

Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD), an X-linked disorder, is the most common muscular dystrophy in children, presenting in early childhood and characterized by proximal muscle weakness and calf hypertrophy in affected boys. Patients usually become wheelchair-bound by the age of 12 years, and die of cardiorespiratory complications in their late teens to early twenties. Advances in the management of DMD, including treatment with corticosteroids and the use of intermittent positive pressure ventilation have provided improvements in function, ambulation, quality of life and life expectancy, although novel therapies still aim to provide a cure for this devastating disorder. The clinical features, investigations, and management of DMD are reviewed, as well as the latest in some of the novel therapies.

Key words: Continuous positive airway pressure, corticosteroids, creatine kinase, Duchenne muscular dystrophy, gene therapy, muscle disease, pediatric

Introduction

Duchenne muscular dystrophy (DMD), an X-linked disorder, is the most common muscular dystrophy in children, presenting in early childhood and characterized by proximal muscle weakness and calf hypertrophy in affected boys. Patients usually become wheelchair-bound by 12 years and die in their late teens to early twenties. Advances in the management of this disorder with supportive therapy, corticosteroids, as well as novel therapies hope to change the outcomes of this disorder.

Clinical Features

Presenting features

The incidence of DMD is approximately 1 in 3500 live male births.^[1]

The most frequent presenting symptoms are motor

delay or an abnormal gait. Affected boys may present with difficulty in running or getting up from the ground, frequent falls, or toe-walking. Most present between three to five years. Less frequent presentations include language or global developmental delay, or incidentally raised serum creatine kinase or transaminase levels when these investigations are performed for other reasons.

Weakness

Proximal weakness affects the lower before the upper extremities, with progression to the point of wheelchair dependence. Eventually distal lower and then upper limb weakness occurs. Weakness of neck flexors is often present at presentation, and most patients with DMD have never been able to jump. Patients have a waddling gait, calf enlargement, and lumbar lordosis which disappears on sitting. Most become wheelchair-bound by age 11-12 years^[2] [Figure 1].

Cardiomyopathy

Cardiac disease consists of dilated cardiomyopathy due to cardiac fibrosis as well as disturbances of rhythm and conduction.^[3-5]

Clinically apparent cardiomyopathy is first evident after 10 years of age, affects one-third of patients by age 14 years, and is present in all patients over 18 years of age.^[5] Preclinical cardiac involvement is seen in 25% of patients under six years of age, with a persistent tachycardia commonly noted.^[5] Atrial and ventricular arrhythmias occur, including premature ventricular beats and more complex or sustained ventricular ectopy, which increase with age and ventricular dysfunction.^[4] Despite the high frequency of cardiac involvement, most patients are relatively asymptomatic due to physical inactivity.^[5]

Respiratory complications

Chronic respiratory insufficiency due to restrictive lung disease is inevitable in all patients. Vital capacity

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increases as predicted until around age 10 years; after this time it starts to decrease at a rate of 8-12% per year.^[6-9] When vital capacity reaches less than 1 liter the risk of death within the next one to two years is relatively high.^[6]

Obstructive sleep apnea is the predominant cause of sleep disordered breathing in the first decade, occurring in up to one-third of patients, with hypoventilation occurring in the second decade.^[10] Four stages of hypercapnic chronic respiratory failure are typically described: Stage 1, sleep disordered breathing without hypercapnia; Stage 2, sleep disordered breathing with hypercapnia during rapid eye movement (REM) sleep; Stage 3, with hypercapnia during REM and Non-REM sleep; and Stage 4, diurnal hypercapnia.^[10-14] At Stage 4, mean survival is less than 12 months without respiratory support.^[15]

Intellectual disability

Intellectual disability is seen in 30% of boys with DMD, with the average intelligence quotient (IQ) being 85, normally distributed one standard deviation below the population norms.^[16-18] Verbal IQ is more impaired than performance IQ.^[16,19] Intellectual disability is not correlated with the severity of weakness.^[16,20,21] Boys with DMD also have a higher incidence of attention deficit hyperactivity disorder.^[22,23]

