transporter molecule might pave the way to an understanding of how various muscle genetic diseases converge toward a similar functional phenotype. The authors' findings suggest a new and testable working hypothesis for the pathophysiology of DMD and other muscular dystrophies. A quick BLAST search reveals that there are uncharacterized *snf-6* homologues in mammals. If *snf-6* mutations in mammalian muscle result in muscular dystrophy similar to that in *mdx* mice and in DMD, and, if snf-6 overexpression can rescue *mdx* mouse muscular dystrophy, this exciting report might point the way to development of new treatment strategies for human DMD and many other conditions that affect cholinergic transmission.

In summary then, these findings suggest that specialized pumps remove some Ach from the synapse, as they do for most neurotransmitters. Second, and perhaps most importantly, they at least raise the possibility that the pathophysiology of DMD in humans could be partially attributed to altered cholinergic transmission or kinetics of acetylcholine at the NMJ. These new results are appealing because they suggest a unifying hypothesis that fits well both with recent *C. elegans* data and with older observations made on vertebrate muscle.

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