Editorial

Gene therapy in myotubular myopathy: promising progress and future directions

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X-linked myotubular myopathy (MTM; OMIM 310400) is a severe congenital myopathy that is classified as one of the centronuclear myopathies (1). MTM is estimated to affect 1/50,000 live born males and is due to loss of function mutations in *MTM1*, which encodes myotubularin, a ubiquitously expressed lipid phosphatase (2,3). The function of myotubularin is still not entirely clear, but a picture is starting to emerge in which myotubularin is a critical maintenance protein of the myofiber sarcotubular system and has a role in excitation-contraction coupling (4-7). The critical role(s) of myotubularin in these specific structural and functional aspects of striated muscle may account for why muscle is so profoundly affected relative to other tissues in myotubularin-deficient MTM patients. Indeed, despite the ubiquity of myotubularin expression, myopathy dominates the clinical picture of affected patients who are born with profound weakness and hypotonia of all skeletal muscles that frequently leads to respiratory failure. Muscle pathology reveals profound myofiber hypotrophy and structural changes such as an increased proportion of muscle fibers with central nuclei. To date intense supportive therapy is the only means of patient management and no effective therapy is available. Despite aggressive care life expectancy is about 2 years in duration and a high proportion of long-term survivors are dependent on life-long mechanical ventilation and require gastrostomy feeding for nutritional support.

A number of different therapeutic approaches have been tested in MTM models; however, since MTM is a monogenetic disorder it was recognized that gene replacement therapy could be effective. Early work showed that local intramuscular injection of *Mtm1* coupled to adeno-associated virus (AAV) in a muscle specific *Mtm1*-knockout mouse model restored myotubularin function and ameliorated the myopathy (6). While this study did not assess the long term effectiveness of the treatment, it did provide an important proof of principle. For MTM gene therapy to move forward improved delivery of the gene therapy vector to muscle tissue and a systemic, rather than a local intramuscular treatment approach, were needed. Furthermore, if gene therapy is to be introduced in clinical care it would be optimal if its effectiveness and potential toxicities could first be longitudinally assessed long-term in a large animal model of MTM.

In a study published in *Science Translational Medicine*, Childers *et al.* addressed many of these needs (8). Childers *et al.* like others working in the field, employed AAV vectors, which are highly suitable and favored for clinical use because they do not cause human disease and are capable of stably expressing its cargo gene over a long period of time. The authors used a muscle-trophic AAV serotype (AAV8) as a vector to deliver a normal copy of *MTM1* and they showed that a single intravascular injection of vector was capable of correcting MTM pathology, strengthening muscles, and improving survival in both a murine and a canine model of MTM long-term. Neither animal model showed evidence of toxicity or an untoward immune response. The importance of this exciting result is amplified by the fact that the experiment was successful in dogs, a large animal model and a first for a monogenetic myopathy.

Childers *et al.* showed that a single tail vein injection of vector in *Mtm1*-knockout mice could correct the

myopathology and reverse the myopathic phenotype whether the mice were treated before the onset of myopathy or late in the disease course. This is a key finding that should not be overlooked since it indicates that MTM pathology is reversible, which is an important aspect of translating this work to patient care. Since the clinical onset of MTM occurs at or near the time of birth patients would undoubtedly be treated when symptomatic. In contrast, gene therapy in animal models of Duchenne muscular dystrophy (DMD), a dystrophinopathy, has not shown the same degree of long-term benefits following a single dose of vector (9,10). Pathologically DMD is very different from MTM. DMD is characterized by inflammation and cycles of myofiber degeneration and regeneration that culminate in myofiber replacement by fibrosis and adipose tissue (4,11,12), while MTM shows myofiber hypotrophy and internal structural alterations, but no significant fibrosis and fat replacement. So then is it reasonable to conclude that the presence of fibrosis and fat replacement impairs the effectiveness of gene therapy in these models? More work is clearly needed to answer that question, but the reversible nature of MTM pathology might mean that gene therapy could be applied to treat other myopathies. Potential good gene therapy candidates appear to include other monogenetic myopathies that are pathologically characterized by minimal fibrosis and fat replacement, including some of the other congenital myopathies such as some of the other forms of centronuclear myopathy, nemaline myopathy, and certain types of core myopathy. More work needs to be done in models of these myopathies but this finding by Childers *et al*. may have broad implications in the treatment of myopathies that extends beyond MTM.

Perhaps the most compelling aspect of the paper by Childers *et al*. is that the experiments were performed using a canine model of MTM. I urge you to acquaint yourself with the story of how the canine model emerged. The story is touching and illustrates the impact a determined and energized mother of an affected son can have on translational research (13). MTM arises in dogs due to an *MTM1* missense mutation that leads to myotubularin protein misfolding and degradation with subsequent loss of function. Affected dogs have a myopathic phenotype that is similar to that encountered in patients and the dogs typically die at about 4 months of age. Using the canine model Childers *et al*. were the first to demonstrate a persistent correction of a monogenetic myopathy in a large animal model following a single intravenous dose of AAV vector.