Orthopedic complications

Scoliosis develops in almost all children with DMD, and impacts on respiratory vital capacity.^[24] It progresses significantly after boys lose ambulation, and maintenance of ambulation slows the rate of progression.^[25] Long bone fractures are common and usually due to falls, affecting 21-44% of boys.^[26,27] Half of the fractures occur in independently ambulatory boys, with 20-40% losing ambulation as a result.^[26,27] Osteoporosis is present in most children with DMD.^[27-29] Loss of bone mineral density begins even when boys are still ambulant,^[27,30] and continues to diminish with age.

Malignant hyperthermia

Whilst the association of malignant hyperthermia with DMD is not clearly established, patients with DMD are thought to have increased risk of malignant hyperthermia, or at least malignant hyperthermia-like reactions if exposed to inhalational anesthetics such as halothane, or succinylcholine.^[31-34]

Pathogenesis and Genetics

DMD is caused by mutations in the *DMD* gene,^[35,36] one of the largest known genes in humans, spanning 2.3 megabases and accounting for 0.1% of the total

human genome.^[37,38] This gene encodes a protein called dystrophin,^[39] which localizes to the cytoplasmic face of the sarcolemma (plasma membrane) of the skeletal muscle,^[40] forming one component of a large glycoprotein complex (dystrophin-associated glycoprotein complex).^[41,42] Dystrophin consists of an N-terminal actin-binding domain, 24 spectrin-like repeat units interspersed by four hinge regions, followed by a cysteine-rich domain and a C-terminal domain.^[39,43] The cysteine-rich domain binds to laminin-2 via alpha and beta dystroglycan, and therefore acts as mechanical link between actin in the cytoskeleton, and the extracellular matrix.^[42,44,45]

The *DMD* gene contains 79 exons, but accounts for only 0.6% of the gene; the rest made of large introns.^[46] The large size of the *DMD* gene makes it susceptible to mutations, with one-third of all mutations arising *de novo*.

Mutations in the *DMD* gene result in loss of function of dystrophin, resulting in a prematurely truncated, unstable dystrophin protein. The reading-frame rule explains the phenotypic differences between DMD and Becker muscular dystrophy (BMD): mutations that disrupt the open reading frame, resulting in an abnormal and truncated dystrophin cause DMD, whilst mutations which maintain the open reading frame, resulting in a shorter lower molecular weight, but partly functional dystrophin, cause BMD.^[47,48] Ninety per cent of cases of DMD and BMD conform to this reading-frame rule.^[48]

The majority of mutations are intragenic deletions, which account for 65-72% of all DMD patients.^[48,49] Most deletions occur in the 'hotspot' region, spanning Exons 45-53,^[50,51] with the most common deletions being of Exon 45 and Exons 45-47.^[48] Single or multiexon duplications are found in 7% of patients,^[48,52] most located in a minor hotspot spanning Exons 2-20.^[50,51] Point mutations, small deletions or insertions account for 20% of patients without deletions or duplications. Most are nonsense, frameshift or splice site mutations; missense mutations are extremely rare.^[48,53]

The precise mechanism of how dystrophin deficiency leads to degeneration of muscle fibers remains unclear. Absence of dystrophin at the plasma membrane leads to delocalisation of dystrophin-associated proteins from the membrane, disruption of the cytoskeleton with resultant membrane instability and increased susceptibility to mechanical stress.^[41,54,55] In addition, altered membrane permeability and abnormal calcium homeostasis are thought to play a role, with increased cytosolic calcium concentration leading to activation of proteases such as calpains.^[55-58] The absence of nitric oxide synthase, delocalized from the subsarcolemmal membrane, may contribute to damage, but is not thought to directly cause dystrophic features.^[59-61]

