Research progress in Duchenne muscular dystrophy

Duchenne (DMD) muscular dystrophy is a muscle wasting condition affecting, in nearly all cases, only boys or young men. However, a few exceptions have been reported where DMD has been diagnosed in girls. DMD is a genetic condition that can be passed down from the parents but can also spontaneously appear without a previous family history.

Finding and characterising the type of mutations has brought vital insight into the underlying biology of this condition as explained below. This knowledge has been crucial for the development of therapeutic approaches and a number of these new technologies are currently tested in clinical trial for their potential to treat DMD.

DMD is usually noticed in children when they are one to three years of age. Boys with DMD in pre-school age generally show difficulty with walking, running, jumping and climbing stairs. As they get older, they will use their hands to help them get up, looking as if they are 'climbing up their legs'. This is called the 'Gower's sign'.

The muscle wasting is progressive and eventually affects all the muscles involved in movement and breathing, as well as the heart. Boys will eventually lose their ability to walk, usually around the age of nine to eleven years of age. Once they are wheelchair users, the complications of DMD including osteoporosis (thinning of the bones), contractures (tightening of joints) and scoliosis (curvature of the spine), become more evident.

In the past, most people with DMD did not live beyond their early twenties. Improvements in the management of the symptoms have meant that life expectancy has increased. At present, average life expectancy for people with DMD is 27 years.

DMD affects around one in 3,500 newborn boys and 100 new cases are being diagnosed each year in the UK. There are thought to be about 2,400 affected individuals in the UK.

The genetic cause of DMD

DMD is caused by mutations in the gene that carries the information for a large muscle protein called dystrophin. These mutations cause complete disruption of the gene.

Dystrophin protein plays a number of vital roles in muscle. It interacts with many other muscle proteins, so that muscle fibres are strongly held together and the muscle contraction is effective. Dystrophin also acts as a shock absorber during movement thereby preventing muscle damage. By interacting with certain proteins, dystrophin has a role in increasing the blood flow to muscle during exercise. In the absence of dystrophin protein, muscle breaks down and wastes away. The muscle damage caused when dystrophin is absent cannot be repaired and muscle is eventually replaced by scar and fat tissue.

DMD is one of the most studied muscle-wasting conditions. However the function of the dystrophin protein is not yet fully understood. There are several animal models available that have been invaluable in investigating the biology of the condition. One of the most used mouse model is the mdx mouse. Much of what we know about the
details of the different events associated with the loss of dystrophin in DMD has been derived from work done on mdx mouse.

The development of treatments for DMD

Besides the interventions offered as the condition progresses (including supportive care, physiotherapy, ventilation for respiratory failure and medications to counter heart problems), steroid treatment is the only therapy routinely used. Steroids can stabilise the strength of DMD muscle for some time so that individuals maintain the ability to walk for one or two years longer into their mid-teens. However continual steroid treatment can cause a number of side effects including weight gain, mood changes and bone thinning.

It is thought that the presence of dystrophin protein might be essential for a treatment to be efficient and the way this can be achieved is by gene and cell therapy. Some of these approaches are mutation specific and therefore have been described as ‘personalised or precision’ medicine.

A first treatment addressing the underlying genetic mutation has received conditional marketing authorisation by the regulatory bodies in Europe. The drug is called Translarna and has been developed by the company PTC Therapeutics and has shown a moderate delay in the progression of the condition in clinical trials.

Translarna targets a specific type of mutation called nonsense mutation which causes cells to stop making the dystrophin protein. Premature stop codons in the dystrophin gene are the underlying cause of DMD in about 13 percent of cases. The strategy used by Translarna is known as stop codon read-through because it causes cells to "read through," or "ignore," erroneous stop signals in the dystrophin gene.

Although the development of Translarna is seen as a breakthrough, there is an urgent need to develop treatments that are more effective and which can be used for boys where the mutation is not a nonsense mutation.

A number of promising therapeutic approaches are currently developed and/or are in clinical trial and are discussed below.

Exon Skipping

Exon skipping uses small DNA fragments (molecular patches) that skip over parts of the mutated dystrophin gene so that a shortened but functional dystrophin protein can be produced. Exon skipping could theoretically be used for 83 percent of all DMD mutations. However as this is a very personalised approach, it would require the testing of a great number of molecular patches.

