

Micro-dystrophin genes bring hope of an effective therapy for Duchenne muscular dystrophy

Kay E. Davies^{1*}, Simon Guiraud¹

¹ MDUK Oxford Neuromuscular Centre, Department of Physiology, Anatomy and Genetics, Oxford OX1 3PT, United Kingdom

*Co-correspondence

Address: MDUK Oxford Neuromuscular Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom

Emails: kay.davies@dpag.ox.ac.uk

Systemic delivery of genes to muscle using vectors based on recombinant adenovirus-associated virus (rAAV) has been explored extensively in animal models of Duchene muscular dystrophy (DMD) to replace the missing dystrophin gene in both skeletal and cardiac muscles (2, 3). A major challenge in bringing this approach to the clinic for DMD is the low gene capacity of rAAV (~5kb) and the large size of the dystrophin mRNA (14kb). Although dystrophin mini-genes and micro-genes were reported almost three decades ago, many lacked important binding sites of the protein. Ramos et al in this issue report the functional evaluation of eight different micro-dystrophin genes whose function is optimised by varying the rod domain structure of dystrophin. This work is significant because although these dystrophin micro-genes are not fully functional, they are showing sufficient promise to be included in clinical trials in DMD patients.

DMD (MIM #310200) is an X-linked recessive disorder affecting 1 in 5000 new born males and caused by mutations which lead to the loss of the large cytoskeletal muscle protein, dystrophin (1). Patients are generally wheelchair bound by the age of 12 years and die in their late twenties or early thirties due to respiratory-cardiac failure. There is currently no effective treatment although promising genetic approaches which extend the ambulatory phase of the disease, such as exon-skipping and stop codon read through, are in the clinic. Nevertheless, these therapies are limited because they are only applicable to a subset of patients with particular mutations.

Dystrophin provides an important structural link between the internal cytoskeleton via F-actin and the dystrophin associated protein complex (DAPC) which lies across the sarcolemma and links to laminin (1). Acting as a shock absorber, dystrophin protects the sarcolemma from damage and stress developed during muscle contraction. Dystrophin has four domains: the N-

terminal region (NTD) contains the binding sites for F-actin, the central rod domain contains 24 spectrin-like repeats interspersed by four hinge regions, the cysteine rich domain (CRD) which binds the dystroglycoprotein complex and the C-terminal domain (CTD) (Figure 1). Loss of either the DAPC or F-actin binding results in DMD but other in-frame deletions of the gene result in milder forms of the disease, Becker muscular dystrophy (BMD, MIM #300376) since the truncated dystrophin molecules are partially functional. The binding of neuronal Nitric Oxide Synthase (nNOS) involves both the N-terminal and rod domains and has been correlated with the severity of disease, although loss of the nNOS localisation is consistent with a mild phenotype in some BMD patients. The concept of a mini-gene being used for the therapy of DMD is based on a very mildly affected BMD patient who had 46% of his dystrophin protein missing with his deletion lying across the central rod domain (4). He presented with muscle weakness in his thirties but he was still able to walk with a stick in his sixties. A biopsy taken from an affected male in another branch of this family confirmed that this deletion results in the localisation of the truncated dystrophin at the sarcolemma. Delivery of a reconstruction of this mini-gene to the *mdx* mouse model of DMD ameliorated the pathology (5) confirming that the mild phenotype is a result of the truncated dystrophin produced rather than genetic background.

Several groups have modified the original BMD patient's dystrophin mini-gene in order to obtain a construct that would fit into an rAAV vector together with the appropriate regulatory sequences, but none show full functional recovery (6, 7). The efficacy of these mini-genes has improved with the addition of nNOS binding sites by including spectrin repeats 16/17 (8) and codon optimisation (9). However, what determines the severity of clinical phenotype is not fully understood. For example, the way in which the spectrin-like repeats assemble relative to each other is more complex than first thought (10), since in-frame deletions of the rod domain result