Various animal models of DMD exist; the most

commonly studied and used is the *mdx* mouse. Several strains of *mdx* mouse have been characterized; the most commonly used strain has a nonsense mutation in Exon 23 of the *DMD* gene.^[62,63]

Investigations

Serum muscle enzymes

The characteristic finding in DMD is a markedly raised serum CK level, at least 10 to 20 times (and often 50 to 200 times) the upper limit of normal before the age of five years. Serum CK concentrations are high even in newborns and prior to any symptoms. The high CK levels at birth can form the basis of neonatal screening for DMD. Levels peak at two to three years of age and then decline with increasing age, due to progressive loss of dystrophic muscle fibers.^[64-66] A serum CK less than 10 times normal in a child with suspected DMD in the first three years of life should raise the question of an alternate diagnosis.^[2]

Serum alanine transaminase and aspartate transaminase levels are raised in DMD and tend to correlate with CK levels.^[67-69] Other enzymes raised in DMD include aldolase and lactate dehydrogenase.^[70-72] Most of these are not specific for muscle and are generally not useful in the diagnosis of DMD.

Electromyography

Electromyography and nerve conduction studies are rarely required in the diagnosis of DMD. Needle electromyography findings are myopathic, with short duration, low amplitude polyphasic motor unit potentials, particularly in proximal muscles.^[2] Abnormal spontaneous activity in the form of fibrillation potentials, positive sharp waves and complex repetitive discharges may be detected due to denervation and some reinnervation in necrotic muscles. This may also result in the presence of satellite motor unit potentials.^[73,74] Over time the motor units become very small and some areas become electrically silent.^[2]

Nerve conduction studies are normal in early DMD. As the disease progresses, compound muscle action potentials decrease in amplitude.

Muscle biopsy

In some centers, muscle biopsy is no longer routine in the diagnostic workup of DMD if genetic testing is positive and the clinical phenotype is consistent. Muscle biopsy is then only performed where genetic testing is negative, or the clinical phenotype is atypical. Others, however, advocate for muscle biopsy to remain a routine investigation in DMD, as it remains the gold standard for diagnosis.^[75]

On light microscopy early changes include degenerating necrotic muscle fibers with invasion by macrophages, as well as clusters of small- to

intermediate-sized regenerating muscle fibers which have basophilic cytoplasm.^[2] Increased variability of muscle fiber size is also seen, initially with larger than normal, then smaller than normal fibers as the disease progresses.^[76] Type 1 fiber predominance is observed, as are hypercontracted muscle fibers. Eventually there is significant replacement of muscle fibers by fat and endomysial connective tissue [Figure 2].

Absent or markedly reduced dystrophin in muscle biopsies of boys with DMD can be demonstrated on immunostaining and/or Western blot analysis, using antibodies directed against different epitopes of dystrophin. Generally, antibodies recognizing the amino-terminus, carboxy-terminus and rod domains are used. Immunostaining using the amino-terminus or rod domain antibodies shows faint sarcolemmal staining in up to 60% of DMD patients. Immunoreactivity to carboxy-terminal antibodies however is absent in DMD and is therefore useful in differentiating DMD from BMD.^[77-79] Western blot analysis allows quantification of the amount of dystrophin protein as well assessment of size of the protein present. In DMD less than 5% of the normal quantity of dystrophin is present when carboxy-terminal antibodies are used, whilst up to 25% of normal dystrophin levels is seen with the use of rod domain antibodies.^[2,80,81] [Figure 3]

Molecular genetic testing

Molecular genetic testing is now the mainstay of diagnosis in most centers. A multiplex polymerase chain reaction (PCR), covering 18 exons at the deletion hotspots developed by Chamberlain and Beggs detected 90-98% of all deletions, although duplications were not identified by this method.^[50,82] More recently, the development of multiplex ligation-dependent probe amplification (MLPA) has provided a more sensitive technique for detecting deletions. All 79 exons are covered by two sets of probes, with individual exons depicted as a single peak. This allows gene dosage abnormalities to be detected, allowing detection of duplications and testing of carrier individuals as well as for deletions.^[83-86] Occasionally, point mutations will also be detected as single exon deletions, with further analysis allowing more specific delineation of the point mutation.^[83]