It is thought that the technology could convert the severe symptoms seen in boys with DMD into milder symptoms observed in individuals with Becker muscular dystrophy (BMD).

This approach is at the advanced stage with two drugs, eteplirsen (Sarepta) and drisapersen (Biomarin) currently in phase III clinical trial to skip exon 51. The difference between the drugs is in the chemical compound that is linked to the molecular patch to ensure efficient delivery to the cells. The clinical trials aim to test
how and to which extend these drugs improve muscle strength and function. A second molecular patch (SRP-4053, Sarepta) to skip exon 53 is also in phase I/II clinical trial.

Completed and ongoing phase III trials have shown that this approach is safe, can lead to dystrophin protein production and in certain cases results in the stabilisation of the condition. Both companies, Sarepta and Biomarin have successfully sought marketing approval for these drugs from the FDA (Food and Drug Administration).

Exon skipping is a complex technology and although the first generation molecular patches are already close to getting to the clinic it is expected that it needs improvements to reach its full potential. Therefore a number of research projects are ongoing to develop the second generation of drugs.

Read more about how exon skipping works.

**Delivering a functional copy of the dystrophin gene to muscle cells using as harmless virus (AAV-microdystrophin therapy).**

This approach is based on the use of a harmless virus called adeno-associated virus (AAV) which has been shown to effectively deliver genes to a range of different types of cells and tissues including muscle. The virus triggers a relatively mild immune response which could allow it to be given more than once. One of the challenges is that the dystrophin gene is too big for the virus to carry. Researchers have made mini- and micro-dystrophin genes.

Following promising results in animal models of DMD, a phase I safety trial in a small number of boys was conducted in the US. However the trial reported an immune reaction to the dystrophin protein produced in this way.

As for exon skipping this is a first generation approach and further research is ongoing to improve the efficiency of the viral system.

**Repairing the mutation in the dystrophin gene by genome editing**

Permanent correction of mutations in patient cells could be achieved using genome editing. This is an exciting, rapidly moving field of research where enzymes are used that act as DNA scissors to cut the dystrophin gene at specific locations. The repair that results can be driven to completely mend the dystrophin gene to restore production of a full-length dystrophin protein. There is the potential to use these DNA scissors directly in patients, but this will require a lot of research and optimisation first. The attraction of gene editing as a therapy is that it would be a one-off permanent treatment and has the potential to provide a cure for people with DMD regardless of their mutation.

This approach is still in its infancy and a lot of preclinical research needs to be carried before clinical trials can be considered.

Read our feature article about genome editing.

**Developing drugs to raise levels of a protein called utrophin to compensate for the missing dystrophin**
Another potential therapy for DMD is to raise the levels of utrophin protein, as a functional substitute for dystrophin. The potential treatment has been developed in the laboratory of Professor Kay Davies in Oxford.

Despite excellent results in animal models, the first clinical trial in healthy volunteers of a first generation drug SMT C1100 lead to the delivery of insufficient amount of the drug to the muscles. SMT C1100 has since been improved and has recently successfully met its primary endpoint of safety and tolerability in a phase I b clinical trial in DMD patients. On the basis of these findings, a second phase I trial tested the drug in boys with DMD when they are on a particular diet. The results showed that a balanced proportion of fats, proteins and carbohydrates increase the levels of SMT C1100 in the blood. A phase II open-label clinical trial to evaluate the safety and longer-term benefits of SMT C1100 on muscle health and function is planned at the end of 2015.

There is now considerable effort ongoing in Oxford to develop second-generation drugs that have the potential to raise utrophin levels further. One such potential drug, SMT022357, has been shown to be distributed along the length of muscle fibres in the mdx mouse. Owing to its physical and chemical properties SMT022357 is likely to be more stable in the body than SMT C1100.

**Developing stem cell therapies**

Although first clinical trials are already taking place, this technology is still in its infancy and is thought to be far from being used in the clinic. The idea behind this approach is to use stem cells to replace the muscle tissue that is lost in DMD.