in differing severities of BMD. Ramos et al report the optimisation of micro-dystrophin genes further by exploring configurations of the rod domain and hinge regions of dystrophin in the *mdx*^{4cv} mouse model. Their previously reported most robust micro-dystrophin gene, μ DysH3, was included for comparison (7). Many of these truncated genes protect the sarcolemma from contraction induced injury and increase force generation, as assayed initially by direct intramuscular injection (IM) to mouse muscle and then by systemic delivery. Micro-dystrophin μ DYs3 (which also contained hybrid spectrin repeats) and μ DYs4 both lacked the nNOS binding site in repeats 16 and 17 and performed less well than μ DysH3 when tested by IM and were not tested further by systemic delivery. These initial studies were undertaken using the CMV promoter but the systemic delivery was performed using a muscle specific promoter smaller in size than CMV which resulted in a smaller overall gene size and better viral titres. The micro-dystrophin genes tested are summarised in Figure. 1, alongside additional micro-dystrophins that have been tested by other groups. As expected, the micro-dystrophins lacking the spectrin repeats 16 and 17 were less effective since they could not localise nNOS. Micro-dystrophin constructs were also tested in mice for the longevity of expression. The configuration μ DYs5 with 5 spectrin repeats was the most functional. Interestingly, no single micro-dystrophin construct performed optimally in all the tests and therefore the choice of the best configuration was a compromise. Functional efficacy of the μ DYs5 gene in the skeletal muscle of the dog model of the disease was recently reported by the same group suggesting that μ DYs5 works well in animals with large muscles and is therefore a good candidate for patient trials (11).

It is also important to consider the ability of these constructs to restore cardiac function. This was not tested by Ramos et al although expression in the heart was observed. Wasala et al have reported the importance of spectrin repeats 16-19 as being protective for the heart (12). It

should be noted that the original BMD patient with the large deletion which includes exons 16-19, lived into his seventies with no obvious cardiac problems. In addition, increased function of the diaphragm has been shown to be beneficial to cardiac function in the mouse and may improve cardiac function in DMD patients (13).

What does this mean for the development of rAAV therapy for DMD patients? The study in this issue together with studies from other groups, suggests that there may not be an optimal micro-dystrophin gene, and that each one is a compromise. The configurations currently in clinical trials are shown in Figure 1. The μ DysH2 configuration is being used by Nationwide/Sarepta with a rAAV-rh74 and by Genethon/Sarepta with a rAAV2/8 in current clinical trials in DMD patients and it remains to be seen whether ringbinden myofibres are a major issue. A Δ R3-19/R20-21/ Δ CTD mini-gene and μ DYs5 both encapsidated within a rAAV9 are in clinical trials (Pfizer and Solid Biosciences). These constructs show excellent functional performance in the dog model of the disease (11, 14). Preliminary data presented at the World Muscle Society conference (<http://investorrelations.sarepta.com/news-releases/news-release-details/sarepta-therapeutics-announces-its-first-rd-day-jerry-mendell-md>) shows expression of the micro-dystrophin in DMD patients after systemic delivery of a micro-dystrophin gene using rAAV. We are at an exciting point in the development of therapy for DMD and eagerly await the final outcome of these trials. However, there will be other challenges in addition to the configuration of the micro-dystrophin. There is a need not only to effectively target skeletal and cardiac muscle, but also to target muscle satellite cells which are less efficiently transduced by certain rAAV serotypes (15). Very high viral titres are required to see a beneficial clinical effect. Timing of delivery may also be crucial as, unlike in the mouse, muscle growth may lead to loss of the virus in young DMD patients, but administration later will be less efficient because of the

significantly reduced muscle mass remaining in which to restore function. Re-administration of virus and an immune response to the dystrophin may also be issues, necessitating the exclusion of some patients with pre-existing immunity. These challenges could be addressed through the delivery of micro-genes modelled on the structure of the dystrophin related protein utrophin (Figure 1) which would not evoke an immune response (16) and the development of non-viral delivery routes. At present, additional obstacles such as producing sufficient high titre virus and the price of treatment are preventing rAAV therapy from becoming a routine therapy for all DMD patients. Nevertheless, the field has come a long way on its journey from gene to therapy even if there is still a long way to go. Patients and their families now have reason to be optimistic since a truly transformative therapy is on the horizon which will improve the lives of all DMD patients.