If MLPA testing is negative, the *DMD* gene can be tested for point mutations. Direct sequence analysis of the *DMD* gene is generally available on a research basis only, due to its labor-intensive and costly nature. Several groups have developed strategies to target exonic regions for direct sequencing after the use of initial screening methods.^[87,88]

A targeted high-density oligonucleotide comparative genomic hybridization microarray that allows high-resolution analysis of the *DMD* gene has also been developed recently, allowing identification of deletions and duplications but also previously unidentified deep

intronic mutations.^[89]

Muscle magnetic resonance imaging

Muscle MRI is usually not performed in DMD for diagnosis, but may be a useful noninvasive tool to evaluate progression of muscle involvement over time. Abnormalities in signal are seen on T1 and T2 images, with initial selective involvement of the gluteus maximus, adductor magnus, quadriceps, biceps femoris, rectus femoris and gastrocnemii muscles.^[90]

Carrier Females

The majority of female DMD carriers are asymptomatic. However, 2-20% of carriers have clinically evident muscle weakness.^[91,92] Weakness is usually mild to moderate, and asymmetric.^[91] Some carriers report myalgia or cramps without weakness.^[91] Creatine kinase levels are raised in 50-60% of known carriers.^[91] Cardiac involvement in carrier females is well recognized, but is usually subclinical, although severe heart failure has been reported.^[93,94] Dilated cardiomyopathy was

present in 8% of carriers in one cross-sectional study, with another 19% of carriers with left ventricular dilatation.^[93] Despite this, however, these carriers do not appear to have reduced life expectancy or increased risk of cardiac death.^[95] The age of onset of cardiomyopathy is unclear, but is thought to be in the early adult years.^[96] There is no association between the degree of muscle weakness and degree of cardiomyopathy.^[91]

Around 20% of carriers have abnormal dystrophin immunostaining on muscle biopsy, with a mosaic pattern of dystrophin-positive and dystrophin-negative fibers present.^[97,98] No association has been found between dystrophin abnormalities and the presence or absence of muscle weakness or cardiomyopathy.^[98]

Management

General management and surveillance

Symptomatic management and surveillance of the orthopedic, cardiac and respiratory complications associated with DMD allows anticipation, early detection and treatment of these complications.



Figure 1: Calf hypertrophy in a boy with DMD

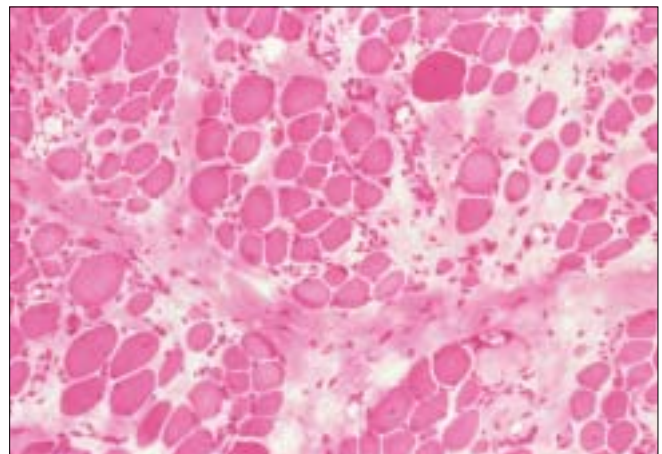


Figure 2: Muscle biopsy of DMD (Hematoxylin and eosin stain) showing marked fibre size variability, degenerating and regenerating fibres, and replacement of muscle by fat and connective tissue



