

Recent Advances in Genetic Muscle Disease – Possible New Treatments in Muscular Dystrophy

Muscular dystrophies are amongst the most important genetic muscle diseases and patients frequently come under the care of a neurologist. Younger onset muscular dystrophies often result in significant disability and cardiorespiratory complications may be fatal. In the last few years there have been significant advances in the area of gene discovery in muscular dystrophy and this has aided diagnosis. Recent research is now moving from gene discovery to molecular therapy and here we have focussed on new gene intervention treatments and new treatments to promote muscle regeneration. Important phase I/II trials in these areas are either in progress or starting.

Diagnosis

For many neuromuscular diseases diagnosis is based on a combination of clinical features and careful evaluation, including EMG, muscle biopsy and, increasingly, gene testing. Standard light microscopy of a biopsy for diagnosis in muscular dystrophy is now inadequate and careful immunohistochemical evaluation using a range of specific antibodies is mandatory to advance to a precise genetic diagnosis - especially in the limb girdle muscular dystrophies. The discovery of causative genes in this group of muscular disorders has led to more accurate diagnosis, clinical management and genetic counselling¹ (Tables 1 and 2). The recognition of important phenotype-genotype relationships allows selection of patients at risk of cardiorespiratory complications, facilitating early intervention which prolongs life. An accurate genetic diagnosis can enable prenatal diagnosis in carefully selected cases.

New treatment trials in muscular dystrophy - Gene therapy and satellite cell stimulation

Recently two new treatment approaches for muscular dystrophy have reached the stage of phase I/II clinical trials. The first group are new gene therapy approaches in Duchenne muscular dystrophy and the second is the modulation of muscle myostatin levels.

Several gene therapy approaches have been developed in Duchenne muscular dystrophy (DMD); these include exon skipping, transfection of truncated dystrophin mini-genes using viral vectors and “read through” of premature stop codons by small molecules that suppress stop codons. Recognition of myostatin as an inhibitor of muscle growth has led to the development of myostatin antibodies that knock down myostatin production in muscle. There is much interest in the potential of myostatin sup-

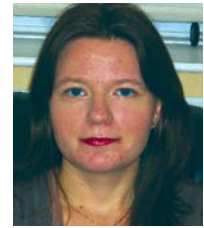
pression as a treatment in a range of genetic muscle diseases including muscular dystrophy.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the commonest muscular dystrophy, affecting 1 in 3 500 live male births.² It is an X-linked recessive disorder due to mutations in the dystrophin gene that encode the subsarcolemmal protein dystrophin leading to a lack of dystrophin expression in skeletal and cardiac muscle. It is a progressive muscle wasting disease and death usually occurs in the third decade from respiratory or cardiac muscle involvement. Animal models which also lack dystrophin expression due to premature stop codons in the dystrophin gene have been used extensively to research this disease. The mdx mouse, although having an almost total lack of dystrophin, has a milder phenotype than that seen in humans, with an almost normal life span.³ Thus some caution has to be used when considering how results of trials in mice may relate to human disease. For this reason the mdx dog is often used in treatment trials as its phenotype is much closer to that seen in boys, with a reduced lifespan resulting from cardiac or respiratory involvement. Furthermore, the muscle mass of the dog is more comparable to that of a child with DMD.³

Exon skipping

Mutations of the dystrophin gene in DMD often disrupt the reading frame, resulting in no production of the key membrane structural protein dystrophin. In contrast mutations in the dystrophin gene found in the milder Becker Muscular Dystrophy (BMD) typically do not disrupt the reading frame and there is some production of a semi-functional dystrophin protein, hence the milder phenotype.⁴ The recognition that a significantly truncated dystrophin gene could still produce a functional protein led to the concept of skipping mutated exons in DMD as a therapeutic strategy. In the last few years morpholino anti-sense oligonucleotides have been developed in DMD to do just this with resultant restoration of the reading frame and increased production of dystrophin. Intramuscular delivery of such oligonucleotides in experiments with the mdx mouse model of DMD confirmed a transient local production of the dystrophin protein with no significant immune response.⁵ More recently, systemic delivery to the mdx mouse has produced dystrophin expression in all skeletal muscles with functional improvement, although expression was not uniformly seen.⁶ To