Considerable research is ongoing into the use of satellite cells which are muscle stem cells that are attached to the muscle fibres. The role of these cells is to repair and replace muscle fibres that are damaged as a result of injury. The hypothesis is that initially these stem cells are active in individuals with DMD or BMD but then lose their ability to repair and replace the damaged muscle cells. The aim of research into satellite cells is to better understand how these cells are regulated and to use them as a way to replace the damaged fibres with healthy ones.

A small scale clinical trial in Italy also tested whether mesangioblasts from healthy donors are safe to use as a treatment for children with DMD. Mesangioblasts are a type of stem cell found in the walls of blood vessels and have the ability to penetrate into muscle tissue. The results of trial have been inconclusive.

Stem cells could be taken from an unaffected donor in which case they have a functional dystrophin gene. However, this would trigger an immune response which would lead to the removal of the cells in the patient’s body. One way to overcome this is to use the patient’s own stem cells. However, before this can be done, the genetic defect causing DMD needs to be corrected.

Another approach is to take skin cells from boys with DMD and genetically re-programme them to become a type of stem cell called induced pluripotent stem (iPS) cells, which could then developed into any cell or tissue type, including muscle. However, this research is still in its infancy.
Even though there are several promising approaches to treat the underlying cause of DMD as described above, scientists believe that a combination of therapeutic approaches will be necessary to efficiently treat affected individuals. This is because the damage caused to the muscle might not be reversed by restoring dystrophin production. Therefore drugs that address complications associated with DMD are also necessary and are being developed. They include the following:

- idebenone which acts on the energy state of the cell and has been shown in clinical trial to delay the respiratory decline seen in DMD patients (recently granted fast track designation from the FDA)
- tadalafil, (a compound that increases the supply of oxygen to the muscles) whose effect on the decline in the walking ability of young DMD boys is being examined (currently in phase I clinical trial)
- eplerenone, a registered heart medication that has very recently been reported to decrease the rate of heart failure in DMD boys when used as part of a long-term combination therapy (currently in phase III clinical trial)
- givinostat, an anti-inflammatory drug (currently in phase I/II clinical trial)
- PF-06252616, an antibody that blocks myostatin which is an inhibitor muscle growth (currently in phase II clinical trial).

The impact of MDUK on DMD research

The charity has supported high quality research into DMD for many years. This has made a considerable fundamental contribution to the technologies that are currently being tested in clinical trial and to recent research advances. However these are first generation treatments and therefore might only have a moderate impact. This is the reason why there is a need to continue to support high quality preclinical research to improve existing technologies.

Exon skipping research

The charity played a pioneering role in the development of exon skipping technology to treat DMD. We started funding research into this potential treatment in the early 1990s and orchestrated the establishment of the MDEX consortium, a group of scientists and clinicians, to advance this technology into clinical trial (2003). We provided crucial support for their successful application to the Department of Health and the Medical Research Council which lead to an award of £2.3M to carry out initial safety and efficacy studies for mutations that are amendable by skipping exon 51. The partner in the pharmaceutical industry was Sarepta Therapeutics who have continued to advance this technology further into phase II/III studies.

The charity also provided funding for the MDEX consortium to apply successfully to the EU FP7 for €5.5M to carry out a phase I trial for mutations amendable by skipping exon 53. We are actively involved in the study to represent the patient’s voice.

The charity is currently funding Professor Matthew Wood – he is part of the MDEX consortium – to further improve and optimize the current exon skipping technology.
He already received a £1.1M Wellcome Trust grant to test this new technology in clinical trial.

We are currently funding research in the laboratories of Professor Matthew Wood in Oxford and Professor Dominic Wells in London to further improve the efficiency of the molecular patches to reach the muscle and the heart.

**Gene therapy**

The charity played a crucial role in the development of a gene therapy approach based on the use of AAVs to deliver a functional copy of the dystrophin gene to the muscle. We funded vital work in the group of Professor George Dickson for the construction of a first generation AAV containing a micro-dystrophin gene. This AAV has been successfully tested in a dog model for DMD in collaboration with the French company Genethon. A first stage clinical trial to test the safety and efficiency is now planned.

Currently we fund two follow up project in Professor Dickson’s laboratory to develop a more efficient AAV that carries a mini-dystrophin gene that is thought to function more efficiently.