Figure 3A: Normal dystrophin immunostaining of muscle

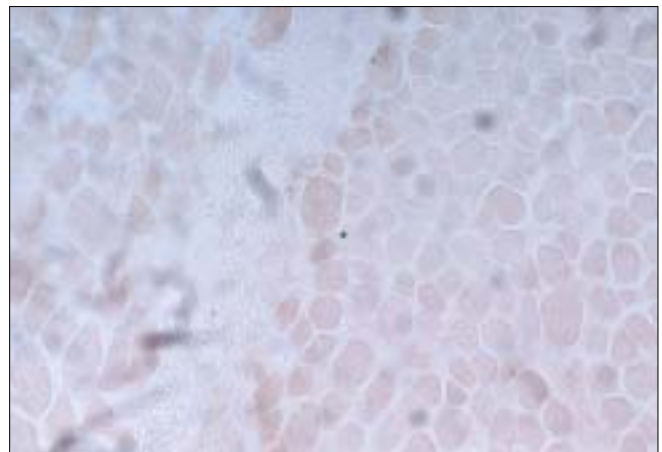


Figure 3B: Absent dystrophin immunostaining in DMD

Cardiac disease

Cardiac surveillance should commence at approximately 10 years of age and continue on an annual or biannual basis. Evaluation should include an electrocardiogram and transthoracic echocardiogram, with consideration of cardiac magnetic resonance imaging in patients with limited echocardiographic acoustic windows. Periodic Holter monitoring should also be considered in patients with known cardiac rhythm abnormalities.^[99]

There is a trend for early treatment of dilated cardiomyopathy with angiotensin converting enzyme inhibitors and beta blockers in DMD, with normalization or improvement in left ventricular dysfunction demonstrated in some patients, thought to be due to cardiac remodeling.^[100,101] Prospective trials are required to evaluate further the optimal management of cardiomyopathy in DMD.

Respiratory disease

Baseline pulmonary function tests and respiratory evaluations should begin at age 8 to 9 years and before ambulation is lost. Ongoing evaluations should occur annually and then biannually after wheelchair confinement. In addition to spirometry, early morning and daytime carbon dioxide levels should be measured. If available, annual polysomnography to detect sleep disordered breathing and nocturnal hypoventilation should also be performed on an annual basis from the time of loss of ambulation. All patients should receive the pneumococcal vaccine and an annual influenza vaccination. Effective airway clearance methods, including the use of manual and mechanical techniques (e.g. mechanical insufflator-exsufflators if available) should be taught.^[102]

Acute respiratory deteriorations due to infections require early management with antibiotics, chest physiotherapy and sometimes respiratory support. Advanced care directives regarding management of these situations should be discussed with the patient and their family from an early stage, providing information about the ventilatory and palliative options available.^[102]

Nocturnal noninvasive intermittent positive pressure ventilation (NIPPV) is a safe and effective treatment for DMD patients with hypercapnia, with early initiation of NIPPV (before the development of daytime hypercapnia) an increasing trend.^[14] Published consenses however, vary on specific recommendations for commencing NIPPV.^[102,103] In some studies nocturnal hypercapnia ($\text{PaCO}_2 \geq 45$ mm Hg) alone has been used as a criterion to commence NIPPV,^[104] whilst in others the presence of clinical symptoms is required. Benefits of NIPPV include increased quality of life, symptom relief, reduction and delay of onset of daytime hypercapnia, and improved life expectancy.^[13,15,104,105] Life expectancy has been

documented to have increased to an average of 25 and even 30 years in patients who receive NIPPV.^[105-107]

Further progression of respiratory failure requires fulltime ventilation, for example with 24 h nasal/mouthpiece ventilation,^[108] or invasive mechanical ventilation via tracheostomy. These types of long-term ventilation are offered in some centers but the ethical, social and economic implications need to be discussed before this therapy is used.