Acknowledgements

We acknowledge the Medical Research Council for funding.

Conflicts of interest

None.

Figure

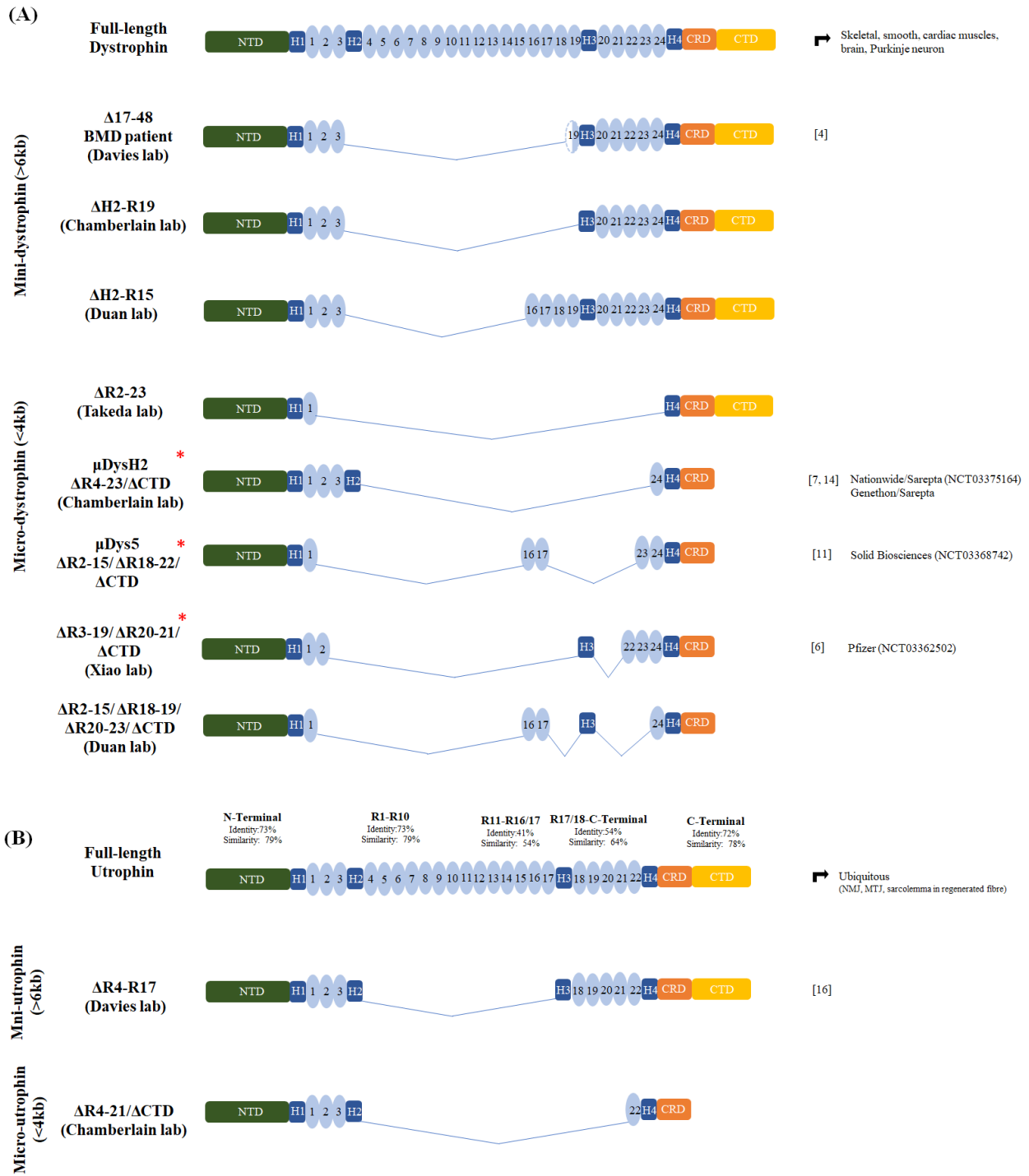


Figure 1. Structures of the full-length dystrophin and utrophin and representative truncated mini and micro-genes

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