However it is expected that an AAV delivering a mini-dystrophin gene to the muscles will only convert the severe symptoms observed in DMD into the milder symptom seen in BMD. Therefore we also support research into a novel gene therapy that would allow delivery of the full-length dystrophin gene to muscle cells. In this project the researchers will use two or three viruses each carrying a different part of the dystrophin gene. In the muscle cell the different parts assemble to form the blueprint to produce a full size dystrophin protein. If successful this approach could be used to treat people with DMD.

**Gene editing research**

Professor George Dickson and his team have developed an innovative technique with the potential to repair the genetic mutation that causes DMD. The ground-breaking technique, described as an application of genome editing, could be the first therapy that offers permanent correction of the genetic mutation in a person’s own DNA. The technique is relevant to all boys and men with DMD.

We awarded a new five-year lectureship to Dr Linda Popplewell at Royal Holloway, University of London who has been working with Professor Dickson for over 10 years on gene based therapies for DMD. Dr Popplewell will use the genome editing technology to remove the mutation causing DMD from a person’s own DNA.

The charity is also funding a project in Professor Francesco Muntoni’s laboratory that uses genome editing to repair duplications in the dystrophin gene.

**Utrophin up-regulation**

The charity has supported the group of Dame Professor Kay Davies at the University of Oxford for more than 25 years for the development of a treatment for DMD based on utrophin up-regulation. Her laboratory identified a first compound, SMT C1100
that has been shown to raise the levels of utrophin in mouse models and is now in clinical trial.

We are currently supporting Professor Kay Davies and Professor Angela Russell to develop more efficient follow-up compounds and to investigate the molecular mechanism of action. A better understanding of how these drugs work will be vital for optimising existing drugs and to identify new therapeutic targets. We have recently awarded a new grant to Professor Davies to continue her search for new compounds that can increase levels of utrophin.

**Stem cell repair**

We currently fund three research projects at University College London investigating the use of stem cells as a therapeutic approach for DMD.

Professor Jenny Morgan is investigating the biological process that leads to muscle fibre death in animal models of DMD. The biological mechanisms that lead to the death of muscle fibres in DMD are not understood, but recent evidence has suggested that a newly discovered process of regulated cell death may play a role. Such a process that is controlled by the cells themselves could offer targets for drugs that could prevent muscle fibre death as a potential therapeutic approach for DMD.

In a second project Professor Morgan is looking into developing new ways to improve the efficiency of stem cell transplantation in damaged muscle using satellite cells.

Dr Saverio Tedesco is working on developing new methods that will allow the repair of a patient’s own stem cells to replace the damaged muscle.

**Identification of DMD biomarkers**

We fund research in the group of Professor Matthew Wood, University of Oxford, who is aiming to identify molecules which could be used as biomarkers for DMD. If successful, these biomarkers could be used to improve diagnosis, measure the progression of the condition more accurately and better assess the benefit of drugs in clinical trials without the painful procedure of muscle biopsies.

**Quality of life**

Evidence suggests that talking about end-of-life care planning can be beneficial for people with life-limiting conditions. Therefore the charity feels it is important to support a project that will increase our understanding of the views men with DMD have about planning end-of-life care. The report that will be produced as a result of such a project will improve care for men with the condition as well as help facilitate discussions about this highly sensitive topic with friends, family members and the wide range of health and social professionals that support men with DMD. We currently support the work of Professor David Abbott at University of Bristol who is investigating the views of men with DMD about end-of-life care and its management as a means to improve quality of life.

**The effect of DMD on the brain**
It is well known that the dystrophin protein acts as a shock absorber and is essential to protect muscle from damage during contraction. However dystrophin protein is also present in the brain and its function is less well understood in this organ. It is clear that boys with DMD have a number of psychological problems such as attention deficit and hyperactivity disorders and obsessive-compulsive disorders as well as learning difficulties and dyslexia. We recently awarded a new grant to Professor Volker Straub at Newcastle University who will investigate the changes that occur in the brain of boys with DMD. This will help understand how the lack of dystrophin affects the brain in DMD and allow scientists to develop better tests and offer more targeted help for families. The research will also enable scientist to understand whether current approaches being developed to treat DMD have a positive effect on the brain.

Summary figure of clinical trials for DMD