Orthopedic issues

Maintenance of ambulation is foremost in the treatment of a child with DMD to prevent development of contractures and scoliosis, and to maintain independence.

In the early part of the disease course passive stretching, particularly of the Achilles tendons, iliotibial bands and hip flexors, and the use of night splints helps prevent development or progression of contractures. Despite these efforts, contractures ultimately develop and surgery is sometimes necessary.

Only prolongation of ambulation and corticosteroid therapy have been shown to delay the onset, and reduce the severity of scoliosis.^[25,109,110] Surveillance radiographs for scoliosis should be performed yearly from age nine years. The average age of scoliosis surgery is 14-15 years. To qualify for surgery, the scoliosis should be greater than 25 degrees, with the vital capacity being greater than 30% of predicted. Baseline cardiac and respiratory function must be assessed prior. Benefits of scoliosis surgery include the prevention of gross deformity and discomfort, and perhaps some improvements to respiratory function.^[111] It has not been shown to increase life expectancy.^[112]

Maintenance of bone density is important in the prevention of fractures, particularly if the patient is on corticosteroid therapy. Monitoring of Vitamin D levels and supplementation of calcium and Vitamin D should be considered in all patients.^[113-115]

General medical and psychosocial issues

Constipation is a common problem related to immobility and should be managed with an adequate bowel regime, high-fiber diet, and the use of laxatives. Behavioral issues can be a significant problem, particularly around the time of loss of ambulation and independence, and assistance from a psychologist or psychiatrist is sometimes required. Depression is probably under-diagnosed, and should be recognized and treated appropriately.

The provision of extra educational and physical assistance in the classroom is important, allowing many boys with DMD to attend a mainstream rather than a special educational school. This change in educational philosophy has been one of the major advances in management since the 1980s. Nevertheless there are

still some children whose physical and intellectual deficits are such that they require a special educational setting. Some adolescents are able to proceed through the normal secondary education stream and undertake tertiary education. Obtaining meaningful employment remains a major issue.

Corticosteroid Therapy

Corticosteroids such as prednisolone, prednisone and deflazacort have been the only drugs shown to be effective to date in DMD. They should be offered to all patients, but only after a balanced discussion of the potential risks and benefits in the short and long term.

The specific mechanisms by which corticosteroids improve strength in DMD are not known, but various possibilities have been proposed. These include alteration of regulation of genes in muscle fibers,^[116] slowing of the rate of skeletal muscle breakdown,^[117] reducing cytotoxic T cells,^[118] lowering cytosolic calcium concentrations,^[119,120] and increasing myogenic repair.^[121]

A number of reviews and consensus statements have been published on the use of corticosteroids in DMD, the largest two being those published by the American Academy of Neurology (AAN), and the Cochrane database.^[122-125] The AAN practice parameter on the use of corticosteroids for the treatment of DMD^[122] identified seven high-quality randomized controlled trials of prednisone/prednisolone,^[126-132] and two of deflazacort.^[133,134] Results from these trials showed that prednisolone/prednisone improved muscle strength within 10 days, which was maximal at three months and maintained up to 18 months. Increases in muscle strength were paralleled by significant improvements in functional testing (e.g. time to arise from supine to standing, time to walk nine meters) and muscle mass, as measured by urinary creatinine excretion.^[126]

The optimum dose of prednisolone/prednisone is felt to be 0.75 mg/kg/day.^[122,123] Lower doses of 0.3 mg/kg/day still result in improvements in strength and function, but to a lesser degree,^[127,128] but may be used if side-effects require a decrease in dose. Higher doses of 1.5 mg/kg/day did not result in additional benefits,^[126] and alternate daily doses of 1.25 mg/kg and 2.5 mg/kg did not achieve the sustained benefits of daily dosing.^[129] Other types of intermittent dosing such as prednisolone 10 days on and 10/20 days off,^[135,136] and twice weekly prednisolone (5 mg/kg/dose)^[137] have also been described to be beneficial.