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Table 1: The Dominantly Inherited Limb Girdle Muscular Dystrophies

LGMD	Chromosome	Gene	Protein	Additional Clinical Features
1A	5q22-q34	Myotilin	Myotilin	Dysarthria Cardiomyopathy
1B	1q11-q21	Laminin A/C*	Laminin A/C	Cardiomyopathy
1C	3p25	Caveolin-3	Caveolin-3	Calf Hypertrophy Rippling muscle disease
1D	6q23	Unknown	Unknown	Cardiac arrhythmias and cardiomyopathy
1E	7q	Unknown	Unknown	Dysphagia No associated systemic features

* Mutations in the Laminin A/C gene are also known to cause Emery-Dreifuss Muscular Dystrophy Type 2, Familial Partial Lipodystrophy and peripheral neuropathy.

Table 2: The Recessively Inherited Limb Girdle Muscular Dystrophies

LGMD	Chromosome	Gene	Protein	Additional Clinical Features
2A	15q15.1-q21.1	Calpain-3	Calpain-3	Distribution of weakness cf. FSHD (scapular, pelvis, trunk). Contractures can occur. Cardiac involvement rare.
2B	2p13	Dysferlin*	Dysferlin	Gastrocnemius wasting. Cardiac involvement rare.
2C	13q12	γ -sarcoglycan	γ -sarcoglycan	Phenotype can be similar to Duchenne Muscular Dystrophy. Respiratory Involvement. Cardiac involvement. Neurosensory hearing loss. Death can occur in 2nd decade.
2D	17q12-q21.3	α -sarcoglycan	α -sarcoglycan	Earlier onset more aggressive than later onset. Cardiomyopathy.
2E	4q12	β -sarcoglycan	β -sarcoglycan	Can be severe phenotype. Can be wheelchair bound in teens. Calf hypertrophy. Cardiomyopathy.
2F	5q33-34	δ -sarcoglycan	δ -sarcoglycan	Can be severe phenotype. Calf hypertrophy. Can be wheelchair bound in teens. Death can occur in 2nd decade. Cardiomyopathy.
2G	17q11-12	Telethonin (Titin Cap)	Telethonin	Foot drop. Cardiac involvement.
2H	9q31-q34.1	TRIM-32	TRIM 32	Variable but generally mild phenotype. Facial weakness.
2I	19q13.3	FKRP gene	FKRP	Respiratory failure. Cardiomyopathy. Calf hypertrophy.
2J	2q31	Titin	Titin	Anterior tibial wasting. No cardiomyopathy reported.
2K	9q34.1	POMT-1	POMT-1	Childhood onset. Contractures. Severe mental handicap.
2L	9q31	Fukutin	Fukutin	Infantile onset. Respiratory and cardiac involvement can occur.
2M	11p13	Unknown	Unknown	Exercise induced myalgia.
2N	19q13	POMT-2	POMT-2	Variable phenotype. Learning difficulties can occur.

*Dysferlin gene mutations are also known to cause Miyoshi Distal Myopathy.

Research is moving from gene discovery to molecular therapy

date none of the experiments with anti-sense oligonucleotides have altered dystrophin expression in the heart. The advantages of this approach are that, in animal models, they have proven to be beneficial without any significant adverse effects or unwanted immune response. The limitations are that the beneficial effects seem to be limited to skeletal muscle and do not include the heart. Due to their transient nature repeated doses are required and the long-term effects of this are not known. The oligonucleotides required to achieve skipping have to be specifically synthesised and tailored to the exact deletion in the dystrophin gene in each patient. This underlines the need to have detailed data bases of carefully genotyped patients as an essential requirement for such translational research efforts. Clinical trials employing intramuscular injections of anti-sense oligonucleotides in boys with DMD are now underway in the UK and Europe.⁷