Initial experiments showed that intramuscular injection of AAV8-canine *MTM1* ameliorated myopathy and improved muscle strength in myotubularin-deficient dogs. Since intramuscular injection may be of limited clinical benefit in limb girdle muscular dystrophy and DMD where it has been used previously (14-16). Childers *et al*. followed-up on the intramuscular injections by delivering AAV8-*MTM1* using an isolated limb perfusion system, which has been shown to yield widespread transduction of muscles in dogs and nonhuman primates (12,17). While the system used by Childers *et al*. is not capable of true systemic vector delivery it can be thought of as the next step-up from local intramuscular injections since it permits the treatment of a large region of tissue, i.e., the hindlimb and this is still an advance in terms of approach. Systemic vector delivery is likely optimal to maximize the effectiveness of gene therapy in treating myopathies as it ensures that all skeletal muscles including respiratory muscles and cardiac muscle can receive a functional copy of the defective gene. This is especially critical in MTM patients as their respiratory muscles are significantly impacted by the myopathy and local injection of each respiratory muscle, including the diaphragm, is simply not feasible. In the past, systemic delivery meant using invasive intravascular delivery methods that were often coupled with the use of toxic compounds to permeabilize target tissues in order to enhance vector uptake. However, the recognition of muscle-trophic AAV serotypes such as AAV8, which was used by Childers *et al*. to deliver a functional copy of *MTM1* permitted the transduction of skeletal and cardiac muscle via a well-tolerated intravascular technique.

While dogs that received intramuscular injections showed improved muscle strength in the injected muscles, dogs treated using the isolated limb perfusion system showed improved strength in both infused and non-infused hindlimbs with near normal strength achieved 6 weeks after infusion. Treated dogs also showed improved respiratory function as measured by normalization of peak inspiratory flow rate, demonstrating that the respiratory muscles received vector. Correcting the dysfunction in respiratory muscles is absolutely critical for gene therapy to be successful in MTM, and this likely played a large role in the enhanced survival observed by Childers *et al*. One of the most exciting results of the studies by Childers *et al*. is that survival was significantly prolonged in the treated dogs and it extended well past 18 weeks when untreated affected dogs lost the ability to ambulate. In fact, treated animals survived well past a year. Histopathologic analysis

of muscle demonstrated improved myofiber architecture and amelioration of, but not a complete reversal of myopathology in some muscles. Myotubularin expression was 21% to 72% that of wild-type control levels in muscle from the infused hindlimbs and 9% to 138% in muscles from non-infused hindlimbs. Necropsy was performed on one dog and myotubularin levels were above control levels in 7 of 13 muscles studied from the infused hindlimb and barely detectable in the contralateral hindlimb or in the forelimbs. Myotubularin expression in the diaphragm and heart of the necropsied dog were 64% and 13% compared to wild-type levels, respectively. These data demonstrate that total reversal of MTM myopathology and the full restoration of myotubularin protein to wild-type levels are likely not required to achieve therapeutic benefit. This indicates that subnormal myotubularin protein levels may be sufficient to ameliorate muscle dysfunction in MTM patients.

The intravascular delivery method was well tolerated by the dogs and none showed evidence of acute or chronic toxicity related to the vector. Liver enzyme levels were normal in all treated animals and liver histology was normal in the necropsied dog. MTM mice treated at the highest dose of vector showed focal inflammation in the heart that was asymptomatic. Cardiac inflammation was not present in the necropsied dog, although myotubularin expression was not fully restored in the heart either, so it may be a question of vector delivery or performance or due to species differences. Since MTM patients are typically affected at birth it will be crucial to develop clinical protocols and practices that are appropriate for infants and young children, which will require a multidisciplinary effort in order to be done properly and safely. Childers *et al*. demonstrated that treated animals showed significant benefit even when myotubularin protein levels were not restored to normal, which raises the issue of how much myotubularin is actually required for proper muscle function and therapeutic benefit? This is a critical question to address and could become important in guiding the development of clinical trials and in managing patient care. More work is needed to estimate the risks of gene therapy in these very young patients and it will be critical to ensure that the proper balance between the risk and benefits of MTM gene therapy and mechanical ventilation and other supportive measures is achieved. Striking such a balance will take time and may need to be done on a case-by-case basis.

Taken together the experiments by Childers *et al*. indicate that intravascular administration of muscle-trophic AAV subtype vectors is likely an efficient and effective method of performing gene replacement therapy in MTM patients. The vector and delivery methods were safe and well tolerated in mice and, more importantly in dogs, a large animal model. Future work will likely focus on developing a well-tolerated and safe means to deliver vector that is truly systemic in extent and capable of transducing all target tissues with a functional copy of the defective gene. Other than supportive care there currently is no treatment for MTM and many patients will die in very early childhood, while those who survive longer require intense medical management and nearly uninterrupted support. This makes the data reported by Childers *et al*. significant and it provides a rationale to start discussing testing gene therapy in a clinical trial for MTM and perhaps even other monogenetic myopathies with the appropriate pathologic features. Their work provides basic scientists new directions for future research, translational-clinical scientists a solid rationale on which to base a clinical trial, and for MTM patients and their families, hope.

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