There are no good data on the optimal age to begin treatment with corticosteroids, or the optimal duration of treatment.^[122] A common regimen is to offer corticosteroids at the time of decline of muscle strength and frequent falls, and to cease treatment when

the child is no longer ambulant. Studies are lacking in the use of corticosteroids in very young children, and in wheelchair-bound patients. Use of intermittent corticosteroid regimens have been reported in children less than five years of age.^[135,138,139]

Deflazacort, an oxazoline analogue of prednisone, is available in some countries as an alternative to prednisolone/prednisone. Treatment with deflazacort as a daily dose of 0.9 mg/kg/day produces similar sustained improvements in muscle strength and function^[140,141] although there are no large randomized controlled trials comparing deflazacort with prednisolone/prednisone.

There are no long-term randomized controlled trials that demonstrate that corticosteroids prolong the time to loss of ambulation in DMD or improve long-term survival, probably because of the large number of patients and long duration required of such a study to demonstrate these effects.^[116] Non-randomized studies of long-term daily corticosteroids however, do suggest that ambulation may be prolonged by up to three to five years,^[110,142-145] and that life expectancy is improved.^[142]

Corticosteroids also appear to have a positive effect on the complications associated with DMD. Randomized and non-randomized studies have shown preservation of respiratory muscle strength and cough efficiency.^[126,127,142,146] Cardiac function is also better preserved, with a lower frequency of dilated cardiomyopathy seen in boys treated with corticosteroids.^[142,147-149] The prevalence and severity of scoliosis is also reduced in treated boys, with a subsequent delay and decrease in the need for spinal surgery.^[110,148,150]

The commonest side-effects of corticosteroids are weight gain and development of a Cushingoid appearance,^[122] with 75-80% of patients showing significant weight gain.^[127,128] Despite this, weight gain did not appear to adversely affect strength or function in these short-term studies. One small randomized trial suggests a lower incidence and severity of weight gain with deflazacort compared to prednisone.^[140] Vertebral fractures have been detected in 32-40% of boys on long-term corticosteroids, although many are incidental and often not a reason to discontinue treatment.^[110,151] Long-bone fractures are also twice as likely compared to steroid-naïve patients.^[110] Growth suppression is also seen after long-term corticosteroid treatment.^[142] Cataracts (usually asymptomatic) appear to occur with high incidence in deflazacort-treated patients.^[141,142]

Other Drug Therapies

Oxandrolone, an anabolic steroid has shown some promise in increasing quantitative muscle strength in a randomized prospective trial, although not with sufficient magnitude to recommend it for routine use.^[152]

Other immunosuppressive agents such as azathioprine and cyclosporine have also been studied. Azathioprine did not provide any benefit,^[128] whilst cyclosporine showed some improvements in strength.^[153] Creatine monohydrate has been associated with mild increases in muscle strength in DMD, but without significant improvements in functional measures.^[154-156] Other drugs which have failed to show effect include nifedipine,^[157] leucine,^[158] selenium and Vitamin E,^[159] and the antiserotonergic drugs methysergide^[160] and pizotifen.^[161]

Novel Therapies

Gene therapy

Viral vectors

The use of recombinant adeno-associated viral (rAAV) vectors that carry critical regions of the DMD gene is being explored, and is still in its early stages.^[162] As only a small transcript size can be incorporated into available viral vectors, microdystrophin and minidystrophin genes have been created, with restoration of dystrophin expression demonstrated in mouse models.^[163,164] Challenges in this form of gene therapy include the potential need for immunosuppression, and optimizing delivery of the vectors to multiple muscle groups.^[162] The use of non-viral plasmid vectors has also been described.^[165,166]