Viral vectors

Another approach to restore some functioning dystrophin to the muscle fibre membrane has been to use viral vectors to deliver 'mini' or 'micro' dystrophin genes. Several viral vectors have been tried but currently adeno-associated viruses are the vectors of choice because of their low pathogenicity in humans and their ability to cross the vascular endothelium and enter skeletal muscle when injected intravenously.⁸ Intravenous administration in the mdx mouse of an rAAV6 – microdystrophin produced uniform distribution of dystrophin not only in

skeletal muscles but also in the heart without provoking an immune response. An improvement in skeletal muscle function and prolonged life expectancy was also observed.⁹ Extension of experiments to canine models however provoked a profound T-cell mediated immunological response to the AAV capsid proteins following IM injection of AAV-mediated transgenes with inhibition of long-term transgene expression.^{10,11} A recent study has indicated that transient immunosuppression may limit the reaction and prevent disruption of transgene expression. This was achieved in canines but required an aggressive immunosuppressant regime of anti-thymocyte globulin, cyclosporine and mycophenolate.¹²

The advantages of the viral vector approach are that uniform expression of dystrophin can be achieved in all skeletal muscles and in the cardiac muscle. There is also prolonged transgene expression, although the exact duration is unclear; it is not considered to be infinite. The major disadvantage is the possibility of a similar immunogenic response that is observed in canines being found in humans. Another potential limitation to clinical use is the current large number of viral particles required to produce a single effective human dose.¹³ Clinical trials are currently underway in France and the USA in limb girdle muscular dystrophy (LGMD) 2C and DMD using viral vectors for transgene delivery.¹⁴

Read through of stop codons

A third approach in the treatment of DMD has

been to consider read through of premature stop codons which could potentially benefit the 10-15% of BMD cases with stop codon mutations. Aminoglycosides are known to achieve this by preventing nonsense mediated mRNA decay leading to increased protein production, but their use is limited by their oto- and nephrotoxicity. This observation however led to the development of PTC124, a new drug that selectively promotes read through of premature stop codons without affecting normal non-premature stop codons.¹⁵ Trials in the mdx mouse confirmed increased dystrophin production in all skeletal muscles tested and the heart with accompanying improvement in muscle function and lack of adverse events. Clinical trials are now underway.

Myostatin

In 1997 a new member of the transforming-growth factor β family of growth and differentiation factors was discovered. Initially labelled as growth/differentiation factor-8 (GDF-8), studies in mice demonstrated a specific effect on skeletal muscle, with null animals exhibiting a uniform dramatic increase in skeletal muscle mass. This negative inhibitor of skeletal muscle growth therefore became known as myostatin.¹⁶ It was predicted that blocking myostatin could be used to increase muscle mass in muscle wasting diseases. Animal studies tested this by weekly intraperitoneal injections of myostatin antibodies in the mdx mouse for three months. Treated mice displayed a significant increase in skeletal muscle mass and strength, with reduc-

tion in serum creatinine kinase to almost control levels.¹⁷

The prospect of myostatin inhibition being used as a therapeutic agent for muscle atrophy from any cause is an exciting one but there may be potential problems with this approach. For example, one recent study evaluating disuse atrophy found that myostatin knockout mice actually lost more muscle mass than control mice.¹⁸ It is clear that myostatin inhibits muscle growth but there are many regulatory factors of myostatin itself and as such its exact role remains to be fully explored.¹⁹ The potential for treatment of muscle atrophy due not only to primary muscle disease but also secondary to systemic illness, drugs or disuse is very attractive, especially for the pharmaceutical industry. However, further work is required to clarify myostatin's specific action in these different mechanisms of muscle damage. The results of a recent Phase II trial in humans with LGMD are awaited.

Conclusion

Recent advances in the genetics of muscular dystrophy have shown that the discovery of causative genes combined with the study of molecular disease mechanisms can identify new therapeutic paradigms. The potential patient benefit of the gene therapy and myostatin pathway manipulation approaches outlined may be significant provided safety can be proved in appropriate trials. Perhaps most promising of all is that some of these approaches, e.g. stop

codon suppression and myostatin pathway manipulation, may have therapeutic potential in a number of different muscular dystrophies and genetic muscle diseases. We are now entering an era of clinical trials for genetic muscle diseases.²⁰

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