Antisense oligonucleotide exon skipping

Antisense oligonucleotides can be used to redirect splicing and induce exon skipping, with the aim of restoring the reading frame and producing a partially functioning dystrophin. This is ideal for the majority of DMD mutations, which are out-of-frame deletions or duplications.^[167] Repeated intravenous administration of antisense oligonucleotides has successfully produced widespread dystrophin production in an *mdx* mouse model.^[168] Successful exon skipping was recently demonstrated after intramuscular injection into the tibialis muscle in four patients.^[169] Limitations to this form of therapy include ensuring sustained and safe beneficial effect of the oligonucleotide, as repeated lifelong administration would be required. In addition, different deletions will require different antisense oligonucleotides, making large-scale production difficult and costly.^[170,171]

Read-through stop codon strategies

Some aminoglycoside antibiotics such as gentamicin cause a relaxation in codon recognition, allowing read-through of nonsense mutations, which occur in around 7% of all DMD patients.^[171] Read-through of the nonsense mutation in Exon 23 of the dystrophin gene in the *mdx* mouse model was demonstrated in 1999.^[172] Subsequent clinical trials, however, have not been particularly successful.^[173,174]

PTC124 is a new orally administered investigative

drug that promotes ribosomal read-through of stop codons, allowing continuation of translation and production of a functioning protein.^[175] PTC124 has been shown to restore dystrophin levels in *mdx* mice, with an associated improvement in muscle function and decrease in CK levels.^[176] Phase 1 studies in healthy adult volunteers showed that the drug is orally bioavailable and well tolerated.^[177] A Phase 2 study of 26 boys demonstrated increased full-length dystrophin expression with PTC124, and decreased CK levels, but without significant improvement in timed functions or muscle strength.^[178] Further clinical studies are underway. The main concern regarding the use of read-through stop codon drugs is the theoretical risk of a global read-through of physiological stop codons.

Stem cell therapy

Whilst early experiments of transplanted myoblasts showed promise,^[179] subsequent studies showed that they were rejected by the host immune response in *mdx* mice and in patients with DMD.^[180,181] Autologous muscle-derived stem cells (myogenic stem cells) which are not at risk of host rejection can undergo *ex vivo* gene correction strategies such as small fragment homologous replacement and chimeraplasty, and be subsequently transplanted.^[182,183] Harvesting sufficient numbers of myogenic stem cells from dystrophic muscle however is a significant problem.^[184,185]

Bone marrow-derived stem cells have been shown to remodel muscle, and may be a renewable non-muscle cell type used to remodel dystrophic muscle.^[186] However, because the number of stem cells with myoremodeling capacity is highly diluted when whole bone marrow is transplanted, strategies to rapidly isolate bone marrow-derived stem cells with myoremodeling capacity in sufficient numbers are required to make this a potential therapy.^[187]

Utrophin

It is thought that utrophin, a protein homologue of dystrophin in the sarcolemma, may compensate for dystrophin deficiency if it is upregulated.^[188] Various factors that increase utrophin expression are being explored, such as heregulin and L-arginine, but most are still in the very early stages.^[189]

Prevention and genetic counseling

All families with an affected male with DMD should be referred for genetic counseling. Detection and counseling of female carriers is an important aspect of management for disease prevention. Prenatal diagnosis is available for carrier mothers, and is most accurate if a mutation is detected. Otherwise, linkage analysis is used. Germinal mosaicism is always a possibility in mothers who have negative molecular testing. The risk for future pregnancies being affected in such cases is generally given as 10-20%.

Carrier females of DMD should also be counseled about risks of cardiomyopathy. Periodic cardiovascular screening is recommended, commencing in early adulthood,^[99,190] with repeat evaluations at a minimum of every five years thereafter.^[99]

Conclusions

Duchenne muscular dystrophy is a devastating condition that continues to affect many boys and their families. Recent advances in symptomatic management, with the careful use of corticosteroids, and respiratory support have increased life expectancy and quality of life considerably. The search for a cure remains elusive, although many promising and novel therapies are in progress, some of which have entered the stage of human trials.

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